



# Radiation for awakening the dormant immune system, a promising challenge to be explored

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Recent advances that have been made in our understanding of cancer biology and immunology show that infiltrated immune cells and cytokines in the tumor microenvironment may play different functions that appear tightly related to clinical outcomes. Strategies aimed at interfering with the cross-talk between microenvironment tumor cells and their cellular partners have been considered for the development of new immunotherapies. These novel therapies target different cell components of the tumor microenvironment and importantly, they may be coupled and boosted with classical treatments, such as radiotherapy. In this work, we try to summarize recent data on the microenvironment impact of radiation therapy, from pre-clinical research to the clinic, while taking into account that this new knowledge will probably translate into indication and objective of radiation therapy changes in the next future.

**Keywords:** immunotherapy, radiotherapy effects, tumor microenvironment, abscopal effect, CTLA-4

## INTRODUCTION

In the last few years, the impact of the specific immune microenvironment in cancer has gained renewed interest, and is actually recognized as one of the major determinants of clinical evolution in a wide range of tumors. In this sense, cells like tumor-associated macrophages (TAM), regulatory T cells (Treg), and myeloid-derived-suppressor cells (MDSC), among others, are being proposed as new prognostic biomarkers that might be taken into account for diagnostic purposes, with the aim to complete classical information that is generally focused mainly on intrinsic characteristics of the tumor cell itself (histopathologic grade, mitosis, etc). Importantly, the effect of antineoplastic treatments on tumor cells may change the composition of microenvironment cells and their functional status, which introduces even more complexity (and importance) to this topic.

Radiotherapy (RT) remains a cornerstone of oncological treatment for many types of tumors. Recently, it has been demonstrated that ionizing radiation may exert interesting effects over the tumor microenvironment, increasing the effectiveness of patients' anti-tumor immune responses in the clinical setting even at distant sites (1). This fact has given rise to the concept of immunogenic death mediated by radiation, which seems largely associated with the immunocompetence status of the host (2).

In this review, we pursue to update and summarize the local and systemic immune effects of RT from the molecular level to the clinical scenario. Finally, some choices for immunotherapy combinatorial approaches based on RT and strategies for monitoring these sorts of response are suggested.

