Immune checkpoint inhibitors with radiotherapy and locoregional treatment: synergism and potential clinical implications

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Purpose of review
Antigens recognized by T cells in tumors include differentiation antigens, overexpressed antigens, cancer-testis, and mutated tumor neoantigens. Ionizing radiation causes damage to multiple biomolecules by direct energy deposition or by generation of free radicals, leading to cell death when the damage cannot be repaired. Tumor cell death induced by radiation will generate specific molecular signals that are sensed by antigen-presenting cells and stimulate their maturation and ability to cross-present tumor-derived antigens to T cells. Immunogenic cell death will complement the activity of immune checkpoint inhibitors. We will provide the emerging information coming from preclinical and clinical testing about the combinations of immunotherapies and radiotherapy.

Recent findings
Radiation induces chemokines that attract effector T cells to the tumor and vascular adhesion molecules that facilitate T-cell infiltration. This process, which has been named 'immunogenic modulation', plays a role not only in regression of the irradiated tumor but also in amplifying and strengthening adaptive antitumor immunity. The ongoing process of killing of tumor cells by cytotoxic T lymphocytes sustains release of more tumor antigens and possibly promotes antigenic spread, that is, activation of a broader T-cell repertoire. Results of several ongoing clinical trials are testing the combination of radiotherapy with immune checkpoint inhibitor treatment. Data support a model whereby 'waves' of tumor cell killing by T cells primed by the initial radiation-elicited antigen release boost the immune response. This process can eventually achieve systemic tumor control.

Summary
Radiation therapy is confirmed to be a sensitizer of tumors to immune checkpoint inhibitors in clinical trials, and its application will be easy to implement and widespread. Conversely, many issues need to be addressed before radiotherapy can become such a valid immunogenic tool. An area of increasing importance will be the development of suitable biomarkers that will be able to reliably assess 'immunogenic tumor cell death', immune effector stimulation, and adaptive immunity. Such an immune profile of biomarkers will aid in searching for an optimal combination of radiotherapy and immunomodulation and allows patient selection and response prediction.

Keywords
abscopal effect, immunotherapy, radiotherapy, tumor immunity

INTRODUCTION

Tumor cell transformation prompts activation of adaptive and innate immune responses, which had a crucial role in eliminating and controlling early cancer growth. Over the past 10 years, there has been a greater understanding of the immune response to tumors, which has led to the development of a huge number of immunotherapeutic strategies [1,2]. Agents such as the immune checkpoint inhibitors have demonstrated to induce a response in a number of solid malignancies [3], but their therapeutic benefit has not been seen in all cancer types. Efforts to develop multimodal strategies that can extend therapeutic benefit to a broader population of patients with a variety of

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tumors are in progress. As part of this strategy, radiotherapy could represent a potentially ideal partner for immune checkpoint inhibitors. This review aims to provide the emerging information coming from preclinical and clinical testing about the combinations of immunotherapies and radiotherapy.

EFFECT OF RADIOTHERAPY ON CANCER IMMUNE RESPONSE

Radiotherapy, in addition to a direct cytocidal effect on cancer cells, has also immune modulatory properties; in fact, it causes immunogenic cell death (ICD) of cancer cells, modulates antigen presentation by cancer cells, and most importantly alters the microenvironment within the irradiated field [4,5▪] (Fig. 1). The ICD of cancer cells involves a multistep process, including the release of 'find-me' signals (such as fractalkine, nucleotides, and ATP) that attract phagocytes or dendritic cells, the expression of 'eat-me' signals (such as calreticulin) that facilitate recognition by phagocytes or dendritic cells, and, finally, the release of danger-associated molecular patterns [such as high-mobility group box 1 protein (HMGB1) and ATP] that enable dying tumor cells to lose the propensity to induce tolerance and to stimulate powerful anticancer immune responses [6–8]. Since the resident dendritic cells within tumors maintain tolerance, ICD alone may not be sufficient to elicit a strong antitumor-immune response [9]. Some preclinical studies showed that radiotherapy overcame the suppressive action of tumor resident dendritic cells by engaging new myeloid-derived dendritic cells that have not undergone the regulatory effects of the tumor microenvironment [10,11]. In summary, radiotherapy mediates naïve dendritic cell recruitment, and may, at least in part, alter the immune-tolerant microenvironment characteristic of tumors. In addition, radiotherapy catalyzes the engagement of effector T cells to tumors through the induction of chemokines such as CXCL16 that correlates with improved survival and increased numbers of tumor-infiltrating lymphocytes in some tumors [12–15]. Radiation also has effects on the tumor vascular endothelium, inducing cell adhesion molecules, which further promote recruitment of antitumor cytotoxic T lymphocytes [16].

