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New Methods and Techniques

Supplement

Management of Nonmelanoma Skin Cancers

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Introduction

Original Articles

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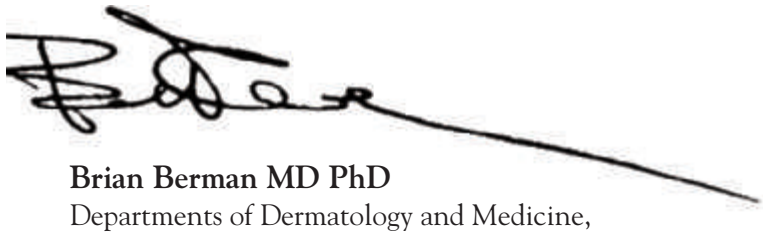
INTRODUCTION

Approximately 1 million cases of basal cell carcinoma (BCC) are expected to occur in the US this year. The current standard treatment of superficial BCC, curettage and desiccation (C&D) alone, is associated with scarring and recurrences. These disadvantages have led investigators to explore the concomitant use of immunotherapy for the treatment of BCC.

Imiquimod (5% cream) is an immune response modifier that has been used alone for the treatment of BCC. When applied topically after C&D, imiquimod appears to reduce the likelihood of recurrence of BCC because imiquimod destroys residual BCC cells and may even induce long-term immune memory directed against tumor cells. Evidence has also been presented that imiquimod may also control excessive postsurgical scarring, a major concern following C&D.

My colleagues and I offer 4 papers in which we describe our experience with the use of imiquimod for the treatment of BCC. My introductory paper summarizes the rationale for combining imiquimod and surgical extirpation; Drs. Tillman and Carroll report their 36-month study of clearance rates, adverse effects, and cosmetic outcome associated with combining curettage and imiquimod; Dr. Rigel reports the results of his use of imiquimod after curettage without electrodessication for the treatment of nodular and superficial BCC; and Drs. Li and Li describe their exploration of imiquimod's ability to stimulate antiangiogenic cytokines, downregulate the expression of proangiogenic factors, upregulate the expression of endogenous inhibitors, and induce cell apoptosis.

Oncologists have long embraced the concept of combining surgery with other therapeutic modalities, including radiotherapy, hormone treatment, and immunotherapy. We hope you find the results of our studies useful in your search for an effective, well-tolerated combination treatment of BCC.



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Scientific Rationale: Combining Imiquimod and Surgical Treatments for Basal Cell Carcinomas

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Abstract
The number of basal cell carcinomas (BCCs) occurring in the US in 2007 has been conservatively estimated to be 1 million. Surgical extirpation alone is the standard for the treatment for BCCs, which results in scarring and is associated with recurrences. We review the rationale for combining surgical extirpation and immunotherapy with topically applied imiquimod 5% cream for the treatment of BCCs.

Immune Response Modifiers

Interferon is the grandfather of all immune response modifiers. Historically, interferon is an antiviral agent, but over the past 25 to 30 years, it has become clear that interferons also possess antiproliferative activities, antiblood vessel forming activities (antiangiogenic), as well as immunomodulatory activities. It is beyond the scope of this article to discuss in any detail the various mechanisms of actions through which the interferons actually exert their various activities, but 2 important mechanisms of actions are worth mentioning. An important ability of interferons is to upregulate tumor suppressor genes, specifically the p53. In addition, interferons are able to enhance apoptosis. Apoptosis is a naturally programmed cell death, which is helpful in certain situations. It is the nature of cells to grow, function at their specific job, reproduce, and then die off. If they lived in perpetuity, in effect one would have a tumor cell line. Nature has included within normal healthy cells multiple pathways to undergo apoptosis and just naturally die off. Unfortunately, certain tumor cells, as well as some virally infected cells, lose this natural ability to apoptose. Interferons, however, are able to enhance and restore the process of apoptosis and allow these abnormal cells to die off naturally.

Imiquimod is another immune response modifier that interacts with monocytes and dendritic cells, including Langerhans cells, via toll-like receptor 7 (TLR-7) and, in so doing, activates the monocytes to elaborate interferon-α (IFN-α).[1,2] IFN-α interacts with lymphocytes of the Th1 subtype activating them to release IFN-γ and interleukin-2 (IL-2), ultimately enhancing antiviral activity, as well as enhancing cell-mediated immunity.[1] In examining the interaction of imiquimod with antigen-presenting dendritic cells and Langerhans cells, the first step is recognizing that the imiquimod molecule comes through an interaction with TLR-7. Upon that interaction, a signal is sent into the cytoplasm of the antigen-presenting cell via MyD88.[2] It is a signal to phosphorylate an inhibitory component of NFκB. The inhibitory protein that becomes phosphorylated is IκB. When IκB is bound to NFκB, NFκB cannot leave the cytoplasm and enter the nucleus to undertake its function in the nucleus; however, once phosphorylated via the indirect action of imiquimod, the IκB protein becomes a substrate or proteolytic cleavage by a proteosome, releasing it from being bound to the NFκB and allowing for the NFκB to enter the nucleus and bind to the promoter region of a variety of genes, including those genes that encode for inflammatory cytokines. The DNA can now be read and transcribed into m-RNA, which then leaves the nucleus, enters the cytoplasm, and is translated into proteins on ribosomes and, ultimately, the proteins (eg, interferon) are secreted.

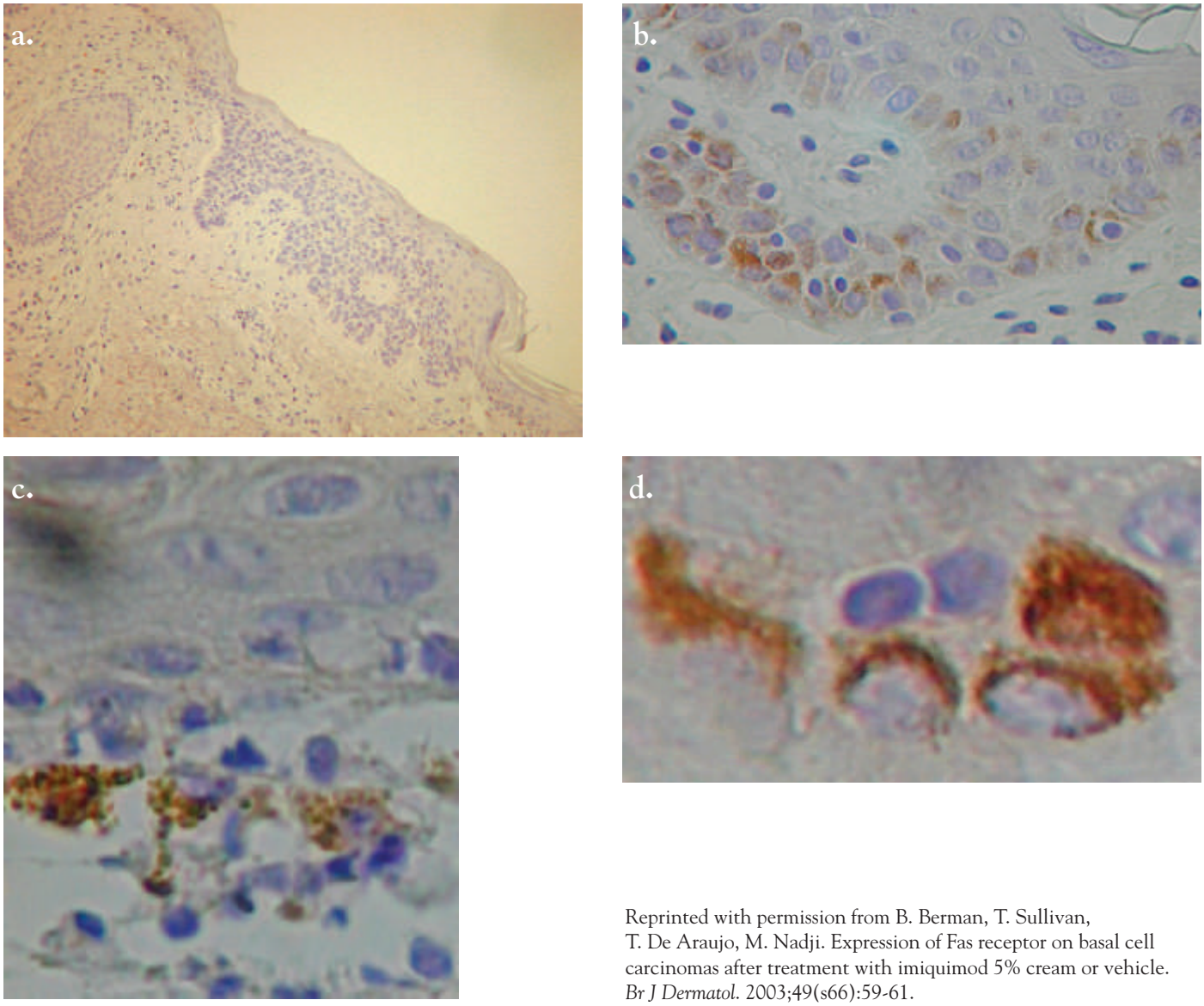
Basal Cell Carcinoma

The cells comprising basal cell carcinomas (BCCs) are capable of escaping immune recognition. One mechanism of avoiding detection is that BCC cells normally do not express Fas receptor (FasR), which is a death receptor. However, when exposed to IFN-α the BCC cells are capable of expressing this death receptor that, when bound to Fas ligand (FasL), present on the surface of tumor infiltrating T-lymphocytes allowing the tumor cells to undergo apoptosis. BCC cells, which normally have the FasL expressed on their cell surface, now may actually undergo BCC cell suicide by the interaction of the interferon-induced FasR and the FasL, which then induces apoptosis and death of the BCC cell. The rationale behind using topically applied imiquimod for the treatment of BCC is based, at least in part, on its ability to interact with dendritic cells to have them elaborate interferon, which then would induce the FasR on the surface of BCC cells, allowing the tumor cells to undergo naturally programmed cell death. Indeed intralesional injection of IFN-α2b ($1.5x10^6$ international units 3 times per week for 3 weeks)[3] is reported to induce complete clearance of BCCs. To answer the question of whether treatment of BCCs with topically applied imiquimod 5% cream can indeed induce the expression of FasR, we undertook a study.[4] Ten patients with biopsy confirmed BCCs were treated in a matched patient blinded fashion 5 times per week for up to 2 weeks with either a 5% imiquimod cream or vehicle cream. The treated area was then excised and the tissue examined for the presence or absence of FasR by immunoperoxidase detection with rat anti-human FasR antibody. As shown in Figure 1, following treatment with vehicle cream, FasR IFN-α-2b was absent;

however, after 6 applications of imiquimod 5% cream, the presence of FasR on the BCC cells was detected.