## LOCAL IMMUNE EFFECTS OF RT

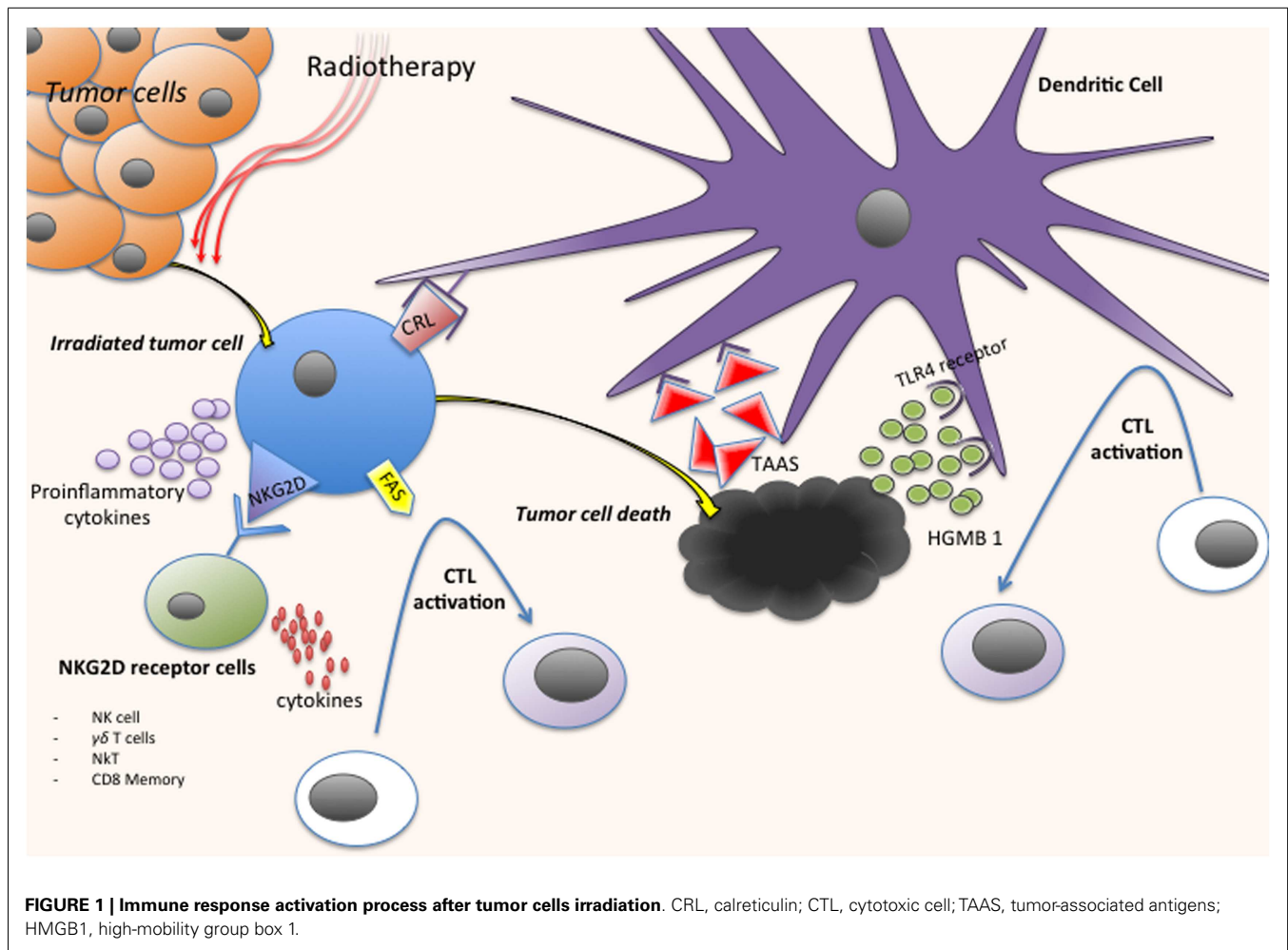
The radiation-induced biological response exerts pro-inflammatory and immunomodulatory effects against tumor cells,

and nowadays the modulation of the acquired immune response after irradiation is gaining increased interest (1).

The radiobiological model considers that DNA damage after radiation induces different types of biological response, this has been classically described as the 5 Rs of radiobiology (intrinsic radiosensitivity, reoxygenation, redistribution in the cell cycle, repair of sublethal damage, and accelerated repopulation) (3). In this model, the radiobiological effects are caused by direct damage on DNA by tumor cells or indirectly after the induction of free radicals. It is worthy of consideration that this fact may determine a variable type of cell death through the phenomena of apoptosis, autophagy, necrosis, or mitotic catastrophe (4). Most cells survive a limited period of time after irradiation and, during this time, they generate molecular signals that induce the overexpression of specific genes that control the expression of growth factors, cytokines, chemokines, and cell surface receptors. Cell survival depends on this response and its ability to repair damaged DNA, being these phenomena of primary importance in radiation treatments, as they may determine the final effects over the surrounding microenvironment (5).

Increasing evidence has revealed that RT can change its recognition level, making the tumor vulnerable to the immune system. Furthermore, it has been described that the radiobiological response causes the activation of different T-cell lines, generating the "switch-on" of the adaptive immune response (6). These findings have led the scientific community to explore the immunotherapy and the RT effects together, as synergic tools in cancer treatment strategies (7).

