ELSEVIER
Seminars in RADIATION ONCOLOGY

Radiotherapy and Immunogenic Cell Death

Encouse B. Golden, MD, PhD,[*] and Lionel Apetoh, PhD[†,‡,§]

Advances in understanding the mechanisms that underlie the interplay between radiation-invoked immune responses and tumor regression are underway. Emerging applications of local radiotherapy as an immunologic adjuvant have provided radiation oncologists with a method for converting malignant cells into endogenous anticancer vaccines. The dispersion of radiotherapy-induced immune-stimulating tumor antigens released from dying tumor cells into the surrounding milieu (known as immunogenic cell death, Fig. 1), is one such exploitable process that contributes to the propagation of antitumor immunity. Downstream components of the immune system may suppress, promote, or ambiguously affect antitumoral responses. Additionally, host, tumor, and treatment-related characteristics govern the significance of these signals, thereby dictating therapeutic outcomes. Herein, we review the process of radiotherapy-induced immunogenic cell death and its role in generating an in situ vaccine to help refine radioimmunotherapy-based protocols.

Introduction

The central dogma of traditional radiobiology states that the cytotoxic effects of radiation on tumor cells are primarily due to the production of DNA double-strand breaks followed by some form of cell death, including apoptosis, necrosis, autophagy, mitotic catastrophe, or replicative senescence. In accordance, DNA damage and subsequent tumor cell kill has been ascribed to 4 basic principles (known as the 4 "Rs" of radiobiology): reassortment of tumor cells into radiosensitive phases of the cell cycle (G2/M), reoxygenation of hypoxic areas within a tumor, repair of sublethal DNA damage, and repopulation of surviving tumor cells; whereby, the manipulation of each factor alters tumor cell radiosensitivity. However, in the context of the tumor microenvironment and host antitumor immunity, the scope of this aforementioned traditional view is limited. Immunogenic cell death (ICD), a newly defined form of cell death, may involve the recruitment of the host's immune system as a contributor of the "in-field" response to radiotherapy, thereby resulting in immune memory and advantageous systemic effects.[1] Thus, radiation-induced immune-mediated tumor rejection can be considered an alternative radiosensitizing modality, referred to as a fifth radiobiologic principle.[2]

The abscopal (ab-scopus, away from the target) effect is a term used to describe radiotherapy-induced tumor regression in lesions distant from a targeted site (Fig. 1). Its occurrence provides a proof of principle for the involvement of the immune system with radiotherapy. Recently, a renewed interest in this phenomenon was sparked by a case report involving a patient with metastatic melanoma treated with local radiotherapy in combination with a cytotoxic T-lymphocyte antigen-4 (CTLA4, an inhibitor of cytotoxic T-cell activation)–blocking antibody (ipilimumab). This treatment resulted in a dramatic abscopal response.[3] An additional report was published that described the first abscopal response in a patient with chemotherapy and radiotherapy-refractory metastatic non–small cell lung cancer (NSCLC) treated with a similar regimen, resulting in a sustained complete clinical and radiographic response.[4] Not only are these reports of abscopal effects a *cause célèbre*, but they were also foreshadowed by work completed over the past decade by Formenti and Demaria.[5-7] They posited that an irradiated tumor itself represented an opportunity to establish an endogenous vaccine.[8]

Formenti and Demaria first established an abscopal tumor model to better understand the mechanisms involved to harness and systematically reproduce its beneficial effects.[5] Using immunocompetent mice bearing a syngeneic mammary carcinoma in both flanks, they showed that local irradiation to

*Department of Radiation Oncology, New York University School of Medicine, New York, NY.
†INSERM U866, Dijon, France.
‡Université de Bourgogne, Dijon, France.
§Centre Georges François Leclerc, Dijon, France.
The authors declare no conflict of interest.
Address reprint requests to Encouse B. Golden MD, PhD, Department of Radiation Oncology, New York University School of Medicine, 160 E 34th St, New York, NY 10016. E-mail: Encouse.golden@nyumc.org

