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## Abstract

Immunotherapy is radically changing the clinical management of patients affected by an increasingly wide array of tumors. However, a limited percentage of patients achieve long-term clinical benefits from immunotherapy employed as a standalone treatment, calling for the development of combinatorial regimens. Radiation therapy (RT) stands out as a particularly promising candidate in this setting, reflecting not only its established safety profile, but also the potential ability of RT to mediate robust immunostimulatory effects that may synergize with immunotherapy in systemic tumor control. However, optimal radioimmunotherapy regimens may call for the redefinition of conventional RT doses and fractionation schedules. Here, we discuss current approaches to improve the efficacy and reduce the toxicity of radioimmunotherapy for the management of cancer.

## Introduction

More than half of cancer patients are treated with radiation therapy (RT), which has become a mainstay for the management of several, locally advanced solid tumors. Over the past few decades, significant technical improvements fostered the widespread dissemination of multiple variants of RT, including intensity modulated RT (IMRT), image-guided RT, as well as intra- or extracranial stereotactic RT.[1] These developments rapidly translated into the possibility of local dose escalation, resulting in increased cure rates in patients affected with some tumors like prostate and cervical cancer.[2] Moreover, it has become possible to deliver high doses of RT with unprecedented anatomical precision, allowing for partial breast irradiation and long-term local control of brain and lung metastases.[3,4] Alongside, the decreased collateral damage to radiosensitive organs enabled by IMRT has reduced the risk of long-term sequelae, including xerostomia for patients with head and neck squamous cell carcinoma (HNSCC), bowel toxicity for subjects with gynecological tumors, and poor cosmetic outcome for women with breast cancers, just to mention a few examples.[5,6]

While these technical ameliorations have spread rapidly over the recent years, the development of therapies to complement RT has somehow stagnated since the 1990s, when several clinical trials demonstrated that chemoradiation – *i.e.*, the association of RT and cytotoxic chemotherapy – can result in improved local tumor control, organ preservation and patient survival in some oncological settings.[7] Since the dissemination of these results, no major trial has successfully challenged the idea that cisplatin, 5-fluoruracil, mitomycin C and temozolomide should be used to improve the therapeutic efficacy of RT. Indeed, contrasting with the expectations nourished by preclinical studies, combinatorial regimens involving targeted anticancer agents and RT failed to provide superior clinical benefits as compared to conventional chemoradiation.[8-10] There are multiple possible causes for these deceptive clinical

results including, on a case-by-case basis, the validity of preclinical proofs of concepts, the impact of combinatorial therapies on normal tissues, the absence of reliable biomarkers for patient selection, and technical aspects linked to radiation delivery. These shortcomings are likely to be overcome by an improved communication among radiation oncologists, medical oncologists, statisticians, pharmacologists, and experts in imaging and translational research.

The realization that the immune system can provide a major contribution to therapeutic responses to RT[11] provided a strong rationale in support of clinical trials combining (multiple variants of) immunotherapy with (various forms of) RT. Such an intensive wave of investigation on radioimmunotherapy has recently culminated in the approval of chemoradiation plus the immune checkpoint blocker (ICB) durvalumab for the treatment of unresectable stage III non-small cell lung carcinoma (NSCLC),[12] which turned out to be effective and well tolerated despite a previous report alerting about potential lung toxicity.[13] Currently, more than 150 clinical trials are open to explore the combination of standard, full-dose (chemo)radiation plus immunotherapy,[14] mostly in the form of ICBs targeting CD274 (best known as PD-L1), like durvalumab itself, avelumab and atezolizumab, programmed cell death 1 (PDCD1, best known as PD-1), like pembrolizumab, nivolumab and cemiplimab, or cytotoxic T lymphocyte-associated protein 4 (CTLA4), like ipilimumab (source https://clinicaltrials.gov/). It can be anticipated that many of these combinatorial regimens will enable superior therapeutic responses as they subside toxicities, hopefully both in the short and long term, ultimately resulting in the cure of patients with previously untreatable tumors, especially in the setting of oligometastatic disease (**Figure 1**).

Classical radiation biology has investigated the efficacy of RT mostly *in vitro* and in xenograft models, *i.e.*, in immunodeficient mice bearing human cancer cell lines, two models that completely neglect potential contribution of the immune system to therapeutic effects.[15] This has imposed a largely cancer cell-autonomous view of the biology of RT, positing that the

response to RT (and hence the likelihood of complete disease eradication) are directly proportion to the radiation dose and the consequent degree of DNA damage overcoming the repair capability of the cell[16]. This model has fostered a large number of dose-escalation studies, which were successful in some indications, such as prostate and cervical cancer,[2] but failed to meet expectation in many others, such as NSCLC, esophageal tumors and brain cancers.[17] The modern conceptual paradigm rather postulates that RT is particularly efficient when its elicits tumor-targeting immune responses.[18] The major corollary of this shift in perspective is that RT should be administered in doses and schedules that are optimally suitable for eliciting anticancer immunity. As the maximum tolerated dose (MTD) often inhibits or kills immune cells involved in tumor control alongside malignant cells, RT delivered at the MTD generally fails to induce efficient immune responses against cancer, implying a non-linear dose-effect relationship.[19] Thus, the implementation of modern radioimmunotherapy regimens calls for novel strategies to improve efficacy and limit toxicity, which we discuss in this review.

