Trial Watch
Anticancer radioimmunotherapy

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Radiotherapy has extensively been employed as a curative or palliative intervention against cancer throughout the last century, with a varying degree of success. For a long time, the antineoplastic activity of X- and γ-rays was entirely ascribed to their capacity of damaging macromolecules, in particular DNA, and hence triggering the (apoptotic) demise of malignant cells. However, accumulating evidence indicates that (at least part of) the clinical potential of radiotherapy stems from cancer cell-extrinsic mechanisms, including the normalization of tumor vasculature as well as short- and long-range bystander effects. Local bystander effects involve either the direct transmission of lethal signals between cells connected by gap junctions or the production of diffusible cytotoxic mediators, including reactive oxygen species, nitric oxide and cytokines. Conversely, long-range bystander effects, also known as out-of-field or abscopal effects, presumably reflect the elicitation of tumor-specific adaptive immune responses. Ionizing rays have indeed been shown to promote the immunogenic demise of malignant cells, a process that relies on the spatiotemporally defined emanation of specific damage-associated molecular patterns (DAMPs). Thus, irradiation reportedly improves the clinical efficacy of other treatment modalities such as surgery (both in neo-adjuvant and adjuvant settings) or chemotherapy. Moreover, at least under some circumstances, radiotherapy may potentiate anticancer immune responses as elicited by various immunotherapeutic agents, including (but presumably not limited to) immunomodulatory monoclonal antibodies, cancer-specific vaccines, dendritic cell-based interventions and Toll-like receptor agonists. Here, we review the rationale of using radiotherapy, alone or combined with immunomodulatory agents, as a means to elicit or boost anticancer immune responses, and present recent clinical trials investigating the therapeutic potential of this approach in cancer patients.

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Abbreviations: 3D-CRT, 3D-conformal radiation therapy; ACT, adoptive cell transfer; AGER, advanced glycosylation end product-specific receptor; APC, antigen-presenting cell; CRT, calreticulin; CT, computed tomography; CRC, colorectal carcinoma; CTLA4, cytotoxic T lymphocyte-associated protein 4; DAMP, damage-associated molecular pattern; DC, dendritic cell; EBRT, external-beam radiation therapy; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; GM-CSF, granulocyte macrophage colony-stimulating factor; HMGB1, high mobility group box 1; HNC, head and neck carcinoma; ICAM1, intercellular cell adhesion molecule 1; ICD, immunogenic cell death; IFN, interferon; IFRT, involved-field radiation therapy; IGRT, image-guided radiation therapy; IL, interleukin; IMRT, intensity-modulated radiation therapy; MIP-1α, macrophage inflammatory protein 1α; MRI, magnetic resonance imaging; PANX1, pannexin 1; PET, positron emission tomography; SBRT, stereotactic body radiation therapy; SIRT, selective internal radiation therapy; SRS, stereotactic radiosurgery; RIPK1, receptor-interacting protein kinase 1; RCC, renal cell carcinoma; RNS, reactive nitrogen species; ROS, reactive oxygen species; TBI, total body irradiation; TGFβ1, transforming growth factor β1; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular cell adhesion molecule 1; VCAM1, vascular endothelial growth factor

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Introduction

Origins and use of radiation oncology. In 1895, the German physicist Wilhelm Conrad Röntgen was the first to produce and detect an electromagnetic radiation with a wavelength corresponding to modern X-rays, marking the beginning of a decade that witnessed several pioneering discoveries in the same field, including that of natural radioactivity by the French physicist Antoine Henri Becquerel (in the same year) and that of radium as a natural source of γ-rays by the Polish physicist and chemist Marie Curie (in 1898).1 As soon as in 1903, the Royal Swedish Academy of Sciences awarded Henri Becquerel, Marie Curie and her French husband Pierre the Nobel Prize in Physics, “in recognition of the extraordinary services they have rendered by their joint researches on the radiation phenomena.”1 Incredibly, no more than 60 d after Röntgen’s discovery, the American physician Emil Grubbe employed X-rays to treat a woman bearing recurrent breast carcinoma, de facto establishing the field of radiation oncology.1

