International Journal of Radiation Oncology biology • physics
www.redjournal.org

Introduction

The Promise of Combining Radiation Therapy With Immunotherapy

Justin C. Jagodinsky, BA, Paul M. Harari, MD, and Zachary S. Morris, MD, PhD

Department of Human Oncology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

Received Jan 14, 2020, and in revised form Mar 30, 2020. Accepted for publication Apr 13, 2020.

The development of immunotherapy in oncology builds upon many years of scientific investigation into the cellular mechanics underlying interactions between tumor cells and immune cell populations. The past decade has brought an accelerating pace to the clinical investigation of new immunotherapy agents, particularly in the setting of metastatic disease. The integration of immunotherapy into phase 3 clinical trial design has lagged in settings of advanced locoregional disease, where combination with radiation therapy may be critical. Yet, such may be the settings where immunotherapies have their greatest potential to affect patient survival and achieve curative outcomes. In this review, we discuss the interaction of radiation with the immune system and the potential to augment antitumor immunity through combined-modality approaches that integrate radiation and immunotherapies. The dynamics of cellular and tumor response to radiation offer unique opportunities for beneficial interplay with immunotherapy that may go unrecognized with conventional screening and monotherapy clinical testing of novel pharmaceutical agents. Using immune checkpoint blockade as a primary example, we discuss recent preclinical and clinical studies that illustrate the potential synergy of such therapies in combination with radiation, and we highlight the potential clinical value of such interactions. For various immunotherapy agents, their greatest clinical effect may rest in combination with radiation, and efforts to facilitate systematic investigation of this approach are highly warranted.

Introduction

Radiation therapy is a mainstay of cancer therapy, with more than 60% of patients receiving radiation in the form of definitive, adjuvant, or palliative treatment. Growing evidence dating to the 1970s demonstrates that the immune system contributes to the antitumor effect generated by radiation.[1] Although classically thought of as a locoregional therapy, radiation has the potential to generate out of field "abscopal" antitumor responses,[2] with current evidence suggesting that immunologic mechanisms underscore this effect.[3] Although the abscopal effect is

Note—An online CME test for this article can be taken at https://academy.astro.org.

Corresponding author: Zachary Morris, MD, PhD; E-mail: zmorris@humonc.wisc.edu

Disclosures: J.C.J. has a patent P190102US01 pending to WARF. Z.S.M. reports personal fees from Archeus Technologies, Seneca Therapeutics, and ViewRay outside the submitted work. In addition, Z.S.M. has a patent App No. 15/652,400 with royalties paid to WARF, a patent App No. 15/658,535 with royalties paid to WARF, a patent P180116US01 pending to WARF, a patent App No. 62/728645 pending to WARF, and a patent P190102US01 pending to WARF. The author's work is supported in part by grants from NIH P30 CA014520 (P.M.H., Z.S.M.), NIH P50 DE026787 (P.M.H., Z.S.M.), NIH 1DP5OD024576 (Z.S.M.), NIH T32GM008692 (J.C.J.), and NIH TL1TR002375 (J.C.J.).

Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

Int J Radiation Oncol Biol Phys, Vol. 108, No. 1, pp. 6–16, 2020

https://doi.org/10.1016/j.ijrobp.2020.04.023

exceedingly rare with radiation therapy alone,[4] these observations of immune-mediated effects of radiation have led to growing enthusiasm for the potential of immunotherapies to augment the locoregional efficacy of radiation therapy and conversely for radiation therapy to help prime a more effective systemic antitumor response to immunotherapies.

Immunotherapies are cancer treatments that seek to engage the patient's own immune system to eradicate tumor cells. The historical development of immunotherapy shares many parallels with that of radiation therapy. As with radiation, initial clinical application of an immunotherapy was reported in the late 19th century. William Coley pioneered the use of a bacterial preparation termed *Coley's toxin* in the 1890s. Although the clinical effect was modest, Coley's toxin provided an early demonstration of the potential to generate an antitumor response by harnessing the immune system. Mirroring radiation, immunotherapies gained prominence as a component of standard cancer treatment in the mid- to late-20th century, albeit with considerable toxicities. This included the origins of cell therapies with the development of bone marrow transplant by Fritz Bach and others in the 1960s[5] and the production, testing, and clinical approval of high dose interleukin 2 (IL-2) for metastatic renal cell carcinoma[6] and melanoma in the 1990s.[7]