It seems that one of the main effects of RT to unleash an effective immune response is the induction of a strong "danger signal," which is a concept postulated by Polly Matzinger in 1994, related to the stress signals generated by the damaged tissue



(8). Dying tumor cells after irradiation induce danger signals like endogenous ligands called “alarmins” with immunogenic properties. Various mechanisms with different peptides, cytokines, and cells are involved in this process (**Figure 1**):

- (1) Calreticulin: Radiation causes the translocation of calreticulin (CRL) from the endoplasmic reticulum to the cell surface, inducing the apoptotic cell antigen presentation to antigen presenting cells (APCs), in particular dendritic cells (DC), and stimulating specific anti-tumor T-cell responses (9, 10).
- (2) High-mobility group box 1 (HMGB1): Another immunogenic determinant of cell death is the pro-inflammatory factor HMGB1. HMGB1 is a nuclear protein that is released after necrotic cell death and from dying cells during late stage apoptosis. After cell death induced by RT, HMGB1 may be released to the stroma and act as a neo-antigen, which in turn acts as an immunogenic endogenous “danger signal,” initiating an inflammatory response through binding Toll-Like Receptor 4 (TLR4) on DC (9, 11).
- (3) NKG2D receptor: NKG2D acts as an activating receptor on NK cells,  $\gamma\delta$  T cells, NKT cells, and memory and activated CD8<sup>+</sup> T cells (12). RT induces the expression of NKG2D ligands that,

- after engagement with its receptor, seems to increase cytokine production in order to stimulate CTL (12, 13).
- (4) Upregulation of death receptors: RT may activate the extrinsic pathway of apoptosis, upregulating the expression of the FAS death receptor on tumor cells, which induces the activation of CTL via FAS ligand expressed on their surface (14).
- (5) Release of tumor antigens: Apoptotic and necrotic tumor cells after RT are a big source of tumor antigens, commonly tumor-associated antigens (TAAs), which can be efficiently taken up by DC that subsequently present them to CTL (15) (this topic is amplified on section “Conclusion”).
- (6) Release of pro-inflammatory cytokines: Pro-inflammatory cytokines like IL-1 $\beta$ , TNF- $\alpha$ , or prostaglandin E2 are up-regulated in tumor cells after the cellular lesion post-irradiation, and they represent ultimately danger signals due to the tissue stress induced by RT (16).

Immune-modulating effects of radiation are influenced by several factors. In this sense, the dose of radiation has been correlated with different responses. Low radiation doses seem to activate innate immune cells and fail to induce cell death. This situation develops a tumorigenic effect mediated by the cells of the immune

microenvironment (17, 18). On the contrary, high radiation doses seem to induce an immunogenic effect. Schaeue et al. studied tumor specific immune response in mice bearing murine melanoma irradiated with 15 Gy administered in different sizes per fraction. The authors concluded that a single dose of 7.5 Gy or higher, but not lower than 5 Gy, was immunostimulatory (19). These findings agreed with the results of Lee who compared single dose of 20 Gy against 5 Gy  $\times$  4 given over 2 weeks in a pre-clinical study. Ablative radiation of 20 Gy dramatically increased T-cell activity and tumor control, whereas the fractionated radiation showed less tumor growth inhibition (20). However, the inflammatory balance in the tumoral environment is quite complex. Radiation could promote – directly or indirectly – negative regulators such as TGF- $\beta$ . It is known that latent isoform of TGF $\beta$ 1 is activated due to reactive oxygen species liberate the latency-associated peptide after RT (21). Furthermore, increased levels of TGF- $\beta$  are detected as a consequence of M2 macrophage release after exposition to apoptotic cancer cells (22). However, until now, there is little evidence about the best radiation schedule to obtain an optimal immunogenic response.

