Abstract

Over the prior two decades, imiquimod, a toll-like receptor 7 agonist, has been applied to nearly fifty clinical settings. Due to its immunomodulatory role, the topical cream today for the first time, is being applied to cutaneous breast cancer in pre-clinical models and in a Phase 2 clinical trial.

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In this issue of Clinical Cancer Research, two sets of authors from Demaria’s group, Dewan et al. (1) and Adams et al. (2) detail in companion papers the anti-tumor efficacy of imiquimod in first, a preclinical breast cancer model in combination with radiation (1), and second, a Phase 2, breast cancer clinical trial assessing safety and immunologic activity (2).

Fifteen years after first approval by the Food and Drug Administration (FDA), imiquimod remains the only approved, active toll-like receptor (TLR) agonist (3). Interestingly, imiquimod’s activity was first demonstrated 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 non-nucleoside, 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 loxoribine 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 eighteen hundred PubMed citations, as well as clinical investigations, including approximately two hundred clinical trials have provided extensive insight into the mechanism and clinical activity of imiquimod. Over fifty 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, Human Papilloma Virus (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 (Figure). The majority of activity results from gene expression reprogramming secondary to movement of transcription factor nuclear factor kappa B (NFkB) to the nucleus. The broad cascade downstream of NFkB leads to upregulation of local pro-inflammatory cytokines such as interferon-α, tumor necrosis factor-α (TNF), as well as interleukins-6, -8 and -12 (IL). Interferon-α, the major cytokine responsible for a cell's viral defense, contributes to imiquimod’s efficacy in 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 (APC) are activated with a subsequent robust T cell infiltrate, specifically a T-helper (Th1) cellular immune response. The Th2 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 adenosine receptor (AR) signaling pathway (6), are also inhibited. Given the pleiotropic effects of TLR stimulation, including preclinical models demonstrating 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 metastatic breast cancer patients. Therapies are limited to surgical resection and post-operative radiation, however high rates of recurrence require development of new treatments. Building on current standard therapies, Dewan and colleagues...
evaluate 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’s lymphoma and mycosis fungoides (9, 10). Dewan demonstrates 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 did not induce complete regression with responses 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, though 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 demonstrated in a neu-transgenic mouse breast cancer model (11). CD4 T cell depletion improved efficacy, suggesting regulatory T cells play a role in inhibition of the immune response. Given the pleiotropic effects of imiquimod, Dewan and colleagues could not conclude if 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 2 clinical trial is presented in this issue of Clinical Cancer Research by Demaria (2). In direct translation, Adams and colleagues performed a prospective, non-randomized, Phase 2 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 two patients with the remaining experiencing progression (2), no response (1), or stable disease (5). Elegantly, serial biopsies of the tumor were performed 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-1β, RANTES, IL-6, IL-10, and IL-17. Despite the extensive corollary work performed, all immunohistochemical and cytokine endpoints failed to demonstrate consistent trends pre- versus post-treatment. 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 post-treatment in one of two responders and decreased local cytokine production of IL-6 and IL-10 in the second responder. Interestingly, these observations support augmentation of a Th1 response and down-modulation 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 increases dendritic cell infiltrate nor inflammatory cytokine profiles across patients treated with imiquimod. Significant heterogeneity in pre-treatment tumor microenvironments were observed which illustrates that baseline immune profile is likely relevant to response to immunomodulatory therapy. Though multiple potential explanations for these observations exist, the series is too limited in number to draw conclusions. Intriguingly, two 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 is now beginning its next phase, applying imiquimod in combination with radiation therapy.
References


Figure Legend

Direct and indirect anti-tumor effects of imiquimod therapy on tumor and immune cell subsets including dendritic, natural killer (NK), cytotoxic T, and B cells.
Figure 1:

- **B cell**
  - Proliferation and Ab production

- **NK cell**
  - TLR-7 and TLR-8
  - myD88
  - NF-κB
  - AP-1
  - Transcription of IFN-α, TNF-α, IL-1, IL-6, IL-8, IL-12 and others

- **Tumor**
  - bcl-2 death receptor
  - caspase-8
  - Apoptosis
  - Antitumor cytotoxicity

- **Dendritic cell**
  - TLR-7 and TLR-8
  - Adenyl cyclase and A2A
  - myD88
  - NF-κB
  - AP-1
  - cAMP
  - Transcription of IFN-α, TNF-α, IL-1, IL-6, IL-8, IL-12 and others

- **Cytotoxic T cell**
  - Activation and perforin induction
  - Present tumor Ag

**Notes:**
- Activation, migration, inflammatory mediators and apoptosis
- Antitumor cytotoxicity
- Activation, migration, inflammatory mediators
- NF-κB, AP-1, cAMP
- Transcription of IFN-α, TNF-α, IL-1, IL-6, IL-8, IL-12 and others
- bcl-2 death receptor
- caspase-8
- Apoptosis
- Antitumor cytotoxicity
- Activation, migration, inflammatory mediators
- Surrounded by: TLR-7 and TLR-8

**References:**
- TLR-7 and TLR-8
- AP-1
- Transcription of IFN-α, TNF-α, IL-1, IL-6, IL-8, IL-12 and others
- NF-κB
- AP-1
- cAMP
- Activation and perforin induction
- Present tumor Ag
Breast cancer treatment with imiquimod: Applying an old lotion to a new disease

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