Trial Watch: Immunotherapy plus radiation therapy for oncological indications

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Trial Watch: Immunotherapy plus radiation therapy for oncological indications

Erika Vaccelli,a,b,c,d,e,* Norma Bloya,b,c,d,e,* Fernando Arandaf, Aitziber Buquieg, Isabelle Cremer,a,b,c,g Sandra Demaria,a, Alexander Eggermonta,g, Silvia Chiara Formentih, Wolf Hervé Fridmana,b,c,g, Jitka Fucikovai,j, Jérôme Galonb,c,k, Radek Spisek,j,l, Eric Taroutu,b,m,n, Laurence Zitvogele,o, Guido Kroemera,b,c,d,e,p,q,r,**, and Lorenzo Galluzzi,a,b,c,e,n,*,**

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ABSTRACT
Malignant cells succumbing to some forms of radiation therapy are particularly immunogenic and hence can initiate a therapeutically relevant adaptive immune response. This reflects the intrinsic antigenicity of malignant cells (which often synthesize a high number of potentially reactive neo-antigens) coupled with the ability of radiation therapy to boost the adjuvanticity of cell death as it stimulates the release of endogenous adjuvants from dying cells. Thus, radiation therapy has been intensively investigated for its capacity to improve the therapeutic profile of several anticancer immunotherapies, including (but not limited to) checkpoint blockers, anticancer vaccines, oncolytic viruses, Toll-like receptor (TLR) agonists, cytokines, and several small molecules with immunostimulatory effects. Here, we summarize recent preclinical and clinical advances in this field of investigation.

Abbreviations: CTL, cytotoxic T lymphocyte; CTLA4, cytotoxic T lymphocyte associated protein 4; DC, dendritic cell; EBRT, external beam radiation therapy; FDA, Food and Drug Administration; GM-CSF, granulocyte macrophage colony-stimulating factor; HNSCC, head and neck squamous cell carcinoma; ICD, immunogenic cell death; IDH, isocitrate dehydrogenase (NADP+) 1, cytosolic; IDO1, indoleamine 2,3-dioxygenase 1; IL, interleukin; mAb, monoclonal antibody; NK, natural killer; NSCLC, non-small cell lung carcinoma; TAA, tumor-associated antigen; TAM, tumor-associated macrophage; TGFβ1, transforming growth factor β1; TNF, tumor necrosis factor; TLR, Toll-like receptor

Introduction

Ionizing irradiation constitutes one of the pillars of modern cancer therapy.1-4 According to current estimates, indeed, at least 50% of subjects with cancer (all confounded) have received or will receive radiation therapy in the course of their disease.5,6 For a long time, radiation therapy was believed to operate in a merely cell-intrinsic manner, i.e., by promoting the death or permanent proliferative arrest of malignant cells upon the establishment of oxidative damage to macromolecules including DNA.7-12 More recently, however, it has become clear that the antineoplastic effects of ionizing irradiation also involve a considerable cell-extrinsic component. Irradiated cancer cells release a wide panel of biologically active mediators that act locally to promote the death of bystander cells.13-15 These factors include not only reactive oxygen and nitrogen species,16-18 but also various potentially cytotoxic (and immunomodulatory) cytokines such as interleukin (IL)-6,19 IL-8,20 transforming growth factor β1 (TGFβ1),21-24 and tumor necrosis factor (TNF).25 Moreover, radiation therapy can promote a particularly immunogenic form of cell death that eventually stimulates the activation of a tumor-targeting immune response with systemic therapeutic potential.26-32 The capacity of ionizing irradiation to stimulate anticancer immunity upon the induction of immunogenic cell death (ICD) explains the so-called abscopal or out-of-field effect, i.e., the relatively rare but sometimes very pronounced clinical response to radiation therapy that can manifest in distant, non-irradiated lesions.33-35 Finally, some forms of radiation therapy promote the normalization of the tumor vasculature, hence improving the access of
chemotherapeutic agents and immune effector cells to malignant lesions.\textsuperscript{39-41}