http://dx.doi.org/10.1016/j.semradonc.2014.07.005

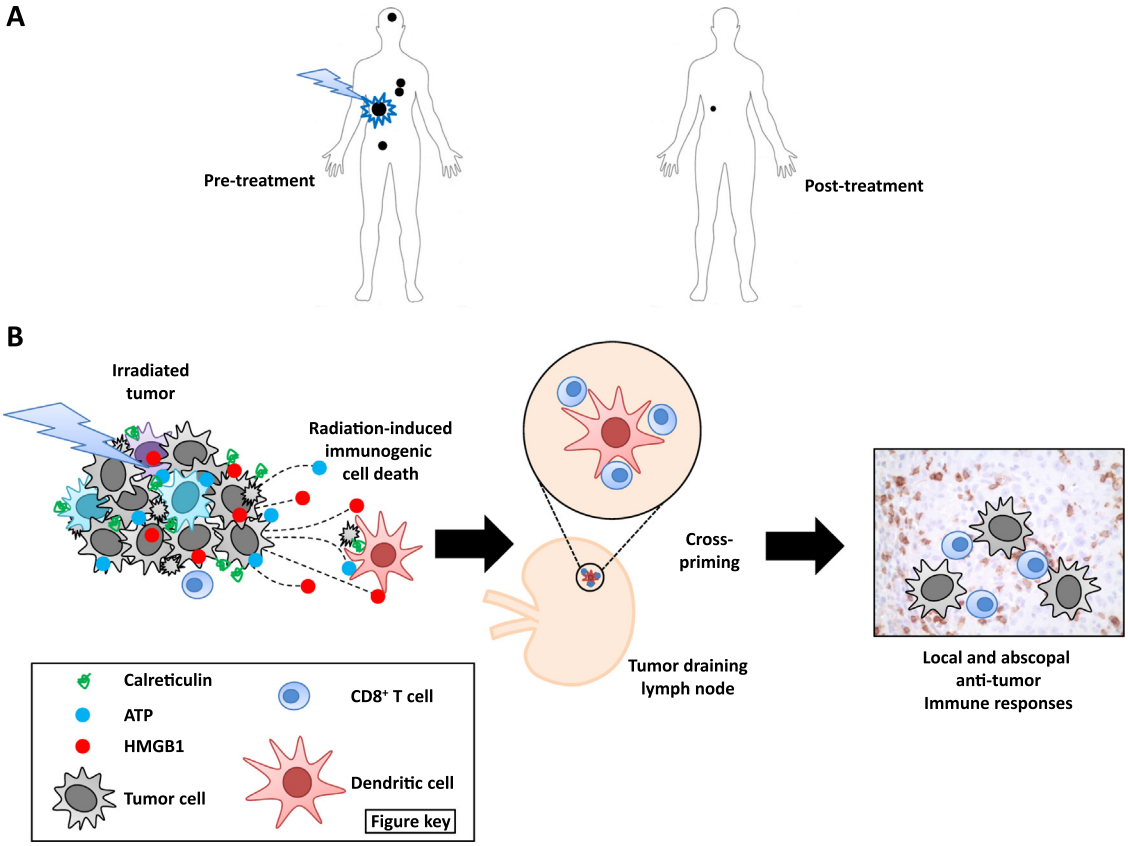

Figure 1 Radiotherapy and antitumoral immunity. (A) Radiotherapeutic treatments were initially designed to selectively kill tumor cells within the irradiated field. However, emerging evidence indicates that radiotherapy harnesses the host's immune system to attack the remaining tumor cells. This immune-driven effect of radiotherapy contributes to the elimination of residual tumor cells at not only locally irradiated sites but also distant sites of disease. (B) Radiotherapy triggers ICD, resulting in the translocation of CRT to the cell surface (a DC "eat-me" signal) and the release of danger signals such as HMGB1 and ATP, which are essential for the promotion of $CD8^+$ T-cell anticancer responses. Primed $CD8^+$ T cells contribute to subsequent residual tumor cell elimination in the tumor bed as well as nonirradiated tumor deposits at distant sites of disease.

only 1 tumor combined with administration of Flt3-L could impair tumor growth of not only the irradiated tumor but also the nonirradiated tumors outside the radiation fields. Interestingly, this abscopal effect was dependent on the presence of T cells as it was not observed in T-cell–deficient nude mice. Their model thus showed that an intact immune system was necessary to recapitulate abscopal responses. They later hypothesized that these responses may involve a radiotherapy-induced immunogenic type of cell death, responsible for immune activation de novo.[1,8] Their in situ vaccination hypothesis asserted that tumor-associated antigens released from irradiated tumor cells are taken up by dendritic cells (DCs) and used to stimulate downstream effector T cells capable of recognizing and lysing tumor cells both locally and at distant sites.[8]

In parallel, Zitvogel and Kroemer helped to elucidate and operationally define ICD.[9] They showed that ICD by some cytotoxic agents elicit tumor-specific immune response in immunocompetent hosts. Although the intricate details of the ICD machinery continue to be revealed, 3 necessary components (calreticulin [CRT] cell surface translocation and HMGB1 and adenosine triphosphate [ATP] release) were well characterized by Zitvogel and Kroemer. Mostly, ICD was shown to intensify DC phagocytosis of tumor cells, DC processing of tumor-derived antigens, and DC secretion of IL-1β, resulting in DC maturation and cross-priming of $CD8^+$ cytotoxic T lymphocytes.[9]

