## Advances in radioimmunotherapy 5

# Optimising efficacy and reducing toxicity of anticancer radioimmunotherapy

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**Immunotherapy is radically changing the clinical management of patients affected by an increasingly wide array of tumours. However, not all patients achieve long-term clinical benefits from immunotherapy as a standalone treatment, calling for the development of regimens that combine various interventions. Radiotherapy stands out as a particularly promising candidate in this setting, not only because of its established safety profile, but also because radiotherapy has the potential ability to mediate robust immunostimulatory effects that could synergise with immunotherapy in systemic tumour control. However, optimal radioimmunotherapy regimens might call for the redefinition of conventional radiotherapy doses and fractionation schedules. In this Series paper, we discuss current approaches to improve the efficacy and reduce the toxicity of radioimmunotherapy for the management of cancer.**

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This is the fifth in a Series of five papers about advances in radioimmunotherapy

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## Introduction

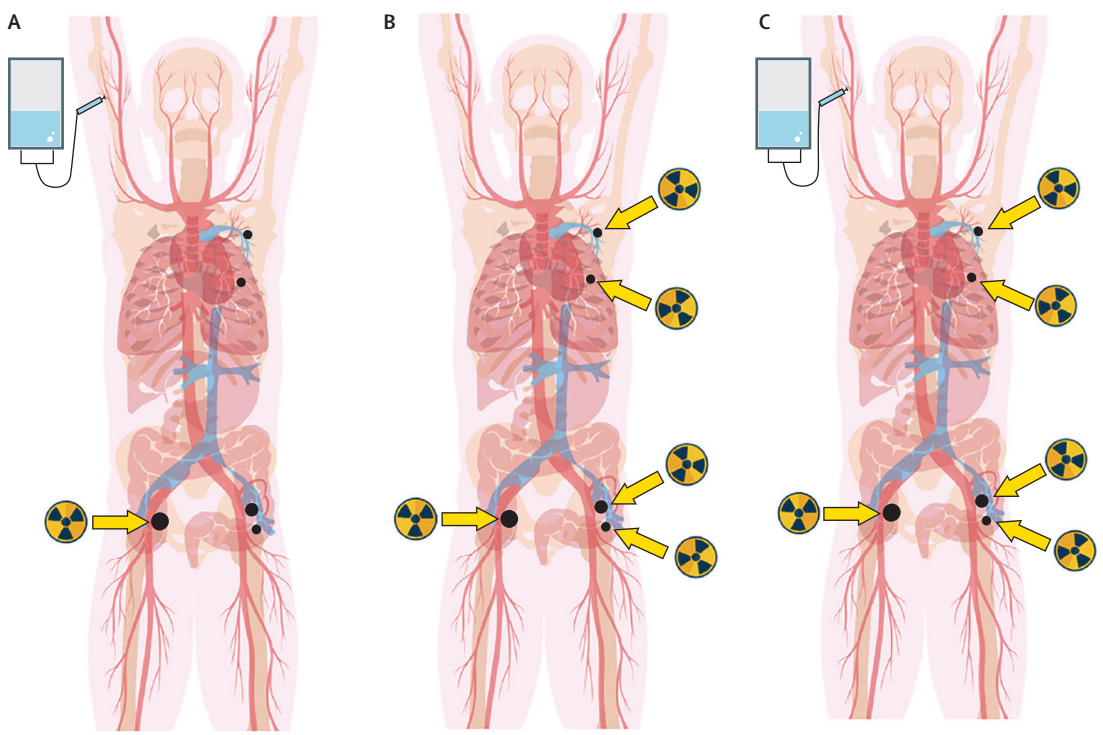
More than half of patients with cancer are treated with radiotherapy, which has become a mainstay for the management of several locally advanced, solid tumours. Over the past few decades, substantial technical improvements have fostered the widespread dissemination of multiple variants of radiotherapy, including intensity-modulated radiation therapy (IMRT), image-guided radiotherapy, and intracranial or extracranial stereotactic radiotherapy.[1] These latest developments in radiotherapy treatment have rapidly translated into the possibility of local dose escalation, resulting in improved disease outcome in patients with particular tumours, such as prostate and cervical cancer.[2] Moreover, it has become possible to deliver high doses of radiotherapy with unprecedented anatomical precision, allowing for partial breast irradiation and long-term local control of brain and lung metastases.[3,4] Additionally, 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 patients with gynaecological tumours, and poor cosmetic outcomes for women with breast cancer.[5,6]

Although these technical ameliorations have spread rapidly over recent years, the development of therapies to complement radiotherapy has stagnated since the 1990s, when several clinical trials showed that chemoradiotherapy—ie, the combination of radiotherapy with cytotoxic chemotherapy—can result in improved local tumour 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, fluorouracil, mitomycin C, and temozolomide should be used to improve the therapeutic effectiveness of radiotherapy. Indeed, contrasting with the expectations nourished by preclinical studies, combination regimens involving targeted anticancer agents and radiotherapy have failed to provide superior clinical benefits when compared with conventional chemoradiotherapy.[8–10] There are multiple possible causes for these deceptive clinical results, including, on a case-by-case basis, the validity of preclinical studies, the effect of combination therapies on normal tissues, the absence of reliable biomarkers for patient selection, and technical aspects linked to radiotherapy delivery. These shortcomings are likely to be overcome by improved communication among radiation oncologists, medical oncologists, statisticians, pharmacologists, and experts in imaging and translational research.

