

IMIQUIMOD AS AN ANTIANGIOGENIC AGENT

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Abstract

Imiquimod (imidazoquinoline 5%) is a topical immune response modifier agent that inhibits angiogenesis, the growth of new blood vessels. In addition to its stimulation of cell-mediated immunity, imiquimod's antiangiogenic activity contributes to its clinical efficacy by interfering with pathological neovascularization that promotes disease progression. The antiangiogenic mechanisms of imiquimod are due to its: 1) induction of cytokines that themselves inhibit angiogenesis (interferons, IL-10, IL-12); 2) local up-regulation of endogenous angiogenesis inhibitors (TIMP, TSP-1); 3) local down-regulation of pro-angiogenic factors (bFGF, MMP-9); and 4) promotion of endothelial cell apoptosis. This report discusses these mechanisms and the rationale for imiquimod's use as an antiangiogenic agent. Key principles of antiangiogenic therapy are presented to describe how imiquimod may be applied in a well-tolerated fashion to treat a broad range of angiogenesis-dependent dermatological conditions, including actinic keratosis (AK), basal cell carcinoma (BCC), squamous cell carcinoma (SCC), lentigo maligna, hemangiomas, Kaposi's sarcoma, pyogenic granuloma, and external genital warts.

Introduction

Imiquimod (imidazoquinoline 5%) is a topical immune response modifier agent indicated for the treatment of actinic keratosis (AK), basal cell carcinoma (BCC), and external genital warts. Because imiquimod does not exert direct anti-proliferative or antiviral effects on cells in culture, its mechanism of action is reported to rely upon cytokine induction through activation of toll-like receptor-7 (TLR7) associated signal transduction in dendritic cells and peripheral blood mononuclear cells.¹ TLR7 activation leads to cellular production of endogenous interferons (IFN- α , - β , - γ) and interleukins (IL-10, IL-12, IL-18), which enhance cell-mediated immunity and induce apoptosis.² We first hypothesized that imiquimod has antiangiogenic activity due to these cytokines. Recent studies provide experimental evidence that imiquimod is indeed a potent inhibitor of angiogenesis, the growth of new blood vessels.³ This mechanism is significant because antiangiogenic therapy is a new treatment strategy for diseases associated with pathological neovascularization, including cancer and age-related macular degeneration. Imiquimod is the first topical antiangiogenic agent that can be used by dermatologists for treating angiogenesis-dependent disorders of the skin (Table 1). This review discusses the specific evidence supporting imiquimod as an antiangiogenic agent and describes how this mechanism can be exploited to treat skin diseases.

Angiogenesis in Skin Disease

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, with one of the lowest mitotic rates in the body.⁴ This non-proliferating state is governed by the balanced effects of endogenous stimulators and inhibitors of angiogenesis present in healthy tissue (Table 2). Positive regulators of angiogenesis include fibroblast growth factors (FGFs), vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), 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 (α , β , γ), interleukins (IL-10, IL-12, IL-18), and thrombospondins (TSP-1, TSP-2), among other factors. Physiological angiogenesis occurs during wound healing, anagen hair growth, and the female reproductive cycle, where local up-regulation and release of positive factors stimulates brief periods of neovascularization. Angiogenic growth factors activate the endothelium in local venules to undergo a cascade of molecular and cellular events: 1) receptor-binding and signal transduction; 2) endothelial proliferation; 3) directional migration; 4) tubular morphogenesis; 5) arterial-venous patterning; 6) recruitment of bone marrow-derived

Table 1. Angiogenesis-Dependent Neoplasms of the Skin Successfully Treated with Imiquimod.

Condition	References
Actinic keratosis	Newell, et al. <i>Br J Dermatol</i> 2003;149:105 Lee, et al. <i>Dermatol Surg</i> 2005;31:659
Basal cell carcinoma	Newell, et al. <i>Br J Dermatol</i> 2003;149:105 Schulz, et al. <i>Br J Dermatol</i> 2005;152:939
Bowen's disease	Nijsten, et al. <i>Br. J Dermatol</i> 2004;837 Nouri, et al. <i>J Drugs Dermatol</i> 2003;2:669
Squamous cell carcinoma	Loggini, et al. <i>Pathol Res Pract</i> 2003;199:705 Nouri, et al. <i>J Drugs Dermatol</i> 2003;2:669
Melanoma	Ribatti, et al. <i>Oncol Rep</i> 2005;14:81 Ray, et al. <i>Int J Dermatol</i> 2005;44:428
Hemangioma of infancy	Kraling, et al. <i>Am J Pathol</i> 1996;148:1181 Hazan, et al. <i>Pediatr Dermatol</i> 2005;22:254

endothelial progenitor (stem) cells; 7) vascular maturation; and 8) initiation of blood flow.⁵ New blood vessels deliver oxygen and micronutrients, and endothelial cells release paracrine survival factors to growing tissues. When the need for neovascularization is fulfilled, positive regulators are down-regulated and the constitutive state of angiogenesis balance is restored.