In support of the in situ vaccine hypothesis and the role of ICD, Formenti and Demaria found that interventions that promote the functionality of DCs (Flt3 ligand) or improve $CD8^+$ cytotoxic T-cell activation (CTLA4 blockade) were conditions responsible for establishing radiotherapy-induced antitumor immunity.[5-7,10] However, the genetic background of the host, the immunogenicity of the tumor being treated, and the radiation dose and fractionation schedule were later shown to also contribute to therapeutic outcomes (Fig. 2).[7,11,12] Thus, once better understood and properly exploited, the strategy of combining radiotherapy with an immune modulator may prove to be a watershed event that transforms the role of radiation from a local therapy to an endogenous vaccine generator, leading to dramatic systemic antitumor immune responses in patients with aggressive tumors.[1,8,13] Herein, we discuss the role of ICD and its contribution to the propagation of radiotherapy-induced antitumor immunity.

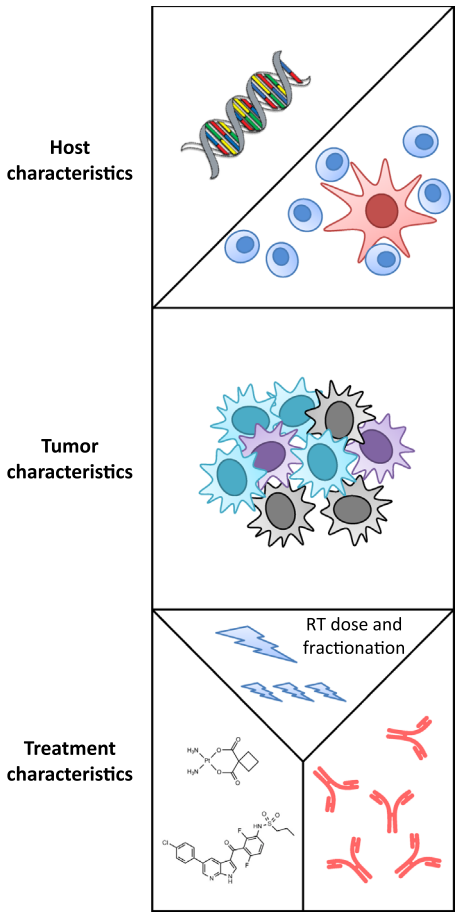

Figure 2 Host, tumor, and treatment-related factors responsible for radiotherapy-induced immunologic responses. The design of successful radiotherapy-based treatments should not only rely on tumor characteristics (tumor type and immunogenic vs nonimmunogenic tumors) but also take into account the immune status of the host (SNPs and pretreatment immunologic status). The careful implementation of combinatorial radiotherapy-based regimens (dose and fractionation and sequential vs concurrent radiotherapeutic regimens) will further enhance the efficacy of radiation via improved antitumor immunologic responses.

Immunogenic Cell Death

As defined by Zitvogel and Kroemer, 3 distinct arms orchestrate ICD in dying tumor cells and are required for immune priming and activation: (1) the cell surface translocation of CRT (an endoplasmic reticulum [ER]-residing protein chaperone and potent DC "eat-me" signal), (2) the extracellular release of HMGB1 (a DNA-binding protein and TLR4-mediated DC activator), and (3) the extracellular release of ATP (an activator of the DC P2XR7 purinergic receptor that triggers DC inflammasome activation, secretion of IL-1β, and subsequent priming of interferon-γ-producing $CD8^+$ T cells).[9] Radiotherapy has been shown to induce all the 3 arms of ICD; whereby, the net effects of all the 3 arms act to promote DC phagocytosis of tumor cells, processing of tumor-derived antigens, and DC-associated cross-priming of $CD8^+$ CTLs (Fig. 1).[14]

CRT cell surface exposure on tumor cells acts as an "eat-me" signal for DCs and involves the coordinated activation of 3 specific modules: ER stress, apoptosis, and CRT-ERp57 translocation.[15] The ER stress module requires eIF2 phosphorylation (a marker for ER stress and translation inhibition). The apoptotic module requires caspase-8 activation, Bap31 cleavage, and Bax-Bak activation. Lastly, the translocation module requires anterograde ER-Golgi trafficking and extracellular exposure of CRT or ERp57.[14,16,17]

