Harnessing the Potential of Radiation-Induced Immune Modulation for Cancer Therapy

Mansoor M. Ahmed¹, James W. Hodge², Chandan Guha³, Eric J. Bernhard¹, Bhadrasain Vikram¹, and C. Norman Coleman¹

Abstract
The conventional use of radiotherapy is for local tumor control. Radiotherapy of the primary tumor can prevent the development of distant metastases, but this modality is generally not effective for treating preexisting systemic disease. However, radiation-induced tumor destruction may be considered a novel strategy for in situ cancer vaccination, in which tumor antigens released from dying tumor cells may be presented in an immunostimulatory context. Moreover, radiation has been demonstrated to induce immunogenic modulation in various tumor types by altering the biology of surviving cells to render them more susceptible to T cell-mediated killing. Finally, radiotherapy typically has a favorable toxicity profile and is associated with the absence of systemic immunosuppression. Together, these properties suggest that radiotherapy may serve as an important component of combinatorial immunotherapies aimed at augmenting systemic antitumor immunity. Here, we provide an overview of the radiation-induced modulations of the immune system that may be harnessed for cancer therapy. Cancer Immunol Res; 1(5); 280–4. ©2013 AACR.

Introduction
More than 50% of patients with solid tumors receive radiotherapy as part of their disease management. When current treatment modalities fail to control the primary tumor, local and distant disease recurrence pose a major challenge for cancer therapy. One promising approach against recurrent disease involves immunotherapy, particularly antibody blockade of negative T-cell checkpoints such as cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) and programmed death 1 (PD-1).

It is evident that cancer cells express diverse tumor antigens that include mutated oncoproteins, fusion proteins derived from chromosomal translocations, and developmentally regulated proteins that are aberrantly expressed during tumorigenesis (1). Radiotherapy impacts innate and adaptive immunity through a variety of mechanisms, thereby shaping the outcome of the host antitumor response: Radiotherapy elicits tumor cell death that provides a source of tumor antigens; radiotherapy is a potent inducer of cytokines, which alters the profile and function of immune infiltrates; and radiotherapy remodels the stromal and angiogenic compartments of the tumor microenvironment. In addition, tumor cells surviving radiotherapy are more sensitive to immune-mediated killing. Here, we present a brief narrative of the major steps through which radiotherapy sculpts the antitumor immune response.

Radiotherapy-induced tumor cell death
A schematic view of the radiotherapy-induced immune modulations is presented in Fig. 1. In an initial step, tumor-associated antigens (TAA) are released from dying tumor cells following radiotherapy. These TAAs and other tumor cell debris may be captured in the tumor microenvironment by immune cells such as macrophages, neutrophils, and dendritic cells for antigen processing and presentation (2). Dendritic cells are professional antigen-presenting cells (APC) that survey tissues for infection or injury and are organized into networks in the skin and mucosal surfaces (3, 4). Following engulfment, APCs process the tumor antigens into short peptides that are presented on the cell surface in the context of MHC molecules. If appropriate “danger” signals are also present, the dendritic cells may become activated and migrate to the draining lymph nodes, where they interact with recirculating T helper cells (Th0). Binding to CD40 ligand (CD40L) expressed on the surface of CD4⁺ T cells stimulates further maturation of dendritic cells (5). Additional costimulatory interactions between mature dendritic cells and Th1 cells and the provision of IFNs and interleukin (IL)-12/18 may stimulate a Th1 response, which in turn supports the proliferation and differentiation of antitumor CD8⁺ CTLs (6).

Mature dendritic cells not only present tumor antigens to CD4⁺ T cells through the MHC class II presentation pathway but may also cross-present tumor antigens to CD8⁺ T cells via

Authors’ Affiliations:
1 Radiation Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, Rockville; 2 Recombinant Vaccine Group, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland; and 3 Department of Radiation Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York

Corresponding Author: Mansoor M. Ahmed, Radiotherapy Development Branch, Radiation Research Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, 9609 Medical Center Drive, Room 3W224, Rockville, MD 20850. Phone: 240-276-5700; Fax: 240-276-5827; E-mail: ahmedmm@mail.nih.gov

doi: 10.1158/2326-6066.CIR-13-0141
©2013 American Association for Cancer Research.
the exogenous MHC class I presentation pathway (7–9). Activated antigen-specific CTLs may traffic systemically to infiltrate primary and metastatic tumors, and mediate tumor cell killing. In this context, recent studies have shown that exposure of various subtypes of carcinomas to sublethal doses of radiation can render them more susceptible to T cell–mediated killing (10–12). This radiation-induced immunomodulation contributes to the overall immune response that includes the upregulation of TAAs, MHC class I, ICAM-1, and Fas, and highlights a complementary mechanism by which radiation can enhance the efficacy of immunotherapy.