Pathological angiogenesis occurs in a number of conditions, including cancer, arthritis, diabetic retinopathy, and a number of skin disorders. 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 , the diffusion distance of oxygen, from the structure. Beyond this zone, epidermal are keratinized and ultimately die.⁶ Tumor cells are also subject to growth restriction defined by oxygen diffusion except that they are capable of releasing high concentrations of multiple growth factors that induce tumor angiogenesis and override this control mechanism. This concept of tumor angiogenesis-dependency was pioneered by Judah Folkman in the 1970s, and new blood vessel formation is now recognized as a critical pathway in tumor biology.⁴ Certain stimuli promote a pro-angiogenic microenvironment. For example, acute ultraviolet (UVB) damage (ie, a sunburn) causes dramatic changes in growth factor cytokines in normal skin. Angiogenic stimulators such as VEGF and FGF

become significantly up-regulated within days, resulting in increased skin microvessels observed histologically. In addition, there is a significant local reduction in endogenous angiogenesis inhibitors such as IFN- β and TSP-1.^{7,8}

Sun damage due to chronic (UV) radiation induces DNA damage (a so-called "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 are the hallmarks of skin cancer, with a 50% to 60% frequency in BCCs, and 60% to 90% frequency in AKs and SCCs.^{9,11} The accumulation of multiple "hits" leads to pre-neoplastic and neoplastic transformation in the skin. Transformed lesions are capable of growth up to 2 millimeters in diameter (500,000 to 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.¹² So called "precursor lesions," such as actinic keratosis (AK) and atypical melanocytic nevi are already angiogenic and exhibit capillary densities greater than surrounding normal tissue.¹³ The progression from hyperplasia to neoplasia is then accompanied by further intensification of angiogenesis. 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.¹⁴ In non-melanoma skin cancer, a similar increase in angiogenesis is found between precursor

Table 2. Angiogenesis Regulatory Molecules

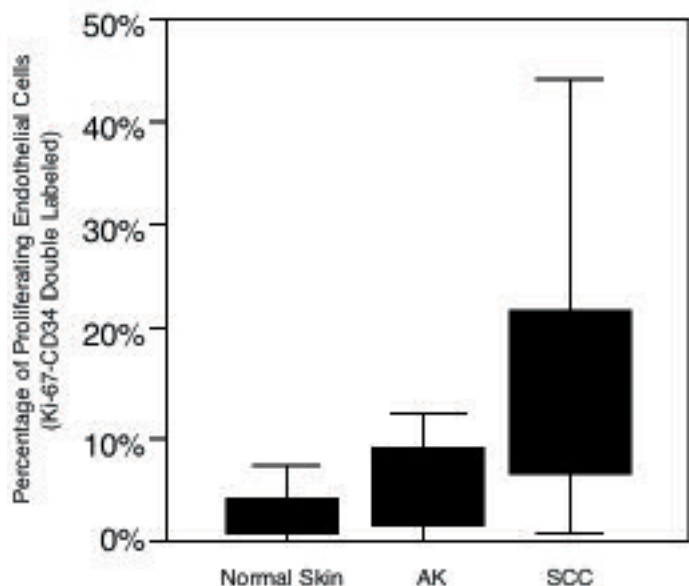
Endogenous Stimulators	Endogenous Inhibitors
Adrenomedullin	Angiostatin
Angiogenin	Canstatin
Angiopoietin-1	Endostatin
Angiopoietin-related growth factor (AGF)	Fibronectin 20 Kd Fragment
Brain-derived neurotrophic factor (BDNF)	Interferons (α, β, γ)
Corticotropin-releasing hormone (CRH)	Interferon-inducible protein-10 (IP-10)
Cyr16	Interleukins (IL-4, IL-10, IL-12, IL-18)
Erythropoietin (Epo)	Kringle 5
Fibroblast growth factors (FGFs)	Maspin
Follistatin	Meth-1, -2
Granulocyte colony stimulating factor (G-CSF)	2-methoxyestradiol (2-ME)
Hepatocyte growth factor (GSF)	Pigment epithelium-derived factor (PEDF)
Interleukins (IL-3, IL-8)	Plasminogen activator inhibitor (PAI)
Midkine	Platelet factor-4 (PF-4)
Nerve growth factor (NGF)	Prolactin 16Kd Fragment
Neurokinin A	Tetrahydrocortisol-S
Neuropeptide Y	Thrombospondin-1 (TSP-1)
Placental growth factor (PIGF)	Tissue inhibitors of matrix proteases (TIMPs)
Platelet-derived endothelial cell growth factor (PD-ECGF)	Troponin-1
Platelet-derived growth factor (PDGF)	Tumstatin
Pleiotrophin	Vasostatin
Progranulin	
Proliferin	
Secretoneurin	
Substance P	
Transforming growth factor-alpha (TGF- α)	
Transforming growth factor-beta (TGF- β)	
Tumor necrosis factor-alpha (TNF α)	
Vascular endothelial growth factor (VEGF)	
VG5Q	

AK lesions and SCC, by paired analysis (Figure 1).¹⁵ Basal cell carcinoma (BCC) exhibits a 5-fold increase in angiogenesis compared to normal skin.¹³ 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.^{16,17} Benign growths such as warts are also angiogenic, with increasing vascularity observed between HPV-negative to HPV-positive warts. Indeed, pinpoint hemorrhagic capillaries are a gross manifestation of the neovascularization that accompanies wart growth and persistence.¹⁸ Thus, skin diseases for which imiquimod has shown clinical efficacy share angiogenesis-dependency as a pathological feature.

