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. 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. 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. This nonproliferating state is governed by the balancing effects of endogenous stimulators and inhibitors of angiogenesis present in healthy tissue. Positive regulators of angiogenesis (proangiogenic) 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-a, -b, -g), 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. Hemangiomas were the first human tumors to be successfully treated with antiangiogenic therapy using interferon-a-2a, based on the recognition that they overexpress angiogenesis stimulators (FGF-2, VEGF) during the proliferative phase. Conversely, during their involutional phase, endogenous angiogenesis inhibitors (tissue inhibitor of metalloproteinase-1 [TIMP-1], interferon-b [IFN-b]) are upregulated.

Benign growths, such as warts, are also angiogenic in nature. Increasing vascularity is observed between HPV-negative and HPV-positive warts; pinpoint hemorrhagic capillaries are a gross manifestation of the neovascularization that accompanies wart growth and persistence. 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 (a so-called “hit”) in keratinocytes. Clonal expansions of p53 mutant cells occur in photodamaged skin, with as many as 40 mutant clones per cm² 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 (PIGF), Ang-2, and αβ integrins are upregulated in cutaneous malignant melanoma. Melanomas greater than 1 mm in thickness have significantly increased microvessel density (MVD) compared to normal dermis and even severely atypical melanocytic nevi. 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. 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. It has also been demonstrated that dormant melanoma micrometastases lack significant vascularity compared to clinical macrometastases, despite comparable rates of proliferation and apoptosis.

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, multi-targeting 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.

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 (Tarceva) 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. 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.

<table>
<thead>
<tr>
<th>Target/Pathway</th>
<th>Intervention</th>
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<tr>
<td>COX-2</td>
<td>Celecoxib, Diclofenac gel</td>
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<tr>
<td>Sonic Hedgehog</td>
<td>Cycloamine</td>
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<tr>
<td>m-TOR</td>
<td>Sirolimus and analogues</td>
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<td>Growth Factor/Receptor of Tyrosine Kinase</td>
<td>Bevacizumab, Sorafenib, Erlotinib, Gefitinib, Polyphenon E ointment</td>
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<td>Toll-Like Receptor</td>
<td>Imiquimod cream</td>
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which could be blocked by COX-2 specific small interfering RNA. 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 types of solid tumors. Oral celecoxib (20 mg/kg/day) suppresses experimental murine SCC and melanoma growth introduced surgically into mice. 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. 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.

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. Cyclopamine is a steroid alkaloid that acts as a HH antagonist by binding directly to Smoothened. In experimental models, cyclopamine inhibits VEGF and capillary formation in models of ocular neovascularization. Aberrant activation of the HH pathway is known to be associated with the development of BCC. 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. 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- and B-cell activation. Sirolimus also inhibits hypoxia-inducible factor (HIF-1), VEGF expression, and endothelial cell proliferation. 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. In one study, when renal transplant patients were converted to sirolimus, remission of nonmelanoma skin cancer was observed in 37 out of 53 patients. 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. 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. 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. 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. Green tea or purified EGCG, when administered to mice in their drinking water, inhibited angiogenesis in the in vitro Matrigel sponge model and restrained tumor growth. Topical EGCG (1 mg/cm²) 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. EGCG is a component of Polyphenon E 15% ointment, approved by the FDA to treat external genital warts. 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. TLRs are expressed in the skin in keratinocytes and Langerhans cells, macrophages, T 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.

**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 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. The interfer pr-ducible protein-10 (IP-10) is itself an angiostatic protein. Interferon-β 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. 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. The antiangiogenic mechanism of IL-10 is not known, but is correlated to increased expression of the angiogenesis inhibitors TSP-1 and TSP-2.

The antiangiogenic activity of imiquimod in vivo has been profiled in both animal and human subjects. 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.

In human patients, we have successfully used imiquimod as an antiangiogenic agent to regress vascular proliferative lesions such as hemangioma of infancy, pyogenic granuloma, hemangiosarcoma, and Kaposi’s sarcoma. 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. The lesion showed a partial 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). 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-
erved erythema (vasodilation) and mild epidermal desqua-
mation, which are commonly asymptomatic. Although mi-
roscopic 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 and discomfort of inflammation, as described by 
Celsius as rubor (redness), calor (warmth), tumor (swelling), 
and dolor (pain).

While it has been reported that there are statistically sig-
nificant 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 treat-
ment success.9 The evidence from targeted molecular ther-
apies 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 treat-
ment efficacy, the paradigm of an antiangiogenesis approach 
that targets the tumor's vasculature demonstrates that cyto-
toxicity 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 max-
imizing dosing frequency. Data generated from our practice 
indicate that there is tremendous heterogeneity of dose 
response among individual patients at which imiquimod in-
duces 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 un-
derirable 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 inflamma-
tion.30 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 suc-
cessful outcomes.

Summary

Antiangiogenesis inhibition is now validated for the treatment 
of a number of tumor types using a variety of approved bio-
logic, 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 anti-
angiogenic agent, is an immune response modifying agent that 
also stimulates antiangiogenic cytokines, downregulates the 
expression of proangiogenic factors, upregulates the expres-
sion of endogenous inhibitors, and induces endothelial cell 
apoptosis. Thus, topical imiquimod impedes pathological 
tissue growth by interfering with its supporting microcircu-
lation 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 in-
hibitory activity, imiquimod can be applied in an efficacious 
and well-tolerated fashion for skin cancer.

Disclosure

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

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