Throughout the last century, along with huge technological advances that allowed for the increasingly more accurate (in both anatomical and quantitative terms) delivery of X and γ-rays, or charged particles (i.e., protons and electrons) to malignant lesions, irradiation has been extensively employed as an antineoplastic intervention, either alone or in combination with other therapeutic modalities, with a variable degree of success.1,2 Thus, according to current estimates, approximately 50% of all cancer patients have already received or will receive some form of radiation therapy, either as a curative intervention (i.e., aimed at eradicating a tumor and/or preventing recurrence) or as a palliative maneuver (i.e., intended to relieve the pain/discomfort caused by neoplastic lesions at specific anatomical locations, such as bones as well as peri-esophageal, peri-spinal, and cerebral areas).3,4 Nowadays, ionizing irradiation is frequently administered in the context of chemotherapeutic regimens and/or surgery, either as a neo-adjuvant, an intraoperative, or an adjuvant intervention. In particular, radiotherapy is employed prior to surgery as a means to reduce tumor size, hence (1) allowing for, or minimizing the anatomical/esthetic impact of, the procedure; and (2) reducing the likelihood of disease recurrence owing to residual neoplastic cells. Intraoperative radiation therapy is advantageous (1) since it sometimes allow for the treatment of neoplastic lesions that are anatomically too close to healthy tissue/organs; and (2) as nearby normal tissues can be properly shielded from irradiation. Finally, adjuvant radiotherapy frequently represents an efficient means of reducing the risk of recurrence, for instance among breast carcinoma patients subjected to lumpectomy of radical mastectomy.1,6

Types of radiotherapy. Depending on how the source of radioactivity is employed to specifically target malignant lesions, radiotherapy can be roughly sub-divided into 2 large groups: (1) external-beam radiation therapy (EBRT), including a wide range of technical variants; and (2) internal radiotherapy, which can be further discriminated into brachytherapy and systemic radiation therapy.2,7 EBRT most often involves a linear accelerator, i.e., a machine that generates X- or γ-rays that are collimated on malignant lesions across the intact skin. Conversely, brachytherapy relies on the seeding of tiny radioactive pellets within the tumor mass (interstitial brachytherapy), in a adjacent cavity—be it surgical or natural—via needles or catheters (intracavitary brachytherapy), while systemic radiation therapy is based on the oral or intravenous administration of a radionuclide, frequently coupled to a tumor-targeting monoclonal antibody.7,8 A peculiar type of intracavitary brachytherapy is represented by plaque radiotherapy, which is frequently employed for the management of uveal and choroidal melanoma.9-11 This approach involves a thin, concave metal plate containing radioactive seeds (often of 125I) that is sewn to the outer surface of the ocular globe. For how it is constructed (with radioactive seeds facing inward), this medical device allows for the relatively focused delivery of ionizing radiation to the eye (and hence the tumor) while protecting other tissues from exposure.9-11

Common types of EBRT include (1) 3D-conformal radiation therapy (3D-CRT), which relies on a sophisticated computer software to deliver X- or γ-rays to precisely defined target areas;12,13 (2) intensity-modulated radiation therapy (IMRT), using hundreds of (stationary or mobile) collimators to treat different zones of the malignant lesions or nearby tissues (see below) with different beam intensities;14,15 (3) image-guided radiation therapy (IGRT), during which repeated scans by computed tomography (CT), positron emission tomography (PET), or magnetic resonance imaging (MRI) are performed to monitor changes in the size and precise location of the tumor, allowing to adjust dose and patient’s position if required;16-18 (4) tomotherapy, a peculiar type of image-guided IMRT based on a machine that can completely rotate around the patient, thus resembling a normal CT scanner;19 (5) stereotactic radiosurgery (SRS), which uses an extremely accurate image-guided tumor targeting and patient positioning to deliver high doses of X- or γ-rays to small neoplastic lesions;20-22 (6) stereotactic body radiation therapy (SBRT), delivering X- or γ-rays to small, isolated tumors, often in fewer sessions while using smaller radiation fields and higher doses than 3D-CRT;23,24 (7) involved-field radiation therapy (IFRT), which specifically targets tumor-affected tissues (a radiotherapeutic mode frequently employed for the treatment of lymphoma patients);25,26 and (8) proton or electron therapy, in which neoplastic lesions are treated with charged particles (photons) instead of a purely electromagnetic wave.27,28 Along similar lines, brachytherapy can be performed in various modalities, for instance as a low vs. high dose-rate treatment, or based on temporary or permanent sources of radioactivity. The MammoSite® system (commercialized by Hologic, Inc.) is a well-known device for the delivery of high dose-rate, temporary brachytherapy to breast carcinoma patients.29 Finally, systemic radiotherapy can be based on naked radionuclides such as 131I, which is specifically taken up by thyrocytes and hence can be used for the treatment of thyroid carcinoma,30 or on radionuclides coupled to tumor-targeting monoclonal antibodies. Two prominent examples of this approach—which is also known as selective internal radiation therapy (SIRT)—are provided by 90Y-ibritumomab tiuxetan and 131I-tositumomab, 2 radionuclide-coupled anti-CD20 monoclonal antibodies that are currently approved for the treatment of lymphoma patients.31,32 Each of these radiotherapeutic modalities
is associated with specific advantages and drawbacks that render it particularly suitable for the treatment of a specific subset of tumors. A detailed discussion of these aspects largely exceeds the scope of the present Trial Watch and can be found in refs. 2 and 7.