Antiangiogenic Therapy

Antiangiogenic therapy is now a validated strategy for cancer directed at proliferating endothelial cells. One approach has been to target growth factors (eg, VEGF, PDGF, EGF, FGFs), matrix proteases, or vascular adhesion molecules (eg, integrins, cadherins). Bevacizumab (Avastin) is a monoclonal antibody that neutralizes VEGF-A, and was the first specific angiogenesis inhibitor to become FDA approved.¹⁹ A second approach has been pharmacological delivery of endogenously occurring angiogenesis inhibitors, such as interferons, endostatin, IL-12, and TIMPs.²⁰⁻²² A third approach has been antiangiogenic immunotherapy and vaccines directed against epitopes expressed by activated endothelial cells.^{23,24} Because antiangiogenic agents are based on biological targeting of

Figure 1. Increasing angiogenesis occurs during skin transformation from normalcy to AK to SCC in patients.



(Reprinted with permission from: Nijsten T, Colpaert CG, Vermeulen PB, Harris AL, Van Marck E, Lambert J. Cyclooxygenase-2 expression and angiogenesis in squamous cell carcinoma of the skin and its precursors: a paired immunohistochemical study of 35 cases. *Br J Dermatol.* 2004;151:837-45.)

proliferating vasculature, these agents are dosed below their cytotoxicity level.²⁵ The normal vasculature remains unaffected because their endothelia are not proliferating. Clinical experience suggests that antiangiogenic therapy may play a useful role for both early intervention and prevention, as well as for managing advanced disease in the front-line, neoadjuvant, and adjuvant settings.

Antiangiogenic Mechanisms of Imiquimod

We first identified imiquimod's antiangiogenic activity in 1998 based on its induction of interferons, IL-10, and IL-12. Each of these cytokines inhibits angiogenesis independently of their immunomodulatory function. Interferons decrease cellular production of several pro-angiogenic factors (bFGF, IL-8, urokinase plasminogen activator), inhibit vascular motility and invasion, and induce endothelial cell apoptosis.²⁶⁻³¹ The interferon-inducible protein-10 (IP-10) is itself an angiostatic protein.³² Interferon- $\alpha 2$ is already used clinically for its angiogenesis inhibitory activity to treat and regress hemangiomas of infancy, pediatric giant cell tumors, and pulmonary hemangiomatosis.³³⁻³⁵ IL-12 inhibits endothelial proliferation and tube formation *in vitro* and angiogenesis *in vivo*. Its mechanisms include

upregulation of IFN- γ , decreasing production of VEGF and bFGF, and inhibiting endothelial migration and invasion.^{36,37} The antiangiogenic mechanism of IL-10 is not known, but is correlated to increased expression of the inhibitors TSP-1 and TSP-2.^{38,39}

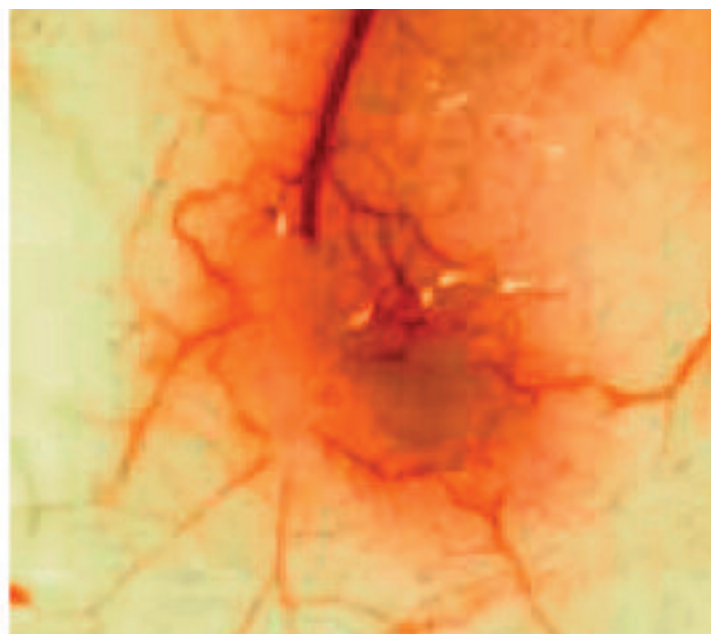
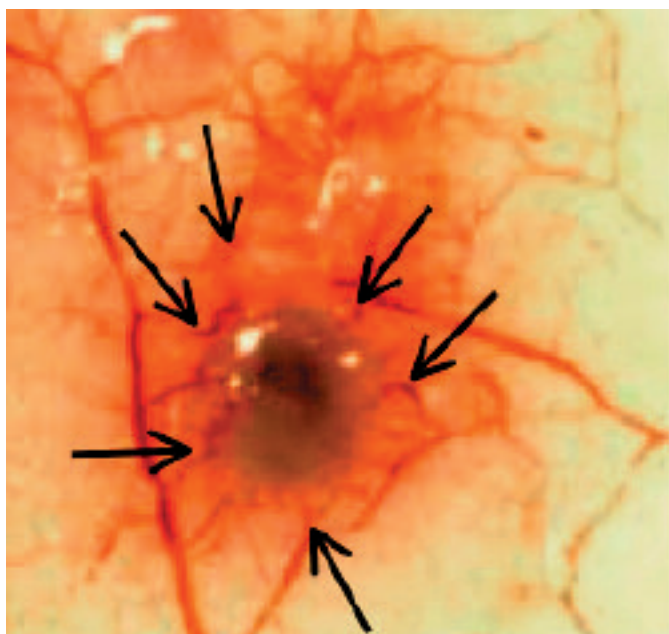
The antiangiogenic activity of imiquimod *in vivo* has been profiled in animal and human subjects (Table 3).^{3,40} Mice bearing tumors formed by Skv keratinocytes derived from human Bowenoid papulosis (HPV16) or from murine L1 lung sarcoma were treated with topical imiquimod. To delineate antiangiogenic from immunomodulatory effects, mice were immunosuppressed with 600R total body irradiation. Subsequently, 50,000 to 100,000 tumor cells were injected intradermally and these 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 (Figure 2).³ These effects were abrogated by administering neutralizing antibodies against IL-18 or IFN γ , 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 matrix metalloproteinase-9 (MMP-9) activity, thereby altering the balance of angiogenesis regulators in favor of inhibitors over stimulators.⁴¹