THE RATIONALE OF THE COMBINATION OF IMMUNOTHERAPY WITH RADIOTHERAPY

Greater understanding of radiation therapy's effect on tumor cells and components of the tumor microenvironment has in turn evidenced the central role of the immune system, as highlighted by Lee *et al.* [17], who described that in a mouse model, radiotherapy needs the presence of $CD8^+$ T cells for postradiotherapy tumor control. The interaction of host immune system and proper antitumor activity can lead to immune-mediated rejection of nonirradiated metastatic lesions after irradiation of the primary lesion in a process known as the abscopal effect. The abscopal effect of radiation therapy is an event by which a primary tumor is irradiated and a response is observed at distant metastatic sites externally of the field of the radiation [18]. Preclinical evidence supported the hypothesis that

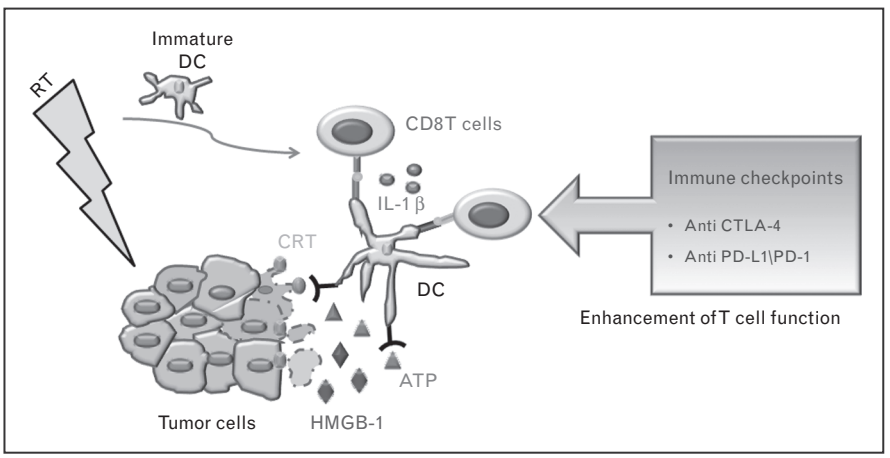

FIGURE 1. Immunogenic cell death involves the cell surface exposure of calreticulin (CRT), and the release of high-mobility group box (HMGB)1 and adenosine triphosphate (ATP) that triggers dendritic cell (DC) engulfment of dying cells, antigen presentation, and production of interleukin (IL)-Iβ, ultimately leading to activation of CD8 T cells. RT mediates also naive DC recruitment. Immune checkpoint inhibitors stimulate T-cell function. CTL-4, Cytotoxic T Lymphocyte Antigen-4; RT, radiotherapy.