In addition, the physical sequence of events after RT seems to be critical to mount a successful immune response. Pre-clinical models have shown that DC loaded with tumor antigens migrate toward the draining lymph nodes. This process leads to an activation of T cells that had not been previously exposed to specific tumor antigens, spreading the immune response against the tumor (20, 23). Activated CTL are guided by the chemokine gradient induced by radiation. This fact has been ascertained, for example, by the CXCL16 chemokine, which is able to recruit effector T cells to the site of the irradiated tumor area (24). In a murine model of metastatic breast cancer, the CXCL16 proved to be essential in the synergism between RT and CTLA-4 blocking (25, 26). In addition, tumor vessels have multiple barriers – through an abnormal architecture or a lower expression of endothelial adhesion molecules – that hinder the infiltration of T cells (27). At this point, RT allows the upregulation of cell adhesion molecules (CAM), which facilitates the transit of lymphocytes to tumor cells (23, 28). In a murine model of squamous cell carcinoma, blockade of CD11b – ligand for ICAM-1 – reduced the radiation-induced infiltration of myeloid cells into irradiated tumors and diminished tumor regrowth (29). VCAM-1 is up-regulated in melanoma in a process requiring IFN- $\gamma$  production (28). Once the CTL are found in the tumor, radiation therapy may again influence and boost the anti-tumor immune response by the death of new tumor cells leading to the increased expression of death receptors on tumor cells such as Fas/CD95, MHC-R, CEA, NKG2D, and other costimulatory molecules (30–32). This fact facilitates the recognition and destruction of tumor cells by CTLs. The death of tumor cells mediated by FAS represents a mechanism independent of the T-cell receptors (TCR), so that if TCR affinity is low FAS has a potent cytotoxic role (14). Therefore, radiation modifies the characteristics of the tumor microenvironment, making it more accessible to the immune system, which supports and extends the response to RT, not only by direct and indirect injury from ionizing radiation, but also by immunomodulatory mechanisms.

The potential immunogenicity of RT is heavily influenced by the differentiation of immune cells in the tumor

microenvironment. Conventional fractionated radiation therapy has traditionally been considered immunosuppressive (33). This is due, in part, to the early apoptotic death occurring in lymphocytes following low doses of radiation (34). However, lymphocyte subsets have distinct radiosensitivity. In this regard, immunosuppressive cells like macrophages are considered essentially radioresistant, whereas final effects of RT in Treg are still unclear (17, 29, 35). Results from studies by pioneering labs in this field showed that sublethal whole-body irradiation of mice bearing tumors may result in absence of responses in nude mice, and in partial or complete tumor regression in those with complete immunity. Authors considered that RT-induced tumor regression by activation of the immune response through downregulation of Treg (36, 37). However, evidence at this point is controversial, since other groups have recently postulated that Treg radioresistant behavior might lead to a percentual increase of these cells. These phenomena should deteriorate RT-induced anti-tumor immunity. In this sense, Schuler et al analyzed Treg in tumor tissues and peripheral blood of head and neck squamous cell carcinoma patients treated with chemoradiotherapy. Their results suggest that chemoradiotherapy favor survival and suppressor functions of Treg, and thus, this combinatorial approach might induce disease recurrence or even development of secondary cancers (38, 39). With respect to macrophages, local low-dose irradiation seems to induce proliferation of iNOS<sup>+</sup> M1-macrophages in tumor microenvironment. These macrophages may release pro-inflammatory molecules such as TNF and facilitate cell infiltration by tumor-reactive effector T cells, improving local immune response against cancer (33). Nevertheless, it has been demonstrated that cell death after radiation may also result in M2 macrophage activation and induce immune suppression (40). The suppressive response of M2 macrophages is a key feature of inflammatory resolution, which serves to repair inflammatory destruction following control of infections by laying down supportive matrix, establishing vascular structures, and terminating adaptive immune responses (22). This change in the macrophage phenotype in tumors from M1 to M2 macrophage has been associated with early tumor growth *in vivo*. Macrophages from irradiated tumors express higher levels of iNOS, arginase-I, and COX-2, and promote tumor growth (41).