## Immunological parameters affecting acute and late RT effects

The side effects of RT generally manifest in a biphasic manner, reflecting both the intensity of the treatment and the organs exposed to direct or scattered irradiation.[20] The acute phase, which is characterized by local atrophy and inflammation, originates from a wave of cell death driven by RT in tissues with a high mitotic index, such as the epithelium of the oral cavity and the gastrointestinal trait or the hematopoietic system, and hence tends to resolve a few weeks after treatment.[21] The chronic phase typically starts around 3 months post-RT and involves vascular alterations, persistent oxidative stress, chronic hypoxia, fibroblasts activation potentially leading to fibrosis, and permanent loss of tissue function.[22] Numerous preclinical studies suggest that the immune system not only participates in the therapeutic effects of RT, but also contributes to both acute and chronic RT toxicities.[23] Consistent with this notion, deletion of intercellular adhesion molecule 1 (*Icam1*), which codes for an endothelial cell surface molecule involved in leukocyte extravasation, inhibits both acute inflammatory responses and chronic fibrotic changes in the lungs of mice subjected to thorax irradiation.[24] A similar anti-fibrotic response can be achieved upon depletion of interstitial macrophages with a monoclonal antibody specific for colony stimulating factor 1 receptor (CSF1R).[25] Moreover, radiation-induced pneumonitis occurring in mice after total body irradiation (TBI) can be suppressed by thymectomy and further restored by the adoptive transfer of splenocytes.[26] Thus, multiple immune effector cells can contribute to the toxicity of RT. Of note, macrophage depletion with CSF1- or CSF1R-targeting agents has also been shown to improve anticancer immune responses driven by RT (especially in the context of PD-L1 blockade), which the tendency of RT (especially at low doses) to favor the polarization of macrophages towards an immunosuppressive phenotype.[27,28] Taken together, these observations suggest that targeting macrophages may provide a dual benefit to patients receiving RT: an improved therapeutic response couple to limited toxicity.

The recruitment of immunosuppressive cells to irradiated organs and their activation is orchestrated by several immunomodulatory molecules released in response to RT. Transforming growth factor beta 1 (TGFB1, best known as TGF-β1), a cytokine with pleiotropic effects, is pivotal in this context.[29] TGF-β1 signaling increases consistently in irradiated tissues, reflecting not only an increased secretion of the latent form of the protein, but also an increased availability of bioactive TGF-β1.[29] On binding to its cognate receptor, which is expressed by a variety of cell types including immune cells and fibroblasts, TGF-β1 mediates robust immunosuppressive effects and favors the secretion of collagen, hence favoring fibrosis.[29] In line with this notion, strategies aimed at inhibiting TGF-β1 synergize with ICBs[30] and RT[31] in the control of experimental tumors in mice, at least in part by improving the access of lymphocytes to malignant lesions. Along similar lines, chemical inhibitors of TGF-β1 signaling attenuate lung fibrosis in mice receiving RT to the thorax.[32] Thus, although efficacy in early clinical trial testing this paradigm was rather deceptive,[33] perhaps reflecting the ability of TGF-β1 to inhibit epithelial cell proliferation,[34] it may be interesting to introduce TGF-β1 inhibitors into multimodal treatments including radioimmunotherapy.

Irradiated tissues also expose or secrete large amounts of damage-associated molecular patterns (DAMPs), a heterogeneous group of endogenous molecules that are normally invisible to the immune system owing to their intracellular localization.[35] On release into the extracellular microenvironment, however, DAMPs can bind to a variety pattern recognition receptors (PRRs) involved in the innate immune response to invading pathogens, including Toll-like receptor (TLRs), hence favoring the establishment of local inflammation.[36] DAMPs such as the non-histone chromatin-binding protein high mobility group B1 (HMGB1) and mitochondrial DNA (mtDNA) have been attributed an etiological role in inflammatory conditions including some cardiomyopathies and systemic inflammatory response syndrome (SIRS), largely reflecting their ability to recruit and activate granulocytes.[37] Consistent with this notion, extracellular

mtDNA is increased in patient with idiopathic pulmonary fibrosis,[38] and chemical antagonists of C-X-C motif chemokine receptor 1 (CXCR1) and CXCR2 (which prevent the accumulation of neutrophils in irradiated lung) limit RT-induced fibrosis in mice.[39] Further corroborating a role for DAMP signaling in the acute and late toxicity of RT, multiple TLR5 agonists have been shown to inhibit RT-driven mucositis, dermatitis, pneumonitis and fibrosis in mice[40]. The precise mechanisms whereby TLR5 agonism (rather than antagonism) mediates radioprotective effects remain elusive.

RT also increases the amounts of MHC Class I and II molecules exposed on the plasma membrane, hence rendering irradiated cells more susceptible to immune recognition.[41] Supporting a role for this mechanism in the side effects of RT, patients receiving TBI can develop auto-immune disorders accompanied by autoreactive lymphocyte infiltration of the irradiated tissue.[42] Importantly such immune reactions cannot originate from overt tissue damage and abundant DAMP release, as the RT doses employed are too low to cause cell death.[42] That said, both TBI and thoracic RT have also been shown to promote the expansion of immunosuppressive $CD4^+CD25^+FOXP3^+$ regulatory T ($T_{REG}$) cells that potentially attenuate autoimmune reactions driven by $CD4^+$ helper T cells[43,44], a mechanism that may depend (at least partially) on epidermal Langerhans cells.[45] Intriguingly, an immunosuppressive effect that could not be linked to the extent of lymphopenia was documented in mice receiving total lymphoid irradiation (TLI) plus TBI (as compared to TLI alone as early as in 1984, when little was known about the immunobiology of $T_{REG}$ and Langerhans cells.[46] The possibility to harness the immunosuppressive activity of $T_{REG}$ cells to limit the inflammatory side effects of RT has attracted considerable attention in the past,[44,47] but is progressively being abandoned. Conversely, efforts remain focused on conventional radioprotectors, including reactive oxygen species scavenger (*e.g.*, amifostine), molecules that favor epithelial reconstitution (*e.g.*, palifermin), and systemic anti-inflammatory drugs (*e.g.*, aspirin, celecoxib).[48]