the abscopal effect is mediated by the immune system [19], but the effect of radiation appears to be relatively weak and is rarely seen in clinical practice. Recently, there have been an increasing number of case reports showing the appearance of abscopal effects when radiotherapy is concomitantly administered with immune checkpoint inhibitors [20,21▪▪,22], underlining that the radiotherapy treatment can lead to an immune response which is augmented by the immune-modulating agents [23▪]. Therefore, the combination of radiotherapy and immune modulation represents a possible new paradigm shift in the management of advanced malignancy. The interaction between the immune system and radiation is intricate and multifactorial; it may rely on the radiation dose/quality and immune cell types [24]. The critical role of radiation is to induce the release of tumor immunogenic antigens responsible for the augmented pool of intracellular peptides for cross-presentation; in this way, the radiation creates an 'in-situ vaccination' [25]. During combinatorial treatment between radiotherapy and immunotherapy, the tumor-specific immune response is elicited and intensified subsequently. Preclinical models showed that radiation can augment tumor-specific antigen–Major Histocompatibility Complex complexes, upregulate antigen cross-presentation in the draining lymph node, and increase T-cell infiltration into tumors [26▪▪].

COMBINATION OF RADIOTHERAPY WITH CHECKPOINT RECEPTOR BLOCKADE

Despite the huge numbers of proimmunogenic effects of radiation, often they are insufficient to shift the immunosuppressive tumor microenvironment to obtain tumor rejection, and systemic antitumor responses following local radiotherapy are extremely rare. Likewise, only a small fraction of cancer patients have objective response from available immunotherapies. A plan to increase both the likelihood and duration of systemic antitumor immunity in response to immunotherapy is to add radiotherapy to sustain the immune response. An effective approach could be to improve effector T-cell function by blocking the immune checkpoint [27]. The aim of this dual approach is to improve the effector phase of immune response by activating T cells. The cytotoxic CD8+ T cells have a predominant role in inhibiting tumor growth. CD8+ T cells are remarkably augmented in combinatorial therapy than radiotherapy or immunotherapy alone [8,24,25,26▪▪]. Deng *et al.* [21▪▪] demonstrated that the decrease of CD8+ T cells significantly restrain the effectiveness of the combinatorial treatment. These data prompt that CD8+ T cells are necessary for combinatorial therapy [21▪▪]. Apart from this, another study demonstrated that natural killer cells can also contribute to local tumor control following combinatorial treatment [28▪▪]. These results strengthened the hypothesis that the antitumor effect of radiotherapy combined with immune-modulating antibodies is mostly mediated by the activated immune response. Therefore, agents which stimulate T-cell function, by blocking immune checkpoints, have an emerging clinical interest. Checkpoint receptors, including Cytotoxic T Lymphocyte Antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1), are up-regulated on activated T cells and transmit inhibitory signals, which suppress T-cell proliferation and function [29]. CTLA-4 is a member of the CD28:B7 immunoglobulin superfamily, and it is normally expressed at low levels on the surface of naive effector T cells and regulatory T cells (Tregs) [30]. After stimulation of a naive T cell, CTLA-4 is up-regulated and competes with CD28 for B7 and, finally, leads to suppression of T-cell activity. Anti-CTLA-4 monoclonal antibody facilitates T-cell proliferation and activation, and abrogates the suppressive function of Tregs [31]. In addition to CTLA-4, PD-1 is a key immune checkpoint protein expressed on chronically stimulated T cells, which leads to the suppression of T-cell activity through interaction with its ligands, PD-L1 and PD-L2 [32▪]. Antibodies targeting PD-L1 or PD-1 have been shown to promote cytotoxic T-lymphocyte expansion [33] and tumor regression in many mouse tumor models [34–37]. In mouse models, localized radiation therapy when combined with systemic CTLA-4 or PD-L1 blockade resulted in the inhibition of systemic metastases [21▪▪,38]. Many case reports, subsequently, confirmed these preclinical findings showing that in patients treated with anti-CTLA-4 therapy, localized radiation therapy can induce regression not only of irradiated but also of distant lesion [39–42]. A retrospective analysis revealed that 52% of 21 patients with progressing metastatic melanoma, after ipilimumab treatment and subsequent radiotherapy treatment, had an abscopal response, and the median overall survival (OS) for these patients was significantly longer when compared with patients without abscopal response [43▪]. Clinical trials using a combinatorial approach with radiotherapy and immune checkpoint inhibitors have been recently published. An open-label phase I/II trial, conducted in 33 men with metastatic castration-resistant prostate cancer, checked escalated doses of ipilimumab from 3 up to 10 mg/kg with or without a single 8-Gy dose of radiotherapy directed at one to three osseous metastases. The highest dose of ipilimumab was well tolerated, and an