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.⁴⁰ The lesion showed a partial clinical

Table 3. Imiquimod—Antiangiogenic Mechanisms of Action.

- Production of antiangiogenic cytokines: IFN- α , - β , - γ ; IL-10; IL-12; IL-18
- Down-regulation of pro-angiogenic factors (bFGF, MMP-9)
- Up-regulation of endogenous angiogenesis inhibitors (IFNs, IP10, TIMP, TSP-1)
- Induction of endothelial apoptosis

Figure 2. Topical imiquimod inhibits tumor-induced angiogenesis. Intradermally-injected human tumor keratinocytes stimulate intense angiogenesis with new vessels converging upon the tumor (a, arrows). Topical treatment with imiquimod 5% x 3 days markedly suppressed new and tumor vessel growth (b).



(Reprinted with permission from: Majewski S, Marczak M, Mlynarczyk B, Benninghoff B, Jablonska S. Imiquimod is a strong inhibitor of tumor cell-induced angiogenesis. *Int J Dermatol.* 2005;44:14-9.)

response to topical therapy with prolonged stabilized disease. At the tissue level, melanoma treated with imiquimod markedly decreased bFGF and MMP-9 expression by 76% and 84%, respectively, compared to baseline. Concurrently, imiquimod treatment upregulated gene expression of the endogenous angiogenesis inhibitors IFN α , TIMP-1, and TSP-1 by 202%, 399%, and 278%, respectively, in the melanoma tissue shifting the balance of forces towards angiogenesis inhibition in the responding malignancy.

The clinic has successfully used imiquimod as an antiangiogenic agent to regress vascular proliferative lesions such as hemangioma of infancy, pyogenic granuloma, hemangiosarcoma, and Kaposi's sarcoma.⁴² The response of these vascular lesions to imiquimod confirms its antiangiogenic activity.

Clinical Application of Imiquimod as an Antiangiogenic Agent

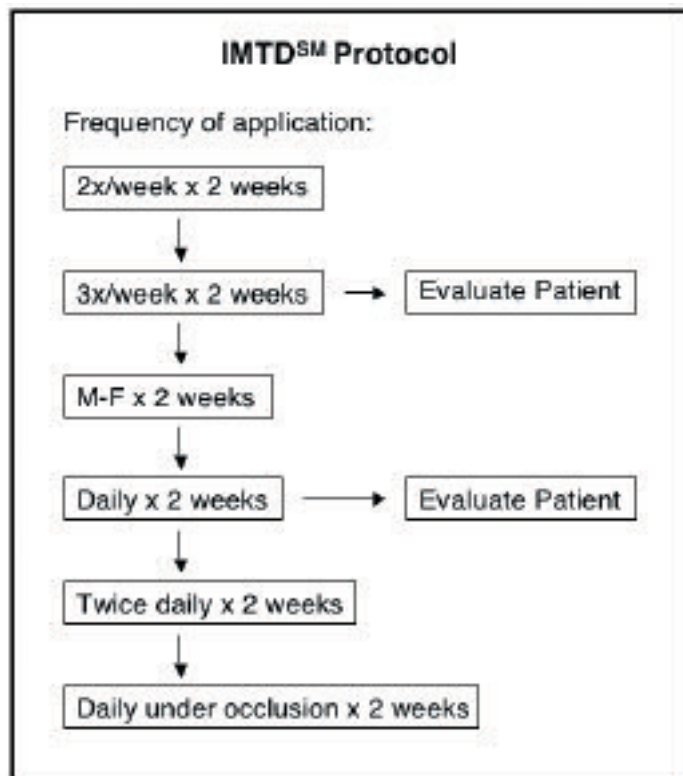
To understand how imiquimod's angiogenesis inhibitory activity may be applied in dermatology practice, several key therapeutic principles of antiangiogenesis merit discussion.