For the purpose of this Trial Watch, radiation therapy can be broadly subdivided into two major therapeutic paradigms: external-beam radiotherapy (EBRT) and internal radiotherapy.\textsuperscript{3,4} In the former setting, malignant lesions are treated across the intact skin, according to collimation procedures that can concentrate the irradiation energy on very specific areas of the tumor.\textsuperscript{42,43} In the latter setting, radionuclides are brought in direct contact with transformed cells, either as pellets that are deposited within the tumor mass (a strategy that is known as brachytherapy), or upon conjugation with (or encapsulation within) tumor-targeting agents, including monoclonal antibodies (mAbs).\textsuperscript{44-46} Both types of radiation therapy are associated with acute and chronic side effects.\textsuperscript{47-50} Acute side effects stem from the unavoidable (but ever more limited, thanks to the technological advances in modern irradiators for clinical use) damage temporarily imposed by irradiation on particularly radiosensitive healthy tissues (like the skin) and often resolve in a few days/weeks after interruption.\textsuperscript{44,51} On the contrary, the chronic toxicity of radiation therapy originates from the permanent damage possibly imposed by considerable radiation doses to stem cell compartments like intestinal crypts,\textsuperscript{44,51} coupled to the establishment of dysregulated chronic inflammatory processes.\textsuperscript{52} Moreover, radiation therapy has been linked to a small but non-negligible increase in incidence of secondary, treatment-related malignancies later in life.\textsuperscript{53-55}

Throughout the past five decades, several strategies have been conceived to improve the therapeutic index of radiation therapy by either improving efficacy (radiosensitization) and/or by selectively limiting toxicity to normal tissues (radioprotection).\textsuperscript{2,56-58} Multiple molecules have been shown to mediate consistent radiosensitization or radioprotection in rodent models of radiation therapy.\textsuperscript{42} However, the antioxidant amifostine (also known as Ethyol\textsuperscript{59}) remains the only agent that is licensed by the US Food and Drug Administration (FDA) for use as a radioprotector in humans.\textsuperscript{59,63} One of the most common practices in radiation oncology is dose fractionation, i.e., the delivery of the total irradiation dose in multiple fractions (therapy sessions spaced by at least 6 h) over several days or weeks.\textsuperscript{64,65} Fractionation exploits the improved capacity of normal over malignant tissues to repair the damage imposed by irradiation, hence maximizing its therapeutic window.\textsuperscript{64,65} Importantly, total dose and delivery schedule have a prominent impact on the ability of radiation therapy to promote ICD and hence drive the establishment of a therapeutically relevant anticancer immune response.\textsuperscript{28,64,66-67}

Classically, radiation therapy has been employed in the context of combinatorial treatment regimens (involving surgery and chemotherapy), either with a curative objective (i.e., with the aim to eradicate primary neoplasms or prevent recurrence) or with a palliative intent (i.e., to limit the pain/discomfort caused by malignancies at specific anatomical locations).\textsuperscript{5,6} Along with the recognition that radiation therapy can mediate potent immunostimulatory effects, considerable interest has been attracted by combinatorial regimens involving EBRT plus one (or more) immunotherapeutic agent(s),\textsuperscript{68-71} including checkpoint blockers,\textsuperscript{72-75} immunostimulatory antibodies,\textsuperscript{72-76} recombinant cytokines,\textsuperscript{77-79} anticancer vaccines,\textsuperscript{80-84} indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors,\textsuperscript{85,86} adoptively transferred cells,\textsuperscript{87-89} oncolytic viruses,\textsuperscript{90-93} Toll-like receptor (TLR) agonists,\textsuperscript{94,95} and various small molecules that operate on the immunological tumor microenvironment. In this Trial Watch, we summarize recent preclinical and clinical advances in the development of combinatorial anticancer regimens based on EBRT plus immunotherapy.