### SYSTEMIC IMMUNE EFFECTS OF RT, THE ABCOPAL EFFECT

In recent decades, RT effects in distant sites away from the original irradiated area have introduced a new concept of the highest interest, which is the ability of RT to exert systemic anti-tumoral responses. The first description of this effect was made by Robin H. Mole in 1953 (42), and this is currently denominated as the abscopal effect. The etymological definition comes from the Latin *ab* (outside) and *Scopus* (target). The abscopal effect provides new insights into the mechanisms of RT activity (43, 44).

The abscopal effect may have a dual role in the RT activity. Firstly, unwanted side events, such as the onset of inflammatory phenomena at a distance, can be generated. These side effects may produce pneumonitis or other serious phenomena like genomic instability resulting in leukemia or other neoplasms (17, 45). Secondly, the abscopal effect can have therapeutic consequences, with the reduction of distant metastasis after RT of primary tumors or localized metastases with palliative purposes as proof of principle

of this phenomenon. Although proving irrefutable evidence of the abscopal effect is a difficult task, this event has been postulated in several types of tumors including melanoma, lymphoma, and hepatocellular or renal cell carcinomas (46, 47).

In the previous section of this review, it was noted that RT induces changes in the tumor microenvironment, transforming the irradiated tissue into an immunogenic hub, which serves to the immune system as a source for the identification of tumor cells. Therefore, the immune system can recognize tumor cell lines out and away from the irradiated zone. Some authors have described this “vaccine effect” by the sensitization process that generates the body, which recognizes previously unnoticed tumor cells (48). Nevertheless, the immunological mechanism underlying the abscopal effect is still unknown (49). The primary hypothesis focuses on a kind of “systemic cytokine storm” after irradiation, with the release of cytokines like TNF, IL-4, IL-18, IL-2, and GM-CSF (50–54). These cytokine may induce an anti-tumor humoral immune effect and subsequently an immune cell response against the tumor, ultimately mediated by T lymphocytes.

In the clinical setting, the abscopal effect has been studied in patients with low grade B cell lymphoma after intratumoral injection of a Toll-like receptor 9 (TLR9) agonist (CpG) during treatment with RT. Previously, authors detected the recognition and response of cytotoxic lymphocytes against B lymphoma cells *in vitro* (55). Furthermore, *in vivo* studies with murine models combining RT and immunostimulants, such as anti-CTLA antibody and the growth factor of DC (Flt-3) showed the reduction of tumor growth outside the irradiation fields (56).

To date case reports related to the abscopal effect are relatively scarce. One of the main reasons might be underdiagnosis due to the lack of knowledge of this phenomenon. As we have mentioned before, RT alone in some circumstances might develop immune response to control the upgrowth of distant metastases. Nowadays, this likelihood is getting higher, due to the addition of immune drugs to RT that might lead to a better recognition of remote tumor cell by the immune system. Nevertheless, some well-documented case reports have been described recently. In this sense, a case of regression of non-irradiated metastases from melanoma NY-ESO<sup>+</sup> after receiving palliative RT combined with immunotherapy (anti-CTLA-4/ipilimumab) has been recently described by Postow et al. (57). After combined treatment (palliative RT and ipilimumab), metastatic lesions showed marked regression. Biological biomarkers were of great interest at this point with the observation of an increase of NY-ESO-1-specific antibodies, CD4<sup>+</sup> ICOS high, NY-ESO-1-specific interferon-gamma-producing CD4<sup>+</sup> cells and HLA-DR-expressing CD14<sup>+</sup> monocytes, after RT. Simultaneously, MDSC levels decreased sharply (57). Another well described case report in a metastatic melanoma patient treated with palliative RT and immunotherapy (anti-CTLA-4/ipilimumab) has been recently published (58). In this case, the regression of non-irradiated in transit metastases after RT of the primary tumor was achieved. Again, a biomarker of immune activity was studied. In this case, autoantibodies against melanoma antigen A3 (MAGEA3) titers were measured demonstrating a systemic anti-tumor immune response (58).