Treatment of superficial BCC (sBCC) with imiquimod 5% cream 5 times per week for 6 weeks resulted in complete histological clearance in 82% of patients in comparison with a 3% clearance histologically in the group treated with the control vehicle alone.[5] Standard of care for the treatment of sBCC is curettage and desiccation (C&D). However, there are 2 important downsides to the treatment of BCC with C&D. First, the presence of residual BCC cells immediately after the treatment, allowing for the development of BCC recurrences; and second, the development of postsurgical firm, white scars (Figure 2). The rationale for adding imiquimod 5% cream to the treatment of BCC with C&D is that imiquimod alone is able to treat BCC; therefore, its addition would allow for reduction of any post-C&D residual BCC cells and reduce the chance of recurrences. In addition, there are data to support the possibility that imiquimod may induce long-term immune memory directed against tumor cells; its use would further reduce the chance of local recurrences or even possibly the *de novo* development of additional BCCs.[6] Bladder carcinoma cells injected in mice proliferate resulting in the death of the mice. If the animals are treated with imiquimod, the injected tumor cells are ultimately killed and the animals survive. This is not surprising in light of imiquimod's induction of interferon with its antitumor activity. The interesting phenomenon is that if one examines the animals that have been cured of their bladder carcinoma after exposure to imiquimod and reinject new tumor cells in the absence of additional imiquimod, these animals reject these tumor cells, suggesting the development of a long-term immune memory directed against this tumor.

Figure 1. Fas receptor expression on BCC treated with imiquimod 5% cream or vehicle (5x/wk x 1-2 wks).[2]

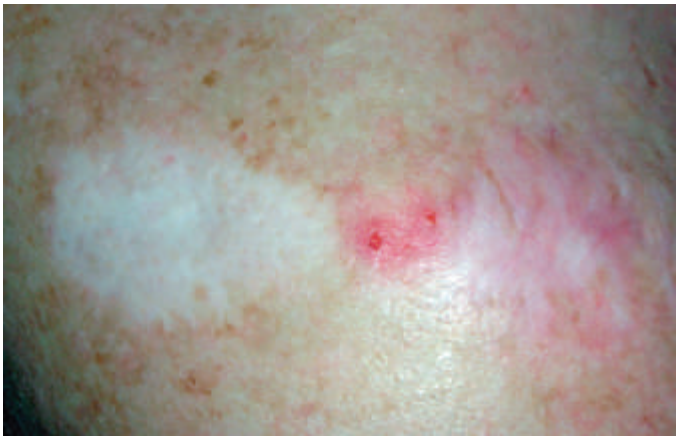


Reprinted with permission from B. Berman, T. Sullivan, T. De Araujo, M. Nadji. Expression of Fas receptor on basal cell carcinomas after treatment with imiquimod 5% cream or vehicle. *Br J Dermatol.* 2003;49(s66):59-61.

Does long-term immune memory develop in humans after treatment with imiquimod? Long-term memory may explain some of the data of a study by Carrasco et al[7] examining recurrence rates of anogenital warts following surgical removal or treatment with imiquimod 5% cream, or the combination of imiquimod 5% cream and then surgical removal. The investigators found that the recurrence rate of anogenital warts following surgery alone was 65% whereas the recurrence rate of warts completely responding to 5% imiquimod was 15%. The interesting finding was that if a wart was treated with imiquimod and it partially resolved (suggesting an immune response but not complete clearance) and then any residual wart surgically removed, the recurrence rate was 20%. This is similar to imiquimod alone and less than the recurrence rate of 65% seen with surgery alone. This suggests that the treatment with imiquimod 5% cream induced a long-term memory directed against the wart virus, which allowed for a recurrence rate that was less than surgery alone, since a simple surgical excision would not induce a long-term immune memory directed against the wart.

The other adverse event associated with C&D for the treatment of BCC is the development of scarring and there is a body of evidence pointing to the ability of imiquimod to have a beneficial effect on postsurgical scarring. What is the rationale for the use of immune response modifiers for the control of excessive scarring? Following tan insult to the skin, and as dermatologists insult the skin every day with the scalpel, the dermal fibroblasts undergo an activation that allows these activated fibroblasts to elaborate greater amounts of extracellular matrix components including collagen, fibronectin, and glycosaminoglycans (GAGs) to repair the defect caused by the surgical insult. It appears that the interferons are a naturally occurring signal for activated fibroblasts to curtail their excessive biosynthetic activity and return to normal. In the case of the fibroblasts that have been activated to induce keloidal scarring, interferon treatment *in vitro* resulted in reduction of the excessive amount of collagen to be normalized, the excessive amount of GAGs to be normalized, and the subnormal level of collagenase activity which breaks down pre-existing collagen to be normalized.[8] In addition, there are data to suggest that activated keloidal fibroblasts express mutations in the p53 tumor suppressor protein, which under normal conditions is responsible, at least in part, for normal apoptosis. However, with mutations in the p53, there is less apoptosis; therefore, the keloidal fibroblasts that are elaborating excessive amounts of extracellular matrix components do not die off naturally. Interferons are able to induce native functioning p53 and allow for restoration of apoptosis to allow these activated fibroblasts to die off naturally.[9] Taken together, it appears that interferon is a potent antifibrotic agent capable of restoring biosynthetic normalcy to activated fibroblasts as well as restoring their ability to apoptose and die. We have demonstrated that the injection of excision sites with IFN-α2b following the removal of keloids surgically reduces the ultimate recurrence rates to 18.7%, while 51.1% of those keloids having been excised without interferon injections recurred ($P<.025$).[10] Treatment of keloid excision sites with imiquimod 5% cream nightly for 2 months resulted in no recurrence (0/11) of the keloidal scarring at 6 months.[11]

Figure 2. Downsides of C&D for BCC.



There are now data that point to the ability of imiquimod 5% cream to help in avoiding, or at least optimizing, postsurgical scarring in patients who do not have keloidal diathesis. We undertook a prospective, double-blinded, randomized, vehicle-controlled study of patients 12 to 50 years of age. Each patient had 2 clinically diagnosed melanocytic or dysplastic nevi in a similar anatomic area that were excised, closed with sutures, and then treated for 4 weeks in order to determine the tolerability of postsurgical excisions in nonkeloidal patients to imiquimod 5% cream or the control vehicle cream. At week 8, there was no dehiscence of any of the 36 surgical sites of the 18 patients completing the study, no evidence of infection at any surgical site, no clinically relevant differences between the 2 sites with respect to pain or tenderness; however, at 8 weeks the sites treated with imiquimod 5% cream were more red when compared to the sites treated with the vehicle cream ($P=.004$). This study points to the tolerability of sites of recent excision to the application of imiquimod 5% cream.[12] Data from the plastic surgery literature points to the cosmetic effectiveness of topical treatment of scars with imiquimod 5% cream.[3] Fifteen patients had undergone bilateral breast augmentation or reduction. One of their bilateral scars was treated with imiquimod 5% cream every 3 to 4 days for 8 weeks starting 2 months after surgery while the other scar was either left untreated or treated with petrolatum. Within the first 4 weeks, all the imiquimod treated scars became inflamed and treatment was suspended for 1 week, then restarted without further inflammation.

This erythematous response is reminiscent of the response mentioned earlier in our study. However, at 24 weeks after surgery, the scars treated with imiquimod were less severe than the surgical sites treated with petrolatum or left untreated as assessed by the Strasser or Beausang scales for determining severity of breast scars. The imiquimod treated scars were significantly better than control scars ($P<.001$) and this was true for both scales and whether the evaluator was the surgeon, the nurse, or a plastic surgeon blinded to the treatments.

A pilot, double-blind, vehicle-controlled study was undertaken by Spencer[14] in which the administration of imiquimod after C&D was investigated to determine if the combination regimen would reduce the frequency of residual tumor compared with C&D alone in patients with primary nodular BCC on the face and ears. Each patient received 3 cycles of C&D and either topical imiquimod (n=10) or placebo (n=10), nightly for 1 month, beginning the same night after surgery. The tumor area was excised 2 months after C&D (one month after completion of daily imiquimod or placebo use). At 8 weeks, the proportion of patients with residual tumors was substantially decreased with imiquimod therapy (10%) compared to vehicle (40%). Wounds in the vehicle group healed more quickly than those in the imiquimod group, although by 8 weeks, all excision sites were healed. Eighty percent of the patients in the vehicle group had atrophic and/or hypopigmented scars whereas only 30% of patients in the imiquimod group had atrophic and/or hypopigmented scars. The imiquimod-treated sites were consistently better in scar height, pliability, vascularity, and pigmentation, except for erythema because the sites were more pink.[14]

Conclusion

In summary, surgical extirpation followed by treatment with imiquimod 5% cream 5 times per week for 6 weeks is a reasonable combination of treatment for BCC. The patient is informed that he or she has a sBCC and is motivated to have their cancer removed immediately. As dermatologists, we are trained and motivated to perform a C&D. Follow-up treatment with 5% imiquimod will reduce the chance of recurrences due to residual BCC cells remaining after the surgical procedure. In addition, treatment of the surgical site with imiquimod 5% cream can optimize the cosmesis of the postsurgical scar. This rational combination use of imiquimod 5% cream will be detailed in the presentations of Drs. Rigel and Tillman.

Disclosures

The indications for imiquimod 5% cream are off FDA-approved labeling except for sBCCs 5 times per week for 6 weeks and AKs 2 times a week for up to 4 months. Dr. Berman is on the speaker's bureau, conducts departmental research, and is a consultant for Schering-Plough, Graceway Pharmaceuticals LLC, and 3M.

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A 36-MONTH CLINICAL EXPERIENCE OF THE EFFECTIVENESS OF CURETTAGE AND IMIQUIMOD 5% CREAM IN THE TREATMENT OF BASAL CELL CARCINOMA

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Abstract

Background: Electrodesiccation and curettage is commonly used for the treatment of basal cell carcinomas (BCCs). Does the addition of imiquimod 5% cream improve clearance rates and cosmetic outcomes?

Objective: To evaluate a 3-year clinical experience of the effectiveness of curettage combined with imiquimod cream in the treatment of BCC.

Methods: Patients were enrolled into the study in the first 10 months of 2003. All patients had biopsy-confirmed BCCs and were treated with curettage followed by imiquimod 5% cream 5 times weekly for 6 weeks.

Results: Ninety patients with 101 tumors were treated; a clearance rate of 96% was obtained. Twenty-five sites were rebiopsied at 6 weeks after therapy, regardless of clinical findings. Two of these biopsies showed persistent BCC. The remaining 76 sites were followed clinically and only rebiopsied for clinical signs of reoccurrence. Two additional BCCs reoccurred at 23 months and 25 months, respectively. All patients were followed a minimum of 13 month with an average of 36 months. There were minimal cutaneous side effects and no systemic side effects.