Antiangiogenic Scheduling

A hallmark of antiangiogenic agents is their biological activity against proliferating blood vessels at doses below their cytotoxic threshold. Certain systemic cytotoxic chemotherapy agents (eg, cyclophosphamide, methotrexate, vincristine) can even be delivered using "low-dose metronomic" scheduling to achieve angiostatic and anti-tumor activity without direct cytotoxicity on tumor cells or systemic side effects.⁴³ This concept represents a paradigm shift in cancer treatment, which has traditionally held efficacy to be eponymous with cytotoxicity. Oncologists are now delivering antiangiogenic agents according to their optimal biological dose. Dermatologists prescribing imiquimod frequently dose the drug until a severe skin reaction occurs, viewing this as a surrogate indicator of tumor response. In our experience, so-called "high doses" of imiquimod (ie, frequent scheduling or its use under occlusion) are not required to achieve efficacy. Antiangiogenic activity and clinical response may be obtained without erosion and gross inflammation.

We reported a dose-response protocol called IMTD (Individualized Maximum Tolerated Dose) designed to

Figure 3. Individualized Maximum Tolerated Dose (IMTD) protocol for titrating application of imiquimod to find optimal biological dose for individual patients. (Source: Angiogenesis Center Clinic, Boston)



achieve a therapeutic response based on antiangiogenesis rather than inflammation (Figure 3).⁴⁴ In this protocol, patients apply imiquimod to their lesion using 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 observed erythema (vasodilation) and mild epidermal desquamation, which are commonly seen and often asymptomatic. Although microscopic inflammation and infiltration of mononuclear cells are invariably present in imiquimod-treated tumors and pre-malignant lesions, use of the IMTD protocol can avoid the classic signs of true inflammation, first described by Celsus over 2,000 years ago as rubor (redness), calor (warmth), tumor (swelling), and dolor (pain) occurring together.

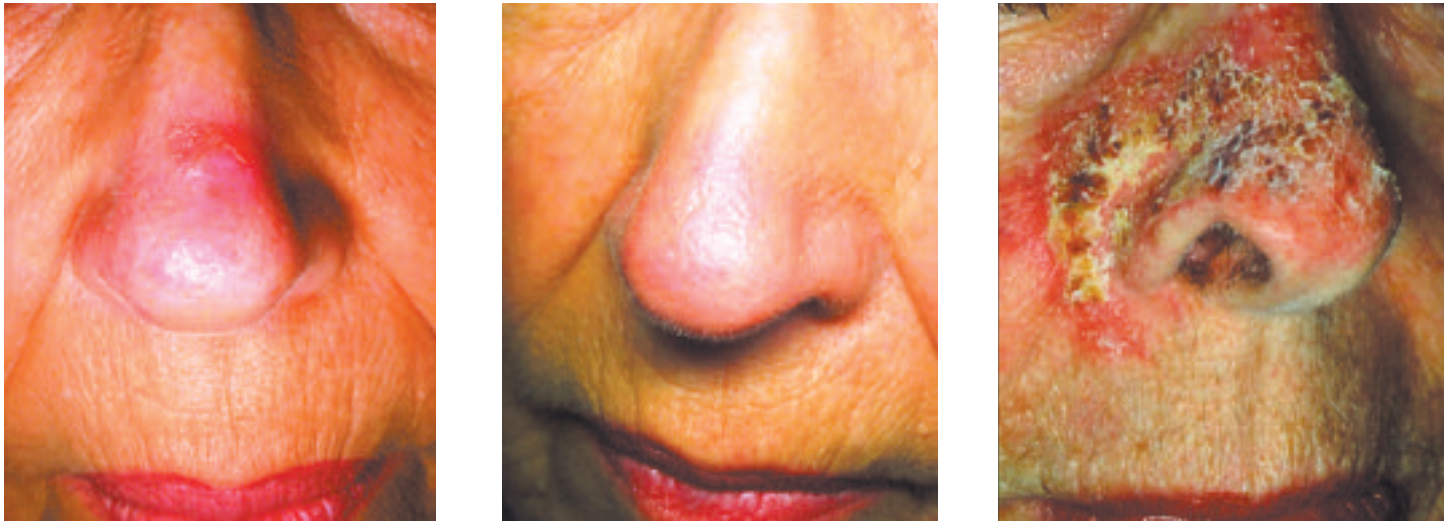
While it has been reported that there are statistically significant higher rates of histologic clearance when more intense erythema, erosion, or scabbing/crusting are observed at the treatment site of superficial BCC, it is important to question whether a severe inflammatory

reaction is *obligatory* for treatment success.⁴⁵ 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 optimizing dosing frequency. Data generated from our practice indicate that there is a tremendous heterogeneity of dose response among individual patients that determines at which dose imiquimod induces a severe local inflammatory reaction.⁴⁶ In the absence of knowing *a priori* which patients will respond to any given dosing frequency, the IMTD allows for a titration to reach the highest frequency at the lowest possible dose to avoid undesirable local skin reactions. In a case series of 56 lesions (AK, SCC *in situ*, and BCC) successfully eradicated with imiquimod, the IMTD protocol resulted in complete response without any gross inflammation.⁴⁶ Dosing frequency ranged from 3x/week (50% of patients) to 5 days/week (4% of patients) to daily (38% of patients) to twice daily (5% of patients). This wide variability implies that a single dosing schedule is likely not applicable to all patients. In our practice, hundreds of patient lesions have been treated using IMTD with excellent tolerability and similar successful outcomes.

