The TLR7 agonist imiquimod as an adjuvant for radiotherapy-elicited in situ vaccination against breast cancer

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Abbreviations: DC, dendritic cell; ICAM1, intercellular adhesion molecule 1; IL-10, interleukin-10; TLR, Toll-like receptor.

Radiotherapy can convert malignant cells into an in situ anticancer vaccine, but is often inadequate at generating sufficient pro-inflammatory signals to optimally activate innate and adaptive immune responses. Topical imiquimod is a powerful pro-inflammatory agent with clinical activity against superficial skin cancers. These two modalities appear to complement each other, hence achieving local and systemic tumor control.

Advanced breast carcinoma can disseminate and generate metastases in multiple organs. While cutaneous metastases are not themselves responsible for the death of breast carcinoma patients, they can profoundly affect their quality of life and remain a therapeutic challenge. Because of their accessibility, cutaneous metastases provide a unique opportunity to straightforwardly monitor the activity of local antineoplastic agents.1

A local treatment commonly employed against cutaneous metastases of breast carcinoma is radiotherapy. The widespread use of ionizing radiation in oncology has been driven by its ability to damage the DNA causing death of rapidly proliferating malignant cells. However, accumulating evidence indicates that radiotherapy exerts multiple immunostimulatory effects, supporting a paradigm change according to which the main goal of radiotherapy would be to convert neoplastic cells into an in situ anticancer vaccine.2 By inducing immunogenic cell death, radiation stimulates dendritic cells (DCs) to cross-present tumor-associated antigens to tumor-specific CD8+ T lymphocytes, hence priming an adaptive immune response.3 These T cells, which are recruited to irradiated tumors by chemokines,4 can easily recognize and reject residual cancer cells (i.e., those that did not die in response to radiotherapy) as they express increased levels of MHC class I molecules, intercellular adhesion molecule 1 (ICAM1) and stress-inducible ligands for killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D).5,6 The precise degree to which radiation-elicited tumor-specific T cells contribute to disease control by radiotherapy in cancer patients remains to be determined. However, like most other anticancer vaccines, radiotherapy is rarely sufficient by itself to elicit an anti-tumor immune response strong enough to achieve systemic tumor control.

The most effective way to improve the immune response induced by vaccination is to identify an effective immune adjuvant. Toll-like receptors (TLRs) are danger sensors that activate innate immunity and initiate adaptive immune responses upon stimulation by a variety of pathogen-derived and endogenous ligands.7 Therefore, synthetic TLR agonists are under intensive investigation as immune adjuvants. The TLR7 agonist imiquimod is commercially available for topical use in patients affected by a bunch of skin neoplasms. Indeed, imiquimod provides a convenient modality to treat the often extensive surface of skin involved by the recurrences of breast carcinoma that involve the chest wall. We have previously shown that topical imiquimod enhances immune response to concomitantly administered tumor-associated antigens.8 More recently, we have reported that imiquimod, employed as a standalone therapeutic intervention, mediates antineoplastic effects as it alters the immunological profile of the tumor microenvironment, inducing a partial response in 20% of patients bearing cutaneous metastases of breast carcinoma.1

We have recently demonstrated that the TLR7 agonist imiquimod mediates adjuvant activity when combined with local radiotherapy in a mouse model of...
cutaneous breast carcinoma. Similarly to what observed in clinical settings, topical imiquimod applied to TSA mouse mammary carcinomas had a partial antineoplastic effect, reducing to some extent tumor growth as compared with placebo. Imiquimod-treated murine tumors exhibited increased infiltration by T cells, similar to the neoplastic lesions of patients responding to this immunotherapeutic, as well as by dendritic cells. When imiquimod was applied topically to neoplastic lesions subjected to local radiotherapy, complete tumor regression was observed in a majority of animals. Conversely, radiotherapy alone delayed tumor growth but did not induce complete disease regression. Tumor-specific effector CD8+ T cells that produced interferon γ in response to antigenic stimulation were detected in the tumor-draining lymph nodes of animals subjected to this combinatorial immunotherapeutic regimen. Thus, imiquimod and local radiotherapy exerted synergistic antineoplastic effects, hence mediating local disease control.

Skin metastases, which frequently manifest after mastectomy and typically present as multiple nodules along the mastectomy scar, can progress to diffusely infiltrating lesions across the chest, back, abdomen and ipsilateral arm. To determine if the delivery of irradiation and imiquimod to one neoplastic lesion, the "vaccination site," could induce antitumor responses that would also be effective against non-irradiated tumors (abscopal effect), mice were injected with TSA cells at two separate sites and—once tumors became palpable—only one of them was treated with imiquimod plus radiotherapy. Indeed, we observed an abscopal effect that was markedly enhanced by the topical application of imiquimod to the non-irradiated tumor. Imiquimod improved the expression of MHC class I molecules and ICAM1 on the surface of transformed cells (TCs).

Figure 1. Ionizing radiation and the Toll-like receptor 7 agonist imiquimod cooperate in converting malignant cells into an in situ anticancer vaccine. Radiation-induced immunogenic cell death allows for the release of tumor-associated antigens that are taken up by dendritic cells (DCs), which abun-
dantly infiltrate imiquimod-treated tumors. Activated DCs loaded with tumor-associated antigens migrate to tumor-draining lymph nodes (TDLN), where they activate naïve tumor-specific T cells. Activated tumor-specific T cells traffic to both irradiated and non-irradiated tumors. The cytotoxic activity of T cells is facilitated by the imiquimod- or radiation-induced upregulation of MHC class I molecules and intercellular adhesion molecule 1 (ICAM1) on the surface of transformed cells (TCs).
Of note, in mouse tumor models the powerful pro-inflammatory activity of imiquimod can activate a feedback immunosuppressive loop based on interleukin-10 (IL-10) that significantly reduces the persistence of antitumor T-cell responses. The use of monoclonal antibodies that block IL-10 or the administration of a single immunomodulatory dose of cyclophosphamide was shown to limit IL-10 levels, promoting sustained anticancer immune responses. The precise role of IL-10 in imiquimod-treated patients remains to be determined, although no consistent increase in the circulating levels of this cytokine has been observed in a previous trial. Ongoing clinical studies will provide critical insights into the therapeutic benefits of imiquimod combined with radiotherapy and, possibly, identify additional targets for optimizing this combinatorial regimen. Most importantly, by monitoring the response of distant metastases, we will assess the potential of this immunotherapeutic strategy to induce systemic antitumor immune responses that not only achieve local disease control but also increase patient survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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