The realisation that the immune system can make a major contribution to therapeutic responses to radiotherapy[11] provided a strong rationale for clinical trials combining multiple variants of immunotherapy with different forms of radiotherapy. Such an intensive wave of investigation of radioimmunotherapy has culminated in the approval of chemoradiotherapy plus the immune checkpoint inhibitor durvalumab for the treatment of unresectable stage III non-small-cell lung cancer (NSCLC),[12] which was shown to be effective and well tolerated despite a report alerting about potential lung toxicity.[13] More than 150 clinical trials are exploring the combination of standard, full-dose (chemo)radiotherapy plus immunotherapy.[14] These immunotherapies are mostly immune checkpoint inhibitors targeting PD-L1 (eg, durvalumab, avelumab, and atezolizumab), PD-1 (eg, pembrolizumab, nivolumab, and cemiplimab), or CTLA-4 (eg, ipilimumab). These combination regimens ought to enable superior therapeutic responses because they could reduce toxic effects, both in the short and long term, ultimately resulting in improved outcomes for patients with previously untreatable tumours, especially in the setting of oligometastatic disease (figure 1).

Classic radiation biology has investigated the efficacy of radiotherapy mostly in vitro and in xenograft models with immunodeficient mice bearing human cancer cell

lines, two models that completely omit the potential contribution of the immune system to therapeutic effects.[21] This omission has imposed a largely autonomous view of cancer cells on the biology of radiotherapy, positing that the response to radiotherapy (and therefore the likelihood of complete disease eradication) is directly proportional to radiation dose and the consequent degree of DNA damage overcoming the repair capability of the cell.[22] This model has fostered many dose-escalation studies, which produced positive results in some indications, such as prostate and cervical cancer,[2] but did not meet expectations in many others, such as NSCLC, oesophageal tumours, and brain cancers.[23] The modern conceptual framework instead postulates that radiotherapy is particularly efficient when it elicits tumour-targeting immune responses.[24] The major corollary of this shift in perspective is that radiotherapy should be administered in doses and schedules that are optimally suitable for eliciting anticancer immunity. As the maximum tolerated dose often inhibits or kills immune cells involved in tumour control alongside malignant cells, radiotherapy delivered at the maximum tolerated dose generally fails to induce efficient immune responses against cancer, implying




| Strategy | Abscopal: immunotherapy plus radiotherapy | Debulking gross disease: radiotherapy alone | All-site combination: immunotherapy plus radiotherapy |
|---|---|---|---|
| Immunotherapy | Yes | No | Yes |
| Radiotherapy<br>Sites<br>Volumes<br>Doses | One or few sites<br>Intermediate to high doses<br>Small volumes | All sites<br>Ablative doses<br>Extended volumes | All sites irradiation plus immunotherapy<br>Immunostimulating therapy<br>Extended volumes |
| Clinical demonstration | Formenti et al (2018)[15]<br>Luke et al (2018)[16]<br>Golden et al (2015)[17] | Nguyen et al (2018)[18]<br>Hong et al (2018)[19]<br>Parker et al (2018)[20] | Clinical demonstration pending |
| Therapeutic benefit | +++ | ++ | ++ |

***Figure 1:*** **Radioimmunotherapy strategies for the management of oligometastatic disease and therapeutic benefits**
The therapeutic effects expected from integrating radiotherapy and systemic immunotherapy might 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 with debulking strategies based on radiotherapy alone (B), or to combinatorial approaches involving the systematic irradiation of all disease sites (C). +=the extent of therapeutic benefit.

a non-linear dose–effect relationship.[25] Therefore, implementation of radioimmunotherapy regimens requires novel strategies to improve efficacy and limit toxicity, which is the subject of this Series paper.

## Immunological parameters affecting acute and late radiotherapy effects

The side-effects of radiotherapy generally manifest in a biphasic manner, reflecting both the intensity of the treatment and the organs exposed to direct or scattered irradiation.[26] The acute phase, which is characterised by local atrophy and inflammation, originates from a wave of cell death driven by radiotherapy in tissues with a high mitotic index, such as the epithelium of the oral cavity and the gastrointestinal tract or the haemopoietic system, and hence tends to resolve a few weeks after treatment.[27] The chronic phase typically starts around 3 months after radiotherapy, and involves vascular alterations, persistent oxidative stress, chronic hypoxia, fibroblast activation potentially leading to fibrosis, and permanent loss of tissue function.[28] Preclinical studies suggest that the immune system not only participates in the therapeutic effects of radiotherapy, but also contributes to both acute and chronic radiotherapy toxicities.[29] Consistent with this notion, deletion of *Icam-1*, which codes for an endothelial cell surface molecule involved in leucocyte extravasation, inhibits both acute inflammatory responses and chronic fibrotic changes in the lungs of mice subjected to thorax irradiation.[30] A similar antifibrotic response can be achieved using a monoclonal antibody specific for CSF-1-R to deplete interstitial macrophages.[31] Moreover, radiation-induced pneumonitis occurring in mice after total body irradiation can be reduced by thymectomy and restored by the adoptive transfer of splenocytes.[32] Thus, multiple immune effector cells can contribute to the toxicity of radiotherapy. Of note, macrophage depletion with agents that target CSF-1 or CSF-1-R also improves anticancer immune responses initiated by radiotherapy (especially in the context of PD-L1 blockade), in which radiotherapy (especially at low doses) favours the polarisation of macrophages towards an immunosuppressive phenotype.[33,34] Taken together, these observations suggest that targeting macrophages might provide a dual benefit to patients receiving radiotherapy: an improved therapeutic response coupled with few toxic effects.