Conclusions: Curettage followed by the application of imiquimod 5% cream resulted in clearance rates of 96% at an average 36 months follow-up. The treatment was well-tolerated and appears to produce a favorable cosmetic outcome.

Introduction

During the last several decades, several studies have reported substantial increases in the incidence of nonmelanoma skin cancer worldwide.[1-4] Moreover, it is estimated that more than 1 million new cases of basal cell carcinoma (BCC) will be diagnosed this year in the US. These increases are also seen in younger persons.[5,6]

Standard treatment options for BCC include electrodesiccation and curettage (ED&C), surgical excision, and Mohs micrographic surgery. Other treatment options include radiation therapy, cryotherapy, and other topical therapies.[7]

Electrodesiccation and curettage is a technique commonly used by dermatologists for the treatment of BCC. It has universal acceptance as a treatment modality because it is a quick, relatively painless office procedure, and does not require multiple office visits for dressing changes or suture removal. The procedure conserves more healthy tissue than surgical excision and is a good choice for those with multiple skin cancers[8,9] or other health issues.

Recurrences rates for BCC treated with ED&C are highly variable ranging from 3% to over 20% depending histology patterns, lesion diameter, and high-risk anatomical sites.[10-14] Lesions located in high-risk areas (ie, nose, perinasal, nasal labial groove, ears, chin, perioral, periocular) had the highest rates of recurrence. Silverman et al[11] reported that the 5-year recurrence rate for tumors larger than 6 mm located in high-risk areas is 17.5%, compared to a 3.3% recurrence rate for tumors located in low-risk areas (eg, extremities and trunk). Consequently, ED&C is not indicated for larger tumors, those in high-risk areas, or for recurrent BCC.

Imiquimod 5% cream has been approved for the treatment of BCC on the trunk. A series of phase II and III, multicenter, randomized studies and open-label studies have shown complete histological clearance rates ranged from 69% to 100% for superficial basal cell carcinoma (sBCC) and from 42% to 100% for nodular BCC, depending on the length of application, use of occlusion, and dosing schedules.[15-22] Few studies have been conducted to determine long-term clinical clearance rates. In an ongoing, 5-year, follow-up study in Europe, evaluating recurrence rates with imiquimod in sBCC, Gollnick et al[23] showed a clinical clearance rate of 87.1% at 3 months, which declined to 79.5% at 24 months.

When imiquimod 5% was used as a monotherapy, these efficacy rates did not confer any advantage over more traditional treatment approaches, except perhaps in patients who declined a surgical approach to treatment for personal or medical reasons.

A small number of investigators proposed using imiquimod after ED&C. This would allow mechanical debulking of the tumor with the curette, with the additive benefits of using an immune response modulator. Following ED&C, residual tumor rates ranged from 8.3% to 49%.[14,24] Salasche reported

that tumors located on the central face have residual tumor rates of 30% after ED&C.[25] However, residual tumor does not always lead to recurrence. Some investigators have theorized that an immune response is responsible for the observed 5-year recurrence rate that is approximately 2 to 5 times less than the residual tumor rate.[26] A nonspecific inflammatory response and a specific antitumor cellular or humoral response activated by curettage have been suggested as sources of the immune response. Theoretically, imiquimod should attenuate the innate immune response and enhance tumor clearance. In addition, imiquimod may help reduce scarring.

Two studies have been published that determine the beneficial effects of adding imiquimod to curettage. Spencer[27] treated 20 patients with confirmed nodular BCC with curettage followed by imiquimod application. Residual tumor was present in 40% of patients in the vehicle group compared with 10% of patients in the imiquimod group.[27] Wu et al[28] treated 17 patients with 34 nodular BCC on the trunk and extremities with curettage followed by the application of imiquimod daily for 6 to 10 weeks (maximum of 30 applications). They reported a histological clearance rate of 94%. Both studies noted an improved cosmetic outcome. While treatment was successful in the short-term, the studies were not designed to evaluate long-term follow-up results. Rigel et al,[29] in a multicenter study, reported 100% clinical clearance of 57 tumors at 12 months; they also noted improved cosmetic outcome.

This single center, open-label, ongoing study was designed to determine the efficacy and safety of curettage followed by topical application of imiquimod 5% cream for the treatment of BCC. The efficacy endpoint was to determine if clearance rates with this combination treatment were comparable to surgical excision and Mohs micrographic surgery. The goal of this study was to establish an alternative treatment method combining curettage with topical imiquimod for the treatment of BCC.

This combination therapy may allow ED&C to become less operator-dependent, relying less on electrodesiccation for the eradication of tumors. The application of imiquimod 5% cream after debulking the tumor with a curette may stimulate the natural host defense mechanisms to destroy residual tumor without removing healthy skin. Theoretically, this would result in less scarring as less electrodesiccation is used, and diminish the potential interaction with implanted cardiac devices. This technique may also prove to be more cost-effective. If long-term clearance rates approach those of surgical excision, it may become a treatment of choice for those individuals who cannot tolerate a surgical approach or who would prefer a less aggressive treatment approach for BCC.

Methods

Patients 18 years and older with histologically-confirmed BCC were eligible for this study and enrolled during the first 10 months of 2003 from a single study center. Patients in this study included those who refused surgical excision or could not tolerate surgery due to advancing age. Others had comorbid conditions, a history of significant scarring from previous treatments, or had difficulty with traveling. Patients with larger or higher risk tumors were included if underlying medical conditions or comorbid conditions existed. There were also those who preferred a more conservative treatment approach for their BCC and were willing to try the combination treatment. All patients were required at least 12 months of follow-up.

Prior to treatment, lesions were measured, photographed, and a 3-point location measurement was obtained. All lesions were biopsied in a shave fashion. Bleeding was controlled with 20% aluminum chloride or very light electrodesiccation. After BCC was confirmed via the biopsy, patients returned to the office for treatment with curettage. Thorough curettement was performed until normal dermis remained. No patients had tumor extending through the dermis and into the adipose tissue. Eighteen (18%) of lesions were treated with curettage at the time of biopsy. These individuals typically had other issues such as transportation or health problems, which prompted treatment at the time of the biopsy.

Patients were instructed to puncture one side of the sachet of imiquimod 5 % cream with a pin and then apply a small amount of the cream topically to the lesion area with their finger. This technique was expected to provide 5 to 7 applications per sachet of imiquimod 5% cream. To encourage compliance, patients were given a calendar, which included directions and dates for application. The dosing regimen prescribed was the topical application of imiquimod 5% cream once daily 5 days per week for 6 weeks. During the initial visit, expected local skin reactions were reviewed and photographs of anticipated reactions were shown. Patients who had significant reactions were allowed treatment rest periods; however, rest periods did not extend the total treatment period. Patients were seen 2 to 3 weeks after starting imiquimod treatment, at the conclusion of 6 weeks of treatment, and after 6 to 8 weeks of treatment-free follow-up. Patients were then followed every 2 to 3 months for the first 24 months, and every 3 to 6 months thereafter. Photographs of the treatment area were taken at each clinic visit, and local skin reactions were assessed.

Results

Of the 90 patients with 101 tumors enrolled in this study, 64 (71%) were male and 26 (29%) were female; the mean age of patients was 71.9 years (range 34 to 94 years). Most patients had at least one prior BCC (mean 5.3 BCC); 56% of the patients had 3 or more. During the 36-month follow-up period, this group of 90 patients developed 395 additional new skin cancers (288 BCC and 107 squamous cell carcinoma [SCC]). Overall, the mean size of treated BCC tumors was 1.01 cm (range 0.3 to 2.0 cm; SD±0.3669) (Table 1). Most tumors were located in high-risk anatomical sites (66%), with the nose being the most frequent site (35%) (Table 2). Most BCCs were nodular (61%); others included infiltrative (13%), mixed (13%), superficial (7%), and re-

current (7%). The recurrent tumors were included in this study; all had their primary tumor treated earlier with ED&C.

Most patients were treated with topical imiquimod cream within 2 weeks (mean 11 days) of biopsy. Patients were instructed to treat the BCC area with imiquimod once daily, 5 days per week for 6 weeks. The mean number of imiquimod applications was 27; most patients used 4 to 5 sachets of imiquimod cream. The mean treatment duration was 6.36 weeks, with a range of 2.5 to 12 weeks (SD±1.38).

Figure 1. An 81-year-old wheelchair-bound, oxygen-dependent male with infiltrative BCC.

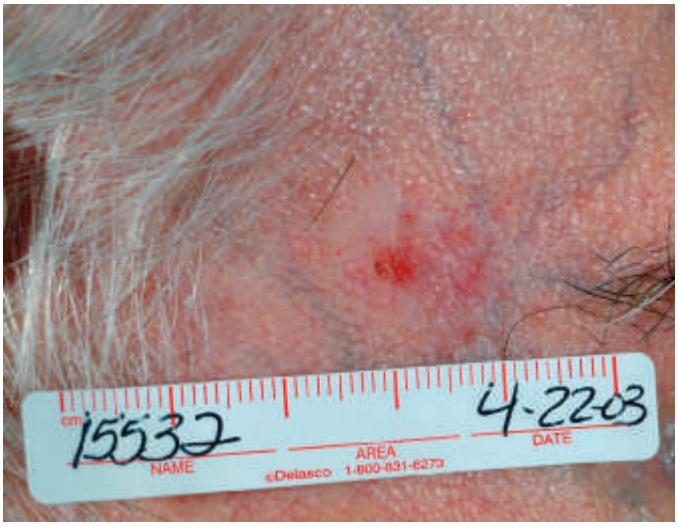


Figure 2. Area treated with biopsy and curettement.

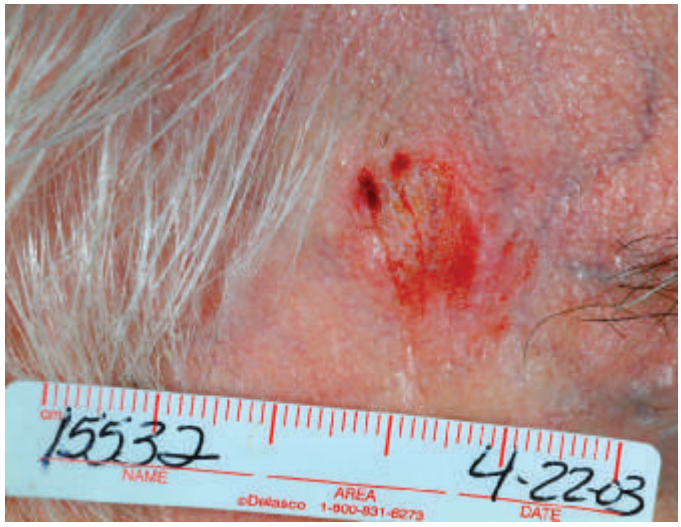


Table 1. Basal cell carcinoma characteristics 101 tumors.