In contrast to CRT, the cell surface marker, CD47 (a DC "do-not-eat-me" signal), is widely expressed in solid and hematogenous tumor cells.[18] CD47 was discovered on newly formed circulating red blood cells and shown to prevent red blood cell clearance by the splenic reticuloendothelial system.[19] CD47 blockade of tumor cells is associated with immune-mediated tumor rejection.[18,20] Interestingly, radiotherapy was shown to reduce the amount of CD47 expression in a dose-dependent manner in human papillomavirus–positive tumor cells of the head and neck. This reduction in CD47 levels resulted in improved immune-mediated tumor clearance by radiation.[21]

HMGB1 is an evolutionary conserved nuclear protein that is expressed by almost all cells (cells with an intact nucleus) and is important for the regulation of transcription.[22] When released from dying cells, it acts as a cytokine and danger-associated molecular pattern protein that mediates responses to infection, injury, and inflammation; thus, HMGB1 has been called by Lotze and Tracey[22] the immune system's "nuclear weapon." HMGB1 is passively released from tumor cells previously exposed to radiotherapy and undergoing necrosis.[9,11,14,22] Passively released HMGB1 signals through RAGE, TLR2, and TLR4, where it promotes the transcription of proinflammatory genes in immune cells.[9,11,14,22]

ATP release is yet another important ICD component. It involves the autophagic machinery, where knock down of ATG7 and ATG5 blocks ATP release.[23] Recently, radiotherapy has been shown in several models to cause the release of ATP from dying tumor cells and activation of immune cells via the P2XR7 purinergic receptor pathway.[12,14,24,25] This pathway involves ATP-P2XR7-receptor stimulation followed by upregulation and activation of the DC inflammasome (a large multiprotein complex composed of NLRP3, cardinal, the adapter ASC, and procaspase-1).[12,23] DC inflammasome activation results in the synthesis and secretion of IL-1β, where secreted IL-1β initiates further proinflammatory events.[12,23,26]

In addition to the classic components of ICD, other molecules upregulated in response to radiotherapy have been shown to be involved in antitumor immunity. For example, major histocompatibility complex Class I molecules present endogenous peptides to cytotoxic T lymphocytes and allows for the recognition of tumor cells by cytotoxic T lymphocytes, resulting in efficient tumor cell kill. Radiotherapy has been shown to increase cell surface expression of major histocompatibility complex Class I molecules on tumor cells in a dose-dependent manner.[27] Additionally, radiation was shown to increase the cell surface expression of RAE-1 on tumor cells, a ligand for natural killer cell group 2D, which supports the recruitment of $CD8^+$ T cells and the subsequent formation of productive immunologic synapses.[28] These findings illustrate the necessity of further work to understand the importance of

both classic and nonclassic ICD mechanisms involved in establishing antitumor immunity with radiotherapy.

Host-Related Factors

TLR4

A single nucleotide polymorphism (SNP) in the human TLR4 gene was found to reduce the binding of HMGB1 to human TLR4 receptor; thus, inhibiting HMGB1-dependent DC cross-presentation. Interestingly, patients with breast cancer who carry a TLR4 loss of function allele relapsed faster than patients with a normal TLR4 allele after treatment with anthracycline-based chemotherapy and radiation.[11] These results exemplify the clinical relevance of ICD and HMGB1-TLR4 DC signaling with this regimen.[11]

P2XR7

ATP released from dying tumor cells acts on the P2XR7 purinergic receptors of DCs and triggers the NLRP3 or caspase-1 activation complex (inflammasome), allowing for the secretion of IL-1-β. In the absence of the IL-1 receptor or in the presence of an IL-1 receptor antagonist, DCs fail to cross-prime $CD8^+$ T cells. Moreover, DC cross-priming of $CD8^+$ T cells failed in NLRP3 or Casp-1-deficient mice, unless exogenous IL-1β was provided. A loss of function SNP in the P2XR7 gene (nucleotide position 1513 [1513 A>C] by changing a glutamic acid to alanine at amino acid 496 [Glu496Ala]) was evaluated, retrospectively in 225 women diagnosed with breast cancer treated adjuvantly with an anthracycline-based chemotherapy and radiation. Similar to the TLR4 loss of function SNP, the P2XR7 loss of function allele demonstrated a significant negative prognostic effect on metastatic disease–free survival (log rank test, $P = 0.02$).[12]