Side effects of radiotherapy. As all other antineoplastic agents, radiotherapy is associated with both acute and chronic side effects, stemming from the inevitable (though increasingly more controlled) irradiation of healthy tissues.33-36 Acute side effects mainly reflect the damage of highly proliferating cells that reside in anatomical regions exposed to irradiation, in a majority of cases including the skin. In addition, irradiated patients often experience fatigue, regardless of the exposed part of the body, and nausea/vomiting, especially when the abdomen and brain are treated.7,47 All these side effects generally resolve within a few days/weeks upon the interruption of radiation therapy. In some cases, however, the damage to highly proliferating cell compartments is permanent, potentially resulting in chronic diarrhea, intestinal bleeding, and infertility. Moreover, radiotherapy is associated with a quantifiable increase in the risk of developing a secondary, treatment-induced cancer later in life.38,48 Such a risk is generally highest among patients who have been exposed to radiation therapy as children or adolescents.41,42 Of note, a small rim of normal tissue immediately surrounding neoplastic lesions is always irradiated on purpose, for 2 reasons: first, to accommodate for some degree of displacement of the tumor mass that may normally result from breathing or from the physiological movement of internal organs; and second, to reduce the likelihood of disease recurrence owing to malignant cells that have invaded adjacent tissues.1,43

Throughout the last 50 y, several approaches have been developed to minimize the acute and chronic side effects of radiation therapy, including technical/strategic improvements as well as chemicals that operate as “radiosensitizers,” thus exacerbating the propensity of malignant cells to succumb to irradiation, or “radioprotectors,” shielding non-transformed cells side from the cytotoxic effects of radiotherapy.2,44-46 The common aim of all these strategies is to minimize the amount of damage experienced by normal tissues (to limit side effects), while maximizing that experienced by malignant cells (to improve efficacy), i.e., to widen the therapeutic window of radiation therapy.46,47 By far the most commonly employed means to achieve this goal is fractionation, i.e., the delivery of radiotherapy in multiple sessions (spaced by at least 6 h) over several weeks, which is advantageous mainly as (1) it increases the likelihood of malignant cells to be exposed to irradiation when they are more vulnerable to it (i.e., not in the S phase of the cell cycle, see below); (2) it efficiently compensates for accelerated repopulation, i.e., the propensity of the neoplastic cells that survive radiotherapy to proliferate at increased rates; and (3) it allows time to normal cells for repairing irradiation-induced damage.1 In addition, several distinct molecules have been demonstrated (in preclinical models) to efficiently sensitize cancer cells to the cytotoxic effects of radiation therapy, including DNA-damaging agents, cell cycle checkpoint inhibitors, and chemicals that increase oxygenation (see below). Along similar lines, a consistent experimental effort has been dedicated to the development of distinct strategies for radioprotection, including the (local) administration of radical scavengers (which minimize radiotherapy-induced damage at the molecular level, see below),48-51 apoptosis inhibitors (to arrest the cellular demise of irradiated normal cells),52-54 growth factors (which stimulate tissue reconstitution),55-58 and immunomodulatory agents (to prevent the establishment of a cytotoxic inflammatory milieu).59-63 This said, amifostine (a radical scavenger also known as Ethyol®) is the only drug currently approved by FDA for use in humans as a radioprotector.64-66

How radiation therapy works. Irradiated cells (be they malignant or normal) absorb high amounts of energy in the form of photons or charged particles, promoting some extent of direct macromolecular damage as well as the generation of highly diffusible reactive oxygen and nitrogen species (ROS and RNS, respectively), which de facto underpin the cytotoxic potential of radiation therapy.33,67 Indeed, both free radicals and molecular oxygen appear to be required for the stabilization of DNA damage, a concept known as the “oxygen fixation” hypothesis.68,70 Thus, a good level of oxygenation is a conditio sine qua non for neoplastic cells to respond to radiotherapy, in vitro and in vivo.71-75 Oxygen concentrations less than 0.02% (0.15 mmHg) decrease the vulnerability of cancer cells to ionizing radiation by 2- to 3-fold,76 and even milder degrees of hypoxia (oxygen concentration 1%, 8 mmHg)—which are commonly found in human tumors—produce an appreciable level of radio- and chemo- resistance.77 In line with this notion, various strategies have been developed in the attempt to radiosensitize neoplastic lesions by means of an increased supply of oxygen, including the ventilation of irradiated patients with hyperbaric oxygen (most often a 95% O₂, 5% CO₂ mix)78,79 and the administration of drugs that reduce the binding of oxygen to hemoglobin, such as eparprol.80,81 Both these approaches exert radiosensitizing effects as they reduce the so-called “hypoxic fraction,” i.e., the percentage of tumor cells exposed to subphysiological oxygen tensions. Alternatively, radiosensitization has been achieved with compounds that selectively target hypoxic cells, such as the 5-nitroimidazole nimorazole and tirapazamine analogs.82-84