Basal Cell Carcinoma	Characteristic	Number	Percent
Diameter (cm)*	<0.5	12	12
	0.6-1	48	48
	1.1-2	41	41
Location	Head and Neck	88	88
	High-risk Areas†	66	66
	Mid-risk Areas‡	22	22
	Trunk	8	8
	Extremities	5	5
Type	Nodular	61	61
	Infiltrative	13	13
	Mixed	13	13
	Superficial	7	7
	Recurrent§	7	7

*Mean = 1.01 (SD±0.3668).
†High-risk areas include central face, periorbital, pre/post-auricular, and ear.
‡Mid-risk areas include cheek, forehead, scalp, and neck.
§All primary tumors treated with ED&C.

Treatment location reactions were graded as mild, moderate, or severe. The majority of patients had reactions reported as mild (43%) or moderate (30%), while 27% of patients had reactions reported as severe. Eighteen patients with severe reactions required either rest periods or reduced application frequency. Most patients complained only of mild itching and burning at the application site; none had any serious side effects, nor did any discontinue therapy due to systemic side effects.

Twenty-five tumors were rebiopsied 6 weeks after imiquimod therapy. The treatment site was identified by 3-point measurements and the entire area was removed in a shave fashion. Of these, 2 patients had residual BCC: a 76-year-old female with an infiltrative BCC on her nose and an 82-year-old male with a 1.2-cm nodular lesion on his finger. The remaining 76 tumor sites were rebiopsied only if suspicious clinical evidence was present. Two additional patients had recurrence of their BCC at 23 and 25 months; both of these lesions were

Figure 3. Significant reaction after 15 applications.

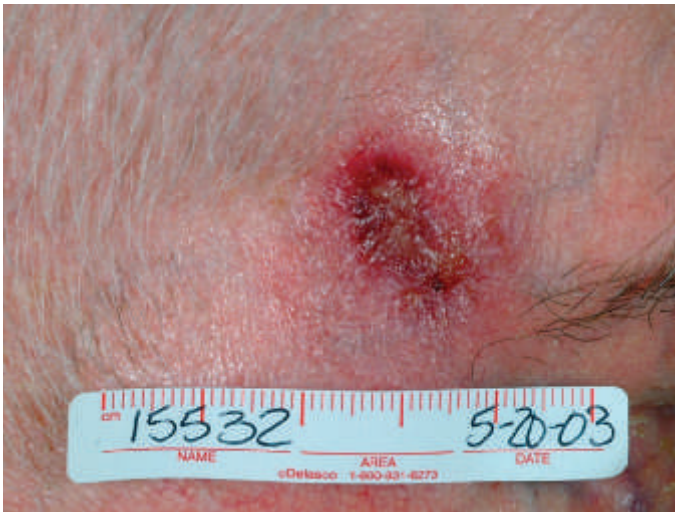


Figure 4. Remains clear at 14 months.

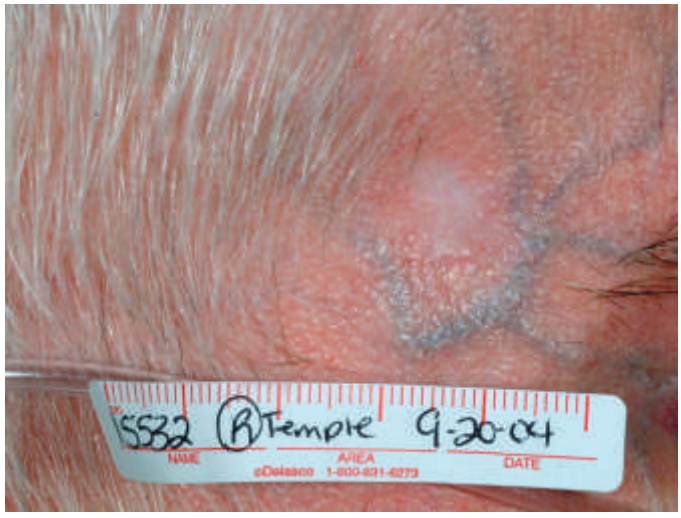


Table 2. Basal cell carcinoma location.

Basal Cell Carcinoma	Location	Number	Percent
High-risk Areas	Nose	35	35
	Temple	8	8
	Lip	6	6
	Pre- and Postauricular	5	5
	Ear	4	4
	Lat. Canthus	2	2
	Chin	2	2
	Glabella	2	2
	Eyebrow	2	2
Mid-risk Areas	Forehead	8	8
	Scalp	6	6
	Cheek	6	6
	Neck	2	2

in high-risk areas: an 81-year-old male with a 0.6-cm nodular BCC on his lip and a 63-year-old female with a 1-cm nodular BCC on her nose.

Figure 5. An 87-year-old male with an infiltrative BCC.

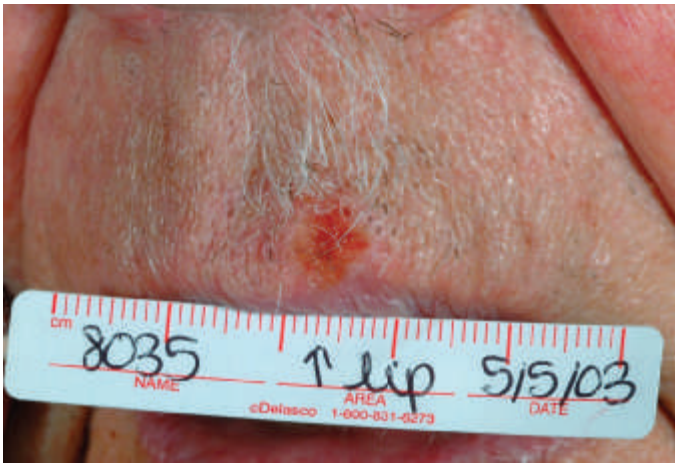


Figure 6. An 87-year-old male with an infiltrative BCC.

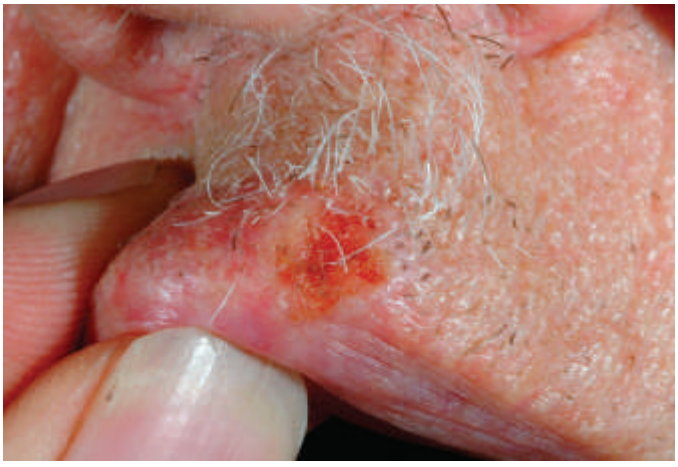
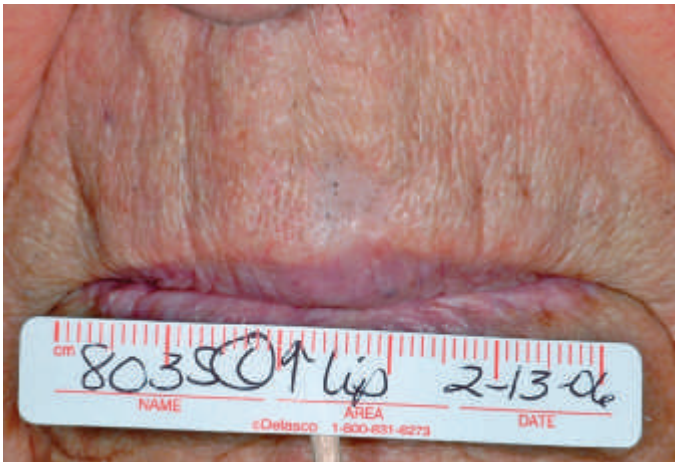


Figure 7. At 33 months after imiquimod treatment.



All patients were followed a minimum of 13 months; 10 patients died of unrelated causes during the follow-up period. One patient was lost to follow-up with no signs of recurrence at the last visit at 18 months. All remaining patients were followed in the office for at least 24 months at regular bimonthly intervals initially and then less frequently (mean 12 visits during the 36-month follow-up period). The mean follow-up was 35.7 months (SD±6.7).

Figures 1 to 4 represent the treatment course of an 81-year-old wheelchair-bound, oxygen-dependent male patient with an infiltrative BCC on the right temple. Figures 5 to 7 represent the treatment course of an 87-year-old male with an infiltrative BCC on the lip, who had 40 months tumor-free follow-up.

Discussion

In this prospective, 36-month, ongoing study using a combination of curettage followed by application of 5% imiquimod cream for 6 weeks, BCC clearance rates were 96%. Multicenter, randomized, open-label studies in Australia, the US, and Europe evaluating the efficacy of imiquimod 5 % cream as monotherapy have shown efficacy rates that range from 71% to 90%.[15,17-21] Curettage to debulk the tumor in combination with imiquimod 5% cream seems to have increased the clearance rate more than if either treatment was used individually.

In a retrospective study looking at the effectiveness of curettage alone as treatment for BCC, Barlow et al[30] reported a 5-year cure rate of 96%. However, these tumors were predominantly in low-risk areas (87%) and under 1.0 cm in size (77%). Similarly, most studies using imiquimod to treat BCC were on lesions less than 1 cm in diameter, located in low-risk areas (trunk and extremities) with low-risk morphology. While this study was not designed to treat high-risk tumors, 66% of the treated tumors were located in high-risk areas and 20% had high-risk morphology. Treated tumors were also large; 89% of tumors were larger than 0.6 cm and 41% were larger than 1.1 cm. (mean 1.01 cm.). Yet, clearance rates were 96%.

We rebiopsied nearly 25% of the tumor sites, regardless of clinical findings at the 3 months posttreatment visit and found residual tumor present in only 2 patients (8% of those biopsied). Based on previously reported studies with ED&C alone, we expected to see a much higher residual tumor rate.[14,24,25] Our findings were consistent with others who have used curettage combined with imiquimod therapy.[27,28] More importantly, of the remaining 76 tumors, only 2 patients (2.6%) had a recurrence at 23 and 25 months, respectively. Both BCCs were in high-risk areas (nose and lip). We believe high-risk tumors should be treated with Mohs; however, many patients in this current study had difficultly with traveling as well as comorbid conditions, making access to Mohs surgery difficult.

As a rule, recurrent BCCs are not generally retreated with ED&C due to recurrence rates of at least 18%;[11] nevertheless, 7 patients included in the study had their primary BCC

treated with ED&C alone. Of these patients, 6 of the 7 were initially treated by the primary investigator (DKT). Most tumors had recurred within an average of 24 months (range 6-75 month) after traditional ED&C. None of these tumors treated with combination therapy have recurred. The average follow-up of this group is 36.2 months (range 13-42 months). In this small number of patients (n=7), the use of imiquimod in conjunction with curettement has provided a longer term clearance rate. Since the technique was the same, the only difference was the addition of imiquimod.