The recruitment of immunosuppressive cells to irradiated organs, as well as the activation of these cells, is coordinated by several immunomodulatory molecules released in response to radiotherapy. TGFB1 (best known as TGFβ1), a cytokine with pleiotropic effects, is pivotal in this context.[35] TGFβ1 signalling 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 TGFβ1.[35] On binding to its cognate receptor, which is expressed by a variety of cell types, including immune cells and fibroblasts, TGFβ1 mediates robust immunosuppressive effects and causes the secretion of collagen, resulting in fibrosis.[35] In line with this notion, strategies aimed at inhibiting TGFβ1 synergise with immune checkpoint inhibitors[36] and radiotherapy[37] in the control of experimental tumours in mice, at least in part by improving the access of lymphocytes to malignant lesions. Similarly, chemical inhibitors of TGFβ1 signalling attenuate lung fibrosis in mice receiving radiotherapy to the thorax.[38] The results of early clinical trials testing this treatment have not been very positive,[39,40] possibly as a result of the duality of TGFβ1 function that inhibits epithelial growth while promoting the progression of advanced tumours.[41] However, TGFβ1 inhibitors stand out as promising tools to simultaneously improve the efficacy and reduce the toxicity of radioimmunotherapy, especially in the context of multimodal treatments.

Irradiated cells also release large amounts of damage-associated molecular pattern molecules (DAMPs), a heterogeneous group of endogenous molecules that are normally invisible to the immune system, owing to their intracellular localisation.[42] On release into the extracellular microenvironment, however, DAMPs can bind to a variety of pattern recognition receptors involved in the innate immune response to invading pathogens, including TLRs (toll-like receptors), thereby causing local inflammation.[43] DAMPs such as the non-histone chromatin-binding protein HMG-1 (high-mobility group 1) and mitochondrial DNA (mtDNA) have been attributed a causal role in inflammatory conditions, including some cardiomyopathies and systemic inflammatory response syndrome, largely reflecting the ability of these DAMPs to recruit and activate granulocytes.[44] Consistent with this notion, extracellular mtDNA is increased in patients with idiopathic pulmonary fibrosis,[45] and chemical antagonists of CXCR-1 and CXCR-2, which prevent the accumulation of neutrophils in irradiated lung tissue, limit radiotherapy-induced fibrosis in mice.[46] Further corroborating a role for DAMP signalling in the acute and late toxicity of radiotherapy, multiple TLR5 agonists inhibit radiotherapy-driven mucositis, dermatitis, pneumonitis, and fibrosis in mice.[47] However, the precise mechanisms whereby TLR5 agonism (rather than antagonism) mediates radioprotective effects remain elusive.

Radiotherapy also increases the amounts of MHC class I and II molecules exposed on the plasma membrane, rendering irradiated cells more susceptible to immune recognition.[40] Evidence that this mechanism has a role in the side-effects of radiotherapy includes the fact that patients receiving total body irradiation can develop autoimmune disorders, accompanied by autoreactive lymphocyte infiltration of the irradiated tissue.[48] Importantly, such immune reactions cannot originate from overt tissue damage and abundant DAMP release, as the radiotherapy doses used are too low to cause cell death.[48] That said, both total body irradiation and thoracic radiotherapy also promote the expansion of immunosuppressive regulatory T cells (Treg) that are positive for

CD4, CD25, and FOXP3 that potentially attenuate autoimmune reactions driven by CD4 T helper cells,[49,50] a mechanism that might depend (at least partially) on epidermal Langerhans cells.[51] An immunosuppressive effect that could not be linked to the extent of lymphopenia was documented in mice receiving total lymphoid irradiation plus total body irradiation (as compared with total lymphoid irradiation alone) as early as 1984, when little was known about the immunobiology of Treg and Langerhans cells.[52] The possibility of harnessing the immunosuppressive activity of Treg cells to limit the inflammatory side-effects of radiotherapy has attracted considerable attention in the past,[50,53] but is progressively being abandoned. Conversely, efforts remain focused on conventional radioprotectors, including reactive oxygen species scavengers (eg, amifostine), molecules that promote epithelial reconstitution (eg, palifermin), and systemic anti-inflammatory drugs (eg, aspirin and celecoxib).[54]

Importantly, most, if not all, of the molecular and cellular mechanisms that underlie the toxicity of radiotherapy are also responsible for its therapeutic activity. Thus, cancer cells exposed to cytotoxic doses of radiotherapy undergo a potently immunostimulatory cell death variant that has been named immunogenic cell death.[55] Immunogenic cell death is characterised by the activation of multiple adaptive stress responses in dying cells that culminate in the exposure or release of DAMPs that affect the regulation of local and systemic homoeostasis.[40] Such stress responses encompass, but might not be limited to, several mechanisms: first, the unfolded protein response at the endoplasmic reticulum, culminating in the exposure of several endoplasmic reticulum chaperones on the surface of dying cells; second, autophagy, enabling the release of high levels of ATP from dying cells; and third, a pathogen-like response involving the detection of ectopic RNA and DNA molecules, culminating in the secretion of type I interferon (IFN).[56] The spatiotemporally coordinated release of endoplasmic reticulum chaperones, ATP, RNA, DNA, and other DAMPs from cancer cells that succumb to radiotherapy enables the recruitment and activation of BATF3-dependent dendritic cells, ultimately resulting in the cross-priming of tumour-specific cytotoxic T lymphocytes.[42] This response involves freshly recruited cytotoxic T lymphocytes as well as tumour-resident cytotoxic T lymphocytes, and is under tonic inhibition by PD-1 and TGFβ signalling.[57–59] Such a robust response can not only drive the eradication of radioresistant cancer cells in the irradiated lesions, but can also potentially attack non-irradiated metastases (at least in mice and in a small proportion of patients), an effect commonly referred to as an abscopal response.[60] Further evidence for the immunological nature of this process is that abscopal responses can be abolished in mice that lack CD8 T cells, as well as in mice in which type I IFN signalling is inhibited.[11,61] Abscopal responses have also been observed at increased frequency in patients concomitantly treated with immunotherapy.[15,17] Of note, DAMPs are not the only commonality between toxicity and therapeutic activity of radiotherapy: radiotherapy can alter vascular permeability, supporting local inflammation,[62,63] and can increase MHC class I and II expression, both of which are also involved in disease control.[40]