In the 21st century, more selective targeting significantly reduced the toxicities of both radiation and immunotherapies. In radiation oncology, this resulted largely from technological advances, including highly conformal intensity modulated and stereotactic techniques combined with high-precision image guidance.[8,9] Prominent advances with immunotherapies include the development of antibodies directly targeting tumor cells, immune checkpoint inhibitor antibodies, and chimeric antigen receptor T cell therapies.[10] Immune checkpoint inhibitor antibodies have revolutionized the approach to treating metastatic disease in several cancers, including melanoma, non-small cell lung cancer, and renal cell carcinoma, with some patients experiencing complete responses durable to over 5 years.[11,12] Currently there are approved therapies targeting 2 immune checkpoints: CTLA-4 and PD-1/PD-L1. The increasing specificity of modern radiation therapy and immunotherapies has reduced toxicity profiles and facilitated their increasing roles in clinical oncology. This historical convergence in increased clinical safety and advancement of radiation and immunotherapy now makes feasible their inclusion as part of combined-modality treatment approaches (Fig. 1).

Theoretical Basis for Combining Radiation Therapy and Immunotherapies

The Steel hypothesis, first conceptualized in the 1970s, describes mechanisms whereby combined-modality drug/radiation approaches could improve treatment outcomes.[13] A modernization of the Steel hypothesis has been described, highlighting exploitable interactions of radiation and cancer drugs in the molecular era.[14] Under this revised framework, radiation and immunotherapy agents may interact to improve clinical outcomes through 5 distinct mechanisms: (1) spatial cooperation, (2) temporal modulation, (3) biological cooperation, (4) cytotoxic enhancement, and (5) normal tissue protection.

Through mechanisms described in the following, radiation has the potential to increase susceptibility of tumor cells to immune-mediated killing.[15] These radiated tumor cells also upregulate negative feedback elements (eg, checkpoint proteins), which can dampen the immune response.[16,17] Immunotherapy agents blocking this negative feedback may reinvigorate an immune response that was primed by radiation (Fig. 2). This biological cooperation resulting from intersecting cellular and signaling mechanisms has potential to enable spatial cooperation through generation of systemic immune responses mounted against distant, out-of-field tumors and cytotoxic enhancement via increased immune killing of radiated tumor cells.[2,18]

Responses to immunotherapy often are delayed compared with other forms of cancer treatment and may follow a transient increase in tumor burden. This has prompted development of new criteria for evaluation of response to immunotherapies.[19] This raises concerns that, in rapidly proliferating tumors, patients who would otherwise have mounted an effective immune response may succumb to sequela of transient progression (eg, airway obstruction). Radiation can reduce the growth of such lesions, allowing a greater window of opportunity for response to immunotherapy, thereby eliciting temporal modulation. Immunotherapy also has the potential to promote normal tissue protection, and current investigational strategies include antibody-mediated blocking of radiation-induced fibrosis by targeting TGFβ.

As these examples illustrate, the Steel hypothesis provides a framework for conceptualizing the potential cooperative therapeutic interactions between radiation therapy and immunotherapies. In this article, we review these interactions through a discussion of illustrative preclinical and clinical studies that investigate combinations of radiation and immunotherapy.

Bench to Bedside: Combining Radiation With Immunotherapy to Generate In Situ Vaccination

A growing body of eloquent preclinical work describes the immunogenic effects of radiation on the tumor microenvironment. Radiation can induce immunogenic tumor cell death and release of tumor-specific antigens.[20,21] Tumor cells surviving radiation may not escape unscathed and undergo phenotypic changes in the expression of immune susceptibility markers.[15] Effects on the microenvironment