### Radiotherapy-induced antigen presentation

Radiation-induced tumor cell death involves not only the release of TAAs but also the concurrent release of “danger signals,” including HSPs, calreticulin, and HMGB1, all of which can induce dendritic cell maturation and differentiation (13–15). These danger signals along with the TAAs released from dying cancer cells can be taken up by APCs and result in effective tumor antigen presentation to T cells (16). Because antigen presentation by cancer cells is often defective, this radiation-enhanced antigen processing allows a greater number of TAAs to be processed and presented by APCs, thereby increasing the diversity of activated CTLs. Moreover, damaged/dying cancer cells translocate calreticulin from the endoplasmic reticulum to the cell membrane, and this constitutes an “eat me” signal for phagocytic cells including APCs (17), further enhancing antitumor immunity.

Studies have shown that radiation can enhance the susceptibility of tumor cells to immune recognition through increased expression of MHC class I molecules (18), which diversifies the antigen repertoire presented (19). Although the precise basis for these effects remains to be clarified, one possibility is that radiation may elicit transcription of otherwise silent genes and trigger new mutations as a consequence of DNA damage. These neoantigens might in turn serve as potent targets for high-affinity CTLs.

### Impact of radiotherapy on the tumor microenvironment

Several factors that attenuate the endogenous antitumor immune response must be considered when using radiotherapy for immunotherapy. First, many cancer antigens are...
poorly immunogenic because of central and peripheral tolerance mechanisms. However, radiotherapy may enhance the expression of these targets and in some cases their translocation to the cell surface (20). Because the repertoire of potential antigens does include mutated proteins, radiotherapy may also augment the presentation of these neoantigens, as a novel approach for personalized therapeutic cancer vaccination (21). Second, neoantigens may not always be presented efficiently to the immune system because of a relative local deficiency of mature APCs. Efforts to increase the numbers and functions of APCs in conjunction with radiotherapy might therefore enhance antitumor immune reactions. Third, the efficiency by which radiation promotes the maturation of APCs remains to be fully clarified. Fourth, tumors secrete immunosuppressive cytokines, such as TGF-β and IL-10, and particular radiation doses may further increase their production (22), which might enhance local immunosuppressive networks. Therefore, the dose and schedule of radiotherapy are important factors to consider for combination with immunotherapy. Fifth, T-cell function is dampened by signaling through negative costimulatory molecules such as CTLA-4, which are upregulated upon radiotherapy-triggered T-cell activation. Finally, the timing of administering a second form of immunotherapy relative to radiotherapy is critical, as surgical excision or rapid ablation of primary tumor by physical means removes the source of the antigen, thereby impeding sustained immune responses. These considerations may apply to several types of physical tumor ablation, including microwave, cryotherapy, laser ablation, high-intensity focused ultrasound, and radiofrequency ablation (23, 24).

The précis is that potentially each step of the antitumor immune response can be augmented or modulated by specific therapeutic maneuvers. For example, a slow and prolonged release of tumor antigens could be effectuated by tumor irradiation, but not by surgical removal or physical ablation (such as cryotherapy, photodynamic, or thermal therapy) of the tumor. Availability of a sufficient number of circulating dendritic cells could be synchronized with neoantigen release by the administration of specific cytokines at appropriate time points during the course of cancer treatment. Maturation of dendritic cells could be ensured by the administration of CD40L. Moreover, following the initiation of the immune response, the tumor might be "debulked" via physical, chemical, or molecular approaches so that the immune response is not attenuated by local IL-10 secretion. It is also possible that pharmacologic blockade of CTLA-4 on activated T lymphocytes could prolong the APC–T(IL) cell costimulation, thereby resulting in a more intense and sustained immune response. Several components of this scheme can be tested in mouse models of advanced cancer generated by local implantation of tumor cells.