The damage inflicted by radiation therapy to macromolecules, in particular DNA and lipids, generally activates the intrinsic pathway of apoptosis, which executes cell death upon the irreversible permeabilization of mitochondrial membranes.85,86 As an alternative, irradiated cells enter senescence, a permanent proliferative arrest manifesting with a series of stereotyped phenotypic and biochemical traits.87,89 Both these processes can be under the control of p5380-82 and often, but not always, ensue the activation of mitotic catastrophe, an oncosuppressive mechanism ensuring the elimination of mitosis-incompetent cells.53,54 In addition, both DNA damaging agents and oxidative stress have been shown to induce instances of programmed necrosis,93-97 including the receptor-interacting protein kinase 1 (RIPK1)-dependent necrotic modality known as necroptosis.98-100

It is therefore not surprising that for decades the therapeutic effects of ionizing radiation have been entirely (but incorrectly) ascribed to the direct cytotoxic or cytostatic activity of X- and γ-rays.1 However, robust preclinical and clinical evidence indicates that (at least part of) the therapeutic efficacy of irradiation
results from local and long-range bystander effects. The former can originate from the transmission of lethal signals via gap junctions, multimeric pores that allow for the exchange of 1000–1500 kDa molecules, including nucleotides, Ca²⁺ ions and ROS, between adjacent cells. In addition, local bystander effects can ensue the release by irradiated (neoplastic and immune) cells of soluble mediators that exert a direct or indirect cytotoxic activity on non-irradiated neighboring cells, including ROS, RNS, and several cytokines such as interleukin (IL)-6, IL-8, transforming growth factor β1 (TGFβ1), and tumor necrosis factor α (TNFα). Conversely, long-range bystander effects, also known as out-of-field or abscopal effects, are thought to reflect the elicitation of an adaptive immune response against malignant cells. Similar to some chemotherapeutic and photodynamic therapy, ionizing irradiation promotes indeed the immunogenic demise of malignant cells, a process that relies on the spatiotemporally-defined emission of specific damage-associated molecular patterns (DAMPs). Radiotherapy has also been shown to favor the normalization of the tumor vasculature, hence facilitating the delivery of chemotherapeutic agents administered via the systemic route. Radiotherapy can promote the infiltration of malignant lesions by effector (as opposed to regulatory) immune cells. At least in part, the ability of ionizing radiation to stimulate the recruitment of immune cells to the tumor microenvironment reflects the fact that irradiated endothelial cells express increased levels of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) on their surface. Whether the upregulation of adhesion molecules on the surface of irradiated endothelial cells results from a strictly cell-intrinsic mechanism or involves the secretion of autocrine/paracrine mediators remains to be elucidated. Thus, the local (and perhaps also the long-range) therapeutic effects of radiotherapy appear to involve a prominent vascular component.

Following the tradition of our monthly Trial Watch series, here we discuss the rationale of using radiotherapy, alone or combined with immunomodulatory interventions, as a means to elicit or boost antitumor immune responses, and review recent clinical trials investigating the therapeutic potential of this approach in cancer patients. Besides being used as an antineoplastic intervention, high-dose radiotherapy—most often in the form of total body irradiation (TBI)—is routinely employed as a conditioning regimen in patients bearing hematological malignancies allocated to receive hematopoietic stem cell transplantation. The therapeutic value of radiation therapy in this setting stems from the ability of TBI to directly kill neoplastic progenitors in the bone marrow and promote a severe state of immunodeficiency (rather than immunostimulatory effects), which is required for engraftment. Thus, the use of TBI as a pre-transplantation conditioning regimen will not be further discussed here.