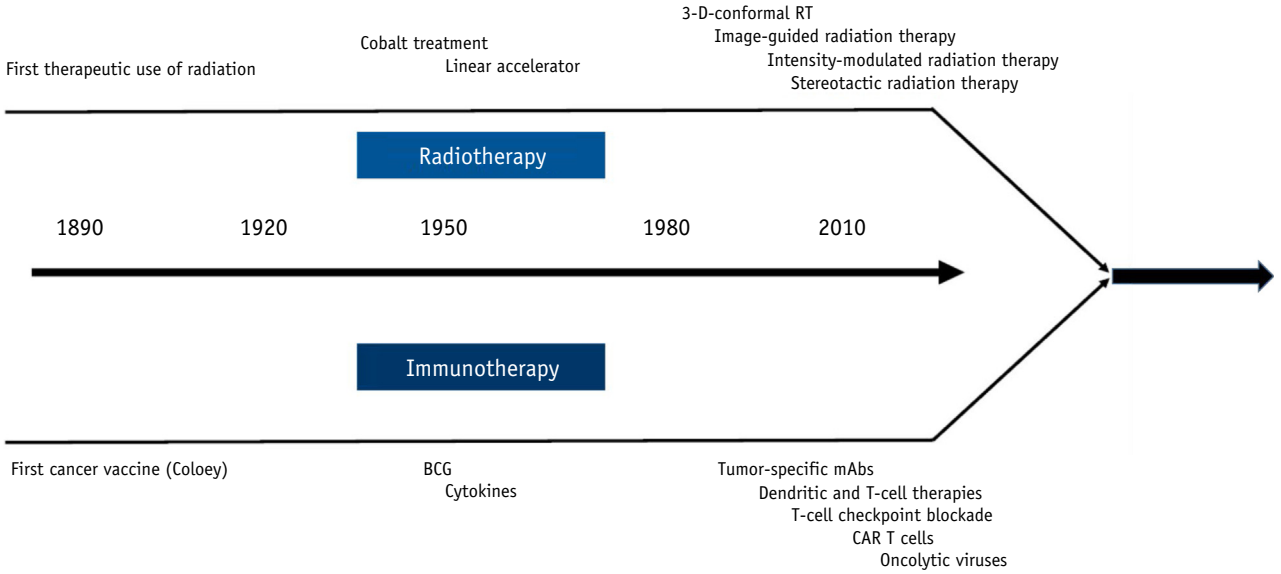

Fig. 1. Historical convergence of radiation therapy and immunotherapy.

include temporary local eradication of radiation-sensitive immune lineages, including suppressor and effector lymphocytes, and local release of inflammatory cytokines and damage-associated molecular patterns resulting in local effects on endothelial cell expression of adhesion receptors, immune cell trafficking, and immune cell activation.[22,23]

On the other hand, radiation also triggers effects in the tumor microenvironment that are potentially detrimental to the development of antitumor immunity. These include delayed increases in tumor infiltration by suppressive regulatory T cells and increased infiltration and activation of inhibitory macrophage and myeloid-derived suppressor cell lineages.[24-28] In addition, certain pathways influenced by radiation can have both positive and negative effects on antitumor immunity and the tumor microenvironment. For example, production of type 1 interferon can induce recruitment of effector T cells and antigen presenting cells, but it can also drive recruitment of myeloid-derived suppressor cells.[29] Additionally, prolonged activation of type 1 and 2 interferon can drive expression of ligands for multiple T cell inhibitory receptors.[30] Targeting such detrimental immunologic effects is an approach whereby immunotherapies may be used to augment the efficacy of radiation therapy.

Dose, fractionation, and volume of radiation influence immunologic effects in the tumor microenvironment. Fractionation of radiation generally enables relative sparing of normal tissues while achieving therapeutic dose delivery to cancer cells. Differences in the capacity and kinetics of DNA damage repair in normal tissues versus tumor cells underlie the rationale for this approach. However, fractionation does not spare adaptive immune cell populations, specifically lymphocytes, which have little capacity for DNA damage repair and undergo apoptosis within hours of exposure to single-fraction doses of just 1 to 3 Gy.[31] Radiation-induced lymphopenia is a negative prognostic factor, and multiple studies indicate that it is positively correlated with field size, dose per fraction, and fraction number.[32,33] Additional clinical data suggest that absolute lymphocyte count is predictive of response to checkpoint blockade and is positively correlated with response rate and duration of response.[34] On the other hand, preclinical studies suggest that despite an initial local depletion of lymphocytes, hypofractionated regimens of radiation may be immune activating.[35] Recent work suggests that standard fractionation and hypofractionation induce expansion of unique immune populations, with standard fractionation favoring a myeloid response and hypofractionation driving a lymphoid response that may be more favorable to adaptive antitumor immunity.[36] Such analyses of fractionation are challenging to control, however, in light of the confounding effects of time and the dynamic nature of changes in tumor-infiltrating immune cells.

