

Toward Precision Radiotherapy for Use with Immune Checkpoint Blockers

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Abstract

The first evidence that radiotherapy enhances the efficacy of immune checkpoint blockers (ICB) was obtained a dozen years ago in a mouse model of metastatic carcinoma refractory to anti-CTLA-4 treatment. At the time, ICBs had just entered clinical testing, an endeavor that culminated in 2011 with the approval of the first anti-CTLA-4 antibody for use in metastatic melanoma patients (ipilimumab). Thereafter, some patients progressing on ipilimumab showed systemic responses only upon receiving radiation to one lesion, confirming clinically the proimmunogenic effects of radiation. Preclinical data demonstrate that multiple immunomodulators synergize with radiotherapy to cause the regression of irradiated tumors and, less often, nonirradiated metastases. However, the impact of dose and fractionation on the immunostimulatory potential of radiotherapy has not been thor-

oughly investigated. This issue is extremely relevant given the growing number of clinical trials testing the ability of radiotherapy to increase the efficacy of ICBs. Recent data demonstrate that the recruitment of dendritic cells to neoplastic lesions (and hence the priming of tumor-specific CD8⁺ T cells) is highly dependent on radiotherapy dose and fractionation through a mechanism that involves the accumulation of double-stranded DNA in the cytoplasm of cancer cells and consequent type I IFN release. The molecular links between the cellular response to radiotherapy and type I IFN secretion are just being uncovered. Here, we discuss the rationale for an optimized use of radiotherapy as well as candidate biomarkers that may predict clinical responses to radiotherapy combined with ICBs. *Clin Cancer Res*; 24(2); 259–65. ©2017 AACR.

Introduction

The era of immune checkpoint blockers (ICB), which started in 2011 with the approval of an antibody targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) on T cells (namely, ipilimumab) for the treatment of metastatic melanoma (1), has brought a new paradigm to cancer therapy whereby the immune system is being harnessed to cure the cancer. Since then, several antibodies targeting another coinhibitory receptor, the programmed cell death 1 (PDCD1, best known as PD-1) or its main ligand CD274 (best known as PD-L1), have been approved for the treatment of multiple cancers (2). These agents have dramatically improved disease outcome in many patients with cancer, as they can lead to responses as sustained as to last, in some cases, for the entire patient's lifetime (reflecting the ability of immunologic memory to prevent tumor recurrence; ref. 3). However, the benefits of ICBs are limited to a subset of patients who have preexisting T-cell responses that can be reactivated by ICBs. For the majority of patients, additional interventions are needed to overcome primary or acquired resistance to ICBs (4).

Tumor-targeted radiotherapy has multiple effects that can overcome at least some of the mechanisms whereby malignant cells intrinsically are or become resistant to ICB-based immuno-

therapy (5). Preclinical evidence has demonstrated that radiotherapy can operate in this sense at least at three different levels: (i) It can generate T cells specific for tumor-associated antigens (TAA) by inducing immunogenic cell death (ICD), an immunostimulatory form of regulated cell death associated with the timely and abundant release of adjuvant-like molecules (6–8); (ii) it can overcome T-cell exclusion from the tumor by promoting the release of chemokines that attract effector T cells as well as by surmounting the vascular barrier to T-cell infiltration (7, 9, 10); and (iii) it can improve the recognition and killing of cancer cells by CD8⁺ cytotoxic T cells (CTL) by promoting antigen presentation on MHC class I molecules, by upregulating death receptors, as well as by promoting the exposure of NK-cell-activating ligands (11–13). Collectively, these effects explain not only the relatively old observation that T cells are important for the therapeutic responses of irradiated tumors (14) but also occasional reports of tumor regression outside of the radiation field (the so-called "abscopal effect"), which we have shown to depend on the immune system in preclinical models (15). However, abscopal effects are very rare in patients receiving radiotherapy alone and have received little attention (16). This has changed when abscopal responses have been documented at increasing rates among patients exhibiting primary or acquired resistance to ICBs before receiving radiotherapy (17–19). Such observations sparked considerable interest in the possibility of using radiotherapy as a means to increase responses to ICBs and other forms of immunotherapy in patients, leading to the initiation of a large number of clinical studies (most of which are currently ongoing; refs. 20, 21). The interaction of several immune modulators with radiotherapy has been extensively investigated in preclinical models, with encouraging results (reviewed in refs. 22, 23). However, the dramatic abscopal responses observed in patients receiving radiotherapy plus ipilimumab (17, 19), which have inspired

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oncologists to pursue combinatorial regimens of this type, have remained relatively rare. Thus, additional insights into the molecular and cellular circuitries whereby radiotherapy favors the elicitation of tumor-specific immune responses are urgently required for improving the efficacy of ICBs in the clinic (24). Here, we discuss recent data describing the molecular links between the cellular response to radiotherapy and signaling pathways normally elicited by viral infection that have a major impact in the immunostimulatory potential of radiotherapy. We are confident that these findings will help the field to move forward toward the development of improved biomarkers for patient selection and to identify optimal radiotherapy doses for use in combination with ICBs.