Importantly, most (if not all) the molecular and cellular mechanisms that underlie the toxicity of RT are also responsible for its therapeutic activity. Thus, cancer cells exposed to cytotoxic doses of RT undergo a potently immunostimulatory cell death variant that has been named immunogenic cell death (ICD).[49] ICD is characterized by the activation of multiple adaptive stress responses in dying cells that culminate with the exposure or release of DAMPs imping on the regulation of local and systemic homeostasis.[41] Such stress responses encompass (but may not be limited to): (1) the unfolded protein response at the endoplasmic reticulum (ER), culminating with the exposure of several ER chaperones on the surface of dying cells; (2) autophagy, enabling the release of high levels of ATP from dying cells; and (3) a pathogen-like response involving the detection of ectopic RNA and DNA molecules, culminating with the secretion of type I interferon.[50] The spatiotemporally coordinated release of these and other DAMPs from cancer cells succumbing to RT enables the recruitment and activation of BATF3-dependent dendritic cells (DCs), ultimately resulting in the cross-priming of tumor-specific cytotoxic T lymphocytes (CTLs).[35] Such a robust response, which involves freshly recruited CTLs as well as tumor-resident CTLs, and is under tonic inhibition by PD-1 and TGF-β signaling,[51-53] can drive not only the eradication of radioresistant cancer cells in the irradiated lesions, but potentially attack non-irradiated metastases (at least in mice and in a limited fraction of patients), a phenomenon commonly referred to as abscopal response.[54] Further supporting the immunological nature of this process, abscopal responses can be abolished in mice lacking $CD8^+$ T cells as well as in mice in which type I IFN signaling is inhibited[11,55] and have been observed at increased frequency in patients concomitantly treated with immunotherapy.[56,57] Of note, DAMPs are not the only commonality between the toxicity and therapeutic activity of RT. Rather, the ability of RT to alter vascular permeability in support of local inflammation,[58,59] and to increase MHC Class I and II exposure are also involved in disease control.[41]

What are therefore the determinants of RT efficacy *versus* toxicity? Accumulating preclinical and clinical data suggest that there at least 3 major parameters at play in this setting: (1) dose and fractionation; (2) irradiated volume; and (3) sequence of administration (in the context of combinatorial regimens).[18] Finally, since both the efficacy and toxicity of RT rely (at least in part) on the immune system, the overall immunological competence of the host and the factors that determine it should not be underestimated. Such factors encompass not only purely endogenous parameters such as sex, age and polymorphisms in immunity-relevant genes, but also elements at the interface between the individual and the microenvironment, such as the composition of the gut microbiome and the presence of an ongoing infection, and purely exogenous influences, such as the concomitant or recent exposure to immunosuppressive drugs.[14]

Optimal radioimmunotherapy regimens should be designed based on all these factors, as delineated below, while critically comparing the effects of radiommunotherapy to immunotherapy alone (as exemplified in the IMPORTANCE trial (NCT03386357) for the treatment of recurrent or metastatic HNSCC.

## Refining radiotherapy modalities to maximize the therapeutic window of radioimmunotherapy.

**Revisiting doses and fractionation.** For decades, RT doses and fractionations have been empirically set by clinicians with sole scope to achieve local tumor control. For multiple indications, such a conventional schedule corresponded to the delivery of 1.8-2 Gy per day, in 5 days per week, during 5-8 weeks. Clinical trials provided rationale for recommending different standard doses for specific tumors, including a total of 70 Gy for HNSCC, 66 Gy for NSCLC and 74-80 Gy for prostate cancer.[17] More recently, the classical dose prescription paradigm has shifted toward higher doses per session and a protracted number of sessions, especially in cases in which RT is delivered to relatively small target volumes. Moreover, precision RT guided by 3D imaging has enabled the delivery of up to 20 Gy in one single dose, a regimen that can be routinely used to eradicate isolated brain metastases with minimal side effects.[60]

Based on these advances, attempts have been made to increase the RT dose up to a threshold dictated by toxicity on normal tissues, resembling prior attempts of 'dose intensification' for cytotoxic chemotherapies.[61] However, such an approach, which is supported by a merely cancer cell-autonomous view of tumor biology, collides with the ever more accepted idea that any form of cancer therapy (conventional chemotherapy, targeted therapy, RT) must induce an anticancer immune response to yield a long-term response beyond treatment discontinuation.[62]

Supporting the notion that high RT doses not always enable systemic disease control, hypofractionated radiation (3 doses of 8 Gy each delivered in 3 consecutive days), but not a single dose of 20 Gy, enables efficient abscopal responses in immunocompetent mice bearing syngeneic mammary carcinoma or colorectal carcinoma cells, when mice are simultaneously treated with a CTLA4-targeting ICB or with a monoclonal antibody neutralizing multiple TGF-

β isoforms.[52,55,63] Abscopal responses driven by RT in the context of TGF-β inhibition can be further boosted by the concomitant administration of a PD-1-targeting antibody, ultimately resulting in the generation of an *in situ* anticancer vaccine that enable long-term disease eradication coupled with protective immunological memory.[52]