Increasing preclinical and clinical data suggest that there are at least three major parameters that influence the determinants of radiotherapy efficacy versus toxicity in this setting: dose and fractionation, irradiated volume, and sequence of administration in the context of regimens that combine various interventions.[24] Finally, because both the efficacy and toxicity of radiotherapy rely, at least in part, on the immune system, the overall immunological competence of the host and the factors that determine this immunological competence should not be underestimated. Such factors include 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 on the basis of all these factors, as delineated in the next section, while critically comparing the effects of radioimmunotherapy with those of immunotherapy alone, as exemplified in the IMPORTANCE trial (NCT03386357) for the treatment of recurrent or metastatic HNSCC.

## Refining radiotherapy methods to maximise the therapeutic window of radioimmunotherapy

### Dose and fractionation

For decades, radiotherapy doses and fractionation have been empirically set by clinicians with the single aim of achieving local tumour control. For many indications, such a conventional schedule corresponded to the delivery of 1·8–2 Gy per day, on 5 days per week, for 5–8 weeks. Clinical trials provided rationale for recommending different standard doses for specific tumours, including a total of 70 Gy for HNSCC, 66 Gy for NSCLC, and 74–80 Gy for prostate cancer.[23] More recently, the classic dose-prescription framework has shifted towards higher doses per session and an increased number of sessions, especially in cases in which radiotherapy is delivered to relatively small target volumes. Moreover, precision radiotherapy guided by three-dimensional imaging has enabled the delivery of up to 20 Gy in a single dose, a regimen that can be routinely used to eradicate isolated brain metastases with minimal side-effects.[64]

On the basis of these advances, attempts have been made to increase the radiotherapy dose up to a threshold dictated by toxic effects on normal tissues, resembling

prior attempts of dose intensification for cytotoxic chemotherapies.[65] However, such an approach, which is supported by the cancer-cell autonomous view of tumour biology described earlier, conflicts with the increasingly accepted idea that any form of cancer therapy (conventional chemotherapy, targeted therapy, or radiotherapy) must induce an anticancer immune response to yield a long-term response beyond treatment discontinuation.[66]

Supporting the notion that high radiotherapy doses do not always enable systemic disease control, hypofractionated radiotherapy (three doses of 8 Gy each, delivered on 3 consecutive days), but not a single dose of 20 Gy, leads to efficient abscopal responses in immunocompetent mice bearing syngeneic mammary carcinoma or colorectal carcinoma cells.[58,61,67] These responses occur when the mice are simultaneously treated with a CTLA-4-targeting immune checkpoint inhibitor or with a monoclonal antibody that neutralises multiple TGFβ isoforms.[58,61,67] Abscopal responses driven by radiotherapy in the context of TGFβ 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 brings about long-term disease eradication, coupled with protective immunological memory.[58]

Abscopal responses also rely on dynamic activation of immune cells and their recruitment into irradiated tumours.[68,69] These immune cells include BATF3-dependent dendritic cells, whose recruitment requires the local secretion of type I IFN downstream of TMEM173 (best known as hSTING) activation.[61,70] Hypofractionated radiotherapy results in the accumulation of endogenous DNA in the cytosol of irradiated cells, and hence efficiently drives hSTING signalling, a process that is inhibited as a consequence of the upregulation of cytosolic nuclease TREX1 (three-prime repair exonuclease 1) when radiotherapy doses higher than a 10–12 Gy threshold (depending on cell type) are used.[61] Importantly, cytosolic DNA accumulating in cancer cells that are responding to hypofractionated radiotherapy can be efficiently shuttled to dendritic cells via exosomes, and this further contributes to type I IFN production.[71] Clinical evidence is emerging in support of the notion that hypofractionated radiotherapy (five fractions of 6 Gy) in combination with immune checkpoint inhibitors targeting CTLA-4 can generate robust abscopal responses.[15]

Similarly, a low radiotherapy dose (a single dose of 2 Gy) efficiently boosts the ability of chimeric antigen receptor (CAR) T cells to eliminate cancer cells that escaped conventional recognition by losing expression of the CAR target antigen.[72] In this setting, low-dose radiotherapy appears to sensitise antigen-negative cancer cells to the lethal effects of TNFSF10 (tumor necrosis factor receptor superfamily member 10), or TRAIL, which is produced by CAR T cells upon CAR-dependent activation, but mediates T-cell receptor-independent effector functions.[72] 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-negative malignant cells, exhibited systemic disease clearance 1 month after treatment.[72] However, this patient relapsed massively starting 2 months after treatment, with the only exception of the left thigh, which had received low-dose palliative radiotherapy (four fractions of 5 Gy) before CAR T-cell infusion and persisted disease-free up to 6 months after treatment.[72]

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

### Irradiation of smaller volumes of tissue

In addition to the total radiation dose, the volume of irradiated tissue has an effect on the side-effects of radiotherapy. Thus, even when high-dose radiotherapy is administered in a few sessions to a small tissue volume, as is typically achieved by brachytherapy and stereotactic radiotherapy, side-effects remain relatively mild. These irradiation techniques offer the advantage that the volume of tissue to which radiotherapy is delivered is sharply delimited, reducing collateral damage to adjacent organs, including lymph nodes.[73]