**Published literature—highlights**


Among these reports, we found of particular interest (and at least partially related to immunotherapy) the works of: (1) Boelens and collaborators (from the University of Pennsylvania, Philadelphia, PA, US), who identified an exosome-dependent mechanism linked to antiviral signaling\textsuperscript{96} whereby stromal cells improve the resistance of breast cancer cells to radiation therapy;\textsuperscript{97} (2) Leder and colleagues (from the University of Minnesota, Minneapolis, MN, US), who developed a mathematical model of platelet-derived growth factor (PDGF)-driven glioblastoma that allowed for the identification of optimal radiation dosing schedules;\textsuperscript{98} (3) Tavora et al. (from the Queen Mary University, London, UK), who identified protein tyrosine kinase 2 (PTK2; best known as FAK)\textsuperscript{99} within the endothelial (not malignant) tumor compartment as a prominent player in the resistance of neoplasms of DNA-damaging agents including radiation therapy;\textsuperscript{100} (4) Tollini and co-workers (from the University of North Carolina at Chapel Hill, Chapel Hill, NC, US), who demonstrated that the capacity of MDM2 to tag tumor protein p53 (TP53; best p53)\textsuperscript{101-103} for proteasomal degradation is dispensable during embryogenesis and development, but essential for normal cellular responses to DNA damage;\textsuperscript{104} (5) Ceccaldi and collaborators (from the Harvard Medical School, Boston, MA, US), who identified in polymerase (DNA) theta (POLQ)\textsuperscript{105} a key regulator or DNA repair in homologous recombination (HR)-deficient tumors;\textsuperscript{106} (6) Moding and colleagues (from the Duke University Medical Center, Durham, NC, US), who showed that ATM (a kinase with a key role in the DNA damage response)\textsuperscript{107} in malignant cells, but not in endothelial cells, is required for the eradication of experimental sarcomas by stereotactic body radiation therapy;\textsuperscript{108} (7) Osswald et al. (from the University Hospital Heidelberg, Heidelberg, Germany), who identified cellular networks involving malignant astrocytes that underlie (at least in part) the pronounced radio- and chemoresistance of astrocytomas;\textsuperscript{109} (8) Reid and coworkers (University of California at San Diego, La Jolla, CA, US), who demonstrated that the radiosensitizer RX-001 (a hypoxia-inducible agent)\textsuperscript{110} is well tolerated by patients with advanced solid tumors and appears to mediate clinical activity (at least to some extent);\textsuperscript{111} (9) Tarish and collaborators (Karolinska Institute, Stockholm, Sweden), who demonstrated that the response of prostate cancer patients to radiation therapy is...
exacerbated by chemical castration\textsuperscript{112} (at least in part) as a consequence of deficient DNA repair in malignant cells;\textsuperscript{113} and (10) Zhang and colleagues (University of Michigan, Ann Arbor, MI, US), who reported that the haploinsufficient tumor suppressor F-box and WD repeat domain containing 7 (FBXW7)\textsuperscript{114-116} may constitute a promising target for radiosensitization owing to its role in non-homologous end-joining\textsuperscript{117} DNA repair.\textsuperscript{118}

Moreover, approximately 600 PubMed entries of those mentioned above contained the keyword “immunotherapy,” dealing (from an experimental or theoretical perspective) with the possibility to combine radiation therapy with anticancer immunotherapy in vitro, in vivo or in patients (source http://www.ncbi.nlm.nih.gov/pubmed). Of these studies, we found of special interest the work of: (1) Deng and colleagues (from the University of Chicago, Chicago, Illinois, US), who not only demonstrated that radiation therapy and checkpoint blockade with antibodies specific for CD274 (best known as PD-L1)\textsuperscript{119} synergize to promote antitumor immunity in mice, but also reported that transmembrane protein 173 (TMEM173; best known as STING)\textsuperscript{120-122} signaling in dendritic cells (DCs) is essential for the elicitation of antitumor immune responses by radiation therapy,\textsuperscript{123,124} (2) Denham and collaborators (from the University of Newcastle, Newcastle, Australia), who showed that zoledronic acid, an immunostimulatory agent that targets immunosuppressive tumor-associated macrophages (TAMs),\textsuperscript{125-129} synergizes with radiation therapy and intermediate-term androgen deprivation in the treatment of patients with locally advanced prostate carcinoma;\textsuperscript{130} (3) Vantourout et al. (from the King’s College, London, UK), who confirmed that irradiation increases the immunological visibility of tumors also by promoting the upregulation of killer cell lectin-like receptor K1 (KLRK1; best known as NKG2D)\textsuperscript{131-134} ligands in epithelial cells, hence favoring natural killer (NK) cell activation;\textsuperscript{135,136} (4) Surave and colleagues (from the University of Zurich, Zurich, Switzerland), who involved the complement system in radiation therapy-driven antitumor immune responses\textsuperscript{137} and (5) Twyman-Saint Victor and collaborators (University of Pennsylvania, Philadelphia, PA, US), who identified in the upregulation of PD-L1 a common mechanism whereby human and murine tumors become resistant to radiation therapy plus checkpoint blockers specific for cytotoxic T lymphocyte-associated protein 4 (CTLA), and demonstrated that anti-PD-L1 antibodies can be efficiently employed to revert resistance (at least in mice).\textsuperscript{138} Moreover, one of our laboratories provided proof-of-principle clinical evidence in support of the possibility to combine local radiation therapy with recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) to increase the incidence of therapeutically relevant abscopal effects in patients with advanced solid tumors.\textsuperscript{139} Finally, we demonstrated that the so-called immuno-score (a multiparametric biomarker conveying quantitative and spatial information on the immunological tumor infiltrate)\textsuperscript{140} not only conveys prognostic information for patients with rectal carcinoma treated by primary surgery, but also predicts clinical response to preoperative chemoradiation.\textsuperscript{141}

Besides unveiling parts of the mechanism whereby cancer cells may become resistant to the cytostatic and cytotoxic effects of irradiation, these findings lend additional support to the notion that radiation therapy and immunotherapy may be conveniently combined to improve disease outcome in cancer patients.