Immunogenic tumor cell death increases as a function of increasing dose.[37] High-dose radiation also leads to dose-dependent increases in the expression of MHC-1 and death receptors such as Fas, which are critical for T cell killing of tumor cells.[38,39] In contrast, moderate fractional doses of 8 to 12 Gy may be optimal for activating a type I interferon response in tumor cells via a dose-dependent increase in the cytoplasmic leakage of DNA from micronuclei, which activates the cGAS/STING pathway.[17,40] At higher doses, radiation-induced STING activation may decline in part because of induced expression of Trex1 exonuclease, which reduces the accumulation of cytoplasmic DNA, resulting in negative feedback inhibition.[17] In preclinical studies, activation of the cGAS/STING pathway has been essential for generating radiation-

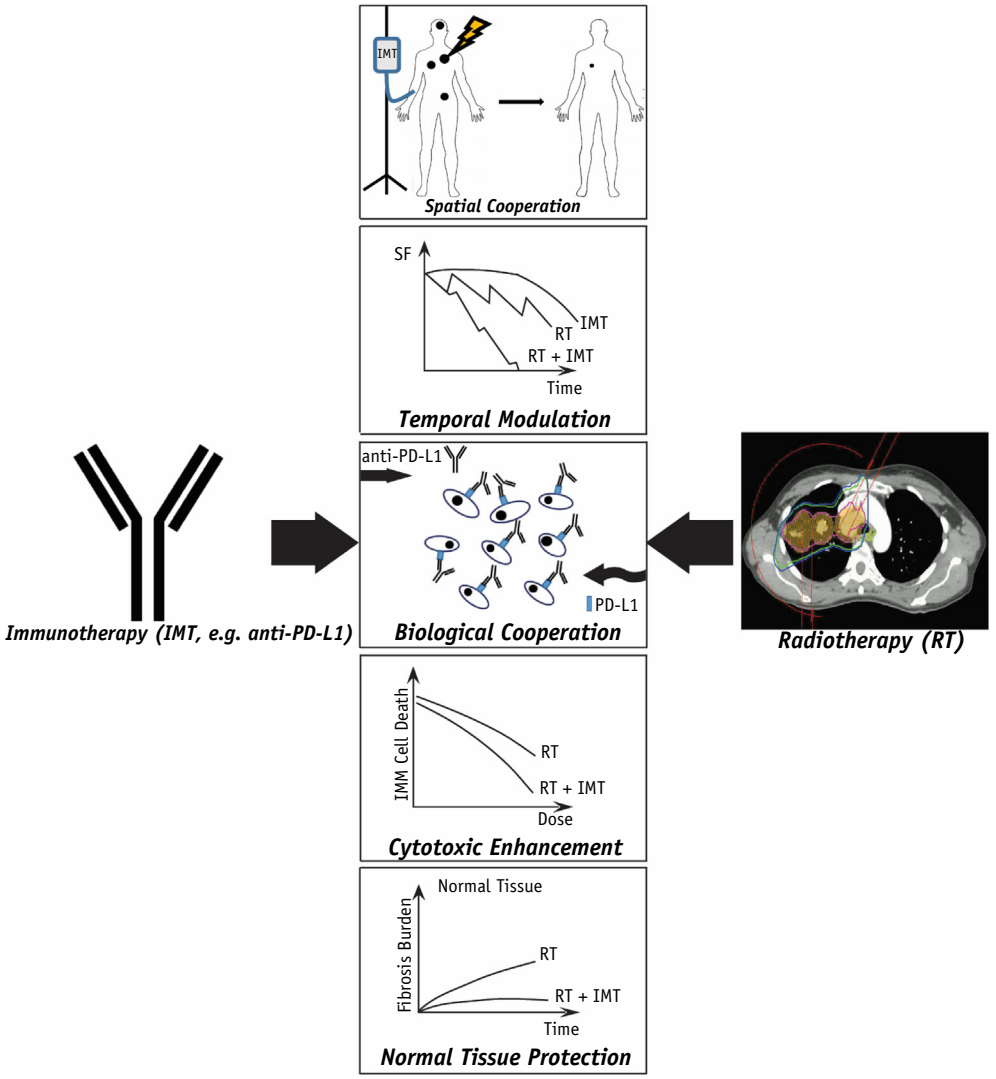

Fig. 2. Schematic illustration of a modernized Steel hypothesis in the era of immunotherapy. The interaction of radiation and immunotherapy is multifaceted, with each component contributing to improved clinical outcomes in the treatment of malignancy. Originally described by Steel in the 1970s, the growing complexity of such interactions prompts revision of this original framework. The potentially exploitable interactions of radiation and immunotherapy include spatial cooperation, temporal modulation, biological cooperation, cytotoxic enhancement, and normal tissue protection. The interaction between anti-PD-L1 therapy and radiation therapy is diagrammed as an illustrative example of biological cooperation. *Abbreviations:* IMM = immunogenic; IMT = immunotherapy; RT = radiation therapy; SF = surviving fraction of cells. Adapted from Morris and Harari[84] and Bentzen et al[14] with permission.

induced adaptive immune responses,[17,41] and the complexity of this interaction extends beyond tumor intrinsic signaling. For example, immune recognition of radiated tumors requires dendritic cell−intrinsic STING activation via cytoplasmic sensing of tumor-derived DNA,[41] which may be mediated in part by uptake of tumor-derived exosomes containing tumor cell DNA fragments.[42] At low doses (2-5 Gy), radiation modulates the tumor microenvironment by inducing the release of cytokines that influence immune cell trafficking and activation.[22,43] At low doses (1-3 Gy), radiation also may modulate the tumor microenvironment by ablating radiation-sensitive immune populations, including suppressive and effector lymphocytes.[44-47] This may create a window of opportunity by locally and temporarily depleting exhausted and suppressive T cells from the tumor microenvironment and allowing reconstitution with a more favorable infiltrate using immunotherapies.