From Local to Abscopal: Jump-starting Systemic Anticancer Immunity with Tumor-Targeted Radiotherapy

Several mechanisms contribute to making an irradiated tumor amenable to immunologic rejection, and many immunostimulatory interventions have been shown to improve the response of irradiated tumors (25). However, the concept of *in situ* vaccination by radiotherapy, whereby a growing tumor can be used as a source of antigens to immunize the host, implies the generation of tumor-specific T cells that mediate abscopal responses. The rejection of nonirradiated lesions presents considerable challenges and thus requires the generation of an antigenically broad and robust antitumor T-cell response to overcome (i) the physical and functional barriers to immune responses that characterize the microenvironment of nonirradiated metastases and (ii) the possible antigenic heterogeneity of different metastases. This explains why abscopal responses mediated by radiotherapy alone are extremely rare, and why even when radiotherapy is combined with ICBs, abscopal responses occur at a low incidence (24, 26, 27).

Most investigations aimed at improving abscopal effects driven by radiotherapy have focused on testing whether the addition of various immunotherapeutics could increase the incidence of abscopal responses achieved with radiotherapy plus a single ICB. Twyman-Saint Victor and colleagues showed that abscopal responses to a single radiotherapy dose of 20 Gy plus an anti-CTLA-4 antibody occurred only in 17% of mice bearing two synchronous B16-F10 melanoma lesions (27). On the basis of the expression of PD-L1 by B16-F10 and on prior data showing that two distinct ICBs improved tumor rejection triggered by an autologous irradiated, GM-CSF-secreting tumor cell vaccine in the same melanoma model (28), these authors went on to test whether adding an anti-PD-1 antibody to radiotherapy plus CTLA-4 blockade would increase abscopal responses. Perhaps unsurprisingly, most B16-F10-bearing mice showed abscopal responses to radiotherapy plus an anti-CTLA-4 and an anti-PD-1 antibody. Together with the success of the double immune checkpoint blockade in patients with melanoma (29), this work has fostered the initiation of clinical trials testing PD-1 and CTLA-4 blockade together with radiotherapy in patients with melanoma and other cancers (<https://clinicaltrials.gov/>). However, double immune checkpoint blockade in patients with melanoma has high activity and significant toxicity, cautioning that the bar is very high for a benefit from radiotherapy to be documentable, whereas the initial results of double immune checkpoint blockade in patients with other cancers are not always as promising (30). In preclinical studies, Rodriguez-Ruiz and colleagues tested the

agonistic antibodies for the coactivatory T-cell receptor TNF receptor superfamily member 9 (TNFRSF9, best known as CD137) with anti-PD-1 to improve abscopal responses to radiotherapy (31). Although this is an effective combination, a recent report raises concerns about the systemic toxicity of anti-CD137 antibodies plus radiotherapy and suggests that tumor targeting of the antibody is required to increase the therapeutic index (32).

Thus, although there is a growing list of immune modulators that should be tested for the ability to synergize with tumor-directed radiotherapy at inducing abscopal responses, this strategy may be time-consuming and of limited yield in the absence of a clear mechanistic understanding of how specific immunotherapies interact with the irradiated tumor microenvironment.

The Essential Ingredients of a Radiation-Induced *In Situ* Anticancer Vaccine

We have taken a different approach to the quest for improving abscopal responses, focusing on how radiation should be administered to generate an effective anticancer vaccination *in situ*.

Both the spontaneous and chemotherapy-driven development of tumor-targeting T-cell responses has been shown to rely on type I IFN (IFN-I), which is essential to recruit and activate BATF3-dependent dendritic cells (DC) specialized in cross-presenting TAAs to CD8⁺ CTLs (33–35). IFN-I is produced by dying cancer cells as a result of endosomal RNA accumulation and Toll-like receptor 3 (TLR3) signaling (36). Tumor-infiltrating DCs (TIDC) secrete IFN-I in response to cancer cell-derived DNA via a mechanism that involves Mab-21 domain containing 1 (MB21D1, best known as cyclic GMP-AMP synthase, cGAS) and transmembrane protein 173 (TMEM173, best known as stimulator of IFN genes, STING; ref. 37). The exact source of the cancer cell DNA and the mechanisms through which it reaches the cytosol of TIDCs have not been defined. Interestingly, Deng and colleagues employed a single dose of 20 Gy to irradiate mouse carcinoma MC38 cells established in immunocompetent syngeneic mice and demonstrated that radiotherapy-mediated stimulation of IFN-I production by TIDCs was critical for the response to radiotherapy, and was dependent on expression of cGAS and STING by TIDCs, whereas the cancer cells did not produce IFN-I (38). These authors also showed that in mice treated with 20 Gy, tumor-targeted radiotherapy tumor response and T-cell priming were enhanced by the intratumoral administration of a STING agonist, suggesting that IFN-I activation by this radiotherapy dose was suboptimal. However, abscopal responses were not tested in this setting.