**Immunogenic Effects of Radiotherapy**

As mentioned above, an increasing amount of preclinical and clinical evidence indicates that the therapeutic potential of ionizing irradiation does not simply reflect the cytotoxic activity of X- and γ-rays, but rather involves local and/or distant bystander effects. Thus, therapeutic (as opposed to unwarranted) abscopal reactions have been documented in patients bearing a wide variety of neoplasms, including (but presumably not limited to) lymphoma, melanoma, primary and metastatic breast carcinoma, adenocarcinoma, sarcoma, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma (RCC), and uterine cancer. Initially, clinicians tended to consider the abscopal effect as a straightforward systemic consequence of the local release of immunostimulatory and cytotoxic cytokines, mainly TNFα and IL-18, by irradiated (malignant and immune) cells. However, it is now clear that—at least under specific circumstances—radiation therapy elicits an adaptive immune response against malignant cells that is capable of mediating robust antineoplastic effects on non-irradiated lesions. At least 3 distinct lines of evidence support this interpretation. First, an elevation in the circulating levels of immunomodulatory cytokines including TNFα and IL-18 would be expected to cause a rather unspecific activation of the immune system, and hence to have a very limited (if any) impact on neoplasms other than melanoma and RCC, which are per se extremely immunogenic and immunosensitive. In line with this notion, the administration of recombinant cytokines (notably IL-2) as standalone therapeutic interventions has been associated with considerable rates of objective clinical responses only among melanoma and RCC patients, while abscopal effects have been documented in individuals bearing several other neoplasms (see above). Second, the abscopal effect can be boosted by various immunostimulatory preparations, including (but presumably not limited to) bone marrow-derived dendritic cells (DCs), IL-2, TGFβ1-blocking strategies, and monoclonal antibodies specific for immunological checkpoint regulators such as cytotoxic T lymphocyte-associated protein 4 (CTLA4).

Third, abscopal effects have been correlated with the induction of interferon γ (IFNγ)-producing CD8⁺ T lymphocytes and have been shown to rely on the presence of both CD4⁺ and CD8⁺ T cells. The mechanisms whereby radiotherapy elicits tumor-specific immune responses have just begun to emerge. Indeed, the (most often) apoptotic demise of irradiated cancer cells has long been viewed as an immunologically silent—if not tolerogenic—event, reflecting the textbook notion that apoptotic corpses are rapidly taken up by professional phagocytes while delivering robust anti-inflammatory signals. Rather, it is now clear that—in response to specific stimuli—malignant cells can undergo apoptosis while emitting a spatiotemporally-defined sequence of danger signals that the immune system translates into a tumor-specific adaptive immune response. Importantly, together with anthracyclines (e.g., doxorubicin, mitoxantrone), oxaliplatin, cyclophosphamide, and hypericin-based photodynamic therapy, ionizing irradiation constitutes a bona fide inducer of immunogenic cell death (ICD), i.e., is capable of killing neoplastic cells while transforming them into a vaccine that efficiently prompts synergetic mice against a subsequent challenge with cancer cells of the same type.
ICD has been shown to rely on (at least) 3 main determinants: (1) the pre-apoptotic exposure of the endoplasmic reticulum (ER) chaperone calreticulin (CRT) on the outer leaflet of the plasma membrane, constituting a prominent “eat-me signal” for professional antigen-presenting cells (APCs), including DCs;199,192-194 (2) the autophagy-dependent and pannexin 1 (PANXI)-mediated secretion of ATP in the blebbing phase of apoptosis, operating both as a “find-me signal” for APCs and as a potent pro-inflammatory cue;195-202 and (3) the post-apoptotic release of the non-histone chromatin-binding protein high mobility group box 1 (HMGB1), which—in its oxidized form—reportedly conveys pro-inflammatory stimuli upon ligation of TLR4 and/or advanced glycosylation end product-specific receptor (AGER).203-206 In addition, dying cells expose or release several other DAMPs that per se are not immunogenic, yet operate as potent adjuvants, such as various mitochondrial products.118 In conditions in which CRT, ATP, and HMGB1 (and perhaps other DAMPs) cannot be properly emitted by dying cancer cells, cannot be sensed by APCs, or cannot be translated into an adaptive immune response, the therapeutic efficacy of various ICD inducers is significantly reduced.115,197,202 Intriguingly, this holds true in some, but not all, preclinical and clinical settings,207 implying that some tumors may be more susceptible than others to immune responses, be them natural or elicited by therapy, and hence to the abscopal effect. The precise impact of specific DAMPs in the therapeutic efficacy of radiation therapy in vivo, however, has not yet been systematically investigated.

Irrespective of this hitherto poorly characterized aspect of the immunogenic demise of cancer cells as induced by X- and γ-rays, these observations indicate that (a large fraction of) the therapeutic efficacy of ionizing irradiation stems from its ability to provoke ICD coupled to the release of potent pro-inflammatory mediators, de facto eliciting a robust tumor-specific immune response.

