Breast Cancer Treatment with Imiquimod: Applying an Old Lotion to a New Disease

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In this issue of Clinical Cancer Research, 2 sets of authors from Demaria’s group, Dewan and colleagues (1) and Adams and colleagues (2), detail in companion articles the antitumor efficacy of imiquimod in first, a preclinical breast cancer model in combination with radiation (1), and second, a phase II, breast cancer clinical trial assessing safety and immunologic activity (2).

Fifteen years after first approval by the U.S. Food and Drug Administration (FDA), imiquimod remains the only approved, active toll-like receptor (TLR) agonist (3). Interestingly, the activity of imiquimod was first shown during a screen for anti-herpes therapy, during which imiquimod reduced herpes pathology in culture and later showed potential to induce complete regression of an HPV lesion. Imiquimod, by chemistry, is a nonnucleoside, heterocyclic amine, and preferentially activates TLR-7 with weak activation of TLR-8. Other members of the imidazoquinoline family include resiquimod with equal TLR-7 and TLR-8 potency, gardiquimod with 10-fold higher potency for TLR-7 than imiquimod and strong activation of TLR-8, and l-nucleoside lorbroluride with preferential activation of TLR-7 and only weak activation of TLR-8. In 1997, imiquimod was approved for the treatment of external genital warts with approvals later including actinic keratosis and superficial basal cell carcinoma, both in 2004. Topical application results in complete clearance of external genital warts in up to 70% of cases, actinic keratosis in 57%, and basal cell carcinoma in 90% of cases (4).

However, as the only approved TLR agonist, extensive preclinical research, including nearly 1,800 PubMed citations, as well as clinical investigations including approximately 200 clinical trials, have provided extensive insight into the mechanism and clinical activity of imiquimod.

More than 50 off-label uses of imiquimod have resulted from these investigations focused on cutaneous infectious and malignant disease. Applications include treatment of infectious diseases such as leishmaniasis, herpes simplex virus (HSV), human papillomavirus (HPV), molluscum contagiosum, orf infections, and tinea pedis. Efficacy in these heterogeneous conditions is mediated indirectly by cytokine induction and subsequent immune response, which is recruited to identify and remove the offending pathogen or lesion. Further preclinical testing has detailed an array of pleiotropic effects and possible mechanisms (Fig. 1). The majority of activity results from gene expression reprogramming secondary to movement of transcription factor NF-κB to the nucleus. The broad cascade downstream of NF-κB leads to the upregulation of local proinflammatory cytokines such as IFN-α, TNF-α, as well as interleukins (IL)-6, -8, and -12. IFN-α, the major cytokine responsible for the viral defense of a cell, contributes to imiquimod’s efficacy in the treatment of HSV, HPV, and other infectious processes. Imiquimod and the cytokines induced, in turn, increase apoptosis of tumor cells via STAT-1. Tumor cell proliferation is also inhibited by increased expression of death receptor CD95 and decreased expression of Bcl-2 (5). Concurrently, local antigen-presenting cells are activated with a subsequent robust T-cell infiltrate, specifically a T-helper (Th1) cellular immune response. The Th1 cellular pathways are also augmented with increased B-cell activity and antibody production. The negative regulatory feedback mechanisms that dampen the inflammatory fire, such as the AR signaling pathway (6), are also inhibited. Given the pleiotropic effects of TLR stimulation, including preclinical models showing both immune recruitment and activation in the tumor microenvironment, as well as induction of tumor apoptosis, clinical investigation in multiple cutaneous cancer histologies, such as melanoma and T-cell lymphoma, has been tested with promising response rates (7, 8).