As a first step in exploring these therapeutic possibilities, we investigated whether the slow release of tumor antigens with local radiotherapy might be synchronized with increased number of dendritic cells elicited with Flt3 ligand (Flt3L; 25, 26). When the 3LL murine lung adenocarcinoma cell line was implanted into syngeneic immunocompetent mice, the treatment of a 3-week established tumor with either surgery or radiation resulted in 60-day survival. Survival was slightly (75 days) prolonged when mice received Flt3L after surgical ablation of the tumor. However, 55% of the mice that underwent radiotherapy together with Flt3L survived long-term (>120 days). These benefits were lost in nude mice, indicating a critical role for T-cell immunity. Histologic examination showed extensive pulmonary metastases in mice that failed to respond to therapy, whereas surviving mice displayed minimal tumor burdens (25). These findings support the hypothesis that slow release of tumor antigens by tumor irradiation, together with the expansion of APCs by the administration of Flt3L, provides the mice with immune protection from subsequent distant metastasis.

On the basis of the earlier observations, follow-up studies were carried out to examine whether the Flt3L-enhanced immune response could be further augmented through enhancing APC maturation with CD40L. Using the same mouse model, the addition of CD40L to the radiation plus Flt3L regimen increased the long-term survival of the treated mice to 70% (27). Incubation of irradiated tumor cells with fluorescence-labeled, bone marrow-derived immature dendritic cells revealed that the irradiated tumor cells were engulfed by the dendritic cells, which was followed by the activation of dendritic cells and the induction of CD86 costimulatory molecules on the cell surface. Furthermore, dendritic cell maturation following incubation with irradiated tumor cells was demonstrated through the switching of chemokine receptor expression from CCR1 to CCR7, a "homing" receptor for activated dendritic cells to migrate to the draining lymph nodes (28, 29).

In summary, these data demonstrate an in situ autologous tumor vaccination strategy in which radiation-enhanced tumor antigen presentation and immunomodulation might serve as a key component of immunotherapy for solid tumors. Because cancer is a chronic disease, the induction of the body's own immune system to fight and potentially eradicate distant occult metastatic disease might help in prolonging patient survival.

**Challenges in using radiotherapy for immunomodulation**

Technical advances have allowed the delivery of conformal radiation by hypofractionation (high doses are delivered in one or a few fractions, thus reducing the number of fractions) or other modifications of standard fractionation (1.8–2.4 Gy per fraction, given in a total of 25–40 fractions per treatment). These modes of radiotherapy may promote immune responses to tumors, but the precise impact of these strategies on T-cell activation and changes in tumor-antigen presentation remains to be fully defined.

The crossroads between radiation biology and cancer immunology highlights several critical issues for further study. Important topics to investigate include the following:

1. Understanding the mechanism through which hypo- and special multifractionated radiotherapy modulate tumor immunogenicity. The impact of radiation dose and
fractionation schedule on immune effector function and survival should be clarified.

2. Determining whether there are differences in the antitumor T-cell responses stimulated with high-dose radiation-induced cell death versus low-dose immunogenic modulation, and whether these impact combinatorial therapies.

3. Delineating the mechanism of radiotherapy-mediated modulation of immune cells such as macrophages and dendritic cells.

4. Developing novel delivery strategies to limit damage to normal tissue with radiotherapy.

5. Characterizing the role of radiation in triggering systemic immunity, which may underlie some forms of the abscopal effect.

6. Developing tumor models to define the best ways in which radiotherapy can be combined with other immunotherapies. These models might also allow definition of settings in which radiotherapy alone is sufficient to generate a potent antitumor immune response.

7. Defining the key preclinical experiments needed to advance promising combination therapies to clinical testing.

In summary, the integration of radiotherapy with immunotherapy holds great promise for intensifying antitumor responses. A deeper understanding of the impact of radiation on tumor cell death, antigen presentation, cytokine production, and the dynamics of the tumor microenvironment will be essential for realizing the potential of this local modality to impact systemic disease.

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

Authors’ Contributions
Conception and design: M.M. Ahmed, C. Guha, B. Vikram, C. N. Coleman
Development of methodology: C. Guha
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Guha
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.W. Hodge, C. Guha
Writing, review, and/or revision of the manuscript: M.M. Ahmed, J.W. Hodge, C. Guha, C. N. Coleman
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Guha
Study supervision: C. Guha, B. Vikram
Supervisor to project: E.J. Bernhard

Received September 4, 2013; accepted September 16, 2013; published online November 4, 2013.

References
Harnessing the Potential of Radiation-Induced Immune Modulation for Cancer Therapy

Mansoor M. Ahmed, James W. Hodge, Chandan Guha, et al.