additional 34 patients were treated with concurrent radiation, with only 25% of patients demonstrating progressive disease [44]. Kwon *et al.* [45▪▪] conducted a double-blind, randomized, multicenter trial in 799 patients with castration-resistant prostate cancer, who progressed on docetaxel. Patients received a single fraction of 8 Gy to one to five sites of osseous metastases and were randomized to following treatment with either 10 mg/kg of ipilimumab or placebo within 2 days of radiotherapy and continued every 3 weeks for up to four doses. The combination was well tolerated, but there was no difference in OS in the population as a whole. However, in subset analysis, there was an improvement in OS of patients with a smaller burden of metastatic disease. Recently, Victor *et al.* [46▪▪] treated, in a phase I trial, 22 patients were affected by advanced melanoma with hypofractionated stereotactic body radiation and ipilimumab. Assessment of nonirradiated lesions using Response Evaluation Criteria In Solid Tumors criteria demonstrated 18% patients had a partial response as the best response, 18% had stable disease, and 64% had progressive disease [46▪▪]. Although responses were observed, the majority of patients in this trial did not respond. To explain the contribution of radiation to immune checkpoint blockade and to discover mechanisms of resistance, the authors used the B16-F10 melanoma mouse model that revealed that resistance was due to up-regulation of PD-L1 on melanoma cells and was associated with T-cell exhaustion. As a consequence, optimal response in melanoma and other cancer types requires a multiple approach with radiation, anti-CTLA4, and anti-PD-L1/PD-1, which could be able to promote response and immunity through distinct mechanisms. Inter and intrapatient factors, both in terms of immune programming and tumor heterogeneity, may be responsible for differential responses. Of particular interest is the combination of anti-PD-1 antibodies with radiotherapy. Supporting data of the efficacy of this combination have been demonstrated in a mouse glioma model, where the combination of a constructed anti-PD-1 therapy and stereotactic radiotherapy led to long-term survival [20], as well as in a murine model of breast and colorectal carcinomas, where augmented tumor control was showed [21▪▪]. Actually, many early-phase clinical trials combining immune checkpoint inhibitors with radiotherapy are ongoing. A number of these studies are examining the combination of stereotactic radiotherapy with PD-1 or PD-L1 inhibitors in patients with oligometastatic disease [47,48]. The optimal timing of administration, the duration, the sequence of the immune-modulating agents with radiotherapy, and the appropriate patient populations are still not elucidated.

RADIOTHERAPY AND IMMUNOTHERAPY: WHERE, HOW, WHEN?