To determine whether radiotherapy dose and delivery schedule might affect the generation of antitumor T cells, we previously compared three regimens that are commonly employed in the treatment of metastatic neoplasms for their ability to cause T-cell-dependent abscopal responses in combination with an anti-CTLA-4 antibody (39). Abscopal responses manifesting with partial or complete regression of nonirradiated synchronous tumors were observed when radiotherapy was administered at single doses of 6 Gy in 5 consecutive days (6 Gy × 5) or 8 Gy in 3 consecutive days (8 Gy × 3), but not 20 Gy given in a single fraction, suggesting that dose and fractionation markedly influence the immunostimulatory potential of radiotherapy. To get insights into the mechanisms underlying such a difference, we performed an unbiased comparison of gene expression in TSA mouse tumors receiving 8 Gy × 3 versus 20 Gy × 1 radiotherapy *in vivo*. This revealed that IFN-I signaling is markedly activated by

8 Gy \times 3 radiotherapy, but not 20 Gy \times 1 radiotherapy, 24 hours after the completion of treatment (40). Further analyses demonstrated an essential contribution of irradiated cancer cells to the production of IFN-I and consequent transactivation of IFN-stimulated genes (ISG), including C-X-C motif chemokine ligand 10 (CXCL10) and other genes that encode chemoattractants for effector T cells (41). *In vitro*, radiation given at 8 Gy \times 3 stimulated IFN β secretion by the TSA carcinoma cells at levels comparable with viral infection. Indeed, we found that the ability of radiotherapy to drive IFN-I secretion by malignant cells depends on the same cytoplasmic DNA-sensing machinery that is activated by viral and retroviral infection in epithelial cells (42, 43). Thus, selective knockdown of cGAS or its downstream adaptor STING abrogated IFN-I secretion by cancer cells exposed to 8 Gy \times 3 radiotherapy and, most importantly, the ability of 8 Gy \times 3 radiotherapy to mediate abscopal effects in combination with anti-CTLA-4 or anti-PD-1 antibodies (40). The critical role of cGAS in radiotherapy-driven IFN-I activation suggested that radiotherapy may favor the accumulation of IFN-stimulatory double-stranded DNA (dsDNA) in the cancer cells' cytosol (44). Consistent with this hypothesis, we documented high levels of dsDNA in the cytosol of TSA and other carcinoma cells responding *in vitro* to 8 Gy \times 3 radiotherapy, but not 20 Gy \times 1 or 30 Gy \times 1 radiotherapy. Interestingly, such an accumulation could be detected after a single radiotherapy dose of 8 Gy. However, *Ifnb1* and various ISGs were modestly upregulated upon 8 Gy \times 1 radiotherapy and markedly increased after only three radiotherapy doses of 8 Gy each (40). Thus, both the dose and the fractionation are key determinants of radiotherapy-driven immunostimulation. Finally, the expression of IFN alpha and beta receptor subunit 1 (IFNAR1), the most abundant subunit of heterodimeric IFN-I receptors, was required on both host and malignant cells for the optimal induction of tumor-specific immunity and durable abscopal responses, suggesting that IFN-I also acts in an autocrine pathway that amplifies the immunogenicity of neoplastic cells responding to radiation (40). This is highly reminiscent of chemotherapy-induced ICD, a scenario in which IFN-I secretion by cancer cells triggers autocrine IFNAR1 signaling and consequent CXCL10 secretion, which is required for the full-blown efficacy of treatment (36).

IFN-I release by cancer cells responding to 8 Gy \times 3 radiotherapy *in vivo* was critical for the recruitment and activation of BATF3-dependent DCs to the tumor, an effect that could not be documented when radiotherapy was given in a single dose of 20 Gy or in control conditions (40). These data indicate that radiotherapy administered in specific doses and according to specific schedules can overcome the relative lack of BATF3-dependent DCs and of proinflammatory cues that characterize poorly immunogenic tumors, hence restoring the priming of tumor-specific T cells that can mediate systemic responses to ICBs (Fig. 1).

Thus, these essential components of an *in situ* anticancer vaccine can be generated only when radiotherapy is used at optimal doses and according to precise fractionation schedules. In most mouse and human carcinoma cells tested so far, cytosolic dsDNA accumulation was observed upon irradiation at single doses above 4 Gy, an effect that plateaued between 8 and 12 Gy. Above this threshold, radiotherapy promoted the upregulation of three prime repair exonuclease 1 (TREX1) that degraded cytosolic dsDNA and hence precluded IFN-I secretion secondary to cGAS/STING signaling (40). Importantly, it is the size of the single dose that determines this threshold, not the cumulative dose: Radiotherapy given

at 8 Gy \times 3 did not induce Trex1 upregulation, whereas 20 and 30 Gy given as a single dose did. Although the mechanisms that control the dose-dependent induction of TREX1 by radiotherapy are currently under investigation, TREX1 is known to play an essential role in autoimmune disorders and in viral escape from innate immune sensing (45–48). Accordingly, *Trex1*^{-/-} mice develop an IFN-I-driven autoimmune disorder, and mutation in *TREX1* in humans is associated with Aicardi-Goutières syndrome, an early-onset inflammatory disorder affecting multiple organs (48, 49). Thus, in physiologic conditions, TREX1 attenuates the immunogenicity of normal cells to prevent autoimmune disorders. Our new findings demonstrate that TREX1 is also a key regulator of the cellular response to radiotherapy that controls the immunogenicity of irradiated cancer cells (40).