ED&C is a meticulous technique that requires an ability to recognize a normal dermal base. We believe that most dermatologists are proficient in the use of this technique; however, there is a wide variability with the use of electrodesiccation. Electrodesiccation is known to cause postoperative hypopigmentation, hypertrophic scars, and keloids.[12,31,32] In addition, electrodesiccation has a potential for interaction with implantable cardiac devices and may delay healing.[33-35] The addition of imiquimod following curettage may provide clearance rates that may reduce if not eliminate the need for electrodesiccation.

Although cosmetic outcome was not specifically evaluated, no evidence of hypertrophic scarring was noted and there appeared to be improved cosmetic outcomes, which is consistent with the work of others.[27-29] Since little to no electrodesiccation was used, less scarring was anticipated; however, imiquimod may have helped to reduce scarring. Berman et al[36] has shown less hypertrophic scarring with the use of imiquimod after excision of keloids, while Prado et al[37] has shown less scarring after breast reconstructive surgery when using imiquimod. Figures 8 to 10 represent a 60-year-old male who has insulin-dependent diabetes mellitus and a BCC on the right antihelix. Previously, he had developed significant keloids after skin grafting for treatment of burns. In the current study, there was no evidence of scar formation following combination therapy with curettage and imiquimod. He remains tumor free at 43 months follow-up.

While imiquimod requires a self-administered therapeutic approach, compliance was not an issue with the patient population described. The majority of our subjects had previous treatment for nonmelanoma skin cancers, with an average of 5.3 previously treated BCCs and 4 previously treated SCC. Patients received detailed instruction regarding treatment and were prepared for expected reactions associated with imiquimod application. Very few of the patients complained regarding their reaction to imiquimod, even those who had severe reactions. Most patients complained only of mild itching and burning, or the cosmetic nuisance of having an inflammatory response. Overall, patients in this study developed 288 additional BCCs. When given a choice on treatment options most of these patients chose combination imiquimod therapy over surgical intervention regardless of the cost and cosmetic nuisance associated with treatment.

Figure 8. A 60-year-old male with BCC on the area of a former keloid.

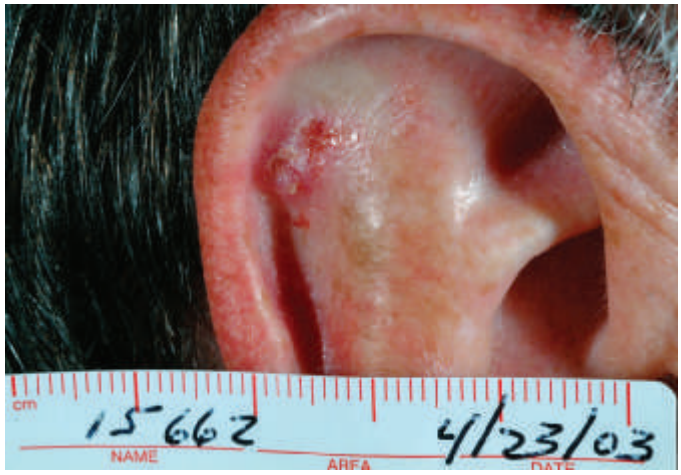


Figure 9. Moderate reaction at the end of 6 weeks of therapy.

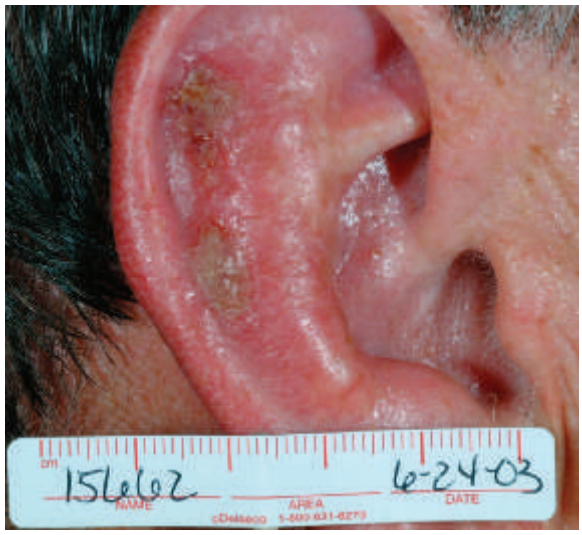
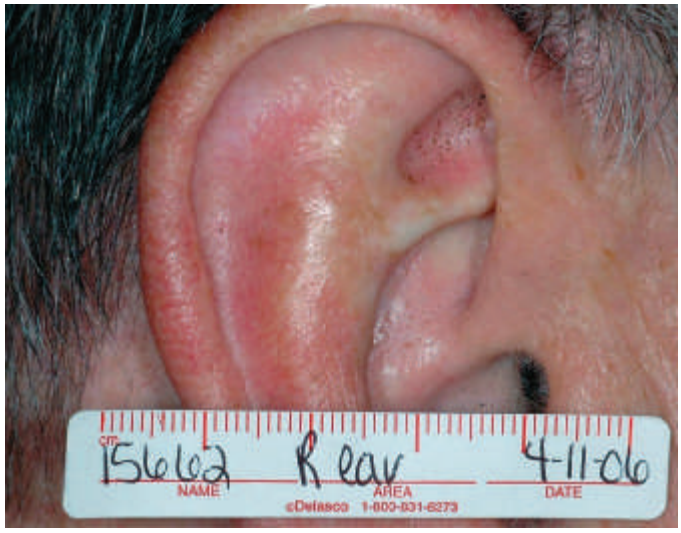


Figure 10. At 36 months after imiquimod treatment.



Conclusion

The incidence of BCC continues to increase in the US and worldwide. Tumors are occurring in younger patients and there is a need to develop additional treatment options for patients who have other medical comorbidities. While surgical excision and Mohs therapy remain the gold standard for treating more aggressive BCC, not all patients are able to tolerate these techniques. This study has multiple limitations in that it was not double-blind or placebo controlled, there was no control group, nor did it have ridged inclusion and exclusion criteria. However, all patients had biopsy-proven BCCs, were followed at least 13 months (mean 35.7 months), and seen an average 12 times during the follow-up period. Since only 2 of the first 25 rebiopsied sites were determined to have residual tumor, the decision was made not to biopsy additional patients unless dermatologic evidence for recurrent tumor existed. During the 36 months of follow-up, only 2 patients developed recurrent lesions. Overall this combination therapy resulted in a 96% clearance rate, which is much higher than expected given the tumor size, location, and type.

Imiquimod therapy was well-tolerated and the expected reaction resolved after completion of therapy. This combination therapy requires active patient participation and commitment to therapy. Comprehensive patient education is essential in combination with regular follow-up during the entire treatment period to monitor compliance and response. Patients who have little or mild reaction need additional monitoring to assure the appropriate response is achieved. Interestingly enough, the 4 patients in this study who had recurrences reported only mild reactions to imiquimod treatment.

While this combination therapy is an alternative treatment modality for certain subsets of the population, treating high-risk tumors with this approach is currently not recommended; Mohs surgery remains the treatment of choice for these tumors. Furthermore, larger multicenter studies are needed to further define the use of imiquimod 5% cream with curettage for the treatment of BCC. In the meantime, the use of imiquimod after ED&C may provide higher clearance rates and potentially better cosmetic results.

Disclosure

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Imiquimod 5% Cream following Curettage without Electrodesiccation for Basal Cell Carcinoma: Preliminary Report

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Abstract

Background: Using more than one therapeutic approach in the treatment of basal cell carcinomas (BCCs) has the potential to enhance cure rates.

Materials and Methods: In this study, 57 nodular and superficial BCCs were curetted without electrodesiccation. One week later, imiquimod 5% cream therapy was initiated once daily 5 times per week for 6 weeks. At 1-year follow-up, 0 of 57 BCCs (0%) had clinical recurrences. Cosmetic results were very good to excellent.

Conclusion: Combination therapy with imiquimod 5% cream followed by curettage represents an effective method for treating BCCs with a high cure rate.

Introduction

It is estimated that there will be more than 1 million newly diagnosed cases of BCC this year in the US. It is estimated that 1 in 5 Americans will get skin cancer within their lifetime.[1] Most of the accepted therapies for this tumor have cure rates of 85% to over 95%.[2]

Electrodesiccation and curettage (EDC) is the most commonly chosen treatment for BCCs in the US.[3] This destructive method is effective but can often lead to a poor cosmetic result with hypertrophic scarring. Topical therapies are almost as effective by themselves and typically lead to good to excellent cosmetic results (even on anatomic sites at higher risk for hypertrophic scarring). Combining one or more therapies for treating BCCs has the potential to increase efficacy and lead to better cosmetic results compared to the individual treatments themselves. The purpose of this preliminary study was to determine if treatment of BCCs by curettage alone followed by topical imiquimod 5% cream resulted in higher cure rates than the individual therapies and also provide excellent cosmetic results.

Material and Methods

The study population consisted of 57 patients with biopsy-proven nodular and superficial BCCs enrolled at 3 clinical centers. Study lesions were located on the lateral face, neck, trunk and extremities. The BCCs were curetted and hemostasis was achieved chemically. One week post curettage, imiquimod 5% cream was applied topically to the curettage site once daily 5 times per week for 6 weeks. Patients were seen at 3 weeks, 7 weeks, 3 months, 6 months, and 12 months. At 1 year posttreatment, 0 of 57 BCCs (0%) had clinical recurrences. The cosmetic results were very good to excellent with only mild hypopigmentation noted at the minority of sites. No hypertrophy was noted. The combination treatment of curettage with imiquimod had superior cosmetic results when compared to curettage with electrodesiccation.

Discussion

Combining multiple modalities for the treatment of BCC has been shown to be more effective than the individual therapies alone.[4] One would expect that the cure rates for utilizing 2 therapies would be as good or better than the cure rate of the better of the 2 treatments alone. In this study, the combination approach yielded a cure rate (100%) that was higher than either of the individual procedures.