Intestinal Microbiota

The amount and type of bacterial colonization may influence an individual's therapeutic outcome.[29] For example, the intestinal microbiota in mice was shown to modulate the anticancer immune effects of cyclophosphamide by reprogramming Th17 cellular responses.[30] Th17 cells are known to have dual effects on tumor cells.[31] However, Th17 cells that secrete IL-17A and express the Th17 transcription factors (Stat3 and RORγt) can be induced from naïve CD4 T cells with IL-1β, IL-6, and IL-23 or with transforming growth factor β and IL-6 to produce TH17 subtypes that result in the elimination or growth of tumor cells, respectively. Cyclophosphamide was shown to disrupt the gut mucosal integrity, resulting in the translocation of gram-positive bacteria into secondary lymphoid organs. Once in the lymphatics, the translocated bacteria stimulated the generation of the Th17 cell subtype responsible for tumor cell elimination. Interestingly, the use of antibiotics to eliminate the gut bacteria abrogated this response and reduced the effectiveness of cyclophosphamide. Given that radiotherapy causes alterations in the barrier function of skin and the gastrointestinal tract, these side effects may lead to transient bacterial translocation and subsequent Th17 antitumor responses. However, the role of the intestinal microbiota and skin flora with radiotherapeutic outcomes has yet to be explored.

Pretreatment Immunologic Status

Individual responses to CTLA4 blockade were shown to depend on the pretreatment immunologic status of the host. For instance, individuals bearing low levels of $CD14^+HLA\text{-}DR^{neg/low}$ myeloid-derived suppressor cells (MDSCs) in the peripheral blood turned out to be more likely to respond to ipilimumab than subjects in whom the circulating amount of these cells were high.[32] Thus, a patient's pretreatment immunologic status may need to be taken into consideration before the administration of radiotherapy with CTLA4-blocking agents. However, further work is needed to evaluate this association with radiotherapy and antitumor immunity.

Tumor-Related Factors

Historically, abscopal responses are a rare occurrence. Few cases have been reported in several tumor types, including melanoma, lymphoma, and NSCLC.[3,33-37] However, with the advent of immune checkpoint inhibitors, such as blocking antibodies to CTLA4, PD-1, and PDL-1, these occurrences are being reported with more frequency. It appears that both immunogenic and nonimmunogenic tumor types may be converted into endogenous vaccines with the addition of immune checkpoint inhibitors or immune adjuvants with radiotherapy.

Breast Cancer

In an in vivo breast cancer model, treatment with imiquimod (a topical TLR7 agonist) in combination with radiotherapy enhanced tumor response compared with either treatment alone.[37-39] The addition of imiquimod to radiotherapy also resulted in growth inhibition of a secondary tumor outside the radiation field. Low-dose cyclophosphamide given before the start of treatment with radiotherapy and imiquimod resulted in further improved tumor inhibition. This combination is currently being tested in patients to determine whether further improved antitumor immune and clinical responses can be achieved (NCT01421017).

Lymphoma

In a pilot study of patients with low-grade non-Hodgkin's lymphoma, 15 were treated with 4 Gy in 2 fractions concurrently with the intratumoral injection of a TLR9 agonist.[40] This in situ vaccination maneuver resulted in 1 patient with a complete response, 3 partial responses, and 2 patients with stable but continually regressing disease. These responses correlated well with the establishment of tumor-reactive $CD8^+$ T cells.[40]

Melanoma

A case of an abscopal effect in a patient with melanoma treated with ipilimumab and radiotherapy was recently reported.[3] Concurrent treatment led to the regression of distant disease in the spleen and mediastinal lymph nodes. Interestingly, the therapeutic response temporarily correlated with an increase in antibody titers targeting epitopes in the central portion of NY-ESO-1 and other tumor-associated antigens, an increase in $CD4^+$ T-cell and myeloid lineage activation, and a decline in the quantity of MDSCs. This case report has led to the initiation of several phase I or II clinical trials in patients with melanoma, recently reviewed by Barker and Postow.[41]

Lung Cancer

An abscopal response was seen in a patient with NSCLC, treated with radiotherapy and CTLA4 blockade. Upon staining with hematoxylin and eosin, lymphocytic infiltration was largely confined to perivascular areas in the pretreatment biopsy, whereas the posttreatment biopsy demonstrated lymphocyte infiltration into the tumor cell nests. On further immunohistochemical analysis, the specimen from the posttreatment biopsy demonstrated a marked increase in $CD8^+$ and TIA^+ cells. Additionally, the ratio of $CD8^+$-$FoxP3^+$ cells was much higher in the posttreatment specimen. Posttreatment tumor regression and tumor marker normalization were also observed.[4] This response underscores the potential therapeutic benefit achievable in both immunogenic and poorly immunogenic tumor types treated with radiotherapy and immune modulation.