Many factors appear to be important to the success of combining immunotherapy and radiotherapy. One variable is represented by the target site of radiotherapy because intratumor heterogeneity within primary tumors and associated metastatic sites could have a relevant impact on the immune response and radiotherapy may lead to a distinct immune response at these different sites. Different mechanisms, such as weakened humoral immune response, T-cell depletion, and clonal exhaustion, should be accountable for the immune tolerance and impaired immune-modulatory activities of radiotherapy. Dose and fractionation are relevant variables in the immunogenicity of radiotherapy. Lymphocytes are extremely responsive to radiotherapy [49]: very high irradiation could destroy antitumor immune activity of the host, whereas very low irradiation could be inadequate to prime an effective antitumor immune response. In preclinical models, low-dose irradiation has been demonstrated to augment T-cell migration into the irradiated field [50]. It has also been prompted that radiotherapy doses from as low as 2 Gy, but up to 20 Gy, may be sufficient to start ICD [23▪]. Also, the fractionation can have a predominant role to trigger the antitumor immunity. Dewan *et al.* [51] reported that when combined with CTLA-4 antibody antagonists, 8 Gy in three fractions or 6 Gy in five fractions are superior to standard fractionation or a single dose of 20 Gy. The explanation of the difference in immune response among disparate fractionation schedules and dose is uncertain, but recent clinical reports, which reported impressive abscopal effects after palliative radiotherapy to a single metastatic site, support the choice of hypofractionated radiotherapy [40,41]. The identification of the most beneficial time point for radiotherapy combined with immunotherapy is another important challenge. In a recent preclinical study combining CTLA-4 blockade with radiotherapy, using a mouse model of breast cancer, the antibody was given at different time points, with the best abscopal response observed when the first dose of antibody was administered during radiotherapy [51]. This result was also observed in a case report in which a patient with nonsmall cell lung cancer obtained an abscopal effect receiving concomitant ipilimumab and radiation [40]. On the contrary, Postow *et al.* [41] reported an abscopal effect in a patient with metastatic melanoma occurred after long-term treatment with ipilimumab prior to radiotherapy. So, the question about the optimal timing of radiotherapy has not yet been addressed.

Table 1. Clinical trials of Immune checkpoint inhibitors and radiotherapy in solid tumors currently recruiting on clinicaltrials.gov

Clinicaltrials.gov Identifier	Phase study	Tumor site/stage	Immune checkpoint inhibitor	RT/dose fraction
NCT01401062	Phase I–II	Metastatic breast cancer	Fresolimumab	7.5 Gy × 3
NCT01689974	Phase II randomized	Metastatic melanoma	Ipilimumab	6 Gy × 5
NCT02221739	Phase I–II	Metastatic nonsmall cell lung cancer	Ipilimumab	6 Gy × 5
NCT01557114	Phase I	Stage III or stage IV melanoma	Ipilimumab	9 Gy × 3, 15 Gy × 5, 18 Gy × 6, 24 Gy × 8
NCT02406183	Phase I	Metastatic melanoma	Ipilimumab	SBRT (24 Gy × 8, 30 Gy × 10, 36 Gy × 12
NCT02311361	Phase I	Unresectable pancreatic cancer	Tremelimumab and/or MEDI4736	SBRT dose escalation (from 1 to 5 doses)
NCT01935921	Phase Ib	Stage III–IVB head and neck cancer	Ipilimumab	IMRT
NCT01711515	Phase I	Locally advanced cervical cancer	Ipilimumab	Extended beam radiation therapy 5 days a week for 6 weeks, and intracavitary brachytherapy for 2 weeks
NCT02107755	Phase II	Metastatic melanoma	Ipilimumab	SART over 2–3 days per week
NCT01449279	Phase I	Metastatic melanoma	Ipilimumab	Palliative RT
NCT02298946	Phase I	Metastatic colorectal cancer	AMP-224	8 Gy for 1 or 3 days
NCT02407171	Phase I–II	Metastatic melanoma or nonsmall cell lung cancer	MK-3475	Starting dose: 3 Gy × 5
NCT02383212	Phase I	Advanced malignancies	REGN2810	Hypofractionated RT
NCT02303990	Phase I	Advanced malignancies	Pembrolizumab	Hypofractionated RT
NCT02303366	Phase I	Oligometastatic breast cancer	MK-3475	SABR (20 Gy in 1 fraction)
NCT02289209	Phase II	Advanced squamous cell carcinoma of the head and neck	MK-3475	1.2 Gy b.i.d. 5 days a week for 5 weeks
NCT02400814	Phase I	Stage IV nonsmall cell lung cancer	MPDL3280A	SART
NCT01703507	Phase I	Melanoma with brain metastases	Ipilimumab	WBRT
NCT02239900	Phase I–II	Advanced solid malignancies	Ipilimumab	SBRT 50 Gy in 4 fractions or 60 Gy in 10 fractions
NCT01970527	Phase II	Stage IV melanoma	Ipilimumab	SBRT
NCT01565837	Phase II	Oligometastatic but unresectable melanoma	Ipilimumab	SART
NCT02115139	Phase II	Melanoma and brain metastases	Ipilimumab	WBRT 3 Gy × 10
NCT01497808	Phase I–II	Metastatic melanoma	Ipilimumab	SBRT
NCT01860430	Phase Ib	Locally advanced head and neck cancer	Ipilimumab	IMRT 70–74.0 Gy in 7–7.5 weeks
NCT01996202	Phase I	Poor prognosis melanoma	Ipilimumab	Standard RT
NCT02097732	Phase I	Melanoma and brain metastases	Ipilimumab	SART
NCT01950195	Phase I	Melanoma brain or spinal metastases	Ipilimumab	SART
NCT02444741	Phase I–II	Stage IV nonsmall cell lung cancer	MK-3475	Conventional RT or SBRT