In EDC, it is the electrodesiccation component of the treatment that leads to poor cosmetic results. Curettage without electrodesiccation has been reported to effectively treat BCCs with a better cosmetic result but with a lower cure rate than with EDC. Reymann et al treated 525 BCCs with curettage alone and followed the treated sites clinically for any recurrences.[5] They found a 15-year recurrence rate of 10.5%. Johnson et al reported on 403 primary BCCs treated with "aggressive" curettage and the treated sites were then excised to determine if all the BCCs had been successfully removed. In all, 64 of the BCCs (15.9%) had residual tumor present following curettage.[6] Morphea BCC had the highest rate of residual tumor with 11 out of 12 having BCC present after treatment.[6] McDaniel et al reported on 328 BCCs treated with curettage alone and the surgical sites followed clinically for 5 years.[7] Any areas suspected of having a recurrence were biopsied.[7] They reported 28 clinical recurrences (9%) and, on a qualitative level, reported that the ultimate cosmetic results of using curettage alone were superior to curettage and electrodesiccation, x-ray therapy, or cryotherapy, and as good as excision.[7] Adding imiquimod to the curettage increased treatment efficacy while having the potential to further improve cosmetic results by decreasing the chance for keloid formation.[8]

Conclusion

The use of curettage followed by imiquimod 5% cream to treat BCC represents an effective method for treating BCCs with a high cure rate and good to excellent cosmetic results. Further study is indicated to see if these high cure rates persist with longer follow-up intervals.

Disclosures

Dr. Rigel is a consultant, investigator, advisory board member, and recipient of grants and honoraria from Graceway Pharmaceuticals. He is also a consultant and receives honoraria from Doak Dermatologics.

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ANTIANGIOGENESIS IN THE TREATMENT OF SKIN CANCER

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Abstract

Angiogenesis is the formation of new capillary blood vessels from existing vasculature. Cancers are dependent upon angiogenesis for their growth. Inhibition of angiogenesis can slow, halt, or regress tumors. Angiogenesis inhibition is now validated for the treatment of cancer using a variety of approved biologic, small molecule, multitargeting, and immunomodulatory agents. In the skin, strategies to inhibit angiogenesis-signaling pathways include blockade of COX-2, m-TOR, sonic hedgehog, growth factor receptor activation, and activation of Toll-like receptors (TLR). The agent with the most clinical experience as a topical antiangiogenic therapy is imiquimod. Imiquimod is a TLR agonist, with immune response modifying properties that also stimulates antiangiogenic cytokines, downregulates the expression of proangiogenic factors, upregulates the expression of endogenous inhibitors, and induces endothelial cell apoptosis. By titrating its dosing for angiogenesis inhibitory activity and not for gross inflammation, imiquimod can be applied in an efficacious and well-tolerated fashion to treat skin cancer.

Tumor Angiogenesis

Judah Folkman's pioneering work in tumor angiogenesis beginning in the 1970s established the field of angiogenesis research.[1] Since then, an enormous body of angiogenesis research has elucidated the growth control mechanisms of the microcirculation, yielding new insights into the critical role of new blood vessel growth in both physiological and pathological conditions.

All solid tumors are dependent upon angiogenesis to grow beyond a few millimeters in diameter.[3] Antiangiogenic therapy for cancer stems from a large body of experimental evidence showing that inhibition of angiogenesis can slow, halt, or regress tumors. Unlike cytotoxic chemotherapy and ionizing radiation, antiangiogenic therapy does not directly kill tumor cells but instead targets the vasculature supporting tumor growth, resulting in a cytostatic effect. This approach represents a paradigm shift for cancer treatment. Clinical benefits of antiangiogenic therapy include prolonged survival, disease stabilization, and improved quality of life, and can often be achieved with less debilitating toxicities than conventional therapies.

Angiogenesis in the Skin

Angiogenesis, the formation of new capillary blood vessels from the existing vasculature, is a tightly regulated physiological process. Under normal circumstances, vascular endothelial cells comprising blood vessels are quiescent and have one of the lowest mitotic rates in the body.[3] This nonproliferating state is governed by the balancing effects of endogenous stimulators and inhibitors of angiogenesis present in healthy tissue. Positive regulators of angiogenesis (proangiogenenic) include fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF; sometimes called vascular permeability factor), platelet-derived growth factor (PDGF), interleukin-8 (IL-8), and more than 30 other proteins. Endogenous angiogenesis inhibitors include endostatin, tumstatin, tissue inhibitors of matrix metalloproteinases (TIMPs), interferons (IFN-α, -β, -γ), interleukins (IL-10, IL-12, IL-18), and thrombospondins (TSP-1, TSP-2), among other factors.

Pathological angiogenesis, typically defined as aberrant or uncontrolled angiogenesis underlying a disease, occurs in a number of skin conditions. The epidermis is an avascular tissue layer separated from underlying dermal capillaries by the basement membrane. Viable epidermal cells are located within 100 to 150 μm from vessels, the diffusion distance of oxygen. Beyond this zone, epidermal cells undergo keratinization and ultimately die and slough. Tumor cells in benign and malignant skin conditions are also subject to growth restriction defined by limits of oxygen diffusion. Unlike normal tissues, however, growing tumors release high concentrations of proangiogenic growth factors that induce capillary growth and override this control mechanism. Tumors can also upregulate growth factor production from host stroma, furthering the angiogenic process.

Skin Cancers

Like all solid malignancies, cancers occurring in skin are highly angiogenic. Vascular tumors of the skin, such as Kaposi's sarcoma, hemangioma of infancy, pyogenic granuloma, and angiosarcoma, are composed of proliferating cells of endothelial origin and are also angiogenesis-dependent.[4,5] Hemangiomas were the first human tumors to be successfully treated with antiangiogenic therapy using interferon-α-2a, based on the recognition that they overexpress angiogenesis stimulators (FGF-2, VEGF) during the proliferative phase.[6,7] Conversely, during their involutional phase, endogenous angiogenesis inhibitors (tissue inhibitor of metalloproteinase-1 [TIMP-1], interferon-β [IFN-β]) are upregulated.[8]

Benign growths, such as warts, are also angiogenic in nature.[9-12] Increasing vascularity is observed between HPV-negative and HPV-positive warts; pinpoint hemorrhagic capillaries are a gross manifestation of the neovascularization that accompany wart growth and persistence.[9] Further, it has been shown that other HPV-associated lesions exhibit increased micro-

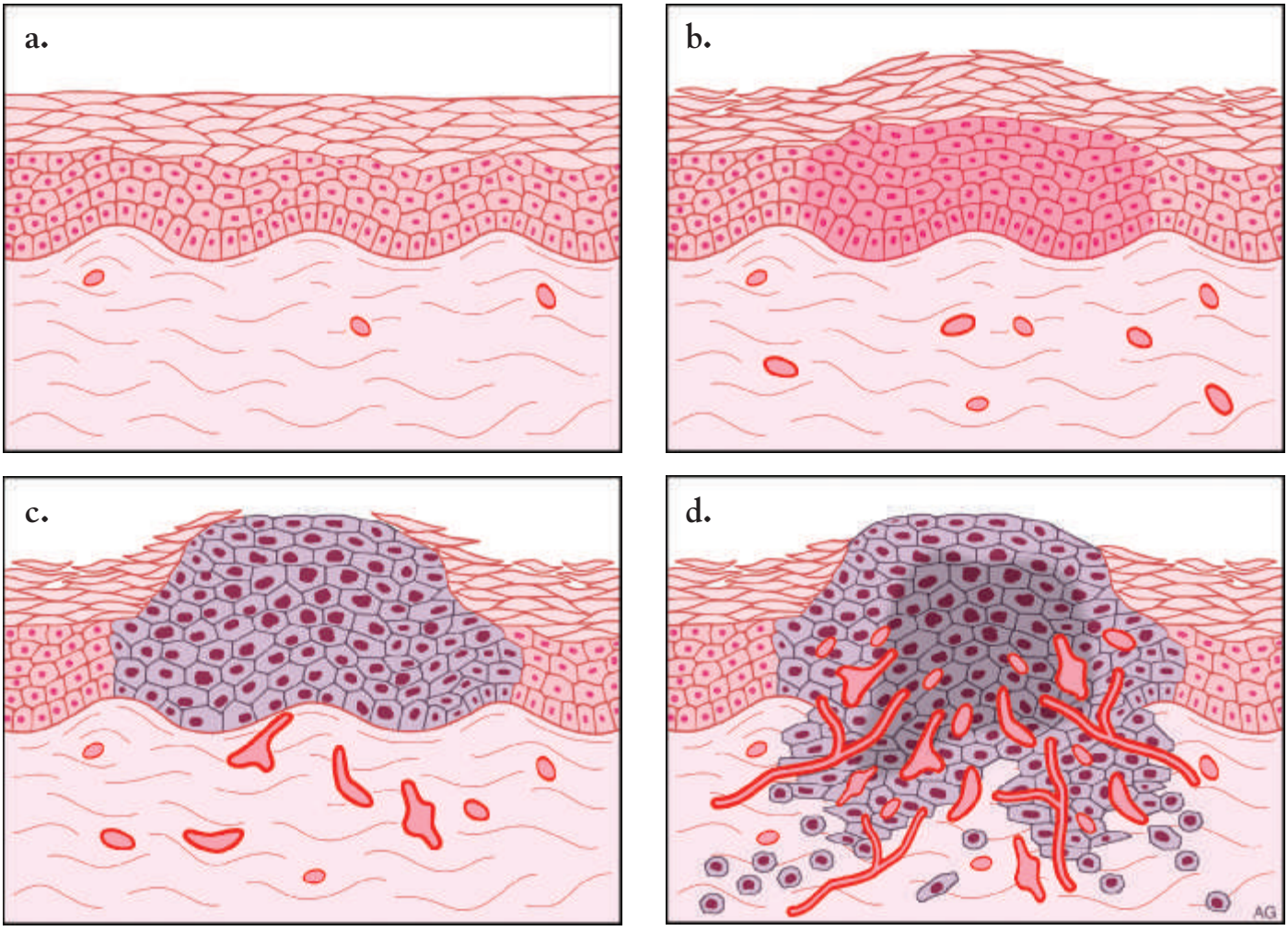
vessel density during the transformation of intraepithelial neoplasia to anal carcinoma and from cervical dysplasia to cervical carcinoma.[10-12]

Angiogenesis-related skin tumors associated with ultraviolet (UV) exposure include actinic keratoses (AKs), melanoma and nonmelanoma skin cancers (basal cell carcinoma [BCC] and squamous cell carcinoma [SCC]).[11-13] Acute ultraviolet (UVB) damage (such as occurs with a sunburn) causes dramatic changes in levels of growth factor cytokines in normal skin. Natural angiogenesis stimulators such as VEGF and FGF become significantly upregulated within days of UV damage, resulting in increased skin microvessel density.[13] In addition, there is a concurrent significant local reduction in endogenous angiogenesis inhibitors such as IFN-β and TSP-1.[14]

Sun damage due to chronic UV radiation also induces DNA damage, referred to as a "hit", in keratinocytes. Clonal expansions of p53 mutant cells occur in photodamaged skin, with as many as 40 mutant clones per cm^2 of skin. Such mutations occur with a 50% to 60% frequency in BCCs and a 60% to 90% frequency in AKs and SCCs.[15-17] The accumulation of multiple "hits" leads to preneoplastic and neoplastic transformation in the skin. These transformed lesions are capable of growth up to 2 mm in diameter (500,000-1,000,000 cells) before their metabolic demands exceed the available blood supply. To expand beyond this limit, the "switch" to the angiogenic phenotype must occur (Figure 1).[18] Hyperplastic skin lesions, including AKs and atypical melanocytic nevi, are already angiogenic and exhibit capillary densities greater than surrounding normal tissue.[19] The progression from hyperplasia to neoplasia is then accompanied by further intensification of angiogenesis. Barnhill and colleagues first demonstrated that microvessel density is increased in AK, SCC *in situ*, and SCC compared to normal skin (Figure 2).[20] Other investigators have found similar increases in angiogenesis between precursor AK lesions and SCC by paired analysis.[21] BCC exhibits a 5-fold increase in angiogenesis compared to normal skin.[19]

The role of angiogenesis in the progression of malignant melanoma is well-documented. Many angiogenic mediators, including VEGF, FGF-2, IL-8, placental-derived growth

Figure 1. Angiogenesis in malignant transformation. **a)** Normal skin; **b)** Hyperplasia; **c)** Dysplasia; **d)** Carcinoma.