Treatment-Related Factors

Dose and Fractionation

Clinically relevant doses of radiotherapy effectively induce the signals for each individual component of ICD, in a dose-dependent manner from 2-20 Gy.[14] Still, in vivo and in the clinic, radiotherapy-produced proimmunogenic effects are often masked by the overwhelming immune-suppressive microenvironment that characterizes established cancers.[8,42] Nevertheless, when some barriers of established immunosuppression are removed, for instance, by adding immune checkpoint inhibitors (such as anti-CTLA4 or anti-PD-1) to local radiation therapy, the proimmunogenic effects of radiotherapy are leveraged and result in immune-mediated tumor rejection.[3,5,7,43]

Dose and fractionation may be important factors regarding the initiation of an in situ vaccine. Antigens produced from radiotherapy-induced ICD may need to be pulsed as opposed to overcoming a certain threshold as a strategy to achieve antitumor immune responses. For example, in a study to investigate the proper radiotherapy regimen to elicit an abscopal response, mice were randomly assigned to 3 distinct regimens of radiotherapy (20 Gy × 1, 8 Gy × 3, or 6 Gy × 5 fractions) with or without CTLA4 blockade.[7] Treatment with CTLA4 blockade alone had no detectable effect. Each of the radiotherapy regimens caused comparable growth delay in the irradiated tumors and was enhanced with the addition of CTLA4 blockade. Interestingly, abscopal effects were seen with the fractionated regimens in combination with CTLA4 blockade. This suggests that other downstream factors, such as pulsatile antigen exposure, may play a role in mediating radiotherapy-induced ICD and antitumor immunity.

Distinct dose and fractionation regimens were shown to elicit abscopal responses in the clinic. For example, Stamell et al,[35] Golden et al,[4] and Postow et al[3] treated patients with metastatic disease with 8 Gy × 3, 6 Gy × 5, and 9.5 Gy × 3 fractions, respectively. Each group achieved abscopal effects with radiotherapy and CTLA4 blockade. Based on the linear-quadratic model for biological effective dose (BED) (a model based on a formula [$BED = nd\,[1 + d/\alpha/\beta]$, where n = no. of fractions and d = dose per fraction] that enables a comparison across different dose and fractionation regimens by applying a specific coefficient [an α/β value] for each tissue irradiated), these regimens employed subablative BEDs ranging between 43.2 and 55.6 Gy_{10}, while achieving both systemic and local tumor control (assuming the α/β value of 10 Gy for established metastases, an "ablative" dose of at least 20 Gy per 3 fractions, corresponding to a BED of 180 Gy_{10} [the subscript 10 refers to the assumed α/β value used to calculate the BED]). This is proof that antitumor immunity may provide an additional benefit to radiotherapy when combined with CTLA4 blockade.

Distinct radiation doses per fraction have a direct effect on the immunologic nature within the local tumor microenvironment. For example, MDSCs and M2 macrophages contribute to an immunosuppressive local microenvironment and support tumor growth.[44] Lower dose radiotherapy, however, has recently been shown to reprogram macrophages toward a $iNOS^+$/M1 phenotype, endowing them with the ability to recruit tumor-specific T cells that promote tumor rejection and hence improve the survival of otherwise immunotherapy-refractory tumor-bearing hosts.[45] This finding highlights the importance of knowing the proper dose and fractionation scheme to modulate the immune system in a way that allows for the rational selection of additional agents to be given concurrently with radiotherapy.

Finally, experimental evidence also suggests that the effects of ionizing radiation on the immune system and in combination with immunotherapy often are model dependent, introducing an additional variable to the challenges of translating preclinical to clinical results.

Concurrent vs Sequential Chemotherapy

Numerous clinical trials suggest that concurrent as opposed to sequential use of radiation and chemotherapy results in improved progression-free survival.[46] However, in the clinical setting, each treatment alone may not quantitatively or qualitatively achieve tumor cell death in the manner that triggers immune-mediated tumor rejection.[47] Formenti and Demaria[1,8,47] hypothesized that these improvements were related to improved immunologic responses. In such settings, ionizing radiation may "reposition" some of the chemotherapies used to optimally induce ICD, a mechanism suggested by

Zitvogel and colleagues.[9] Thus, an alternative approach to achieve ICD may be the concurrent use of radiotherapy and some chemotherapy agents, including carboplatin and paclitaxel to best achieve ICD.[14]