### Ongoing studies

In the period of time elapsing since the publication of the latest Trial Watch dealing with topic (2014 July \textsuperscript{148} through 2016 May 1st, no less than 620 clinical studies testing the safety and efficacy of anticancer therapeutic regimens based on (or at least involving) EBRT have been initiated (source: https://clinicaltrials.gov/). Nearly one-third of these studies (210 trials) investigates the clinical profile of EBRT as a standalone therapeutic intervention, in particular among patients affected by breast carcinoma (34 studies), prostate cancer (44 studies), non-small cell lung carcinoma (NSCLC; 15 studies), and hepatocellular carcinoma (14 studies). Some additional 220 trials initiated between 2014 July 1st and 2016 May 1st assess the safety and efficacy EBRT in combination with various chemotherapeutic regimens, for the most part among individuals with head and neck cancer (34 studies), esophageal cancer (32 studies), pancreatic carcinoma (25 studies), and NSCLC (19 studies). Finally, approximately 70 of these trials evaluate the therapeutic profile of EBRT combined with targeted anticancer agents, including tumor-targeting mAbs such as the epidermal growth factor receptor (EGFR)-specific molecule cetuximab,\textsuperscript{142-145} or with various alternative non-immunotherapeutic interventions, like hyperthermia or nanoparticles. Since all these studies do not involve bona fide immunotherapeutic agents, we will not discuss them in further detail here. Rather, we will focus on 95 clinical trials initiated between 2014 July 1st and 2016 May 1st that aim to evaluate the safety and efficacy of EBRT combined with immunomodulatory mAbs including checkpoint blockers (66 studies), adoptive cell transfer (4 studies), TLR agonists (4 studies), DC-based vaccination (5 studies), recombinant cytokines (4 studies), peptide-based vaccines (3 studies), oncolytic virotherapy (2 studies), or other immunomodulatory agents (10 studies) (source: https://clinicaltrials.gov/).

The safety and efficacy of EBRT combined with the FDA-approved CTLA4-targeting checkpoint blocker ipilimumab\textsuperscript{96,146,147} alone or with ipilimumab plus the experimental TLR9 agonist SD-101\textsuperscript{94,148-150} is being assessed in cohorts of melanoma patients (NCT02406183, NCT02662725, NSCLC patients (NCT02221739),\textsuperscript{151} lymphoma patients (NCT02254772),\textsuperscript{152} and individuals with advanced solid tumors (NCT02239900). EBRT is being tested together with nivolumab, an FDA-approved checkpoint blocker targeting programmed cell death 1 (PDCD1; best known as PD-1)\textsuperscript{153,155} alone or in combination with cytotoxic chemotherapy or targeted anticancer agents, in patients with breast carcinoma (NCT02499367), glioblastoma (NCT02617589, NCT02667587), head and neck squamous cell carcinoma (HNSCC) (NCT02684253, NCT02764593), melanoma (NCT02716948), and NSCLC (NCT02768558). In addition, EBRT plus a combined immunotherapeutic regimen involving both ipilimumab and nivolumab is being assessed for safety and efficacy in individuals affected by melanoma (NCT02659540) or intracranial metastases originated from NSCLC (NCT02696993). The clinical profile of EBRT given in combination with yet another FDA-approved PD-1-targeting checkpoint blocker, i.e.,
Table 1. Clinical trials recently started to investigate the safety and efficacy of EBRT plus immunostimulatory antibodies in cancer patients.