Abscopal responses also rely on the dynamic recruitment and activation of immune cells into irradiated tumors.[64,65] These include BATF3-dependent dendritic cells, whose recruitment requires the local secretion of type I IFN downstream of transmembrane protein 173 (TMEM173, best known as STING) activation.[55,66] Hypofractionated RT favors the accumulation of endogenous DNA in the cytosol of irradiated cells, and hence efficiently drives STING signaling, a process that is inhibited when RT doses higher than a 10-12 Gy threshold (depending on cell type) are used as a consequence of the upregulation of the cytosolic nuclease three prime repair exonuclease 1 (TREX1).[55] Importantly, cytosolic DNA accumulating in cancer cells responding to hypofractionated RT can be efficiently shuttled to dendritic cells via exosomes, and this further contributes to type I IFN production[67]. Clinical evidence in support of the notion that hypofractionated RT (5 fractions of 6 Gy each) in combination with ICBs targeting CTLA4 can generate robust abscopal responses is emerging.[57]

Along similar lines, it has recently been shown that low RT doses (a single dose of 2 Gy) efficiently boost the ability of CAR T cells to eliminate cancer cells that escaped conventional recognition by losing expression of the CAR target antigen.[68] In this setting, low-dose RT appears to sensitize antigen-negative cancer cells to the lethal effects of TNF superfamily member 10 (TNFSF10; best known as TRAIL), which is produced by CAR T cells upon CAR-dependent activation but mediates TCR-independent effector functions.[68] Supporting the clinical relevance of these findings, a patient with diffuse large-cell lymphoma receiving CD19-targeting CAR T-cell therapy despite a large proportion of $CD19^-$ malignant cells exhibited systemic disease clearance 1 month after treatment, yet relapsed massively starting 2 months

after treatment, with the only exception of the left thigh, which had received low-dose palliative RT (4 fractions of 5 Gy each) before CAR T-cell infusion and persisted disease-free up to 6 months after treatment.[68]

These findings comfort the idea that the optimal radiation dose required for radioimmunotherapy may be lower than the MTD, at least in some oncological indications. This insight offers the opportunity to define new therapeutic windows in which RT is administered at lower doses to minimize its toxicity and to maximize its efficacy due to synergistic combination with immunotherapy. These observations are also important for the response of normal tissue to RT. Indeed, even the most sophisticated irradiation plan inevitably involves the exposure of large volumes of normal tissues to low RT doses, causing immunomodulatory effects that are yet poorly defined.

**Irradiation of smaller volumes.** In addition to the total radiation dose, the volume of irradiated tissue has a major impact on the side effects of RT. Thus, even when high-dose RT is administered in a few sessions to a small volume, as typically achieved by brachytherapy and stereotactic RT, side effects remain relatively mild. These irradiation techniques offer indeed the advantage that the volume to which RT is delivered is sharply delimited, reducing collateral damage to adjacent organs including lymph nodes.[69]

Abscopal responses to RT (especially in the context of radioimmunotherapy) illustrate the possibility that anticancer immune responses elicited by RT can propagate outside of the radiation field. This raises the intriguing possibility that RT may elicit robust clinical responses also when parts of the (rather than the entire) tumor is irradiated. Preclinical and clinical evidence in support of this notion has begun to emerge.[70,71] However, caution should be used

when these findings are interpreted, as the areas of the tumor outside of the irradiation field also receive RT, albeit at lower doses, which may be responsible for, or at least contribute to, therapeutic efficacy.[72] Moreover, in the case of radioimmunotherapy, responses outside of the irradiation field can simply originate from immunotherapy, especially in immunosensitive tumors.[72] Adjuvant RT after breast conservation surgery constitutes a welcome alternative to mutilating mastectomy for breast cancer patients, because it not only decreases local relapse rates (as radical surgery does) but also diminishes the risk of distant relapse,[73] pointing to the elicitation of an abscopal response targeting micrometastases. In this setting, accelerated partial breast irradiation (APBI) using multicatheter brachytherapy stands out as a morbidity-reducing alternative to whole breast irradiation for the treatment of low-risk mammary carcinoma,[74] lending further support that larger irradiation volumes do not necessarily translate in improved disease control.

**Sparing draining lymph nodes and the gut.** Conventional RT regimens are generally conceived to deliver a full dose (50-70Gy) to the tumor and prophylactic coverage to tumor-draining lymph nodes (TDLNs) (45-50 Gy), as established by multiple clinical trials in various indications (*e.g.*, HNSCC, cervical cancer, NSCLC). This approach may not be particularly appropriate when RT is delivered in doses and fractionations that support the activation of anticancer immunity, and even less so in the context of radioimmunotherapy. Although TDLNs may not be the only sites of T-cell cross-priming by dendritic cells (which can also occur in intratumoral tertiary lymphoid structures)[75] they constitute major platforms for the initiation of local and systemic antitumor immune responses. Accordingly, TDLNs increase in volume as a consequent of robust $CD8^+$ CTL infiltration when transplantable mouse melanomas and breast carcinomas are exposed to hypofractionated RT in the context of PD-1 blockage.[76] Thus, it may be detrimental to perturb TDLNs with RT. In support of this notion, nasopharyngeal carcinoma

patients with tumor-free TDLNs and treated with TDLN-sparing IMRT exhibited rare locoregional relapse.[77] Similarly, recent clinical data with partial tumor irradiation (which spares TDLNs) combined with pembrolizumab compare positively with previous results involving TDLN irradiation.[71] However, these findings should be interpreted with caution, as multiple patients involved in the trial had tumors that are potentially sensitive to pembrolizumab alone.[72] Clinical trials comparatively evaluating TLDN irradiation *versus* TDLN sparing in the context of radioimmunotherapy are urgently awaited. Of note, optimal therapeutic responses to RT and radioimmunotherapy may also be compromised, at least to some extent, by the irradiation of the small and large intestines. Indeed, bowel irradiation has been shown to alter the composition of the gut microbiota,[78] which in turn impacts on the efficacy of multiple therapeutic regimens including immunotherapy.[79]