By contrast, some chemotherapeutic agents may counteract the immunogenicity of radiotherapy. For example, 5-fluorouracil and gemcitabine have been shown to exert ambivalent effects on anticancer immune responses.[31,48] On one hand, they were shown to selectively kill MDSCs, thereby relieving immunosuppression and enhancing CD8 T-cell-dependent anticancer immune responses. On the other hand, they were shown to trigger activation of caspase-1 in MDSCs, leading to IL-1β release, TH17 cell polarization, and tumor growth.[31,48] Several concurrent regimens employ 5-fluorouracil or gemcitabine in combination with radiotherapy. Thus, the effects of these drugs with radiotherapy may need to be reevaluated, especially now that several small molecular inhibitors that modify Th17 function are undergoing preclinical development.[49] The strategy of adding these small molecule inhibitors may lead to enhanced antitumor immune responses with concurrent chemoradiotherapy regimens already used in the clinic. However, this needs to be further tested.

Total Body Irradiation to Ablate the Immune System

An alternative approach to elicit an in situ vaccine with radiotherapy may be to completely wipe out the immune system and replace it with an immune system capable of recognizing and eliminating the tumor. Total body irradiation (TBI) to deplete both effector and suppressor cells are thought to "create space" for adoptive cell therapy with autologous tumor-infiltrating lymphocytes; thereby, establishing a new immune repertoire as a means to mediate tumor rejection. A study of adoptive cell therapy for metastatic melanoma using nonmyeloblative chemotherapy alone or with TBI (12 Gy in 6 fractions) showed improved objective clinical response rates from 49%-72%, respectively.[50]

Future Directions

The goal of future studies concerned with radiation and antitumor immunity should be to harness the immune system to either eliminate all tumor deposits to completion or to keep tumor cells dormant in perpetuity. Radiotherapy has a role as a systemic therapy capable to reset the immune system by tipping the balance of the tumor microenvironment from being immunosuppressive toward one that incites an anti-tumor immune response. Additionally, immunosuppressive breaks are further removed with the addition of immune checkpoint inhibitors, such as antibodies to PD-1, PDL-1, and CTLA4, to radiation. As such, the role of radiotherapy as a systemic treatment is not limited to just treating localized disease but is applicable to the metastatic setting, as well. Future preclinical and prospective clinical studies will need to pay careful attention to host, tumor, and treatment-related characteristics proficient at inciting ICD. This will help to individualize treatment and guide the selection of beneficial radiation-based regimens in combination with chemotherapies, immune checkpoint–blocking agents, or immune-stimulating agents in a rational manner.

References

1. Formenti SC, Demaria S: Systemic effects of local radiotherapy. Lancet Oncol 10(7):718-726, 2009
2. Golden EB, Formenti SC: Is tumor (r)ejection by the immune system the "5th R" of radiobiology. Oncoimmunology 3(1):e28133, 2014
3. Postow MA, Barker CA, Yamada Y, Yuan J, Kitano S, et al: Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 366(10):925-931, 2012
4. Golden EB, et al: An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 1 (6):365-372, 2013
5. Demaria S, et al: Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 58 (3):862-870, 2004
6. Demaria S, et al: Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res 11(2 Pt 1):728-734, 2005
7. Dewan MZ, et al: Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res 15(17):5379-5388, 2009
8. Formenti SC, Demaria S: Combining radiotherapy and cancer immunotherapy: A paradigm shift. J Natl Cancer Inst 105(4):256-265, 2013
9. Ma Y, et al: Chemotherapy and radiotherapy: Cryptic anticancer vaccines. Semin Immunol 22(3):113-124, 2010
10. Chakravarty PK, et al: Flt3-ligand administration after radiation therapy prolongs survival in a murine model of metastatic lung cancer. Cancer Res 59(24):6028-6032, 1999
11. Apetoh L, et al: Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13(9):1050-1059, 2007
12. Ghiringhelli F, et al: Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 15(10):1170-1178, 2009
13. Formenti SC, Demaria S: Local control by radiotherapy: Is that all there is? Breast Cancer Res 10(6):215, 2008
14. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC: Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. Oncoimmunology 3:e28518, 2014
15. Panaretakis T, et al: Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 28(5):578-590, 2009
16. Obeid M, et al: Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. Cell Death Differ 14(10):1848-1850, 2007
17. Perez CA, et al: Radiation induces an antitumour immune response to mouse melanoma. Int J Radiat Biol 85(12):1126-1136, 2009
18. Willingham SB, et al: The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci U S A 109(17):6662-6667, 2012
19. Khandelwal S, van Rooijen N, Saxena RK: Reduced expression of CD47 during murine red blood cell (RBC) senescence and its role in RBC clearance from the circulation. Transfusion 47(9):1725-1732, 2007
20. Maxhimer JB, et al: Radioprotection in normal tissue and delayed tumor growth by blockade of CD47 signaling. Sci Transl Med 1(3):3ra7, 2009
21. Vermeer DW, et al: Radiation-induced loss of cell surface CD47 enhances immune-mediated clearance of human papillomavirus-positive cancer. Int J Cancer 133(1):120-129, 2013
22. Lotze MT, Tracey KJ: High-mobility group box 1 protein (HMGB1): Nuclear weapon in the immune arsenal. Nat Rev Immunol 5(4):331-342, 2005