<table>
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<th>Antibody</th>
<th>Indication(s)</th>
<th>Phase</th>
<th>Status</th>
<th>Type of RT</th>
<th>Notes</th>
<th>Ref.</th>
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<td>NCT02562625</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>II</td>
<td>Not yet recruiting</td>
<td>3D-EBRT, IMRT</td>
<td>Combined with carboplatin and paclitaxel</td>
<td>NCT02621398</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/I</td>
<td>Recruiting</td>
<td>SBRT, WFRT</td>
<td>None</td>
<td>NCT02444741</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>Not yet recruiting</td>
<td>SFRT</td>
<td>None</td>
<td>NCT02658097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>Recruiting</td>
<td>SBRT</td>
<td>None</td>
<td>NCT02492568</td>
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<tr>
<td>Pancreatic cancer</td>
<td>Recruiting</td>
<td>EBRT</td>
<td>Combined with capecitabine</td>
<td>NCT02305186</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>Not yet recruiting</td>
<td>SBRT</td>
<td>Combined with a genetically-modified allogenic cancer cell-based vaccine and cyclophosphamide</td>
<td>NCT02684282</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
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<td>SBRT</td>
<td>None</td>
<td>NCT02599779</td>
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<td>SCLC</td>
<td>I</td>
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<td>EBRT</td>
<td>Combined with multimodal chemotherapy</td>
<td>NCT02402920</td>
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</tr>
<tr>
<td>Solid tumors</td>
<td>I</td>
<td>Not yet recruiting</td>
<td>SBRT</td>
<td>None</td>
<td>NCT02608385</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Recruiting</td>
<td>HRT</td>
<td>None</td>
<td>NCT02303990</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>I</td>
<td>Recruiting</td>
<td>EBRT</td>
<td>None</td>
<td>NCT02318771</td>
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<td></td>
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<td>I/I</td>
<td>Recruiting</td>
<td>SBRT</td>
<td>None</td>
<td>NCT02407171</td>
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<tr>
<td>Thoracic tumors</td>
<td>Solid tumors</td>
<td>I</td>
<td>Not yet recruiting</td>
<td>EBRT</td>
<td>Combined with paclitaxel</td>
<td>NCT02587455</td>
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<tr>
<td>Tremelimumab</td>
<td>Breast carcinoma</td>
<td>EBRT</td>
<td>Recruiting</td>
<td>SRS, WBRT</td>
<td>None</td>
<td>NCT02383212</td>
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<td>Varilumab</td>
<td>Prostate cancer</td>
<td>Recruiting</td>
<td>EBRT</td>
<td>None</td>
<td>NCT02563925</td>
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Abbreviations: 3D-CRT, 3D conformal radiotherapy; CAPOX, capecitabine plus oxaliplatin; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EBRT, external beam radiation therapy; HIGRT, hypofractionated image-guided radiotherapy; HNSCC, head and neck squamous cell carcinoma; HRT, hypofractionated radiation therapy; HSRT, hypofractionated stereotactic radiation therapy; IFN-β, interferon β; IMRT, intensity-modulated radiation therapy; NSCLC, non-small cell lung carcinoma; SART, stereotactic ablation radiation therapy; SBRT, stereotactic body radiation therapy; SCLC, small cell lung carcinoma; SRS, stereotactic radiosurgery; WBRT, whole brain radiation therapy; WFRT, wide-field radiation therapy. ° initiated between 2014, July 1st and 2016, May 1st; † anti-PD-1 fusion protein.
As for immunotherapies not based on checkpoint blockers and other immunostimulatory antibodies, EBRT is being evaluated in combination with: (1) autologous DCs expanded ex vivo in the presence of tumor cell lysates in children with advanced solid tumors (NCT02496520) or in Grade IV glioma patients (NCT02772094); (2) unmodified autologous DCs re-infused upon expansion ex vivo, in patients with NSCLC concurrently receiving standard-of-care platinum-based chemotherapy (NCT02662634); (3) an autologous DC-based vaccine specific for mutant isocitrate dehydrogenase (NADP⁺) 1, cytosolic (IDH1) (NCT02771301); and (4) vaccines based on TAA-derived peptides or heat-shock protein (HSP)-enriched preparations of tumor lysates in glioma patients (NCT02287428, NCT02722512) or women with cervical carcinoma concurrently receiving cisplatin-based chemotherapy (NCT02722512); (5) FDA-approved or experimental oncolytic viruses in individuals with soft tissue sarcoma (NCT02453191) or children with brain malignancies (NCT02457845) (Table 1).