The aforementioned cases serve as proof of principle for the abscopal effect theory related to the anti-tumor immune response,

supporting evidences extracted from pre-clinical studies (Table 1). Therefore, association of RT and immunotherapy can open new lines of work and research in both fields. Further studies are needed to determine, which are the better RT and immunotherapy schedules and combinations to unleash this immune response against tumors.

## COMBINATION OF IMMUNOTHERAPY AND RT

Immunogenic cell death mediated by RT may serve as the basis of an effective immunogenic host response that can be modulated by other immunogenic strategies.

As previously described, synergism between RT and other immune therapies have a robust biological rationale that, related to the adaptive-cell response and generation of cytotoxic T lymphocytes, may be summarized in the following sequence of events.

- (1) First signal: tumoral associated antigens availability: At this point, the effects of RT inducing an antigenic environment with the release of tumoral antigens after cell death is a fact of the highest importance since it favors the generation of an inflammatory microenvironment around the irradiated tumor. In addition, RT seems to favor antigen presentation via surface MHC-I in APCs to cytotoxic lymphocytes (59). Specifically tumor specific antigens (TSA) identification increases treatment efficacy due to immune response to selective cancer cells. Robbins et al. showed that transferring autologous lymphocytes – previously exposed to mutated cancer proteins – lead to an *in vitro* and *in vivo* tumor regression (59).
- (2) Second signal: co-stimulatory/co-inhibitory molecules: Immune synapse has been revealed as a promising therapeutic target and a set of monoclonal antibodies (mAb) targeting the molecules of this virtual space is under intensive clinical research (60).
  - (a) Cytotoxic T lymphocyte antigen-4 (CTLA-4): mAb anti-CTLA-4 ipilimumab has been approved by the FDA for the treatment of advanced melanoma, after demonstrating an increase in overall statistically significant survival in two randomized phase III trials in the first and second line setting (61, 62). Specifically with RT in pre-clinical models, local RT and CTLA-4 blockade have shown to mediate synergistic effects. In this sense, in mice concurrently challenged with two tumors, the treatment of one tumor with local RT in combination with the systemic administration of anti-CTLA-4 induced significant growth delay in the second tumor that did not receive local RT (63). The exact mechanism underlying the abscopal regression of unirradiated tumors has not been fully explained, but the results are consistent with an increased priming of tumor antigen-specific T cells that subsequently infiltrate the tumor. Such an effect would likely be mediated by blocking the engagement of CTLA-4 on effect or T cells in the context of intensified cross-priming capacity of DCs in the lymph nodes (48). At this point, Dewan et al. reported that a fractional dose of 8 Gy × 3 was optimal for the induction

**Table 1 | Recompilation of case reports on abscopal effect.**

Case reports	Diagnosis	Dose RT/irradiated site	Response to RT	Associated treatments	Specific immune response markers
Antoniades et al. (74)	Stage III non-Hodgkin's lymphoma	30 Gy in 20 fx	Regression of abdominal lymph nodes after mantle's irradiation	No	No
Ohba et al. (50)	Metastatic hepatocellular carcinoma to bone	36 Gy to metastasis	Complete regression of the metastasis and remarkable regression of the hepatic lesions.	No	Increase of TNF- $\alpha$
Wersäll et al. (73)	Metastatic renal cell carcinoma				
	Case report A: metastases in lymph nodes and lung	32 Gy in 4 fx to primary tumor	Complete regression of the lung lesions and an almost complete regression of lymph nodes	No	No
	Case report B: multiple pulmonary metastases	RT only in three pulmonary metastases (no dose mentioned)	All the metastases responded partially or completely	Thalidomide	No
	Case report C: four pulmonary metastases	30 Gy in 2 fx in two lesions in the lungs	Complete regression of treated lesions and partial regression of remaining metastatic lesions	No	No
	Case report D: metastases in lymph nodes	32 Gy in 4 fx to primary tumor	Complete response of all metastases	No	No
Okuma et al. (72)	Hepatocellular carcinoma with metastases in mediastinal lymph node and lung	60.75 Gy in 27 fx to single lung metastasis	Reduction of the mediastinal lymph node and lung metastasis unirradiated	No	No
Cotter et al. (75)	Merkel cell carcinoma with cutaneous metastases	12 Gy in 2 fx to some lesions	Treated and untreated lesions responded partially or completely	No	No
Postow et al. (57)	Metastatic melanoma with pleural-based paraspinal mass, hilar lymphadenopathy, and splenic lesions	28.5 Gy in 3 fx to pleural-based paraspinal mass	All the metastases regressed significantly	Ipilimumab	Increase of NY-ESO-1-specific antibodies, CD4 <sup>+</sup> ICOS high, NY-ESO-1-specific interferon-gamma-producing CD4 <sup>+</sup> cells and HLA-DR-expressing CD14 <sup>+</sup> monocytes Decrease of myeloid-derived-suppressor cells
Stamell et al. (58)	Metastatic melanoma	First RT: 24 Gy in 3 fx to primary tumor.	All metastases had resolved (forehead, scalp, and neck)	Ipilimumab	Increase of MAGEA3
	Development of nodal and brain metastases	Second RT: intracranial stereotactic radiosurgery	Complete remission, including node metastasis		