**Optimizing radioimmunotherapy: sequencing.** The recent demonstration that the adjuvant administration of PD-L1-targeting ICBs after chemoradiation postpones the onset of metastasis and improves the survival of patients with stage III NSCLC[12] may transform the field of oncology. One fundamental question that arises from these results concerns the order in which different therapies should be administered, and if a concurrent administration of RT and immunotherapy would have yielded better results. Unlocking immunological checkpoints before or after, rather than concomitant to, radiotherapy may offer the potential to avoid potentially cumulative toxicities. This becomes even more relevant in light of the probable surge in dual or triple immunotherapies that, especially if combined with radiotherapy or chemoradiation,[80] may demand sequential rather than simultaneous interventions to manage side effects. Unfortunately, clinical data robustly comparing different treatment sequencing are largely missing in the setting of radioimmunotherapy (**Table 1**). It can be speculated though that the increasing use of first-line immunotherapy will result in a situation where radiotherapy

will often be performed after immunotherapy. That said, both existing data plead in favor of the administration of hypofractionated radiotherapy before PD-L1 blockade,[12,81] perhaps because it stimulates tumor infiltration by T lymphocytes.[64] One clinical trial is currently open to define the optimal scheduling of PD-1 and CTLA4 blockade with respect to RT in patients with metastatic cancers (NCT03453892). In this setting, quantitative systems pharmacology models, which include key elements of immuno-oncology and dose-exposure-target modulation features,[82] may assist the understanding of immune cell dynamics within irradiated tumors.

**Optimizing radioimmunotherapy: biomarkers.** A large panel of immunotherapies beyond ICBs have been developed, and will soon enter (or have already entered) clinical practice. These include oncolytic virotherapy, CAR T-cell therapy and a wide array of small molecules to functionally alter the tumor microenvironment toward a immunostimulatory configuration.[14] In this setting, great efforts have been dedicated to the identification of reliable predictive biomarkers of response, reflecting not only safety issues, but also economic considerations.[83] It appears plausible that such biomarkers –be they based on the measurement of immunological parameters in the blood or in the tumor - will guide optimal radioimmunotherapy in the future, not only with respect to the choice of the best immunotherapeutic agent for combinatorial regimens, but also with regard to RT doses and schedule. The local upregulation of PD-L1 in response neoadjuvant chemoradiotherapy of rectal cancers is associated with favorable prognosis[84] and might speculatively predict responsiveness to immunotherapy. Moreover, computational imaging (radiomics) stands out as a promising strategy to non-invasively quantify tumor infiltration by $CD8^+$ T cells,[85] which is an important positive prognostic factor for patients affected by multiple tumors.[86] In particular, the ability of radiomics to provide a longitudinal, non-invasive monitoring of the tumor microenvironment may support the implementation of a personalized radioimmunotherapy, with the ultimate goal that each patient

receives a treatment modality that triggers an effective anticancer immune response. High-dimensional single-cell analyses using genomics, transcriptomics, proteomics and metabolomics approaches are also on the verge of revolutionizing biomarker discovery and clinical practice.[87] This technology might be used as an inclusion/exclusion criterion for clinical trials beyond the mere presence of $CD8^+$ T cells (as in NCT03453892).

Current refinements in radioimmunotherapy trials offer the advantage to collect tumor biopsies after treatment. Such trials constitute a valuable source of samples for characterizing responses and resistance to radioimmunotherapy way beyond murine models. Indeed, standard treatment might *per se* affect the expression of immune-related biomarkers, which may prevent a careful prognostic assessment and/or misguide therapeutic decisions. For instance, rectal tumors exposed to pre-operative chemoradiation alone exhibit increased PD-L1 expression,[84] which offers a strong rationale to combine treatment with adjuvant PD-1/PD-L1 blockage. Similar observations have been made in both the melanoma[88] and HNSCC setting.[89] Alterations in the peripheral blood compartment, including shifts in major T cell populations, may also offer a convenient, non-invasive gateway to monitor responses to RT and radioimmunotherapy in cancer patients.[90]

**Chemoradioimmunotherapy or radioimmunotherapy?** Combination of cytotoxic chemotherapy with RT has demonstrated superiority over RT employed as a standalone intervention at enabling local disease control and sometimes improved organ preservation and survival in patients with multiple tumor types.[91] However, such a therapeutic success often came with an excess in complications and side effects.[92] An in-depth understanding of the tumor microenvironment and its influence on resistance to immunotherapy will be instrumental for defining potent radioimmunotherapy regimens that do not require the addition of cytotoxic chemotherapy for optimal efficacy. Preliminary clinical data from ongoing studies underscore the fact that PD-L1/PD-1 blockade combines safely with concurrent RT and cetuximab alone in HNSCC patients[93,94], as well as with RT plus platinum-based chemotherapy in NSCLC patients [95]. However, robust demonstration of clinical benefits is still awaited. Similarly, while recent evidence suggests that adjuvant PD-L1 blockade improves disease outcome after chemoradiation in NSCLC patients,[12] the vast majority of preclinical evidence supporting the implementation of concurrent radioimmunotherapy in the clinics do not involve cytotoxic chemotherapy,[11,51,53,55,63] suggesting that immunotherapy might *per se* combine favorably with RT. Thus, conventional chemotherapeutics may no longer be required to achieve robust clinical responses in the context of radioimmunotherapy, which may also translate in decreased incidence and severity of side effects. Clinical trials should specifically be designed to investigate this possibility. For instance, it might be investigated whether the superior efficacy of PD-1 blockade plus chemoradiotherapy against NSCLC (Ref. [12]). truly requires chemotherapy to be included in the therapeutic protocol.