Field Therapy to Prevent Lesion Progression

Antiangiogenic therapy may be used to suppress the progression of clinically visible lesions as well as subclinical lesions in the skin. Because photodamage occurs in all exposed skin areas, mutational clones develop throughout UV-exposed body surfaces, resulting in a field of evolving malignancies. One clinical strategy for addressing this problem is to apply imiquimod over large regions of skin, rather than just over individual lesions. We term this approach “field therapy,” defined as the application of medical therapy to treat and suppress disease over the entire region of at-risk tissue. The goal of field therapy is three-fold: 1) to treat clinically visible lesions; 2) to suppress the subclinical lesions; and 3) to regress pre-malignant lesions in order to prevent their progression to malignancy. In this fashion, imiquimod may be used to prevent *in situ* or invasive skin cancer.

Figure 4. Patient with actinic keratosis at baseline (left panel) treated for 8 weeks with topical imiquimod 5% (2x/week) using IMTD dosing has successful clearance of lesion with excellent cosmesis (middle panel). By contrast, topical 5-FU (1x/day) has destructive side effects with undesirable cosmesis at 4 weeks (right panel). (Source: Angiogenesis Center, Boston).



Field therapy is particularly well-suited as a primary approach to treating AK, and offers certain advantages over locally destructive treatment modalities (ie, cryotherapy) or topical chemotherapy (ie, 5-FU) (Figure 4). Cryotherapy destroys only visible lesions, while cytotoxic chemotherapy is accompanied by severe tissue reaction, necrosis, and inflammation that are undesired and poorly tolerated by most patients. By dosing to achieve antiangiogenic but not gross inflammatory activity, field therapy of AKs with imiquimod overcomes both limitations. Unlike with topical chemotherapy, imiquimod is not harmful to normal skin; in fact, during safety studies for the treatment of genital warts, daily use of imiquimod was less irritating than Vaseline intensive care lotion.⁴⁷ This should provide a comfort level to clinicians in recommending application of imiquimod to broad surface areas over a prolonged period of time. In practice, we usually treat patients with AKs 2x/week or 3x/week for 12 weeks or longer.

Field therapy using imiquimod on visible AKs often leads to pharmacological unmasking of nearby subclinical lesions, probably through the diffusion of cytokines throughout the treatment area. A study of 131 patients whose AKs were treated and cleared with imiquimod showed long-term prevention of AK recurrence in a dose-dependent manner.⁴⁸ Importantly, at 16 months after AK regression, there were no long-term adverse changes in

skin quality associated with treatment. Imiquimod field therapy offered a well-tolerated and efficacious strategy for pre-cancerous skin lesions.

Combinatorial Therapy

Combinatorial antiangiogenic therapy, exploiting the synergistic effects of multiple angiogenesis inhibitors, is an important therapeutic approach for skin and other cancers.^{49,50} Imiquimod has been combined with other topical agents possessing antiangiogenic activity in a “cocktail” fashion. We developed and reported the efficacy of a combinatorial regimen (OLCAT-005) consisting of the topical drugs imiquimod, diclofenac, tretinoin, calcipotriene, and hydrocortisone valerate. These were mixed in equal parts and administered topically to biopsy-proven invasive SCC in immunosuppressed patients using the IMTD protocol. OLCAT-005 achieved complete tumor regression of squamous neoplasia (n = 60) in 97% of patients after 12 weeks of treatment, confirmed by surveillance biopsy of the site.⁵¹ Histopathological analysis demonstrated increased angiogenesis in the initial cancer. OLCAT-005 therapy appeared to revert microvessel density back to normal skin levels following complete remission. This protocol is now under investigation for a variety of solid tumors in animals and humans.

Conclusions

Angiogenesis inhibition is a validated new strategy for the treatment of cancer employing biologic, small molecule, and immunomodulatory agents. Imiquimod is a novel, immune response modifying agent that 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. Combined with its effects on cell-mediated immunity, imiquimod's antiangiogenic activity has potent anti-tumor effects. Clinically, imiquimod is indicated for genital warts, AK, and BCC, but it has been used successfully to treat a number of angiogenesis-dependent skin conditions, including SCC, lentigo maligna, hemangiomas, and pyogenic granuloma.^{52,53}

Field therapy is an important application of imiquimod as an antiangiogenic agent is as field therapy aimed at subclinical and clinical angiogenic lesions (AK, BCC, SCC) in areas of sun damaged skin. Long-term studies substantiate imiquimod's effectiveness in treating primary AK and in preventing its recurrence. By titrating its dosing for angiogenesis inhibitory activity, imiquimod can be applied in an efficacious and well-tolerated fashion for AK and non-melanoma skin cancers. Combinatorial therapies incorporating imiquimod with other topical or systemic antiangiogenic agents may be an even more potent approach for invasive or advanced skin cancers. Our clinical experience with OLCAT-005 demonstrates efficacy for invasive SCC and prevention of recurrence. As the fields of immunotherapy and antiangiogenic therapy begin to merge, agents such as imiquimod will assume an even greater role in the medical management of dermatological disorders.