Interestingly, Filatenkov and colleagues (50) showed that irradiation with a single dose of 30 Gy resulted in better local control than a fractionated regimen of 3 Gy \times 10 in mice bearing CT26 mouse colorectal carcinoma implanted in the leg. Rejection of the irradiated tumor required CD8⁺ T cells, but the response was insufficient to mediate abscopal effects, and evidence of a T-cell response capable of rejecting a new tumor challenge was not seen until the irradiated tumors had completely disappeared a few weeks later. Because tumors were infiltrated by suppressive myeloid cells with high expression of PD-L1, it is possible that in this case, the high radiation dose was more effective at eliminating these cells, which allowed infiltration of the tumor by preexisting T cells. In support of this possibility, PD-1 blockade resulted in efficient rejection of CT26 tumors treated with 2 Gy \times 5 radiotherapy (51). Overall, these data highlight the different requirements for rejection of irradiated and abscopal tumors.

Potential Mechanistic Biomarkers to Guide the Use of Radiotherapy as an Enhancer of Clinical Responses to ICBs

Preclinical data on the critical role of IFN-I in the development of therapeutically relevant tumor-specific immune responses (52) are supported by clinical findings documenting the endogenous activation of IFN-I signaling in spontaneously regressing melanoma and other melanocytic lesions (53), and IFN-I-related gene signatures in metastases from melanoma and in other cancers highly infiltrated by T cells (54, 55). Thus, several strategies are being pursued to activate IFN-I signaling within the microenvironment of tumors devoid of T cells to convert them into T-cell-rich tumors that would respond to ICBs (35, 55, 56). Among them, intratumoral delivery of a Toll-like receptor 9 (TLR9) agonist in combination with 2 Gy \times 2 radiotherapy induced abscopal responses in patients with low-grade B-cell lymphoma (57), supporting the hypothesis that sufficient IFN-I activation in the irradiated tumor is key for activation of systemically effective antitumor immunity.

Radiotherapy by itself has the potential to effectively induce the secretion of IFN-I within the tumor microenvironment, but it must be administered at optimal doses and schedules to generate robust antitumor immunity. In relatively immunogenic tumors, IFN-I is spontaneously secreted by TIDCs that take up TAA and IFN-stimulatory DNA (37), a process that is enhanced by radiotherapy doses that do not induce IFN-I production by cancer cells (38, 58). However, poorly immunogenic tumors avoid generation of antitumor T cells by excluding DCs (59). In these tumors, activating the secretion of IFN-I by cancer cells

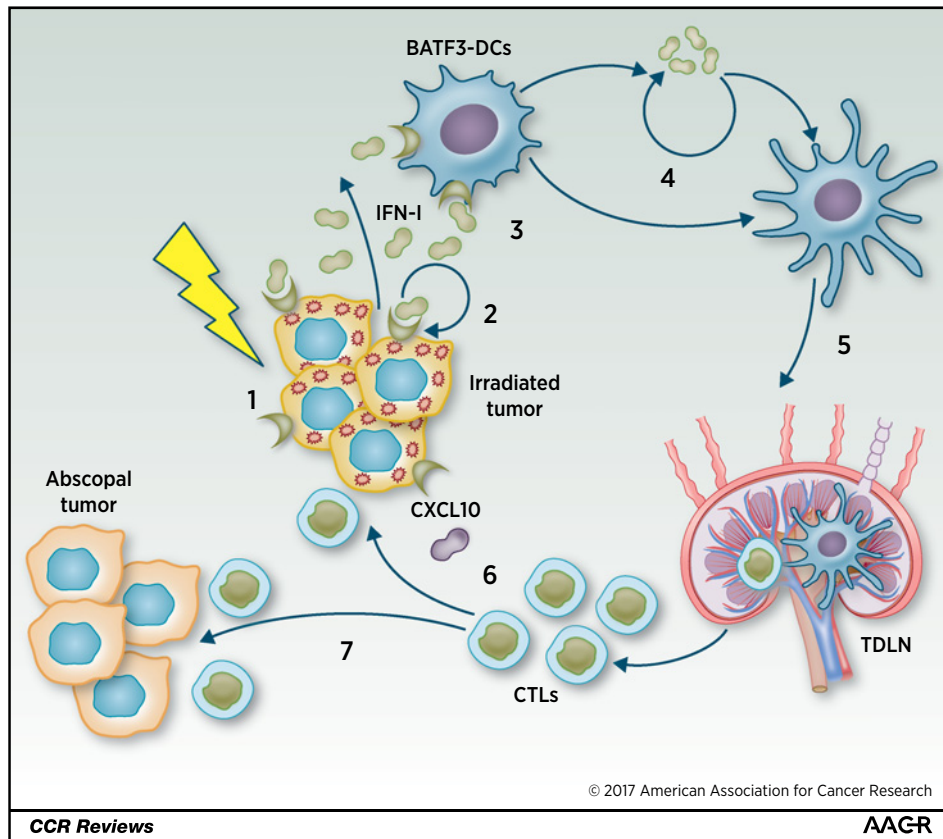


Figure 1.