(*Continued*)

Table 1 (*Continued*)

Clinicaltrials.gov Identifier	Phase study	Tumor site/stage	Immune checkpoint inhibitor	RT/dose fraction
NCT02437071	Phase II	Metastatic colorectal cancer	Pembrolizumab	Radiofrequency ablation or conventional RT
NCT02313272	Phase I	Recurrent high-grade gliomas	Pembrolizumab	HFSRT
NCT02318771	Phase I	Recurrent/metastatic head and neck cancer, renal cell cancer, urothelial cancer, melanoma, and nonsmall cell lung cancer	MK-3475	Conventional RT (8 Gy × 1 or 4 Gy × 5)
NCT02492568	Phase II	Stage IV nonsmall cell lung cancer	Pembrolizumab	SBRT
NCT01853618	Phase I	Liver cancer	Tremelimumab	Radiofrequency ablation or transarterial catheter chemoembolization
NCT01730157	Phase 0	Uveal melanoma with liver metastases	Ipilimumab	Sequential hepatic radioembolization

HFSRT, hypofractionated stereotactic irradiation; IMRT, intensity modulated radiotherapy; RT, radiotherapy; SART, stereotactic ablative radiosurgery; SBRT, stereotactic body radiation therapy; WBRT, whole-brain radiotherapy.

CONCLUSION

The recent success of cancer immunotherapy, particularly checkpoint inhibitors, has turned on an exciting light on new field of applications and combinatorial treatments (Table 1). The synergy of radiotherapy and immunotherapy represents a rising strategy with the potential to better target local irradiated/viable tumor cells, and to give higher control of distant systemic disease. Preclinical and clinical studies have shown the safety and efficacy of radiotherapy combined with immunotherapy. Future trials may investigate combined approaches to immunotherapy that will improve the effect of radiotherapy on antitumor T-cell priming, as well as concur to other steps of immune rejection [52]. It will be crucial to elucidate the appropriate dose and fraction of radiotherapy, the time point of the combination, and most appropriate patient population to improve patient care in the future.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Drake CG, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. Adv Immunol 2006; 90:51–81.
2. Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. Ann Oncol 2012; 23 (Suppl 8):viii6–viii9.
3. Postow MA, Callahan MK, Wolchok JD. The antitumor immunity of ipilimumab: (T-cell) memories to last a lifetime? Clin Cancer Res 2012; 18:1821–1823.
4. Formenti SC, Demaria S. Systemic effects of local radiotherapy. Lancet Oncol 2009; 10:718–726.
5. ■ Thompson RF, Maity A. Radiotherapy and the tumor microenvironment: mutual influence and clinical implications. Adv Exp Med Biol 2014; 772:147–165.

This article describes the role and clinical implications of the tumor microenvironment and radiotherapy.