References

1. Stanley MA. Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential. *Clin Exp Dermatol.* 2002;27:571-7.
2. Megyeri K, Au W-C, Rosztoczy I, et al. Stimulation of interferon and cytokine gene expression by imiquimod and stimulation by sendai virus utilize similar signal transduction pathways. *Mol Cell Biol.* 1995;15:2207-18.
3. Majewski S, Marczak M, Mlynarczyk B, Benninghoff B, Jablonska S. Imiquimod is a strong inhibitor of tumor cell-induced angiogenesis. *Int J Dermatol.* 2005;44:14-9.
4. Folkman, J. In: DeVita VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles & Practice in Oncology*, 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:509-519.
5. Li WW. Tumor angiogenesis: molecular pathology, therapeutic targeting, and imaging. *Acad Radiol.* 2000;7:800-11.
6. Van Scott EJ, Flaxman BA. In: Fleischmajer R, Billingham RE, eds. *Epithelial-Mesenchymal Interactions*. Baltimore, MD: Williams & Wilkins; 1968:280-294.
7. Bielenberg DR, Bucana CD, Sanchez R, et al. Molecular regulation of UVB-induced cutaneous angiogenesis. *J Invest Dermatol.* 1998;111:864-72.
8. Yano K, Kadoya K, Kajiya K, Hong Y-K, Detmar M. Ultraviolet B irradiation of human skin induces an angiogenic switch that is mediated by upregulation of vascular endothelial growth factor and by downregulation of thrombospondin-1. *Br J Dermatol.* 2005;153:115-121.
9. Backvall H, Asplund A, Gustafsson A, et al. Genetic tumor archeology: microdissection and genetic heterogeneity in squamous and basal cell carcinoma. *Mutat Res.* 2005;571:65-71.
10. Melnikova VO, Ananthaswamy HN. Cellular and molecular events leading to the development of skin cancer. *Mutat Res.* 2005;571:91-106.
11. Marks R, Rennie G, Selwood TS. Malignant transformation of solar keratoses to squamous cell carcinoma. *Lancet.* 1988;1:795-7.
12. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell.* 1996;86:353-64.
13. Newell B, Bedlow AJ, Cliff S, et al. Comparison of the microvasculature of basal cell carcinoma and actinic keratosis using intravital microscopy and immunohistochemistry. *Br J Dermatol.* 2003;149:105-10.

14. Li V, Barnhill R. Angiogenesis in dermatology: pathophysiology and clinical implications. *Prog Dermatol* 1995;29:1-12.
15. Nijisten T, Colpaert CG, Vermeulen PB, Harris AL, Van Marck E, Lambert J. Cyclooxygenase-2 expression and angiogenesis in squamous cell carcinoma of the skin and its precursors: a paired immunohistochemical study of 35 cases. *Br J Dermatol*. 2004;151:837-45.
16. Lane BR, Liu J, Bock PJ, et al. Interleukin-8 and growth-regulated oncogene alpha mediate angiogenesis in Kaposi's sarcoma. *J Virol*. 2002;76:11570-83.
17. Frischer JS, Huang J, Serur A, et al. Biomolecular markers and involution of hemangiomas. *J Pediatr Surg*. 2004;39:400-4.
18. Harada K, Lu S, Chisholm DM, Syrjanen S, Schor AM. Angiogenesis and vasodilation in skin warts. Association with HPV infection. *Anticancer Res*. 2000;20:4519-23.
19. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med*. 2004;350:2335-42.
20. Morini M, Albin A, Lorusso G, et al. Prevention of angiogenesis by naked DNA IL-12 gene transfer: angioprevention by immunogene therapy. *Gene Ther*. 2004;11:284-91.
21. Sparano JA, Gray R, Giantonio B, O'Dwyer P, Comis RL. Eastern Cooperative Oncology Group Portfolio of Clinical Trials. Evaluating antiangiogenesis agents in the clinic: the Eastern Cooperative Oncology Group Portfolio of Clinical Trials. *Clin Cancer Res*. 2004;10:1206-11.
22. Kulasegaram R, Giersing B, Page CJ, et al. In vivo evaluation of ¹¹¹In-DTPA-N-TIMP-2 in Kaposi sarcoma associated with HIV infection. *Eur J Nucl Med*. 2001;28:756-61.
23. Wada S, Tsunoda T, Baba T, et al. Rationale for antiangiogenic cancer therapy with vaccination using epitope peptides derived from human vascular endothelial growth factor receptor 2. *Cancer Res*. 2005;65:4939-46.
24. Hou J, Tian L, Wei Y. Immunotherapy of tumor by targeting angiogenesis. *Sci China C Life Sci*. 2004;47:545-52.
25. Li WW. Tumor angiogenesis as a control point for early intervention and cancer prevention. In: Kelloff GJ, Hawk ET, Sigman CC, eds. *Cancer Chemoprevention, Vol. 1: Promising Cancer Chemopreventive Agents*. New Jersey: Humana Press, 2004:611-633.
26. Brouty-Boye D, Zetter BR. Inhibition of cell motility by interferon. *Science*. 1980;208:516-8.
27. Yoshida A, Anand-Apte B, Zetter BR. Differential endothelial migration and proliferation to basic fibroblast growth factor and vascular endothelial growth factor. *Growth Factors*. 1996;13:57-64.
28. Shousong C, Kun L, Toth K, et al. Persistent induction of apoptosis and suppression of mitosis as the basis for curative therapy with S-1, and oral 5-fluorouracil produg in a colorectal tumor model. *Clin Cancer Res*. 1999;5:267-274.
29. Fujita M, Kuwano K, Kunitake R, et al. Endothelial cell apoptosis in lipopolysaccharide-induced lung injury in mice. *Int Arch Allergy Immunol*. 1998;117:202-8.
30. Riedel F, Gotte K, Bergler W, Rojas W, Hormann K. Expression of basic fibroblast growth factor protein and its down-regulation by interferons in head and neck cancer. *Head and Neck*. 2000;22:183-9.
31. Reddy KB, Hocevar BA, Howe PH. Inhibition of G1 phase cyclin dependent kinases by transforming growth factor beta 1. *J Cell Biochem*. 1994;56:418-25.
32. Yang J, Richmond A. The angiostatic activity of interferon-inducible protein-10/CXCL10 in human melanoma depends on binding to CXCR3 but not to glycosaminoglycan. *Mol Ther*. 2004;9(6):846-55.
33. White CW, Sondheimer HM, Crouch EC, Wilson H, Fan LL. Treatment of pulmonary hemangiomas with recombinant interferon alfa-2a. *N Engl J Med*. 1989;320(18):1197-200.
34. Kaban LB, Troulis MJ, Ebb D, et al. Antiangiogenic therapy with interferon alpha for giant cell lesions of the jaws. *Oral Maxillofac Surg*. 2002;60:1103-11.