Abscopal responses to radiotherapy (especially in the context of radioimmunotherapy) show the possibility that anticancer immune responses elicited by such therapy can propagate outside of the radiation field. This raises the possibility that radiotherapy might also elicit robust clinical responses when parts (rather than the entirety) of the tumour are irradiated. Preclinical and clinical evidence in support of this possibility has begun to emerge.[16,74] However, caution should be used when these findings are interpreted, because the areas of the tumour outside of the irradiation field also receive radiotherapy, albeit at lower doses, which might be responsible for, or at least contribute to, therapeutic efficacy.[75] Moreover, in the case of radioimmunotherapy, responses outside of the irradiation field can simply originate from immunotherapy, especially in immunosensitive tumours.[75] Adjuvant radiotherapy after breast conservation surgery constitutes a promising alternative to mastectomy for patients with breast cancer because it not only decreases local relapse rates (as radical surgery does), but also diminishes the risk of distant relapse,[76] pointing to the

production of an abscopal response targeting micrometastases. In this setting, accelerated partial-breast irradiation using multicatheter brachytherapy stands out as a morbidity-reducing alternative to whole-breast irradiation for the treatment of low-risk mammary carcinoma,[77] lending further support to the notion that larger irradiation volumes do not necessarily translate into improved disease control.

### Sparing tumour-draining lymph nodes and the gut

Conventional radiotherapy regimens are generally formulated to deliver a full dose (50–70 Gy) to the tumour and prophylactic coverage to draining lymph nodes (45–50 Gy), as established by multiple clinical trials in various indications (eg, HNSCC, cervical cancer, NSCLC).[78] This approach might not be particularly appropriate when radiotherapy is delivered in doses and fractionations that support the activation of anticancer immunity, and even less so in the context of radioimmunotherapy. Although draining lymph nodes are not the only sites of T-cell cross-priming by dendritic cells (which can also occur in intratumoral tertiary lymphoid structures),[79] they do constitute major platforms for the initiation of local and systemic antitumour immune responses. Accordingly, draining lymph nodes increase in volume as a consequence of robust CD8 cytotoxic T-lymphocyte infiltration when transplantable mouse melanomas and breast carcinomas are exposed to hypofractionated radiotherapy in the context of PD-1 blockade.[80] Thus, it might be detrimental to perturb draining lymph nodes with radiotherapy. In support of this notion, patients with nasopharyngeal carcinoma with tumour-free draining lymph nodes and treated with lymph node-sparing IMRT rarely exhibited locoregional relapse.[81] Similarly, clinical data on partial tumour irradiation sparing draining lymph nodes combined with pembrolizumab compare positively with previous results involving draining lymph node irradiation.[16] However, these findings should be interpreted with caution, as multiple patients involved in the trial had tumours that were potentially sensitive to pembrolizumab alone.[75] Clinical trials comparatively evaluating draining lymph node irradiation versus sparing in the context of radioimmunotherapy are urgently awaited. Of note, optimal therapeutic responses to radiotherapy and radioimmunotherapy might 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,[82] which, in turn, influences the efficacy of many therapeutic regimens, including immunotherapy.[83]

### Optimising radioimmunotherapy: sequencing treatments

The recent finding that the adjuvant administration of PD-L1-targeting immune checkpoint inhibitors after chemoradiotherapy postpones the onset of metastasis and improves the survival of patients with stage III NSCLC[12] could 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 radiotherapy with immunotherapy would have yielded better results than sequential administration. Unlocking immunological checkpoints before or after radiotherapy, rather than concomitant to radiotherapy, might offer the chance to avoid potentially cumulative toxic effects. This becomes even more relevant when considering dual or triple immunotherapy treatments that, if combined with radiotherapy or chemoradiation,[84] could demand sequential rather than simultaneous interventions to manage side-effects. Unfortunately, clinical data robustly comparing different treatment sequences are largely missing in the setting of radioimmunotherapy (table). However, it can be speculated that the increasing use of first-line immunotherapy will result in a situation where radiotherapy will often be performed after immunotherapy. That said, existing data support the administration of hypofractionated radiotherapy before PD-L1 blockade,[12] perhaps because hypofractionated radiotherapy stimulates tumour infiltration by T lymphocytes.[68] The aim of one clinical trial (NCT03453892) is to define the optimal scheduling of PD-1 and CTLA-4 blockade with respect to radiotherapy in patients with metastatic cancers. In this setting, quantitative systems pharmacology models, which include key elements of immuno-oncology and dose-exposure-target modulation features,[93] could assist the understanding of immune-cell dynamics within irradiated tumours.

### Biomarkers for optimising radioimmunotherapy

Many immunotherapies beyond immune checkpoint inhibitors 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, which functionally alter the tumour microenvironment towards an immunostimulatory configuration.[14] In this setting, great efforts have been made in the identification of reliable predictive biomarkers of response, reflecting not only safety issues, but also economic considerations.[94] It appears plausible that such biomarkers, whether they are based on the measurement of immunological parameters in the blood or in the tumour, will guide optimal radioimmunotherapy in the future, not only with respect to the choice of the best immunotherapeutic agent for combination regimens, but also with regard to radiotherapy doses and schedules. The local upregulation of PD-L1 in response to neoadjuvant chemoradiotherapy of rectal cancers is associated with favourable prognosis[95] and might speculatively predict responsiveness to immunotherapy. Moreover, computational imaging (radiomics) stands out as a promising strategy to non-invasively quantify tumour infiltration by CD8 T cells,[96]