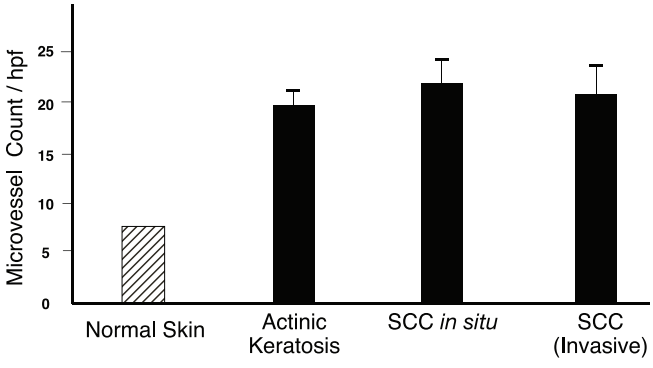
factor (PlGF), Ang-2, and $\alpha_v\beta_3$ integrins are upregulated in cutaneous malignant melanoma.[22,23] Melanomas greater than 1 mm in thickness have significantly increased microvessel density (MVD) compared to normal dermis and even severely atypical melanocytic nevi.[24] For example, there is a 1.5-fold increase in microvessel density between dysplastic nevi and primary melanoma in the vertical (>2.0 mm) growth phase.[25] Increased melanoma tumor thickness correlates with neovascularization, which facilitates hematogenous metastases. In a seminal paper, Breslow described primary melanoma thickness as directly proportional to rate of metastases.[26] It has also been demonstrated that dormant melanoma micrometastases lack significant vascularity compared to clinical macrometastases, despite comparable rates of proliferation and apoptosis.[27]

Era of Antiangiogenic Therapies

Antiangiogenic therapies encompass a spectrum of interventions that inhibit new blood vessel growth in pathological tissues. Presently, numerous antiangiogenic therapies have been either FDA approved or are in advanced clinical trials for many cancer types (colorectal, renal, non-small cell lung, myeloma, and breast, to name a few), ophthalmic conditions (age-related macular degeneration), skin disorders (warts, AK, nonmelanoma skin cancers), and vascular tumors in children (hemangiomas, giant cell tumors).

Approved antiangiogenic therapies for cancer fall into 3 major classes: 1) biologic agents; 2) small molecule, multitargeting agents; and 3) off-label use of drugs with antiangiogenic activity. The biologic agents include monoclonal antibodies directed against specific growth factors, primarily VEGF (bevacizumab, Avastin®) and epidermal growth factor (EGF; erlotinib, Tarceva®). These therapies bind extracellularly to the targeted growth factor, preventing activation of the receptor. Another biologic agent approved in China is a modified endostatin (rh-endostatin, Endostar™), a recombinant protein based on an endogenous angiogenesis inhibitor. Small molecule antiangiogenic agents include inhibitors of multiple receptor tyrosine kinases (sorafenib, Nexavar®; sunitinib, Sutent®) and anticytokine drugs (thalidomide, Thalomid®; lenalidomide, Revlimid®). The third class of agents includes certain older drugs that may exhibit antiangiogenic properties when administered off-label as single agents or as "cocktails" of several drugs. In addition, certain chemotherapeutic agents can be administered over long periods of time at low doses and in a "metronomic" schedule that induces optimal biological activity, thereby suppressing tumor growth through antiangiogenic rather than cytotoxic effects. The use of approved antiangiogenic agents, such as bevacizumab, as maintenance therapy in cancer patients who have experienced remission is also being examined in a number of clinical studies.

Figure 2. Increased microvessel density in epidermal neoplasia.



In this study, we found that malignant transformation of the skin is associated with prominent angiogenesis. This was the first demonstration that the switch to an angiogenic phenotype has already occurred in AK.[20] Methods: skin biopsies from normal donors, AK, SCC *in situ* and SCC (invasive) were stained with Ulex Europeaus agglutinin to highlight the vasculature. The number of microvessels per high-powered field (hpf) were counted using standard methods described in references 81 in AK (N=9), SCC *in situ* (N=10), SCC invasive (N=13).

Antiangiogenic Approaches for Skin Cancer

Dermatology researchers and clinicians are finding new and innovative ways to treat skin cancer by exploiting the antiangiogenic properties of a wide variety of agents. A number of angiogenic growth factor and receptor antagonists already approved for use in solid tumors are currently in clinical trials for skin cancer: gefitinib (Iressa®) and erlotinib (Tarceva) for SCC; sorafenib (Nexavar), bevacizumab (Avastin), and erlotinib (Tarveca) for melanoma. In addition to approved agents, many other agents/substances have been found to interrupt critical angiogenic signaling pathways in skin cancer, including inhibitors of COX-2, hedgehog, m-TOR, growth factors/receptors, and Toll-like receptor signaling (Table 1).

Cyclooxygenase-2 (COX-2) plays a key role in the release of proangiogenic proteins such as prostaglandin E2 that directly stimulate endothelial migration and proliferation. There is also a direct relationship between COX-2 and VEGF expression. COX-2 expression has been found to correlate with microvessel density in nonmelanoma skin cancer.[28] In one experiment, COX-2 transfected into BCC cell lines resulted in increased VEGF-A mRNA and protein and bFGF,

Table 1. Angiogenesis signaling pathways in skin cancer and interventions.

Target/Pathway	Intervention
COX-2	Celecoxib, Diclofenac gel
Sonic Hedgehog	Cyclopamine
m-TOR	Sirolimus and analogues
Growth Factor/Receptor or Tyrosine Kinase	Bevacizumab, Sorafenib, Erlotinib, Gefitinib, Polyphenon E ointment
Toll-like Receptor	Imiquimod cream

which could be blocked by COX-2 specific small interfering RNA.[29] COX-2 overexpressing tumors had a 2-fold increase in microvessel density compared to vector-control tumors. COX-2 inhibitors, originally developed to alleviate pain and inflammation, are now being studied as preventive therapy to reduce the risk developing several different types of solid tumors. Oral celecoxib (20 mg/kg/day) suppresses experimental murine SCC and melanoma growth introduced surgically into mice.[30] Clinical trials of oral celecoxib in human patients with BCC nevus syndrome are currently underway. In addition to the oral formulations, topical celecoxib has been compounded and studied in experimental hairless mice with UVB-induced papillomas/carcinomas. Topical COX-2 inhibition using celecoxib (500 or 2500 µg/ml) has been shown to decrease numbers of p53 positive and proliferative (PCNA-positive) epidermal cells with a corresponding reduction in size and number of UV-induced lesions.[31] Another agent, topical diclofenac gel (3% diclofenac sodium, Solaraze), is a COX-1 and COX-2 inhibitor that is indicated for the treatment of AK.[32]

Sonic hedgehog (HH), a secreted morphogen, is an angiogenic factor that induces capillary morphogenesis and interacts with the Patched-1 transmembrane receptor to induce angiogenic signaling (via upregulation of VEGF-A and angiopoietin-1 and -2) through the pathway activator Smoothened.[33,34] Cyclopamine is a steroid alkaloid that acts as a HH antagonist by binding directly to Smoothened.[35] In experimental models, cyclopamine inhibits VEGF and capillary formation in models of ocular neovascularization.[36] Aberrant activation of the HH pathway is known to be associated with the development of BCC.[37,38] Curis and Genentech have studied a topical form of cyclopamine in a phase I clinical trial for BCC that, while demonstrating histologic clearance in some subjects, appeared to have low transepidermal penetration, which led to discontinuation of the trial. Other HH antagonists under study may have future promise.

M-TOR (mammalian target of rapamycin) is a serine/threonine kinase active in the PI3/Akt cellular signaling pathway, which controls cell proliferation and survival. Alterations in the PI3K/Akt pathway occur in many cancer types and result in increased proangiogenic cell signaling through mTOR and other proteins. It was recently discovered that mTOR regulates Akt phosphorylation in endothelial cells and plays a role in regulating VEGF-A and VEGF-C.[39,40] The drug sirolimus (rapamycin), an inhibitor of mTOR, is a macrolide antibiotic that also functions as an immunosuppressive agent for transplant patients through inhibition of the postreceptor signal transduction of interleukin-2, which blocks T-cell and B-cell activation. Sirolimus also inhibits hypoxia-inducible factor (HIF-1), VEGF expression, and endothelial cell proliferation.[41,42] Following observations that organ transplant patients treated with sirolimus had a decreased incidence of skin tumors, an increasing number of transplant specialists began utilizing sirolimus rather than other immunosuppressants to exploit its antitumor effects.[43] In one study, when renal transplant patients were converted to sirolimus, remission of nonmelanoma skin cancer was observed in 37 out of 53 patients.[44] It was recently discovered in animal studies that continuous dosing of sirolimus, rather than bolus dosing, results in the most effective tumor control in animal studies, which is consistent with its antiangiogenic properties.[45] Analogues of sirolimus, such as temsirolimus (Torisel™) and everolimus (RAD001), are being used or developed as oncology drugs. Temsirolimus was recently approved to treat advanced renal cancer. A derivative of rapamycin, temsirolimus binds to the intracellular protein FKBP-12 to form a complex that disrupts mTOR signaling. Sirolimus is in clinical trials to evaluate its use in the prevention of new nonmelanoma skin cancer in renal transplant recipients. In addition, a topical sirolimus ointment is in phase I clinical trial for patients with BCC nevus syndrome.