**Clinical Development of Radioimmunotherapy**

For a long time, the term “radioimmunotherapy” has been used to specifically denote SIRT, i.e., the use of radionuclides coupled to (tumor-targeting) monoclonal antibodies, such as the FDA-approved agents 90Y-ibritumomab tiuxetan (Zevalin®; Cell Therapeutics Inc.) and 131I-tositumomab (Bexxar®; GlaxoSmithKline LLC).208-211 Along with the recent expansion of anticaner immunotherapy, which embraces a large (and incessantly growing) panel of approaches, this expression has gained an ampler meaning and is nowadays employed to refer to the combinatorial or sequential administration of virtually any immunotherapeutic agent plus irradiation.212 As EBRT is licensed by FDA and other international regulatory agencies as a neoadjuvant, intraoperative or adjuvant intervention against a majority of neoplasms, the number of clinical studies that de facto rely on an radioimmunotherapeutic approach, irrespective of whether this was explicitly envisioned a priori or not, is exponentially growing. Thus, interrogating PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) with the string “cancer AND radiotherapy AND patients AND immunotherapy,” on June 13, 2013, returned a total of 3698 entries, of which more than 15% were published after January 1, 2011. In the same period, i.e., during the last 30 mo, no less than 400 distinct clinical trials have been launched to investigate the safety and antineoplastic potential of radioimmunotherapy, all types confounded (source www.clinicaltrials.gov). Of note, (1) the vast majority of these trials also relies on one or more chemotherapeutic agents and/or surgery; and (2) a consistent fraction of these studies does not specifically aim at evaluating the clinical potential of radiotherapy, but rather involves (most often external-beam) irradiation as part of conventional therapeutic regimens. In this setting, radiation therapy is administered only to the active comparator arm of the trial or to both arms (at least in one of which combined with the immunotherapeutic agent that is under investigation).

As it stands, official sources list no less than 177 ongoing (not withdrawn, suspended, terminated or completed on the day of submission) clinical trials initiated during the last 30 mo (that is, after, January 1, 2011) to assess the safety profile and antineoplastic activity of radioimmunotherapeutic regimens (source www.clinicaltrials.gov). Of these, 77 involve tumor-targeting or immunostimulatory monoclonal antibodies, such as the epidermal-growth factor receptor (EGFR)-specific agent cetuximab213,214 and the anti-CTLA4 antibody ipilimumab;215-217 51 immunogenic chemotherapeutics (i.e., cyclophosphamide, anthracyclines, oxaliplatin),214,119 5 DC-based approaches, including the FDA-approved cell preparation known as sipuleucel-T®;130,218-220 3 immunostimulatory cytokines (e.g., IL-2, IFN-α2b);127,139 4 FDA-approved or experimental TLR agonists, such as imiquimod and SD-101,128,129,140,221 4 adoptive cell transfer (ACT) protocols;126,137 1 peptide vaccines;132,222-224 1 oncolytic viruses,138,225,226 and 31 combinatorial strategies comprising at least 2 distinct types of immunotherapy (Table S1).

Neoadjuvant or adjuvant irradiation is routinely employed in the clinics to treat patients affected by multiple variants of head and neck carcinoma (HNC), alone or in combination with chemotherapeutic regimens based on platinum derivatives, most often cisplatin.227-230 In line with this notion, no less than 35 clinical trials initiated in the last 30 mo are currently evaluating the clinical potential of various radioimmunotherapeutic approaches in HNC patients. With 3 notable exceptions, namely NCT01821495 (in which radiotherapy is combined with the administration of DCs and cytokine-induced killer cells), NCT01584284 (testing EBRT coupled to the intravenous administration of an oncolytic virus) and NCT01728480 (investigating the radioprotective potential of a TLR5 agonist), all these studies involve EGFR-targeting antibodies, most often the FDA-approved drug cetuximab or the hitherto experimental agent nimotuzumab (Table 1; Table S1). Irradiation is a frequent therapeutic choice also for anal and colorectal carcinoma (CRC) patients, especially when primary lesions have already metastasized or are attached to internal organs, rendering their complete removal by surgery virtually impossible.232-234 Accordingly, official sources list 29 ongoing clinical trials initiated after January 1, 2011, to test the safety profile and efficacy of radioimmunotherapy in subjects bearing anal, colorectal, or rectal carcinoma. In this setting, the immunotherapeutic component
of the combinatorial regimen is frequently represented by oxaliplatin (a bona fide ICD inducer) and/or by the vascular endothelial growth factor (VEGF)-blocking antibody bevacizumab, both of which are approved by FDA for use in CRC patients. NCT01839539 (in which radiotherapy is combined with conventional chemotherapeutic agents, DCs, and cytokine-induced killer cells), NCT01539824 (investigating the immunostimulatory potential of SBRT given in combination with IMM-101, a TLR2/TLR4 mixed agonist), and NCT01320683 (involving an ⁹⁰Y-conjugated monoclonal antibody specific for the carcinomaembryonic antigen) constitute prominent exceptions to this trend (Table 1; Table S1). No less than 24 ongoing clinical trials have been launched during the last 30 mo to test the safety and efficacy of radioimmunotherapy in individuals affected by hematological neoplasms (mainly lymphoma). In this case, EBRT is often employed as a consolidative treatment upon the administration of a chemotherapeutic regimen involving, among other drugs, cyclophosphamide and doxorubicin (2 ICD inducers). Alternatively, lymphoma patients are frequently allocated to receive ⁹⁰Y-ibritumomab tiuxetan (which specifically targets CD45) or EBRT in combination with various chemotherapeutic regimens involving the FDA-approved anti-CD20 antibody rituximab (Table 1; Table S1).