| | Indication and selection criteria | Phase | Patients | Treatment modality | Toxic effects | Efficacy |
|---|---|---|---|---|---|---|
| Levy et al (2016)[85] | Solid tumours with ≥5% PD-L1 expression among malignant cells | 1/2 | 10 | Durvalumab 10 mg/kg Q2W plus radiotherapy (median dose 20 Gy in median 5 fractions), given a median of 8·5 days after last durvalumab | 50% of patients had radiotherapy-related grade 2 AEs | In-field: objective response 60% (2/10 had CR, 4/10 had PR); out-of-field: 10/14 had SD, no objective responses; no abscopal responses |
| Antonia et al (2018)[12] | Stage III, locally advanced, unresectable non-small-cell lung cancer | 3 | 709 | Conformal radiotherapy (54–66 Gy) with 2 concurrent platinum cycles plus sequential durvalumab 10 mg/kg Q2W, initiated within 6 weeks after radiotherapy and continued for up to 1 year (or placebo if no PD during radiotherapy) | Serious AEs in 29·1% and 23·1% of patients in the durvalumab and placebo groups, respectively | Median PFS: 16·8 months *vs* 5·6 months (p<0·001); median TTD or distant metastasis: 23·2 months *vs* 14·6 months (p<0·001) |
| Luke et al (2018)[16] | Advanced solid tumours | 1 | 79 | SBRT to 2–4 metastases in doses ranging from 30 to 50 Gy in 3–5 fractions (partial irradiation of metastases >65 mL) plus pembrolizumab initiated within 7 days after SBRT completion | 6 patients had DLT with no radiotherapy dose reductions | Proportion of patients with an objective response: 13·2%; median PFS: 3·1 months (95% CI 2·9–3·4) |
| Tang et al (2017)[86] | Metastatic solid tumours with ≥1 lesion in the liver or lung amenable to SBRT and ≥1 non-contiguous lesion for monitoring | 1 | 35 | Ipilimumab (3 mg/kg Q3W for 4 doses) in 5 radiotherapy groups: concurrent (1 day after the first dose) or sequential (1 week after the second dose) radiotherapy (50 Gy in 4 fractions) to lung or liver, or sequential 60 Gy (in 10 fractions) to lung or liver | 2 patients had DLT and 12 (34%) had grade 3 AEs | Out-of-field responses amongst assessable patients: 3/31 (10%) PR; 7/31 (23%) clinical benefit (PR or SD ≥6 months) |
| Williams et al (2017)[87] | Brain metastases from melanoma | 1 | 16 | WBRT or SRS plus ipilimumab dose escalated from 3 mg/kg to 10 mg/kg Q3W starting at day 3 of WBRT or 2 days after SRS | 21 grade 1–2 neurotoxic effects, with no DLT; 10 additional grade 3 AEs (5 gastrointestinal toxic effects); no grade 4–5 toxic effects | Median PFS: 2·5 months after WBRT, 2·1 months after SRS |
| Kwon et al (2014)[88] | Metastatic castration-resistant prostate cancer with one or more bone metastases progressing after docetaxel | 3 | 799 | Bone-directed radiotherapy (8 Gy in 1 fraction) plus ipilimumab 10 mg/kg or placebo Q3W for up to 4 doses, starting after radiotherapy | Most common grade 3–4 AEs were immune-related (26% of the ipilimumab group, including 4 toxic deaths) | Median OS: 11·2 months *vs* 10·0 months (p=0·053); median PFS: 4·0 months *vs* 3·1 months (p<0·0001) |
| Hiniker et al (2016)[89] | Stage IV melanoma with ≥1 non-irradiated metastasis measuring ≥1·5 cm available for response assessment | 1 | 22 | Radiotherapy to 1–2 disease sites (BED10: 23·3 Gy to 72·9 Gy), initiated within 5 days of ipilimumab initiation (for 4 cycles) | No unexpected toxic effects; three grade 3 AEs | 3 patients (27·3%) achieved systemic CR, and 3 (27·3%) an initial PR |
| Slovin et al (2013)[90] | Metastatic castration-resistant prostate cancer | 1/2 | 33/50* | Ipilimumab Q3W for 4 cycles at 3, 5, or 10 mg/kg, or at 3 or 10 mg/kg plus radiotherapy (8 Gy per lesion) | Common grade 3 immune-related AEs in patients receiving radiotherapy were colitis (16%) and hepatitis (10%); 1 treatment-related death was recorded (in 5 mg/kg group) | 8 PSA declines ≥50% (duration: 3–13 plus months), 1 CR (duration: 11·3 plus months), and 6 SD (duration: 2·8–6·1 months), amongst patients treated with radiotherapy plus ipilimumab 10 mg/kg |
| Golden et al (2015)[17] | Metastatic solid tumours with ≥3 distinct measurable sites of disease | 2 | 41 | Concurrent radiotherapy (35 Gy in 10 fractions over 2 weeks) to 1 metastatic site and GM-CSF (125 μg/m$^2$ subcutaneously daily for 2 weeks, starting during the second week of radiotherapy); course repeated to target a second metastatic site | Most common grade 3–4 AEs were fatigue (6 patients) and haematological (10 patients); 1 patient had grade 4 pulmonary embolism | Abscopal responses in 11 (26·8%, 95% CI 14·2–42·9) patients |
| Twyman-Saint et al (2015)[84] | Metastatic melanoma | 1 | 22 | Escalating doses of SBRT (2–3 fractions) to index lesion, followed 3–5 days later by ipilimumab Q3W for 4 cycles | No DLTs; 15 grade 3 AEs | Evaluation of non-irradiated targets: 18% PR, and 18% SD |
| Seung et al (2012)[91] | Metastatic melanoma or renal cell carcinoma | 1 | 12 | 1–3 doses of SBRT (20 Gy per fraction), last dose administered 3 days before initiation of high-dose interleukin-2 | No DLT attributable to SBRT | Evaluation of non-irradiated targets: 1 CR and 7 PR |
| Bloch et al (2017)[92] | Glioblastoma | 2 | 46 | Surgical resection followed by standard conformal radiotherapy plus autologous vaccine generated from resected tumours and delivered weekly after radiotherapy completion | AEs attributable to the vaccine in 34 patients (74%); but no grade 3–4 AEs related to vaccination | Median OS: 23·8 months (18·0 months for patients with high PD-L1 expression on myeloid cells *vs* 44·7 months for patients with low PD-L1 expression; p=0·007) |