6. Golden EB, Pellicciotta I, Demaria S, *et al.* The convergence of radiation and immunogenic cell death signaling pathways. Front Oncol 2012; 2:1–13.
7. Obeid M, Panaretakis T, Joza N, *et al.* Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. Cell Death Differ 2007; 14:1848–1850.
8. Zitvogel L, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. Cell 2010; 140:798–804.
9. Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol 2004; 4:941–952.
10. Dieu MC, Vanbervliet B, Vicari A, *et al.* Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J Exp Med 1998; 188:373–386.
11. Teitz-Tennenbaum S, Li Q, Okuyama R, *et al.* Mechanisms involved in radiation enhancement of intratumoral dendritic cell therapy. J Immunother 2008; 31:345–358.
12. Luster AD, Leder P. IP-10, a-C-X-C-chemokine, elicits a potent thymus-dependent antitumor response in vivo. J Exp Med 1993; 178:1057–1065.

13. Gutwein P, Schramme A, Sinke N, *et al.* Tumoural CXCL16 expression is a novel prognostic marker of longer survival times in renal cell cancer patients. Eur J Cancer 2009; 45:478–489.

14. Matsushita K, Toiyama Y, Tanaka K, *et al.* Soluble CXCL16 in preoperative serum is a novel prognostic marker and predicts recurrence of liver metastases in colorectal cancer patients. Ann Surg Oncol 2012; 19 (Suppl 3): S518–S527.

15. Matsumura S, Wang B, Kawashima N, *et al.* Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol 2008; 181:3099–3107.

16. Hallahan D, Kuchibhotla J, Wyble C. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. Cancer Res 1996; 56:5150–5155.

17. Lee Y, Auh SL, Wang Y, *et al.* Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood 2009; 114:589–595.

18. Mole RH. Whole body irradiation: radiobiology or medicine? Br J Radiol 1953; 26:234–241.

19. Demaria S, Ng B, Devitt ML, *et al.* Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Radiat Oncol Biol 2004; 58:862–870.

20. Zeng J, See AP, Phallen J, *et al.* Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 2013; 86:343–349.

21. ■■ Deng L, Liang H, Burnette B, *et al.* Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 2014; 124:687–695.

This article provides the rational design of combination therapy with immune modulators and radiotherapy.

22. Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol 1953; 26:234–241.

23. ■ Golden EB, Frances D, Pellicciotta I, *et al.* Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. Onco Immunol 2014; 3:e28518.

This article provides preliminary evidence that ICD contributes to the establishment of a peritumoral pro-immunogenic milieu.

24. Rodel F, Frey B, Gaipl U, *et al.* Modulation of inflammatory immune reactions by low-dose ionizing radiation: molecular mechanisms and clinical application. Curr Med Chem 2012; 19:1741–1750.

25. Sharma A, Bode B, Wenger RH, *et al.* Gamma-radiation promotes immunological recognition of cancer cells through increased expression of cancer-testis antigens in vitro and in vivo. PLoS One 2011; 6:e28217.

26. ■■ Sharabi AB, Nirschl CJ, Kochel CM, *et al.* Stereotactic radiation therapy augments antigen-specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen. Cancer Immunol Res 2015; 3:345–355.

This article provides the rationale for combining radiation with PD-1 blockade.

27. Weber J. Immune checkpoint proteins: a new therapeutic paradigm for cancer–preclinical background: CTLA-4 and PD-1 blockade. Semin Oncol 2010; 37:430–439.

28. ■■ Dovedi SJ, Adlard AL, Lipowska-Bhalla G, *et al.* Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. Cancer Res 2014; 74:5458–5468.

This article reveals the basis for an adaptive response by tumor cells that mediates resistance to radiotherapy.

29. Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clin Cancer Res 2007; 13:5271–5279.

30. Peggs KS, Quezada SA, Chambers CA, *et al.* Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 2009; 206:1717–1725.

31. Khan S, Burt DJ, Ralph C, *et al.* Tremelimumab (anti-CTLA4) mediates immune responses mainly by direct activation of T effector cells rather than by affecting T regulatory cells. Clin Immunol 2011; 138:85–96.