Numerous ongoing trials are designed to induce abscopal responses. Although data are not mature yet, preliminary findings are promising. Combining PD-1/PD-L1 blockade with RT has been associated with durable out-of-field responses in 3/9 (33%) evaluable patients with metastatic triple negative breast cancer unselected for PD-L1 expression.[96] PD-1 blockade

preceded by SBRT resulted in a 2-fold increase in ORR without accrued toxicity in NSCLC patients.[97] In sharp contrast, addition of SBRT to PD-1 blockade in patients with metastatic HNSCC was safe but failed to improve outcome in a randomized evaluation of abscopal responses.[98] A randomized Phase II trial testing PD-1 blockade plus RT in patients HNSCC (IMPORTANCE, Keynote-717) comparatively evaluates standalone PD-1 targeting immunotherapy to radioimmunotherapy. The results of this study will provide useful insights into the relative contribution of radiotherapy to clinical responses to immunotherapy.

**Radioimmunotherapy beyond conventional X-rays.** Data have been generated in support of the notion that very low irradiation doses – in the range of conventional full body CT scans – can mediate robust immunostimulatory effects that may combine favorably with immunotherapy.[99] Densely ionizing particles (protons, carbon ions), which are characterized by a dose deposit in a narrow depth range (leading to minimal exit dose), are being used in an increasing number of indications, with the highest benefit probably being for pediatric patients linked to reductions in both dose-dependent complications and risk for secondary tumors.[100] Protons and carbon ions are generally considered superior to photons (conventional X-rays) for distribution ballistics.[101] Moreover, carbon ions may provide increased biological and clinical effectiveness, at least in specific indications that are generally refractory to conventional RT, such as bone and soft tissue sarcomas of the skull base.[102] Preliminary data suggest that protons resemble photons in their immunological effects.[103] Taken together, these observations suggest that the superior dose distribution offered by protons, which spare a considerably higher amount of normal tissues than photons, provides a good opportunity for radioimmunotherapy combinations. However, whether protons and carbon ions can be favorably combined with immunotherapy remains to be demonstrated.

## Concluding remarks

RT has been used for more than a century for the clinical management of virtually all cancers with often positive results, both in terms of side effects and efficacy. Such a favorable therapeutic window places RT in a privileged position for the development of combinatorial treatment regimens. In line with this notion, the delivery of RT along with cytotoxic chemotherapy has enables improved local disease control in multiple oncological indications, in some cases accompanied by superior organ preservation and patient survival.[104] However, chemoradiation protocols are often associated with increased incidence and severity of side effects, reflecting the fact that both RT and chemotherapy are often used at (or near to) their MTD.[105] It can be anticipated that low-dose molecular targeted radiotherapy using radiolabeled molecules will overcome the problem of toxicity.[106,107] Over the past decade, immunotherapy has largely transformed the management of multiple solid tumors.[14] However, response rates to immunotherapy employed as single therapeutic interventions often are low (with the exception of CAR T-cell therapy, which is associated with response rates >80% for selected indications), calling for the development of combinatorial regimens. In this context, RT stands out as an optimal partner for immunotherapy. However, conventional RT regimens will have to be redesigned for radioimmunotherapy to mediate superior efficacy in the presence of limited side effects (**Figure 2**). Revisiting doses and fractionation schedules, reducing delivery volumes, sparing both TDLNs and the intestine, limiting the concomitant administration of cytotoxic chemotherapeutics, employing radiomics to longitudinally monitor responses, and elucidating the actual therapeutic value of protons and carbon ions are some of the directions that will foster the development of safe and efficient radioimmunotherapy regimens to treat cancer.

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## Legends to Figures

***Figure 1.*** **Radioimmunotherapy strategies to tackle oligometastatic disease, impact on therapeutic benefits.** The therapeutic effects expected from integrating radiotherapy and systemic immunotherapy may vary according to radiotherapy modalities (dose, fractionation, irradiated volume), as well as study design. As it stands, combining focal radiotherapy to one or a few sites with systemic immunomodulation (**a**) stands out as a promising approach as compared to debulking strategies based on radiotherapy alone (**b**), or to combinatorial approaches involving the systematic irradiation of all disease sites (**c**). *In the PACIFIC trial, immunotherapy improved the outcome of non-metastatic patients who had all their macroscopic tumor sites previously irradiated.[20]

***Figure 2.*** **From conventional chemoradiation to radioimmunotherapy.** Improving therapeutic index in the era of radioimmunotherapy requires the redefinition of multiple approaches established in the context of conventional chemoradiation, including (but not limited to) doses, fractionation schedules, as well as irradiation volumes and targets. Clinical trials designed to investigate novel radioimmunotherapy approach should be specifically designed to simultaneously achieve limited toxicity to normal tissues and superior systemic disease control.