Q2W=every 2 weeks. AE=adverse event. CR=complete response. PR=partial response. SD=stable disease. PD=progressive disease. PFS=progression-free survival. TTD=time to death. SBRT=stereotactic body radiotherapy. DLT=dose-limiting toxicity. Q3W=every 3 weeks. WBRT=whole-brain radiotherapy. SRS=stereotactic radiosurgery. OS=overall survival. BED10=biologically effective dose (estimated for the tumour). PSA=prostate-specific antigen. GM-CSF=granulocyte macrophage colony-stimulating factor. *Dose escalation (33 patients); dose expansion (50 patients).

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

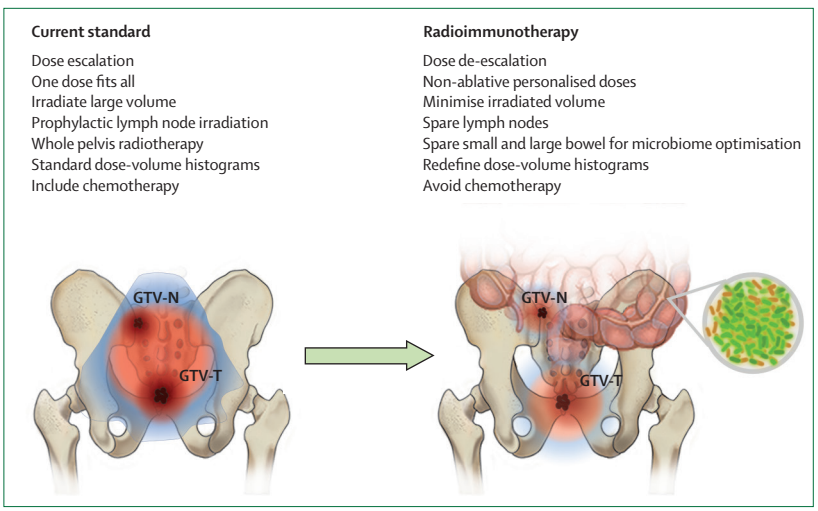



***Figure 2:*** **From conventional chemoradiotherapy to radioimmunotherapy**
Improving the therapeutic index in the era of radioimmunotherapy requires the redefinition of multiple approaches established in the context of conventional chemoradiotherapy, including (but not limited to) doses, fractionation schedules, and irradiation volumes and targets. Clinical trials designed to investigate novel radioimmunotherapy approaches should be specifically designed to simultaneously achieve low toxicity to normal tissues and superior systemic disease control. GTV-T=primary gross tumour volume. GTV-N=lymph node gross tumour volume.

which is an important positive prognostic factor for patients affected by multiple tumours.[97] In particular, the ability of radiomics to provide longitudinal, non-invasive monitoring of the tumour microenvironment could support the implementation of a personalised radio-immunotherapy, with the ultimate goal that each patient receives a treatment modality that triggers an effective anticancer immune response. High-dimensional single-cell analyses using genomic, transcriptomic, proteomic, and metabolomic approaches are also on the verge of revolutionising biomarker discovery and clinical practice.[98] The results of these analyses might be used as inclusion and exclusion criteria for clinical trials beyond the mere presence of CD8 T cells (as in NCT03453892).

Current refinements in radioimmunotherapy trials offer the benefit of collecting biopsy samples from tumours after treatment. Such trials constitute a valuable source of samples for characterising responses and resistance to radioimmunotherapy far beyond murine models. Indeed, standard treatment might per se affect the expression of immune-related biomarkers, which could prevent a careful prognostic assessment, misguide therapeutic decisions, or both. For instance, rectal tumours exposed to pre-operative chemoradiotherapy alone exhibit increased PD-L1 expression,[95] which offers a strong rationale to combine treatment with adjuvant PD-1 or PD-L1 blockade. Similar observations have been made in both melanoma[99] and HNSCC.[100] Alterations in the peripheral blood compartment, including shifts in major T-cell populations, could also offer a convenient, non-invasive gateway to monitor responses to radio therapy and radioimmunotherapy in patients with cancer.[101]

## Chemoradioimmunotherapy or radioimmunotherapy?

A combination of cytotoxic chemotherapy and radiotherapy has shown superiority over radiotherapy employed as a standalone intervention at enabling local disease control, and sometimes improving organ preservation and survival in patients with multiple tumour types.[102] However, this therapeutic success often comes with several complications and side-effects.[103] An in-depth understanding of the tumour microenvironment and its influence on resistance to immunotherapy will be instrumental in defining potent radioimmunotherapy regimens that do not require the addition of cytotoxic chemotherapy for optimal efficacy. Preliminary clinical data reinforce the evidence that PD-L1 or PD-1 blockade combines safely with concurrent radiotherapy and cetuximab alone in patients with HNSCC,[104,105] as well as with radiotherapy plus platinum-based chemotherapy in patients with NSCLC.[106] However, robust demonstration of clinical benefits is still awaited. Similarly, although evidence suggests that adjuvant PD-L1 blockade improves disease outcome after chemoradiotherapy in patients with NSCLC,[12] most preclinical evidence supporting the implementation of concurrent radio-immunotherapy in the clinic does not involve cytotoxic chemotherapy,[11,57,59,61,67] suggesting that immunotherapy might per se combine favourably with radiotherapy. Thus, conventional chemo-therapeutics might no longer be required to achieve robust clinical responses in the context of radio-immunotherapy, which might also translate into decreased incidence and severity of side-effects. Clinical trials should specifically be designed to investigate this possibility. For instance, there could be an investigation into whether the superior efficacy of PD-1 blockade plus chemoradiotherapy against NSCLC[12] truly requires chemotherapy to be included in the therapeutic protocol.