Cancer cell secretion of IFN-I is an essential signal for a radiation-induced *in situ* anticancer vaccine. (1) Radiotherapy used at the optimal dose and fractionation leads to accumulation of cytosolic dsDNA in the cancer cells, which stimulates the production of IFN-I via the cGAS/STING pathway, and the transactivation of ISG, including chemokines such as CXCL10. (2) IFN-I binds to its receptor on the cancer cells, promoting further secretion of IFN-I and expression of several ISGs. (3) IFN-I binds to its receptor on BATF3-dependent DCs (BATF3-DC), promoting their recruitment to the tumor and their activation. (4) Once in the tumor, BATF3-DCs take up TAA and tumor-derived DNA, which further stimulates production of IFN-I via cGAS/STING. (5) Optimally activated BATF3-DCs then migrate to tumor-draining lymph nodes (TDLN) where they can cross-prime CD8⁺ T cells. (6) Once activated, tumor-specific CD8⁺ T cells differentiate into CTLs that express CXCR3. Their homing to the irradiated tumor is facilitated by CXCL10 and other IFN-induced chemokines. CTLs eliminate the residual cancer cells remaining after radiotherapy, leading to durable tumor regression. (7) The CTLs are also capable to home to distant metastatic sites and reject nonirradiated metastases (abscopal effect).

with radiotherapy represents an efficient and clinically viable means to recruit and activate BATF3-dependent DCs and hence prime a T-cell response that overcomes resistance to ICBs and mediates abscopal effects (40).

Cancer cell–intrinsic IFN-I activation in response to radiation is dependent on the expression of cGAS and STING and on a cellular microenvironment that allows for the productive activation of cGAS/STING signaling (40). Recent data indicate that cGAS and/or STING expression is downregulated, often by epigenetic mechanisms, in over a third of colorectal cancers and melanomas, and in some melanoma cells, the ability of STING to activate the transcription factors NF-κB and IRF3 is compromised (60, 61). Thus, it will be important to determine whether cGAS and/or STING expression in malignant cells can serve as a biomarker to identify the patients who may benefit from the addition of radiotherapy to ICB-based immunotherapy. A routine IHC staining can be cost-effectively employed for this purpose (60). Along similar lines, evaluating the methylation status of the *MB21D1* and/or *TMEM173* promoters may inform on the possibility of

using demethylating agents for recovering IFN-I secretion in response to radiotherapy. These evaluations need to be complemented by the assessment of the global functionality of the IFN-I–secreting machinery in cancer cells and the identification of the most immunostimulatory radiotherapy dose and schedule for each specific tumor. In a panel of mouse and human carcinoma cells, the radiotherapy dose threshold for TREX1 induction at levels that are sufficient to degrade cytosolic DNA ranged from 12 to 18 Gy (40), and this may vary further for other types of cancer. We have shown that patient-derived tumor xenografts (PDX) implanted in immunodeficient mice can be used with a small-animal irradiator to provide information on the functionality of the IFN-I pathway and to identify the most immunogenic radiotherapy dose and fractionation for a given tumor (Fig. 2; ref. 40). We are currently assessing whether *ex vivo* irradiation of fresh tumor biopsies could be used as a less expensive and more time-efficient alternative. The advantage of PDX is the possibility to perform additional evaluations of the interaction of the patient's T cells transferred into the mice with the irradiated tumor in the