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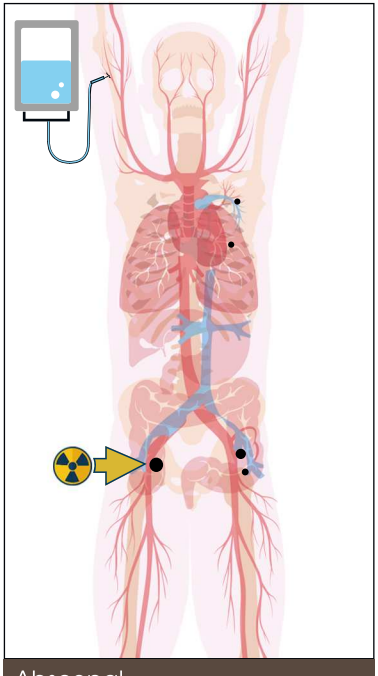
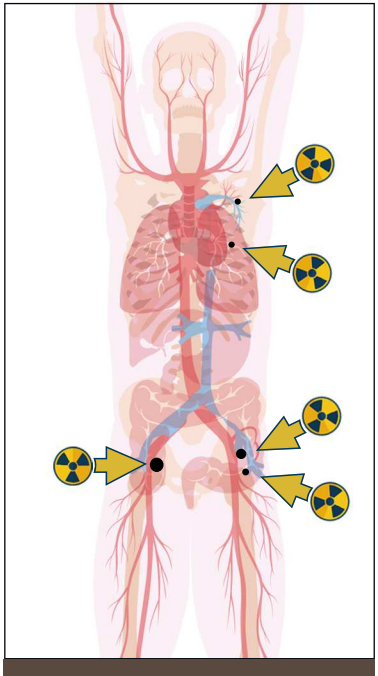
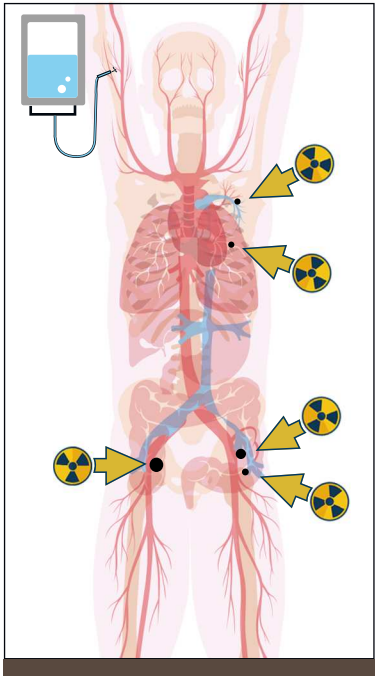
# Combinatorial immunoradiotherapy approaches to tackle the oligometastatic disease, impact on therapeutic ratio



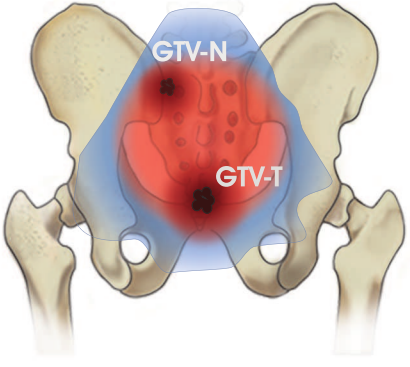


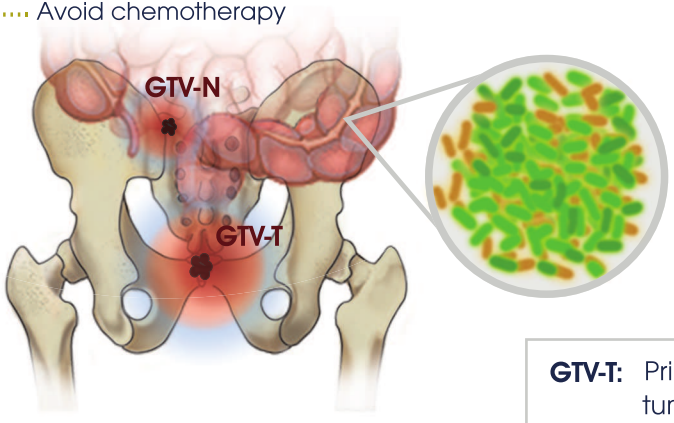


| Strategy | Abscopal<br>Immunotherapy + radiotherapy | Debulking gross disease<br>Radiotherapy alone | Combinatorial<br>Radiotherapy alone |
|---|---|---|---|
| Immunotherapy | YES | NO | YES |
| Radiotherapy<br>1/ Sites<br>2/ volumes<br>3/ dose | 1 or multiple sites<br>Intermediate to high doses<br>Small volumes | All sites<br>Ablative doses<br>Extended volumes | All sites irradiation + immunotherapy<br>Immuno stimulating doses<br>Extended volumes |
| Clinical demonstration | Formenti, Nat. Med. 2018<br>Luke, JCO 2018,<br>Golden; Lancet Oncol 2015 | Palma, ASTRO 2018<br>Gomez, ASTRO 2018<br>Parker, Lancet 2018 | |
| Therapeutic ratio | +++ | ++ | ++ |

# From conventional chemoradiation to radioimmunotherapy radiotherapy

| Current standard | Radio-immunotherapy |
|---|---|
| Dose escalation | Dose de-escalation |
| One dose fits alls | Non-ablative personalized doses |
| Irradiate large volumes | Minimize irradiated volume |
| Prophylactic lymph nodes irradiation | Spare lymph nodes |
| Whole pelvis RT | Spare small and large bowel for microbiome optimization |
| Standard DVH | Refined DVH |
| Include chemotherapy | Avoid chemotherapy |




**GTV-T:** Primary gross tumor volume

**GTV-N:** Lymph node gross tumor volume

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**Table 1. Prospective clinical studies assessing radiotherapy in combination with immunomodulatory regimens.**