Most among the 18 clinical trials initiated after January 1, 2011, to assess the safety profile and efficacy of radioimmunotherapy in subjects affected by neuroectodermal and central nervous system tumors involve EBRT given in combination with temozolomide (an alkylating agent) and/or bevacizumab. Only a few among these studies, such as NCT01798004, NCT01526603, and NCT01857934, were initiated to test EBRT as a consolidative therapy upon autologous stem cell transplantation. Along similar lines, a majority of the 19 clinical studies launched during the last 30 mo to test radioimmunotherapeutic approaches in patients with gastric or esophageal carcinoma relies on EBRT in combination with a tumor-targeting monoclonal antibody, most often cetuximab or panitumumab (another FDA-approved EGFR-specific agent). Alternatively, the immunotherapeutic component of such combinatorial approaches is represented by oxaliplatin (Table 1; Table S1). Sixteen clinical trials initiated in the same period aim at investigating the antineoplastic activity of radioimmunotherapy in individuals affected by pancreatic carcinoma. With a few notable exceptions such as NCT01342224 (which involves a telomerase-targeting peptide vaccine given in combination with the granulocyte macrophage colony-stimulating factor [GM-CSF]),¹²⁵,¹²⁶ and NCT01298401 (testing the safety and efficacy of 3D-CRT combined with conventional chemotherapy and ganitumab, an experimental monoclonal antibody specific for the insulin-like growth factor 1 receptor), all these studies involve an oxaliplatin-containing chemotherapeutic cocktail. Of particular interest in this setting is NCT01581307, testing the clinical activity of small glass microspheres (20–30 μm in diameter) containing ⁹⁰Y (TheraSphere), a preparation that is partially approved by FDA for the treatment of hepatocellular carcinoma)²³⁷,²³⁸ administered (via radioembolization) in the context of second line FOLFOX (5-fluouracil, folinic acid, and oxaliplatin) to patients bearing gemcitabine-refractory pancreatic carcinoma with liver metastases (Table 1; Table S1).

During the last 30 mo, additional 36 clinical trials have been launched to test the safety and anticancer activity of several radioimmunotherapeutic protocols in cohorts of patients bearing breast carcinoma (9 studies), melanoma (8 studies), sarcoma (6 studies), prostate carcinoma (4 studies), or other solid tumors (9 studies). The immunotherapeutic component of clinical studies

| Cancer type | Phase | N° | Notes |
|-------------|-------|----|-------|
| Breast carcinoma | I–III | 9 | A heterogeneous panel of radioimmunotherapeutic strategies is being tested in this clinical scenario |
| CRC | I–III | 29 | In a majority of cases, EBRT is administered in the context of the FOLFOX regimen or together with bevacizumab |
| Gastroesophageal carcinoma | I–III | 19 | EBRT is often employed in combination with anti-EGFR monoclonal antibodies or oxaliplatin-based chemotherapy |
| Hematological tumors | I–III | 24 | EBRT is frequently administered as a consolidation therapy, in combination with immunogenic chemotherapeutics or the CD20-targeting monoclonal antibody rituximab |
| HNC | I–IV | 35 | Most studies combine IMRT with monoclonal antibodies specific for EGFR, such as cetuximab or nimotuzumab. |
| Melanoma | I–II | 8 | The most prominent approach involves one form of EBRT, often SBRT, combined with ipilimumab |
| Neuroectodermal and CNS tumors | I–III | 18 | Many of these studies involve the combination of EBRT with conventional chemotherapy plus bevacizumab |
| Pancreatic carcinoma | I–III | 16 | Patients are often allocated to receive one form of EBRT combined with oxaliplatin-based chemotherapy. |
| Prostate cancer | II | 4 | EBRT is often given in combination with sipuleucel-T* |
| Sarcoma | I–II | 6 | Most often, one variant of EBRT is combined with cyclophosphamide- or oxaliplatin-based chemotherapy |
| Others | I–IV | 9 | ⁹⁰Y-based radioembolization is frequently investigated for the treatment of HCC and cholangiocarcinoma patients |