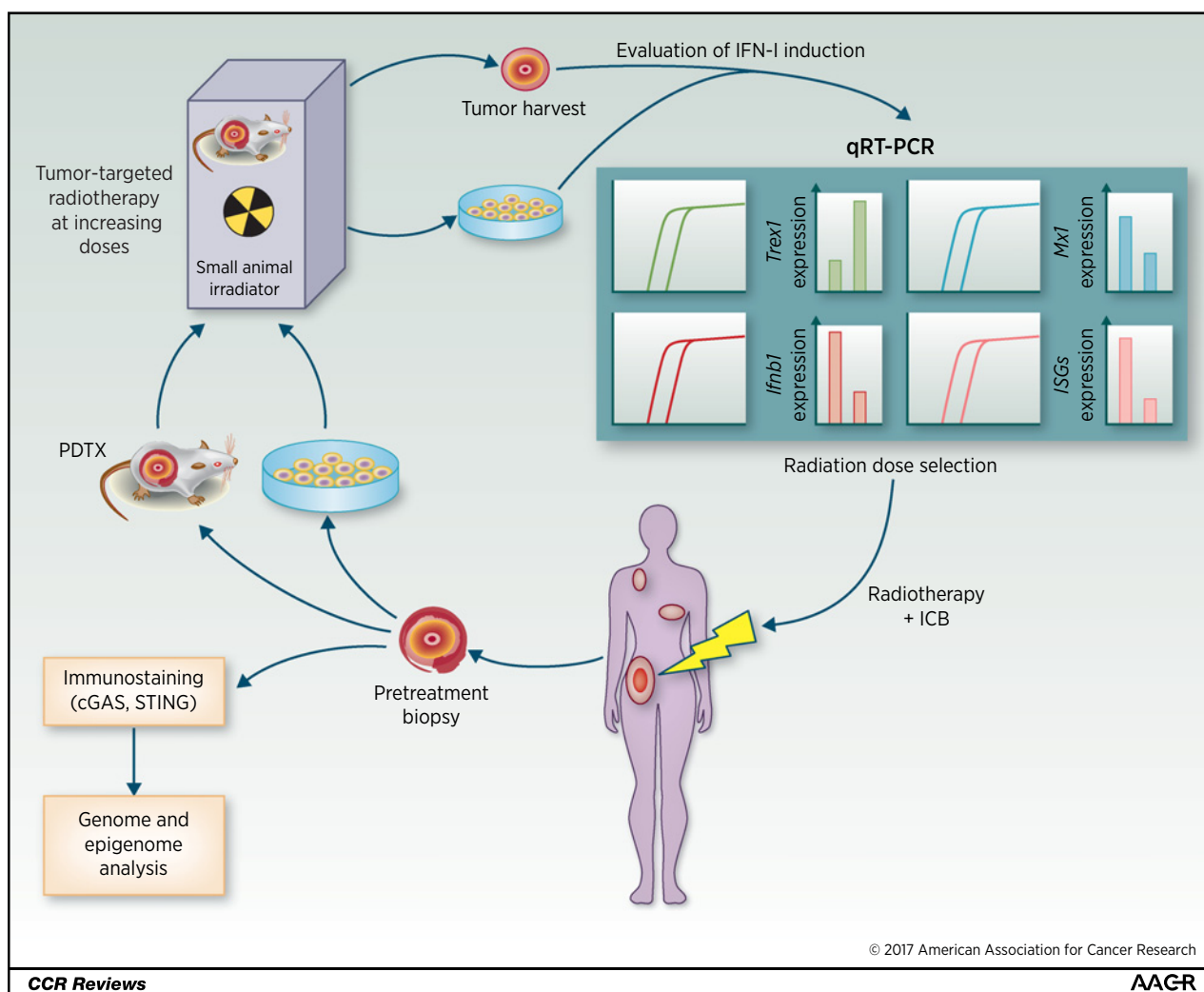


Figure 2.

Proposed strategy for rational selection of the radiation dose to be used in combination with ICBs. A biopsy is performed to test the tumor site selected for irradiation for expression of cGAS and STING by immunostaining. If the expression is weak or negative, the tumor is analyzed for methylation of the promoter of the genes encoding cGAS, STING, and IFN β . If sufficient tissue is available, comprehensive genomic and epigenomic analysis can provide additional information about specific mutations and mutation load, and RNA sequencing (RNA-Seq) can be used to confirm cGAS and STING expression. Another portion of the fresh tumor is used to establish the radiation dose threshold for *Trex1* induction versus the optimal induction of IFN-I and ISGs. This can be accomplished in two ways: (i) If sufficient material is available, tissue fragments are irradiated in *ex vivo* cultures or (ii) alternatively, the tissue is used to prepare seeds (0.1 \times 0.3 \times 0.3 cm) for implantation in a subcutaneous pocket on the flank areas of NOD.Cg-Prkdcscid B2mtm1Unc Il2rgtm1Wjl/SzJ (NSG) mice to generate PDX. Once tumors reach 5 mm in average diameter, they are irradiated. Obtained information is used to choose the radiation dose and fractionation for treatment of the patient in combination with ICBs. In the event that IFN-I is not induced by any radiation dose due to hypermethylation of the promoter of cGAS- and or STING-encoding genes, radiation is preceded by administration of a demethylating agent.

presence or absence of ICBs (62). Collectively, these approaches have the potential to move the field forward not only by providing biomarkers for improved patient selection but also by allowing for the implementation of ever more personalized radiotherapy schedules to boost the efficacy of immunotherapy.

Conclusions

Radiation oncology has evolved over several decades as specific protocols for the delivery of radiation optimized to achieve local tumor control, rather than abscopal responses, have been devel-

oped. The advent of stereotactic body radiotherapy has stimulated the use of radiotherapy at high single doses (63). Despite the growing enthusiasm for immuno-oncology and the tremendous expansion of preclinical and clinical studies testing the hypothesis that tumor-targeted radiotherapy can assume a new role as an immune adjuvant that boosts the efficacy of immunotherapy, many outstanding questions remain unanswered (24).

The findings discussed above highlight the urgent need to revisit our current understanding of the cellular responses elicited by radiotherapy, focusing on their cross-talk with the immune system. Intriguingly, the primary role of CD8⁺ T cells is

to identify and eliminate virally infected cells, which may explain the emerging links between the mechanisms underlying the immunogenicity of cancer cells triggered by radiotherapy or chemotherapy and the processes involved in antiviral defense (36, 40).