| Indication and selection criteria | Phase | N° of patients | Treatment modality | Toxicity | Efficacy | Ref. |
|---|---|---|---|---|---|---|
| Solid tumors with ≥5% PD-L1 expression amongst malignant cells | I/II | 10 | Durvalumab 10 mg/kg Q2W + RT (median dose 20 Gy in median 5 fractions), given a median of 8.5 d after last durvalumab. | 50% of RT-related grade 2 AEs. | In-field: ORR 60% (2/10 CRs, 4/10 PRs); out-of-field: 10/14 SD, no objective responses; no abscopal responses. | 108 |
| Stage III, locally advanced, unresectable non-small cell lung carcinoma | III | 709 | Conformal RT (54-66 Gy) with 2 concurrent platinum cycles + sequential durvalumab 10 mg/kg Q2W, initiated within 6 w after RT and continued for up to 1 y (or placebo if no PD during RT). | Serious AEs in 29.1% and 23.1% of patients in the durvalumab and placebo groups, respectively. | Median PFS: 16.8 mo *vs* 5.6 mo (p<0.001); median TTD or distant metastasis: 23.2 mo *vs* 14.6 mo (p < 0.001) | 12 |
| Advanced solid tumors | I | 79 | SBRT to 2-4 metastases in doses ranging from 30 to 50 Gy in 3 to 5 fractions (partial irradiation of metastases > 65mL) + pembrolizumab initiated within 7 d after SBRT completion. | Six patients experienced DLT with no RT dose reductions. | ORR: 13.2%; median PFS: 3.1 mo (95%CI, 2.9-3.4 mo). | 71 |
| Metastatic solid tumors with ≥1 lesion in the liver or lung amenable to SBRT and ≥1 non-contiguous lesion for monitoring | I | 35 | Ipilimumab (3 mg/kg Q3W for 4 doses) in 5 RT groups: concurrent (1 d after 1st dose) or sequential (1 w after the 2nd dose) RT (50 Gy in 4 fractions) to lung or liver, or sequential 60 Gy (in 10 fractions) to lung or liver. | Two patients experienced DLT and 12 (34%) grade 3 AEs. | Out-of-field responses amongst assessable patients: 3/31 (10%) PR; 7/31 (23%) clinical benefit (PR or SD ≥6 months). | 109 |
| Brain metastases from melanoma | I | 16 | WBRT or SRS + ipilimumab dose escalated from 3 mg/kg to 10 mg/kg Q3W starting at D3 of WBRT or 2 d after SRS. | Twenty-one gr 1-2 neurotoxic effects, with no DLT; 10 additional grade 3 AEs (5 gastrointestinal toxicities); no grade 4-5 toxicity. | Median PFS: 2.5 mo after WBRT, 2.1 mo after SRS. | 110 |
| Metastatic castration-resistant prostate cancer with ≥1 bone metastasis progressing after docetaxel | III | 799 | Bone-directed RT (8 Gy in 1 fraction) + ipilimumab 10 mg/kg or placebo Q3W for up to 4 doses, starting after RT. | Most common grade 3-4 AEs were immune-related (26% of the ipilimumab group, including 4 toxic deaths). | Median OS: 11.2 mo *vs* 10.0 mo ($p$=0·053); median PFS: 4.0 mo *vs* 3.1 mo ($p$<0·0001). | 111 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Stage IV melanoma with ≥1 non-irradiated metastasis measuring ≥1.5 cm available for response assessment | I | 22 | RT to 1-2 disease sites (BED10: 23.3 Gy to 72.9 Gy), initiated within 5 d of ipilimumab initiation (for 4 cycles). | No unexpected toxicity; 3 grade 3 AEs. | Three patients (27.3%) achieved systemic CR, and 3 (27.3%) an initial PR. | 112 |
| Metastatic castration-resistant prostate cancer | I/II | 33/50 | Ipilimumab Q3W for 4 cycles at 3, 5, or 10 mg/kg, or at 3 or 10 mg/kg + RT (8 Gy per lesion). | Common grade 3 immune-related AEs in patients receiving RT were colitis (16%) and hepatitis (10%); 1 treatment-related death was recorded (in 5 mg/kg group). | Eight PSA declines ≥50% (duration: 3-13+ mo), 1 CR (duration: 11.3+ mo), and 6 SD (duration: 2.8-6.1 mo), amongst patients treated with RT + ipilimumab 10 mg/kg. | 113 |
| Metastatic solid tumors with ≥3 distinct measurable sites of disease | II | 41 | Concurrent RT (35 Gy in 10 fractions over 2 w) to 1 metastatic site and GM-CSF (125 μg/m$^2$ *s.c.* daily for 2 weeks, starting during the 2$^{nd}$ week of RT). Course repeated to target a 2$^{nd}$ metastatic site. | Most common grade 3-4 AEs were fatigue (6 patients) and hematological (10 patients); 1 patient experienced grade 4 pulmonary embolism. | Abscopal responses in 11 (26.8%, 95% CI 14.2-42.9) of patients. | 56 |
| Metastatic melanoma | I | 22 | Escalating doses of SBRT (2-3 fractions) to index lesion, followed 3-5 d later by ipilimumab Q3W for 4 cycles. | No DLTs; 15 grade 3 AEs. | Evaluation non-irradiated targets: 18% PR, and 18% SD. | 80 |
| Metastatic melanoma or renal cell carcinoma | I | 12 | 1-3 doses of SBRT (20 Gy per fraction), last dose administered 3 d before initiation of high-dose interleukin-2. | No DLT attributable to SBRT. | Evaluation of non-irradiated targets: 1 CR and 7 PR. | 114 |
| Glioblastoma | II | 46 | Surgical resection followed by standard conformal RT + autologous vaccine generated from resected tumors and delivered weekly after RT completion. | AEs attributable to the vaccine in 34 pts (74%); but no grade 3-4 AEs related to vaccination. | Median OS: 23.8 mo (18.0 mo for patients with high PD-L1 expression on myeloid cells *vs.* 44.7 mo for patients with low PD-L1 expression; $p$=0.007). | 115 |

***Abbreviations.*** AE, adverse event; BED10, biologically effective dose (estimated for the tumor); CR, complete response; DLT, dose-limiting toxicity; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; RT, radiation therapy; SBRT, stereotactic body radiation therapy; SD, stable disease; SRS, stereotactic radiosurgery; TTD, time to death; WBRT, whole-brain RT.