32. ■ Momtaz P, Postow MA. Immunologic checkpoints in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmgenomics Pers Med 2014; 7:357–365.

This article describes the role of immunotherapy with PD-1 blocking antibodies.

33. Strome SE, Dong H, Tamura H, *et al.* B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res 2003; 63:6501–6505.

34. Iwai Y, Ishida M, Tanaka Y, *et al.* Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD- L1 blockade. Proc Natl Acad Sci USA 2002; 99:12293–12297.

35. Pilon-Thomas S, Mackay A, Vohra N, *et al.* Blockade of programmed death ligand 1 enhances the therapeutic efficacy of combination immunotherapy against melanoma. J Immunol 2010; 184:3442–3449.

36. Goding SR, Wilson KA, Xie Y, *et al.* Restoring immune function of tumor-specific CD4þ T cells during recurrence of melanoma. J Immunol 2013; 190:4899–4909.

37. Okudaira K, Hokari R, Tsuzuki Y, *et al.* Blockade of B7-H1 or B7-DC induces an antitumor effect in a mouse pancreatic cancer model. Int J Oncol 2009; 35:741–749.

38. Demaria S, Kawashima N, Yang AM, *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 2005; 11:728–734.

39. Hiniker SM, Chen DS, Reddy S, *et al.* A systemic complete response of metastatic melanoma to local radiation and immunotherapy. Transl Oncol 2012; 5:404–407.

40. Golden EB, Demaria S, Schiff PB, *et al.* An abscopal response to radiation and ipilimumab in a patient with metastatic nonsmall cell lung cancer. Cancer Immunol Res 2013; 1:365–372.

41. Postow MA, Callahan MK, Barker CA, *et al.* Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012; 366:925–931.

42. Sullivan RJ, Lawrence DP, Wargo JA, *et al.* Case records of the Massachusetts General Hospital. Case 21-2013. A 68-year-old man with metastatic melanoma. N Engl J Med 2013; 369:173–183.

43. ■ Grimaldi AM, Simeone E, Giannarelli D, *et al.* Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Onco Immunol 2014; 3:e28780.

This article suggests that local responses to radiotherapy may be predictive of abscopal responses.

44. Slovin SF, Higano CS, Hamid O, *et al.* Ipilimumab alone or in combination with radiotherapy in metastatic castration resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013; 24:1813–1821.

45. ■■ Kwon ED, Drake CG, Scher HI, *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; 15:700–712.

This study investigates the use of ipilimumab after radiotherapy in patients with metastatic castration-resistant prostate cancer.

46. ■■ Victor CT, Rech AJ, Maity A, *et al.* Radiation and dual checkpoint blockade activate nonredundant immune mechanisms in cancer. Nature 2015; 520: 373–377.

This article provides explanation of how PD-L1 on melanoma cells allows tumours to escape anti-CTLA4-based therapy.

47. ClinicalTrials.Gov. AMP-224, a PD-1 inhibitor, in combination with stereotactic body radiation therapy in people with metastatic colorectal cancer. https://clinicaltrials.gov/ct2/show/NCT02298946. [Accessed 02/05/15]

48. ClinicalTrials.Gov, MPDL3280A and stereotactic ablative radiotherapy in patients with nonsmall cell lung cancer. https://clinicaltrials.gov/ct2/show/NCT02400814?term=NCT02400814&rank=1. [Accessed 02/05/15]

49. Wasserman J, Blomgren H, Rotstein S, *et al.* Immunosuppression in irradiated breast cancer patients: in vitro effect of cyclooxygenase inhibitors. Bull N Y Acad Med 1989; 65:36–44.

50. Klug F, Prakash H, Huber PE, *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.

51. Dewan MZ, Galloway AE, Kawashima N, *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; 15:5379–5388.

52. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 2013; 39:1–10.