35. Ezekowitz RA, Mulliken JB, Folkman J. Interferon alfa-2a therapy for life-threatening hemangiomas of infancy. *N Engl J Med*. 1992;326:1456-63.
36. Duda DG, Sunamura M, Lozonschi L, et al. Direct in vitro evidence and in vivo analysis of the antiangiogenesis effects of interleukin 12. *Cancer Res*. 2000;60:1111-6.
37. Yao L, Sgadari C, Furuke K, et al. Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. *Blood*. 1999;93:1612-1621.
38. Kohno T, Mizukami H, Suzuki M, et al. Interleukin-10-mediated inhibition of angiogenesis and tumor growth in mice bearing VEGF-producing ovarian cancer. *Cancer Res*. 2003;63:5091-4.
39. Kawakami T, Tokunaga T, Hatanaka H, et al. Interleukin 10 expression is correlated with thrombospondin expression and decreased vascular involvement in colon cancer. *Int J Oncol*. 2001;18:487-91.
40. Hesling C, D'Incan M, Mansard S, et al. In vivo and in situ modulation of the expression of genes involved in metastasis and angiogenesis in a patient treated with topical imiquimod for melanoma skin metastases. *Br J Dermatol*. 2004;150:761-7.
41. Sidbury R, Neuschler N, Neuschler E, et al. Topically applied imiquimod inhibits vascular tumor growth *in vivo*. *J Invest Dermatol*. 2003;121:1205-9.
42. Marra DE, Haynes HA, Li VW. Antiangiogenic treatment of pyogenic granuloma with imiquimod. *J Am Acad Dermatol*. 2004;50(3):P57.
43. Emmenegger U, Man S, Shaked Y, et al. A comparative analysis of low-dose metronomic cyclophosphamide reveals absent or low-grade toxicity on tissues highly sensitive to the toxic effects of maximum tolerated dose regimens. *Cancer Res*. 2004;64:3994-4000.
44. Zand S, Li WW, Li VW. IMTD: An algorithm-based approach to optimize dose-response in the treatment of skin cancer using imiquimod. *J Am Acad Dermatol*. 2004;50(3):P129.
45. Geisse J, Caro I, Lindholm J, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from two phase III, randomized, vehicle-controlled studies. *J Am Acad Dermatol*. 2004;50:722-33.
46. Li VW, Ahmed S, Marra D, Li WW. What is the optimal dosing of imiquimod. *J Am Acad Dermatol*. 2005;52(3):P150.
47. Meng TC, Shupack JL, Friedman-Kien AE et al. A vehicle controlled safety and efficacy study evaluating 5% imiquimod cream for the treatment of external genital/perianal warts in HIV-positive patients. *Proc International Society for Sexually Transmitted Diseases Congress (Poster 99)*, 1999.
48. Lee PK, Harwell WB, Loven KH, et al. Long-term clinical outcomes following treatment of actinic keratosis with imiquimod 5% cream. *Dermatol Surg*. 2005;31:659-64.
49. Jones PH, Christodoulos K, Dobbs N, et al. Combination antiangiogenesis therapy with marimastat, captopril and fragmin in patients with advanced cancer. *Br J Cancer*. 2004;91:30-6.
50. Minischetti M, Vacca A, Ribatti D, et al. TNP-470 and recombinant human interferon-alpha2a inhibit angiogenesis synergistically. *Br J Haematol*. 2000;109:829-37.
51. Li VW, Ball RA, Vasan N, Li WW. Antiangiogenic therapy for squamous cell carcinoma using combination agents. *J Clin Oncol*. 2005;23(16S):3032.
52. Trotter MJ, Tron VA. Dermal vascularity in lentigo maligna. *J Pathol*. 1994;173(4):341-5.
53. Streit M, Detmar M. Angiogenesis, lymphangiogenesis, and melanoma metastasis. *Oncogene*. 2003;22:3172-9.

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