23. Michaud M, et al: Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 334 (6062):1573-1577, 2011
24. Zappasodi R, et al: Improved clinical outcome in indolent B-cell lymphoma patients vaccinated with autologous tumor cells experiencing immunogenic death. Cancer Res 70(22):9062-9072, 2010
25. Ohshima Y, et al: gamma-Irradiation induces P2X(7) receptor-dependent ATP release from B16 melanoma cells. Biochim Biophys Acta 1800 (1):40-46, 2010
26. Petrovski G, et al: Phagocytosis of cells dying through autophagy induces inflammasome activation and IL-1beta release in human macrophages. Autophagy 7(3):321-330, 2011
27. Reits EA, et al: Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 203(5):1259-1271, 2006
28. Ruocco MG, et al: Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. J Clin Invest 122 (10):3718-3730, 2012
29. Hooper LV, Littman DR, Macpherson AJ: Interactions between the microbiota and the immune system. Science 336(6086):1268-1273, 2012
30. Viaud S, et al: The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 342(6161):971-976, 2013
31. Martin F, Apetoh L, Ghiringhelli F: Controversies on the role of Th17 in cancer: A TGF-beta-dependent immunosuppressive activity? Trends Mol Med 18(12):742-749, 2012
32. Meyer C, et al: Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. Cancer Immunol Immunother 63(3):247-257, 2014
33. Robin HI, et al: The abscopal effect: Demonstration in lymphomatous involvement of kidneys. Med Pediatr Oncol 9(5):473-476, 1981
34. Wersall PJ, et al: Regression of non-irradiated metastases after extracranial stereotactic radiotherapy in metastatic renal cell carcinoma. Acta Oncol 45 (4):493-497, 2006
35. Stamell EF, et al: The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys 85(2):293-295, 2013
36. Sullivan RJ, et al: Case records of the Massachusetts General Hospital. Case 21-2013. A 68-year-old man with metastatic melanoma. N Engl J Med 369(2):173-183, 2013
37. Demaria S, et al: The TLR7 agonist imiquimod as an adjuvant for radiotherapy-elicited in situ vaccination against breast cancer. Oncoimmunology 2(10):e25997, 2013
38. Adams S, et al: Topical TLR7 agonist imiquimod can induce immune-mediated rejection of skin metastases in patients with breast cancer. Clin Cancer Res 18(24):6748-6757, 2012
39. Dewan MZ, et al: Synergy of topical toll-like receptor 7 agonist with radiation and low-dose cyclophosphamide in a mouse model of cutaneous breast cancer. Clin Cancer Res 18(24):6668-6678, 2012
40. Brody JD, et al: In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: A phase I/II study. J Clin Oncol 28(28):4324-4332, 2010
41. Barker CA, Postow MA: Combinations of radiation therapy and immunotherapy for melanoma: A review of clinical outcomes. Int J Radiat Oncol Biol Phys 88(5):986-997, 2014
42. Russell JS, Brown JM: The irradiated tumor microenvironment: Role of tumor-associated macrophages in vascular recovery. Front Physiol 4:157, 2013
43. Golden EB, et al: An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res:. http://dx.doi.org/10.1158/2326-6066.CIR-13-0115
44. Coussens LM, Zitvogel L, Palucka AK: Neutralizing tumor-promoting chronic inflammation: A magic bullet? Science 339(6117):286-291, 2013
45. Klug F, et al: Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell 24(5):589-602, 2013
46. Glynne-Jones R, Hoskin P: Neoadjuvant cisplatin chemotherapy before chemoradiation: A flawed paradigm? J Clin Oncol 25(33):5281-5286, 2007
47. Formenti SC, Demaria S: Effects of chemoradiation on tumor-host interactions: The immunologic side. J Clin Oncol 26(9):1562-1563, 2008. [author reply 1563]
48. Bruchard M, et al: Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nat Med 19(1):57-64, 2013
49. Huh JR, Littman DR: Small molecule inhibitors of RORgammat: Targeting Th17 cells and other applications. Eur J Immunol 42(9):2232-2237, 2012
50. Dudley ME, et al: Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 26(32):5233-5239, 2008