In preclinical and clinical studies, several groups have taken advantage of the favorable immunomodulatory effects of radiation to prime a more effective systemic antitumor immune response.[48-50] This treatment strategy, termed *in situ vaccination,* uses a patient's own tumor as a source of tumor-specific antigen to stimulate and diversify an effective antitumor T cell response. This approach takes advantage of "private antigens," which are induced by

random, patient-specific mutations and differentiation markers in tumor cells. Recent evidence suggests that these mutated proteins are the most important tumor antigens recognized by T cells.[51] Through the capacity to immunomodulate the tumor microenvironment and generate an in situ vaccination effect, radiation may play a role in rendering tumors more responsive to immunotherapies.

Preclinical evidence provides a clear rationale for the combination of radiation with immune checkpoint blockade. Radiation can promote adaptive resistance through upregulation of PD-L1 on tumor cells,[52] and the addition of checkpoint blockade can overcome this resistance mechanism and enhance the generation of abscopal responses.[53] Combination with radiation may be particularly valuable in the treatment of immunologically "cold" tumors, which are characterized by low levels of T cell infiltrate and low mutation burden, resulting in few mutation-created neo-antigens.[54] Such "cold" tumors do not typically respond to immunotherapies such as immune checkpoint inhibitors.[16,18] Even in tumors that are responsive to immune checkpoint blockade or other immunotherapies, radiation may allow for increased depth and duration of response by priming a more diversified adaptive antitumor immune response. For example, in the B16 murine model of melanoma, radiation and checkpoint blockade activated separate immunologic mechanisms: diversification of the repertoire of T cell receptors among tumor-infiltrating lymphocytes and increased clonal expansion of these cells, respectively.[16] These observations have stimulated multiple clinical studies testing combinations of radiation and immune checkpoint inhibitors. Next-generation approaches combining additional classes of immunotherapies with radiation are being developed in preclinical studies to improve upon and further leverage the in situ vaccine effect of radiation to enhance development of antitumor immunity.[55-58]