of an abscopal effect when combined with anti-CTLA-4, whereas an abscopal effect was not observed when tumors were treated with 20 Gy  $\times$  1 or 6 Gy  $\times$  5 alone or in combination with anti-CTLA-4 (63). The mechanic basis for the ability of 8 Gy  $\times$  3 to properly synergize with anti-CTLA-4 was not explored, but nevertheless the

authors pointed out that this dose schedule resulted in the highest level of infiltration and IFN- $\gamma$  production by T cells. The synergism between local RT and the CTLA-4 blocking observed in pre-clinical models appears to translate well into the clinic. As previously mentioned, some clinical reports in melanoma patients have demonstrated



abscopal regression following treatment with local RT and anti-CTLA-4 (ipilimumab) that was associated with elevated immunity to tumor-associated antigens (58, 59).

(b) OX40: Irradiation and anti-OX40 treatment synergistically promote infiltrating CD8<sup>+</sup> T cells (64). OX40 stimulation obtains no inherent capacity to polarize T cells toward one particular effector subset, but in comparison, drives T-cell polarization in the context of the inflammatory ambient. Considering the nature of most tumor-associated antigens, it is important to observe that co-stimulation through OX40 can deliver priming of low avidity T cells and can also reverse T-cell tolerance against self-antigens. Pre-clinical and clinical data employing local ablative RT with OX40 agonistic antibody, systemic IL-2, or anti-CTLA-4 determine that signaling through CD25 and OX40 increase T-cell responses against tumor-associated antigens (64, 65). Future clinical trials involving local RT, anti-CTLA-4, and agonistic OX40 are promising and may hopefully induce impressive results.

(c) Programed death ligand 1 (PD-L1): Evidence in pre-clinical models suggests that a PD-L1 blockade is essential in some situations to fully uncover anti-tumor immunity that is induced by local RT in combination with co-stimulatory receptor engagement. Local RT combined with anti-OX40 and anti-PD-L1 has shown to mediate complete regression in orthotopic AT-3 mammary tumors. (66).

(d) CD137: Agonist antibodies to CD137 and CD137-ligand co-stimulate T cells after TCR stimulation (67). Anti-CD137 mAb immunotherapy has been combined with RT in pre-clinical models with encouraging results. At this point, mAb to CD137 combined with a hypofractionated RT schedule induced up to a 100% rejection rate of orthotopically implanted triple negative mammary tumors (66).

Besides CTLA-4, OX40, PD-1, and CD137, other co-stimulatory and co-inhibitory molecules like CD40 or glucocorticoid induced TNFR (GITR) represent other new emerging targets for immunotherapy (68).