In this issue of Clinical Cancer Research, Dewan and colleagues extend the application of imiquimod to cutaneous breast cancer (1). Skin metastases due to breast cancer represent the second most common cause of cutaneous involvement occurring in up to 10% of patients with...
metastatic breast cancer. Therapies are limited to surgical resection and postoperative radiation; however, high rates of recurrence require development of new treatments. Building on current standard therapies, Dewan and colleagues investigate the combination of TLR-7 stimulation with radiation and chemotherapy. Prior preclinical and early-phase clinical trials support synergy of radiation therapy with TLR-9 stimulation by CpG in fibrosarcoma, non-Hodgkin lymphoma, and mycosis fungoides (9, 10). Dewan and colleagues show that topical imiquimod applied to TSA, a poorly immunogenic breast tumor, effectively reduced tumor size alone or in combination with radiation with minimal toxicity. Imiquimod monotherapy inhibited tumor growth, though it did not induce complete regression with response dependent on the adaptive immune response of CD8 T cells. Similarly, fractionated radiation as monotherapy did not result in complete regression. Complete regression was only observed with combination therapy. Dewan and colleagues investigated a two-tumor model to assess for a systemic immune response, which was minimal following imiquimod alone, although a dramatic effect at the second, untreated tumor was observed with topical imiquimod combined with radiation to the primary tumor. Augmentation of the model’s activity was further increased by depletion of regulatory T cells with low-dose cyclophosphamide. The addition of cyclophosphamide also resulted in more robust IFN-γ responses by tumor-specific cytotoxic T cells and provided protection from tumor rechallenge.

The observed responses at untreated sites support the role of imiquimod in enhancing adaptive immunity, as already shown in a neu-transgenic mouse breast cancer model (11). CD4 T-cell depletion improved efficacy, suggesting that regulatory T cells play a role in inhibition of the immune response. Given the pleiotropic effects of imiquimod, Dewan and colleagues could not conclude whether the modulation of lymphocyte recruitment, angiogenesis inhibition, or T-cell function influenced the observed efficacy.

The first step in testing the triple combination in a phase II clinical trial is presented in this issue of Clinical Cancer Research by Demaria and colleagues (2). In direct translation, Adams and colleagues conducted a prospective, non-randomized, phase II trial of topical imiquimod, 5%,
applied 5 days per week for 8 weeks in a cohort of 10 women with breast cancer skin metastases, assessing safety and immunologic endpoints. All 10 women were heavily pretreated and had failed multiple prior therapies. This approach was well tolerated with limited grade 1 and 2 local and systemic side effects including flu-like symptoms and local injection site reactions. Partial responses were observed in 2 patients with the remaining experiencing progression (2), no response (1), or stable disease (5).

Elegant, serial biopsies of the tumor were conducted before and after treatment to assess surface phenotype of tumor-infiltrating lymphocytes and intracellular cytokines both immediately ex vivo and following culture. Additional corollary studies included a cytokine profile developed from tumor supernatant assaying for IFN-γ, IFN-α2, IL-1b, RANTES, IL-6, IL-10, and IL-17. Despite the extensive corollary work conducted, all immunohistochemical and cytokine endpoints failed to show consistent trends pre-versus posttreatment. Among responding patients, robust tumor-infiltrating lymphocyte populations of CD4 and CD8 T cells were observed with increased local cytokine production of IFN-γ and IFN-α2 posttreatment in one of 2 responders and decreased local cytokine production of IL-6 and IL-10 in the second responder. Interestingly, these observations support the augmentation of a Th1 response and downmodulation of the immunosuppressive cytokine milieu.

In addition to successfully meeting the primary endpoints of safety and feasibility, Adams and colleagues challenge current preclinical models, as their limited clinical series did not observe increased dendritic cell infiltrate or inflammatory cytokine profiles across patients treated with imiquimod. Significant heterogeneity in pretreatment tumor microenvironments was observed, which illustrates that baseline immune profile is likely relevant to respond to immunomodulatory therapy. Although multiple potential explanations for these observations exist, the series is too limited in number to draw conclusions. Intriguingly, 2 patients later received hormone therapy and achieved complete responses, reminiscent of prior observations of sensitized responses to standard chemotherapies following cancer vaccines (12). The promising translational work of Demaria and colleagues is now beginning its next phase, applying imiquimod in combination with radiation therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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