In addition, the safety and efficacy of EBRT combined with immunotherapy is being assessed in the context of (1) adoptive cell transfer, in colorectal cancer patients receiving autologous DCs plus cytokine induced killer (CIK) cells along with FOLFOX (folinic acid plus 5-fluorouracil plus oxaliplatin) chemotherapy (NCT02209298), sarcoma patients treated with autologous CD8⁺ cytotoxic T lymphocytes (CTLs) genetically modified to recognize the TAA NY-ESO-1 (NCT02319824), and hepatocellular carcinoma patients receiving highly purified autologous CD8⁺ CTLs (NCT02678013); (2) TLR stimulation, in soft tissue sarcoma patients receiving the experimental TLR4 agonist glycoporphin A lipid acid stable emulsion (GLA-SE) (NCT02180698), lymphoma patients concurrently administered with the experimental TLR9 agonist SD-1 (NCT02266147), and melanoma patients co-treated with the FDA-approved TLR7 agonist imiquimod (NCT02394132); and (3) relatively unspecific immunostimulation with recombinant IL-2 or GM-CSF in patients with renal cell carcinoma (NCT02306954), glioblastoma (NCT0263440), and NSCLC (NCT02735850), with thymalfasin (a recombinant version of the human Tlr7-1 skewing peptide thymosin α1) in colorectal cancer patients (NCT02535988), lung cancer patients (NCT02542137, NCT02542930), and esophageal cancer patients (NCT02545751), with TAM-targeting agents like trabectedin or zoledronic acid in subjects with soft tissue sarcoma (NCT02275286) or metastatic NSCLC (NCT02480654), with a chemical inhibitor of IDO1 (i.e., indoximod) in children with brain tumors concurrently receiving temozolomide-based chemotherapy (NCT02502708), with chemical inhibitors of the TGFβ1 receptor in breast carcinoma patients (NCT02538471) and rectal carcinoma patients concurrently treated with standard-of-care chemotherapy (NCT026887129), and with
celexcoxib, an inhibitor of the immunosuppressive enzyme prostaglandin-endoperoxide synthase 2 (PTGS2; best known as COX2), in HNSCC patients (NCT02739204).\(^1\) With a single exception, all these studies are ongoing (i.e., their status is or has been “Active, not recruiting,” “Not yet recruiting,” or “Recruiting” by official sources). NCT02662725, a Phase II clinical trial testing stereotactic radiosurgery plus ipilimumab-based immunotherapy in melanoma patients with brain metastases, appears as “Completed.” To the best of our knowledge, however, these results have not yet been disseminated (sources: [https://clinicaltrials.gov/](https://clinicaltrials.gov/); [http://www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed); and [meetinglibrary.asco.org/abstracts](meetinglibrary.asco.org/abstracts)).

**Concluding remarks**

Total-body irradiation has been extensively employed in the clinic as a myelo- and lymphoablating measure to pre-condition hematopoietic stem cell transplantation recipients. Nonetheless, it is now well established that the localized, targeted irradiation of malignant lesions in the context of dose...
fractionation within the standard therapeutic range promotes direct antineoplastic effects while eliciting a therapeutically relevant anticaner immune response.\textsuperscript{234} Thus, radiation therapy currently stands out as an accessible and promising tool for improving the efficacy of immunotherapeutic agents as diverse as checkpoint blockers, immunostimulatory antibodies, anti-cancer vaccines, oncolytic viruses, recombinant cytokines, TLR agonists, and small molecules that repolarize the tumor micro-environment. The clinical activity of all these immunotherapeutic interventions (and presumably that of many chemotherapeutic agents as well)\textsuperscript{235} relies indeed on the activation of a robust and polyclonal tumor-specific immune response, and radiation therapy has been convincingly demonstrated to promote such a response by favoring the release of immunostimulatory signals by dying cancer and stromal cells, hence improving their adjuvanticity.\textsuperscript{31,235} Intriguingly, fractionated radiation appears to be superior to single-dose radiation therapy in its capacity to trigger anticaner immune responses \textit{in vivo}.\textsuperscript{64,236} This has been linked to improved capacity of fractionated radiation (as compared to single-dose radiation therapy) to induce the release of damage-associated molecular patterns (DAMPs) by the tumor.\textsuperscript{237,238} In addition, it may reflect (at least in part) the capacity of fractionated (but not single-dose) radiation to temporarily allow for the survival of malignant cells accumulating genetic and genomic defects that result in exacerbated antigenicity.\textsuperscript{239-241} This intriguing hypothesis has not yet been formally addressed. Irrespectively, by virtue of its well-established efficacy and safety profile, radiation therapy lies together with chemotherapy and immunotherapy at the core of a multimodal therapeutic regimen that holds great promise for the future of clinical tumor immunology.

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No potential conflicts of interest were disclosed.

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