Studies focusing on the mechanisms of natural botanical extracts have also shown that certain extracts can block angiogenesis growth factor signaling. For example, epigallocatechin-3-gallate (EGCG), the major catechin in green tea, possesses antiangiogenic activity *in vitro* and *in vivo*.[46-51] Green tea extracts inhibit the expression of VEGF in squamous epithelial cell lines; this activity is associated with the inhibition of EGF-receptor signaling pathways.[52] EGCG also inhibits VEGF-receptor expression and activity and has been shown to interfere with the activity of key enzymes related to angiogenesis, including urokinase (u-plasminogen activator) and matrilysin, as well as COX-2.[53-56] Green tea or purified EGCG, when administered to mice in their drinking water, inhibited angiogenesis in the *in vivo* Matrigel sponge model and restrained tumor growth. Topical EGCG (1 mg/cm^2) applied to the skin inhibits MMP-2 and MMP-9, increases TIMP-1, and inhibits VEGF expression in a mouse model of UV-induced skin carcinogenesis.[57] EGCG is a component of Polyphenon E 15% ointment, approved by the FDA to treat external genital warts.[58] It is likely that clinicians will utilize this agent in expanded use fashion.

Mammalian Toll-like receptors (TLRs) are members of a family of proteins that resemble the *Drosophila* toll protein, a mediator of antimicrobial immune defenses. Agonists of TLRs exhibit antitumor activity through the induction of cytokines, thereby enhancing the activity of natural killer and tumor-reactive T-cells, altering the tumor microenvironment, and inhibiting angiogenesis.[59] TLRs are expressed in the skin in keratinocytes and Langerhans cells, macrophages, T-cells and B-cells, mast cells, endothelial cells, fibroblasts, and adipocytes. Signaling through TLRs results in the production of cytokines and chemokines, driving adaptive immunity toward a Th1 response, and suppressing neovascularization. Loxoribine, S28690, and 852A are TLR agonists in development for solid tumors and leukemia.[60]

Antiangiogenic Mechanism of Imiquimod

The agent with the most clinical experience as a topical antiangiogenic therapy is imiquimod. Imiquimod 5% cream is a TLR-7 agonist approved for genital warts, AKs, and BCC. We first identified imiquimod's antiangiogenic activity in 1998 based on its induction of interferons and IL-10

and IL-12. Each of these cytokines inhibits angiogenesis independently of their immunomodulatory function. Interferons decrease cellular production of several proangiogenic factors (bFGF, IL-8, urokinase plasminogen activator), inhibit vascular motility and invasion, and induce endothelial cell apoptosis.[61-66] The interferon-inducible protein-10 (IP-10) is itself an angiostatic protein.[67] Interferon-β2 is already used clinically for its angiogenesis inhibitory activity and has been used to treat and regress hemangiomas of infancy, pediatric giant cell tumors, and pulmonary hemangiomatosis.[68-70] IL-12 inhibits endothelial proliferation and tube formation *in vitro* and angiogenesis *in vivo*. Its mechanisms include upregulation of IFN-β, downregulation of production of VEGF and bFGF, and inhibition of endothelial migration and invasion.[71,72] The antiangiogenic mechanism of IL-10 is not known, but is correlated to increased expression of the angiogenesis inhibitors TSP-1 and TSP-2.[73,74]

The antiangiogenic activity of imiquimod *in vivo* has been profiled in both animal and human subjects.[75,76] In one study, mice with tumors formed by Skv keratinocytes derived from human bowenoid papulosis (HPV16) or by murine L1 lung sarcoma were treated with topical imiquimod. To delineate antiangiogenic effects from immunomodulatory effects, the mice were immunosuppressed with 600R total body irradiation. Subsequently, 50,000 to 100,000 tumor cells were injected intradermally; these cells subsequently induced tumor angiogenesis in the cutaneous nodules. The skin overlying the tumor was treated with imiquimod (2.5% or 5%) either once or 3 days in a row. Imiquimod inhibited tumor angiogenesis in a dose- and schedule-dependent fashion. These effects were abrogated by administering neutralizing antibodies against IL-18 or IFN-β, therefore implicating these cytokines in the antiangiogenic mechanism of action. Topical imiquimod also suppressed vascular tumor growth in a mouse model of hemangioendothelioma (EOMA line), inducing a 14-fold increase in apoptosis in these endothelioid cells. In this system, imiquimod stimulated a 14-fold increase in TIMP-1 expression and a 5-fold reduction in MMP-9 activity, thereby altering the balance of angiogenesis regulators in favor of inhibitors over stimulators.[77]

In human patients, we have successfully used imiquimod as an antiangiogenic agent to regress vascular proliferative lesions such as hemangioma of infancy, pyogenic granuloma, and Kaposi's sarcoma.[59] The response of these vascular lesions to imiquimod confirms its antiangiogenic activity. In human melanoma, imiquimod potently influences gene expression of angiogenesis regulators. Pre- and post-treatment biopsies of cutaneous melanoma metastasis from a patient were examined by quantitative real-time reverse transcription-polymerase chain reaction (PCR) to profile angiogenesis markers.[76] The lesion showed a partial clinical response to topical therapy with prolonged stabilized disease. At the tissue level, imiquimod markedly decreased bFGF and matrix metalloproteinase inhibitor-9 (MMP-9) expression in melanoma by 76% and 84%, respectively, compared to baseline. Concurrently, imiquimod treatment upregulated gene expression of the endogenous angiogenesis inhibitors IFN-β, TIMP-1, and TSP-1 by 202%, 399%, and 278%, respectively, in the melanoma tissue, strongly shifting the regulatory balance toward angiogenesis inhibition in the responding malignancy.

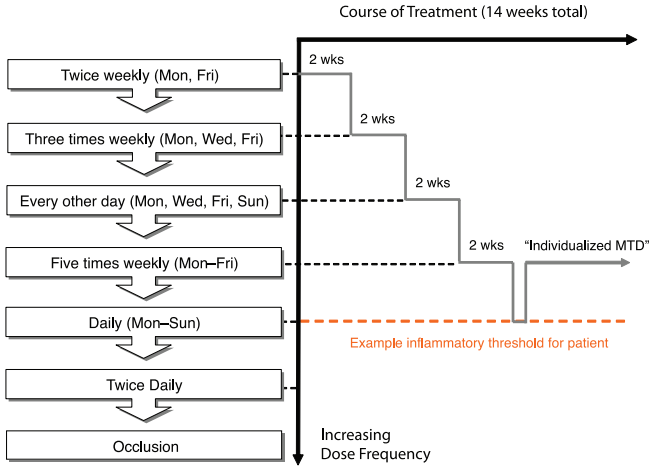
New Concepts and Clinical Practices

A defining feature of certain agents is that they are antiangiogenic when used at doses below their cytotoxic threshold. In the case of imiquimod, dermatologists frequently prescribe this drug at high doses until a severe skin reaction occurs, viewing this as a surrogate indicator of tumor response. Paradoxically, this approach poses a barrier to successful utilization of imiquimod therapy when a patient discontinues therapy due to discomfort and impairment of cosmesis. In our experience, so-called "high doses" of imiquimod (ie, frequent scheduling or its use under occlusion) are not required to achieve efficacy. Rather, antiangiogenic activity and clinical response may be obtained without erosion and gross clinical inflammation.

Optimization of Dose by Antiangiogenic Scheduling

We developed a dose-response protocol called iMTD℠ (Individualized Maximal Tolerated Dose) designed to achieve a therapeutic response based on antiangiogenesis rather than inflammation (Figure 3).[78] In this protocol, patients apply imiquimod to their lesion at a titrating schedule and stop at a dosing frequency just short of inducing true skin inflammation. We use the term "skin activation" to describe the ob-

Figure 3. iMTD℠ (Individualized Maximal Tolerated Dose) schedule.



iMTD is an individualized approach to dosing, guiding each patient to incrementally titrate to their own maximal dose. It is important to note that the goal of therapy is not to reach maximal frequency (eg, twice daily/occlusion), but to reach their own individualized maximal tolerated dose (iMTD), beyond which inflammation and discomfort prevail. Shown is an example case for a patient with an iMTD of 5 times weekly dosing, where daily dosing results in inflammation and discomfort. Different patients will have different thresholds. Reprinted with permission by The Angiogenesis Foundation. © 2007 by The Angiogenesis Foundation. All Rights Reserved.

served erythema (vasodilation) and mild epidermal desquamation, which are commonly asymptomatic. Although microscopic inflammation and infiltration of mononuclear cells are invariably present in imiquimod-treated tumors and premalignant lesions, use of the iMTD protocol can avoid the classic signs and discomfort of inflammation, as described by Celsus as rubor (redness), calor (warmth), tumor (swelling), and dolor (pain).

While it has been reported that there are statistically significant higher rates of histologic clearance when more intense erythema, erosion, or scabbing/crusting is observed at the treatment site of superficial BCC, a central question is whether a severe inflammatory reaction is *obligatory* for treatment success.[79] The evidence from targeted molecular therapies in oncology suggests it is not. Although inducing a severe local skin reaction—similar to the destructive effects of cytotoxic chemotherapy—is clearly associated with treatment efficacy, the paradigm of an antiangiogenesis approach that targets the tumor's vasculature demonstrates that cytotoxicity is not necessary for tumor response, and certainly is not desirable from a patient's quality of life perspective. Nevertheless, high rates of tumor clearance depend on maximizing dosing frequency. Data generated from our practice indicate that there is tremendous heterogeneity of dose response among individual patients at which imiquimod induces a severe local inflammatory reaction. In the absence of knowing *a priori* which patients will respond at what dosing frequency, the iMTD schedule stipulates that patients titrate the dosing frequency only up to the level above which undesirable local skin reactions occur. In a case series of 56 lesions (AK, SCC *in situ*, and BCC), the iMTD protocol resulted in complete response without any gross inflammation.[80] Dosing frequency ranged from 3 days/week (50% of patients) to 5 days/week (4% of patients) to daily (38% of patients) to twice daily (5% of patients), demonstrating that a single dosing schedule is not applicable to all patients. In our practice, hundreds of patient lesions have been treated using iMTD with excellent tolerability and similarly successful outcomes.

Summary

Angiogenesis inhibition is now validated for the treatment of a number of tumor types using a variety of approved biologic, small molecule, and immunomodulatory agents. There are also a number of newer strategies to inhibit angiogenesis signaling pathways in skin cancer via blockade of COX-2, m-TOR, sonic hedgehog, growth factor receptor activation, and activation of TLR. Imiquimod, a proven topical antiangiogenic agent, is an immune response modifying agent that also stimulates antiangiogenic cytokines, downregulates the expression of proangiogenic factors, upregulates the expression of endogenous inhibitors, and induces endothelial cell apoptosis. Thus, topical imiquimod impedes pathological tissue growth by interfering with its supporting microcirculation and the release of endothelial-derived paracrine survival factors. Combined with its effects on cell-mediated immunity, imiquimod's antiangiogenic activity has potent antitumor effects. By titrating its dosing for angiogenesis inhibitory activity, imiquimod can be applied in an efficacious and well-tolerated fashion for skin cancer.

Disclosure

Dr. Vincent Li has received honoraria from 3M Pharmaceuticals. Dr. William Li has no disclosures relevant to this activity.

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