CNS, central nervous system; CRC, colorectal carcinoma; EBRT, external-beam radiation therapy; EGFR, epidermal growth factor receptor; FOLFOX, 5-flurouracil, folinic acid and oxaliplatin; HCC, hepatocellular carcinoma; HNC, head and neck carcinoma; IM rT, intensity-modulated radiation therapy; SBRT, stereotactic body radiation therapy. *Based on clinical trials started after January 1, 2011, and not withdrawn, terminated, or suspended at the day of submission (source www.clinicaltrials.gov). See also Table S1.
involving breast carcinoma patients is relatively heterogeneous, including the FDA-approved ERBB2-specific monoclonal antibody trastuzumab, the TLR7 agonist imiquimod, and ICD inducers such as cyclophosphamide and doxorubicin. Conversely, in this setting melanoma patients are near to invariably allocated to receive one variant of EBRT in combination with ipilimumab. A standalone exception to this trend is represented by NCT01416831, testing whether SBRT can improve the antineoplastic potential of high-dose IL-2. The radioimmunotherapeutic approach is also relatively homogeneous among prostate cancer-related clinical trials, with a majority of studies involving sipuleucel-T. In addition, a rather heterogeneous panel of radiotherapeutic and immunotherapeutic regimens is currently under investigation for the treatment of patients with sarcoma, non-small cell lung carcinoma, pleuropulmonary blastoma, Merkel cell carcinoma, hepatocellular carcinoma, cholangiocarcinoma, and other solid tumors. In this context, some interest appears to be spurred by the use of 90Y-based transarterial radioembolization as an alternative to transarterial chemoembolization for the treatment of hepatocellular carcinoma (NCT01381211; NCT01798160) and cholangiocarcinoma (NCT01798147) (Table 1; Table S1).

Concluding Remarks

Nowadays, combinatorial anticancer therapy is an affirmed clinical practice, reflecting the fact that—perhaps with a few notable exceptions—standalone chemo- or radiotherapeutic regimens are generally unable to control neoplastic lesions. As combining therapeutic agents with dissimilar mechanisms of action potentially results in synergistic antineoplastic effects, this approach presents several advantages over the use of monotherapeutic regimens, including (1) a decrease in the incidence and severity of adverse effects (as in this setting as generally employed at reduced dosages); and (2) a lowered propensity of malignant cells to become chemo- or radioresistant.

Along with the realization that most (if not all) clinically successful anticancer agents operate—at least in part—by eliciting or boosting tumor-specific immune responses, and with the development of efficient means to (re)state anticancer immunity, great interest has been spurred by the possibility to combine chemo-, radio-, and immunotherapeutic regimens. Thus, several preclinical and clinical studies are underway to investigate whether and under which conditions various immunostimulatory agents can be used in combination with each other or with conventional antineoplastic regimens to achieve improved response rates and/or to minimize side effects.

Radioimmunotherapy constitutes a rather peculiar case of this general approach. Indeed, radiation therapy has been extensively used as a conventional anticancer regimen throughout the last century, mostly as it was thought to mediate direct cytotoxic/ cytostatic effects on malignant cells. Preclinical and clinical evidence, however, indicates that the antineoplastic activity of irradiation exceeds that of a merely cytotoxic/cytostatic intervention and rather involves the (re)activation of tumor-specific cellular immune responses. Thus, radiotherapy may per se constitute a combinatorial anticancer regimen, de facto inducing the death of cancer cells while exerting robust immunostimulatory effects. It is therefore tempting to speculate that combining radiation therapy with specific immunomodulatory interventions, such as immunological checkpoint inhibitors or TLR agonists, may result in superior antineoplastic effects, at least in a subset of cancer patients. Well-designed clinical trials are required to formally address this hypothesis.

Some studies indicate that immunosuppressive cells, including CD4+CD25+FOXP3+ regulatory T cells (which potently antagonize antitumor immune responses), may be highly resistant to the cytotoxic effects of ionizing radiation, favoring their preferential increase in the course of therapy. In line with this notion, experimental approaches aimed at specifically depleting regulatory T cells appear to greatly potentiate the antineoplastic potential of radiation therapy in murine tumor models. Several chemotherapeutic agents have been shown to specifically subvert the immunosuppressive functions of regulatory T cells, including gemcitabine and cyclophosphamide (especially when administered according to a metronomic schedule). Thus, it will be particularly interesting to see the results of multiple clinical trials that are currently underway to evaluate the safety and antineoplastic profile of cyclophosphamide-based radioimmunotherapeutic regimens in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental materials may be found here:
http://www.landesbioscience.com/journals/oncoimmunology/article/25595

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