Many ongoing trials are designed to induce abscopal responses. Although data are not mature yet, preliminary findings are promising. Combining PD-1 or PD-L1 blockade with radiotherapy has been associated with durable out-of-field responses in three (33%) of nine evaluable patients with metastatic triple-negative breast cancer unselected for PD-L1 expression.[107] PD-1 blockade preceded by stereotactic body radiotherapy (SBRT) for patients with NSCLC resulted in a doubling of the proportion of patients who achieved an objective response without accrued toxicity.[108] By contrast, addition of SBRT to PD-1 blockade in patients with metastatic HNSCC was safe but failed to improve outcome in a randomised evaluation of abscopal responses.[109] A randomised phase 2 trial testing PD-1 blockade plus radiotherapy in patients with HNSCC (IMPORTANCE, Keynote-717) is comparing standalone PD-1-targeting immunotherapy with radioimmunotherapy. The results of this study will provide useful insights into the relative contribution of radiotherapy to clinical responses to immunotherapy.

### Search strategy and selection criteria

We searched PubMed with the term "radiation and immunotherapy" for articles published between Jan 1, 2010, and Dec 31, 2018, and entries were manually selected for relevance. Additional articles published before and after 2010 were manually selected for inclusion by the authors.

### 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 could combine favourably with immunotherapy.[110] Densely ionising particles (protons, carbon ions), which are characterised by a dose deposit in a narrow depth range (leading to minimal exit dose), are being used in an increasing number of indications.[111] The greatest benefit of using these densely ionising particles is probably for paediatric patients, and is linked to reductions in both dose-dependent complications and risk for secondary tumours.[111] Protons and carbon ions are generally considered superior to photons (conventional x-rays) for distribution ballistics.[112] Moreover, carbon ions might provide increased biological and clinical effectiveness, at least in specific indications that are generally refractory to conventional radiotherapy, such as bone and soft tissue sarcomas of the skull base.[113] Preliminary data suggest that protons resemble photons in their immunological effects.[114] 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 favourably combined with immunotherapy remains to be shown.

## Conclusion

Radiotherapy has been used for more than a century for the clinical management of virtually all cancers, often with positive results, both in terms of side-effects and effectiveness. Such a favourable therapeutic window places radiotherapy in a privileged position for the development of combination treatment regimens. In line with this notion, the delivery of radiotherapy with cytotoxic chemotherapy has enabled improved local disease control in multiple oncological indications, in some cases accompanied by superior organ preservation and patient survival.[115] However, chemoradiotherapy protocols are often associated with increased incidence and severity of side-effects, reflecting the fact that both radiotherapy and chemotherapy are often used at (or near to) their maximum tolerated dose.[116] It can be anticipated that low-dose molecular targeted radiotherapy using radiolabelled molecules will overcome the problem of toxicity.[117,118] During the past decade, immunotherapy has largely transformed the management of many solid tumours.[14] However, the proportion of patients who respond to immunotherapy employed as a single therapeutic intervention is often low, (except for CAR T-cell therapy, which has been associated with more than 80% of patients achieving a response for selected indications.[119] Such promising results call for the development of combination regimens that include different immunotherapeutic approaches. In this context, radiotherapy stands out as an optimal partner for immunotherapy; however, conventional radiotherapy regimens will have to be redesigned for radioimmunotherapy to mediate superior efficacy in the presence of few side-effects (figure 2). Revisiting doses and fractionation schedules, reducing delivery volumes, sparing both draining lymph nodes and the intestine, minimising 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.

#### Contributors

ED, LG, and GK conceived the article. ED and CC prepared the first version of the manuscript and display items with inputs and revisions from LG and GK. LG and GK addressed comments from reviewers. All authors approved the final version of the article.

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ED declares grants Roche Genentech, Servier, AstraZeneca, Merck Seriono, Bristol-Myers Squibb, and MSD, outside the submitted work; and personal fees from Merck Seriono, and Roche Genentech, outside the submitted work. CC has been invited to national or international congresses by Takeda, and operates as (principal) investigator on clinical or preclinical trials for TherAguix, Roche, and Servier. LG declares grants from Phosplatin, and Lytix, outside the submitted work; personal fees from The Luke Heller TECPR2 Foundation, AstraZeneca, OmniSEQ, and VL47, outside the submitted work; and travel support from The Luke Heller TECPR2 Foundation, and Phosplatin, outside the submitted work. GK declares grants from Bayer Healthcare, Genentech, GlaxoSmythKline, Lytix Pharma, PharmaMar, Sotio, and Vasculox, outside the submitted work; personal fees from Bayer Healthcare and Lytix Pharma, outside the submitted work; and is an unpaid member of the Executive Board of Bristol-Myers Squibb Foundation France, and a scientific cofounder of everImmune and Samsara Therapeutics, outside the submitted work. GK's spouse holds positions on Scientific Advisory Boards at Lytix Pharma, EpiVax, NeoVax, and Tusk Pharma; holds a position on the Administrative Board at Transgene; has received grant and research support from GlaxoSmithKline, Merus, Tusk Roche, and Incyte; and is Founder and shareholder of everImmune, all outside the submitted work.

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