(3) Third signal: cytokines: To complete the process of a full effector T-cell response with clonal expansion and differentiation on memory T cells, a third signal provided by cytokines is needed (69). In this step, cytokines that mediate and amplify adaptive immune responses are produced mainly by antigen-stimulated T lymphocytes, and they include, among others, IL-2, IL-4, IL-5, IL-13, and IFN- $\gamma$  (70). Colony stimulating factors (CSFs) are cytokines made by activating T cells, macrophages, endothelial cells, and bone marrow stem cells that stimulate the growth of bone marrow progenitors, thereby providing a source of additional inflammatory cytokines (69). GM-CSF is one of the most important cytokines in cancer microenvironment and among their functions it may induce the maturation of DCs enabling the priming of immune responses and the generation of memory T cells (15). Some preliminary data support an eventual synergism between cytokines and RT. Demaria et al. designed a

trial that recruited metastatic cancer patients that were previously treated with chemotherapy and afterward with RT to one metastatic lesion coupled with GM-CSF s.c for 14 days. Although it was a heavily pre-treated population, a response outside of the RT field was detected in 4 out of 12 patients, serving as proof of principle of the eventual abscopal effect induced by RT and cytokines (71).

A tumor microenvironment is a challenging battlefield where many actors interact. From a clinical point of view, synergy between RT and immune therapies open a new breakthrough for clinical research. Specifically, targeting of immunostimulatory and inhibitory checkpoints with immunomodulatory mAbs can “awake” and promote the systemic effects induced by RT that ultimately may be maintained and boosted by cytokines.

Monitoring immune response generated after RT is another big challenge in this field. A set of different biomarkers in blood and in tissue may aid in detecting the “switching-on” of the immune response, if it finally occurs, and to follow its “real-time” evolution. This may provide valuable information in order to amplify and boost (with cytokines and other strategies) the immune responses detected. Clinical development of anti-CTLA-4 mAb in the last few years has increased the interest in the search and validation of immune biomarkers. At this point, characterization of antigenic-specific immune responses has been performed for several cancer related antigens. Serological and T-cell responses to NY-ESO-1 and MAGEA3 have been detected and prove to be useful to monitor immune responses and clinical evolution in patients with melanoma treated with ipilimumab and with the combination of RT and ipilimumab (57, 58). Some subsets of immunosuppressive cells like Treg, TAM, and MDSC may represent other interesting biomarkers. Furthermore, in the abscopal case reported by Postow et al. with ipilimumab and RT, a decline in the levels of MDSC (CD14 + HLA-DR<sup>low</sup>) was ascertained after RT and these findings were timely correlated with the clinical response detected (57).

Every clinical trial with the aim of studying the synergism between RT and immunotherapy in the future might introduce some of the biomarkers previously cited (or a combination of them) in order to detect accurately if the clinical outcomes eventually observed are related or not to the combination approach. This is a critical point to truly confirm the abscopal effect hypothesis, as noted in some case reports illustrated in **Table 1** (50, 57, 58, 71–75). Furthermore, these biological markers may represent powerful tools to increase not only the quality (duration of responses) but also the quantity of life of cancer patients who will benefit from this approach.

## CONCLUSION

The data available support the hypothesis of a mediated immune anti-tumor activity for RT. Our understanding of this effect at the molecular level has substantially increased in the last few years, and a couple of well-documented clinical cases have been reported recently, which serve as proof of principle of the abscopal effect in the clinical scenario. Therefore, combined strategies of radio-immunotherapy will eventually modulate immune response toward cancer cell destruction leading to meaningful clinical results. Nowadays, several clinical trials

are ongoing exploring the immune consequences of RT, especially with immune checkpoints, and they will probably shed more light to this topic. Interestingly, validation of biomarkers like antibodies against NY-ESO-1 and MAGEA3 or measurement of TAM and MDSC is another very important task in order to facilitate the design of fine tune approaches related to these new immunotherapies and combinations in the coming future.

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