In addition to cytosolic DNA, which plays a dominant role in the radiotherapy-driven secretion of IFN-I by cancer cells and the consequent induction of abscopal responses (40), small endogenous noncoding RNAs may also contribute to IFN-I secretion by irradiated cells. A recent report demonstrates that the small nuclear RNAs U1 and U2 translocate to the cytoplasm after irradiation to form a complex with DExD/H-box helicase 58 (DDX58, best known as retinoic acid-inducible gene-1, RIG-I), one of the three major dsRNA sensors, which activates mitochondrial antiviral signaling protein (MAVS) to initiate IFN-I responses (64). Conversely, it remains to be elucidated whether TLR3 activation contributes to the ability of radiotherapy to promote IFN-I secretion upon recognition of RNA molecules potentially accumulating in endosomes (36). Moreover, the role of RIG-I in the ability of radiotherapy to synergize with ICBs at inducing abscopal responses remains to be ascertained.

Irrespective of these and other unknowns, the identification of the mechanistic bases underlying the immunostimulatory activity of radiotherapy as they pertain to dose, fractionation, and onco-

genic alterations present in a specific tumor represents a critical step forward toward the use of precision radiotherapy in the context of immunotherapy. Timely clinical validation of the findings discussed here is needed to determine their potential impact in improving patients' treatment.

Disclosure of Potential Conflicts of Interest

S.C. Formenti is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Dynavax, Eisai, Elekta, GlaxoSmithKline, Janssen, Regeneron, and Varian. S. Demaria reports receiving commercial research grants from Lytix Biopharma and is a consultant/advisory board member for Eisai, EMD Serono, and Lytix Biopharma. No potential conflicts of interest were disclosed by the other author.

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References

- Ledford H. Melanoma drug wins US approval. *Nature* 2011;471:561.
- Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015;161:205–14.
- Postow MA, Callahan MK, Wolchok JD. The antitumor immunity of ipilimumab: (T-cell) memories to last a lifetime? *Clin Cancer Res* 2012;18:1821–3.
- Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 2017;168:707–23.
- Demaria S, Golden EB, Formenti SC. Role of local radiation therapy in cancer immunotherapy. *JAMA Oncol* 2015;1:1325–32.
- Ma Y, Kepp O, Ghiringhelli F, Apetoh L, Aymeric L, Locher C, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Semin Immunol* 2010;22:113–24.
- Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J Immunol* 2005;174:7516–23.
- Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol* 2017;17:97–111.
- Matsumura S, Wang B, Kawashima N, Braunstein S, Badura M, Cameron TO, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. *J Immunol* 2008;181:3099–107.
- Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, et al. Low-dose irradiation programs macrophage differentiation to an iNOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell* 2013;24:589–602.
- Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 2006;203:1259–71.
- Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN, et al. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol* 2003;170:6338–47.
- Ruocco MG, Pilonis KA, Kawashima N, Cammer M, Huang J, Babb JS, et al. Suppressing T cell motility induced by anti-CTLA-4 monotherapy improves antitumor effects. *J Clin Invest* 2012;122:3718–30.
- Stone HB, Peters LJ, Milas L. Effect of host immune capability on radio-curability and subsequent transplantability of a murine fibrosarcoma. *J Natl Cancer Inst* 1979;63:1229–35.
- Demaria S, Ng B, Devitt M-L, Babb JS, Kawashima N, Liebes L, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* 2004;58:862–70.
- Abuodeh Y, Venkat P, Kim S. Systematic review of case reports on the abscopal effect. *Curr Probl Cancer* 2016;40:25–37.
- Postow MA, Callahan MK, 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* 2012;366:925–31.
- Hiniker SM, Chen DS, Reddy S, Chang DT, Jones JC, Mollick JA, et al. A systemic complete response of metastatic melanoma to local radiation and immunotherapy. *Transl Oncol* 2012;5:404–7.
- Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. *Cancer Immunol Res* 2013;1:365–72.
- Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. *J Immunother Cancer* 2016;4:51.
- Vacchelli E, Bloy N, Aranda F, Buqué A, Cremer I, Demaria S, et al. Trial Watch: Immunotherapy plus radiation therapy for oncological indications. *Oncoimmunology* 2016;5:e1214790.
- Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. *J Natl Cancer Inst* 2013;105:256–65.
- Tang C, Wang X, Soh H, Seyedin S, Cortez MA, Krishnan S, et al. Combining radiation and immunotherapy: a new systemic therapy for solid tumors? *Cancer Immunol Res* 2014;2:831–8.
- Demaria S, Coleman CN, Formenti SC. Radiotherapy: changing the game in immunotherapy. *Trends Cancer* 2016;2:286–94.
- Kwilas AR, Donahue RN, Bernstein MB, Hodge JW. In the field: exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. *Front Oncol* 2012;2:104.
- Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel

- chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014;15:700–12.
27. Twyman-Saints Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015;520:373–7.
 28. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci U S A* 2010;107:4275–80.
 29. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 2013;369:122–33.
 30. Ott PA, Hodi FS, Kaufman HL, Wigginton JM, Wolchok JD. Combination immunotherapy: a road map. *J Immunother Cancer* 2017;5:16.
 31. Rodriguez-Ruiz ME, Rodriguez I, Garasa S, Barbes B, Solorzano JL, Perez-Gracia JL, et al. Abscopal effects of radiotherapy are enhanced by combined immunostimulatory mAbs and are dependent on CD8 T cells and cross-priming. *Cancer Res* 2016;76:5994–6005.
 32. Schrand B, Verma B, Levay A, Patel S, Castro I, Benaduce AP, et al. Radiation-induced enhancement of antitumor T-cell immunity by VEGF-Targeted 4-1BB costimulation. *Cancer Res* 2017;77:1310–21.
 33. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med* 2011;208:1989–2003.
 34. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8a+ dendritic cells. *J Exp Med* 2011;208:2005–16.
 35. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. *Nat Rev Immunol* 2015;15:405–14.
 36. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat Med* 2014;20:1301–9.
 37. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, et al. STING-Dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* 2014;41:830–42.
 38. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* 2014;41:843–52.
 39. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 2009;15:5379–88.
 40. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* 2017;8:15618.
 41. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol* 2014;32:659–702.
 42. Cai X, Chiu YH, Chen ZJ. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Mol Cell* 2014;54:289–96.
 43. Gao D, Wu J, Wu YT, Du F, Aroh C, Yan N, et al. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science* 2013;341:903–6.
 44. Gao P, Ascano M, Wu Y, Barchet W, Gaffney BL, Zillinger T, et al. Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell* 2013;153:1094–107.
 45. Yan N, Regalado-Magdos AD, Stiggelbout B, Lee-Kirsch MA, Lieberman J. The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. *Nat Immunol* 2010;11:1005–13.
 46. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* 2008;134:587–98.
 47. Lehtinen DA, Harvey S, Mulcahy MJ, Hollis T, Perrino FW. The TREX1 double-stranded DNA degradation activity is defective in dominant mutations associated with autoimmune disease. *J Biol Chem* 2008;283:31649–56.
 48. Yang YG, Lindahl T, Barnes DE. Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. *Cell* 2007;131:873–86.
 49. Crow YJ, Manel N. Aicardi-Goutieres syndrome and the type I interferonopathies. *Nat Rev Immunol* 2015;15:429–40.
 50. Filatenkov A, Baker J, Mueller AM, Kenkel J, Ahn GO, Dutt S, et al. Ablative tumor radiation can change the tumor immune cell microenvironment to induce durable complete remissions. *Clin Cancer Res* 2015;21:3727–39.
 51. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, McKenna C, Jones S, Cheadle EJ, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res* 2014;74:5458–68.
 52. Dunn GP, Bruce AT, Sheehan KC, Shankaran V, Uppaluri R, Bui JD, et al. A critical function for type I interferons in cancer immunoediting. *Nat Immunol* 2005;6:722–9.
 53. Wenzel J, Bekisch B, Uerlich M, Haller O, Bieber T, Tuting T. Type I interferon-associated recruitment of cytotoxic lymphocytes: a common mechanism in regressive melanocytic lesions. *Am J Clin Pathol* 2005;124:37–48.
 54. Harlin H, Meng Y, Peterson AC, Zha Y, Tretiakova M, Slingluff C, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res* 2009;69:3077–85.
 55. Woo SR, Corrales L, Gajewski TF. The STING pathway and the T cell-inflamed tumor microenvironment. *Trends Immunol* 2015;36:250–6.
 56. Bald T, Landsberg J, Lopez-Ramos D, Renn M, Glodde N, Jansen P, et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. *Cancer Discov* 2014;4:674–87.
 57. Brody JD, Ai WZ, Czerwinski DK, Torchia JA, Levy M, Advani RH, et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. *J Clin Oncol* 2010;28:4324–32.
 58. Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Res* 2011;71:2488–96.
 59. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* 2015;523:231–5.
 60. Xia T, Konno H, Ahn J, Barber GN. Deregulation of STING signaling in colorectal carcinoma constrains DNA damage responses and correlates with tumorigenesis. *Cell Rep* 2016;14:282–97.
 61. Xia T, Konno H, Barber GN. Recurrent loss of STING signaling in melanoma correlates with susceptibility to viral oncolysis. *Cancer Res* 2016;76:6747–59.
 62. Sanmamed MF, Chester C, Melero I, Kohrt H. Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies. *Ann Oncol* 2016;27:1190–8.
 63. Timmerman RD, Herman J, Cho LC. Emergence of stereotactic body radiation therapy and its impact on current and future clinical practice. *J Clin Oncol* 2014;32:2847–54.
 64. Ranoa DR, Parekh AD, Pitroda SP, Huang X, Darga T, Wong AC, et al. Cancer therapies activate RIG-I-like receptor pathway through endogenous non-coding RNAs. *Oncotarget* 2016;7:26496–515.