Clinical Investigation of Immunotherapy Agents in Combination With Radiation

Retrospective studies analyzing combinations of radiation and checkpoint blockade

Clinical safety is a central concern in translating combination therapies to patients. Early clinical data describing the safety of radiation and immunotherapy combinations stem from retrospective analyses. For example, in 2 separate series of patients with metastatic melanoma who received nonbrain radiation therapy during their course of checkpoint blockade, researchers found that this combination was not associated with higher than expected rates of adverse events.[59,60] Shaverdian et al[61] conducted a secondary analysis of the KEYNOTE-001 trial and found that patients treated with both radiation therapy and pembrolizumab experienced longer progression-free survival and better overall survival than patients who did not have previous radiation therapy, with an acceptable safety profile. To determine the safety of combining stereotactic radiosurgery with immunotherapy, Martin et al[62] analyzed 480 patients with newly diagnosed brain metastases secondary to non-small cell lung cancer, melanoma, and renal cell carcinoma treated with stereotactic radiation. The addition of immunotherapy was associated with symptomatic radiation necrosis, and this association was especially strong in patients with melanoma ($P = .03$). Together, these studies demonstrate the safety of combining immune checkpoint blockade and radiation therapy; however, caution is warranted with stereotactic radiosurgery.

Randomized prospective trials

Several prospective trials have investigated the addition of immune checkpoint blockade to radiation therapy. Based on strong preclinical data in a spontaneous murine model of prostate cancer,[63] Kwon et al[64] conducted a multicenter phase 3 clinical trial that included men with at least 1 bone metastasis from castration-resistant prostate cancer that had progressed after docetaxel treatment. Patients were randomly assigned to receive radiation therapy to a bone metastasis (8 Gy in 1 fraction) followed by either ipilimumab or placebo. Ipilimumab did not increase overall survival compared with placebo ($P = .053$). However, on subgroup analysis, patients with favorable prognostic features (no visceral metastasis, no anemia, normal alkaline phosphatase) who received ipilimumab experienced a statistically significant improvement in survival compared with placebo.

In a phase 1 trial Tang et al[65] enrolled patients with solid tumors having at least 1 metastatic lesion in the liver or lung to receive SABR (50-60 Gy in 4-10 fractions) and ipilimumab either sequentially or concurrently. Response outside the radiation field was assessable in 31 patients, with 3 exhibiting a partial response and 10 experiencing clinical benefit.

Blockade of the checkpoint PD-1/PD-L1 in combination with radiation has demonstrated similar efficacy in prospective trials. The PACIFIC trial randomized patients with locally advanced, unresectable, non-small cell lung cancer who had previously received platinum-based chemoradiation therapy to receive either durvalumab or placebo. This study demonstrated that progression-free survival was significantly longer among patients receiving durvalumab compared with placebo, and secondary endpoints of 12- and 18-month progression-free survival rates, objective response rate, and duration of response also were improved with durvalumab.[66]

The US Federal Drug Administration—approved indications for checkpoint blockade are largely restricted to the metastatic setting. However, emerging evidence suggests a potential role for immunotherapy in nonmetastatic settings. Preclinical work demonstrates the potential for immune checkpoint blockade to prevent metastatic

progression from localized disease.[67] These findings have implications for patients with high-risk, locally advanced tumors. The PACIFIC trial provides strong supporting clinical evidence, and its success represents a clear opportunity and need to investigate the addition of immunotherapy to potentially curative combined modality therapies in nonmetastatic disease settings.

Further prospective studies have attempted to identify mechanisms underlying clinical responses observed with combination therapy. In a clinical trial investigating combining radiation therapy with anti-CTLA-4 in patients with non-small cell lung cancer, objective responses were observed in 18% of enrolled patients, and 31% had disease control.[68] The strongest response predictors were increased serum interferon-β after radiation therapy and early dynamic changes of blood T cell clones, which is in agreement with preclinical mechanistic data. Interrogation of the T cell receptor repertoire in responding patients from this study demonstrated detection and expansion of T cell clones not present at baseline after radiation therapy, consistent with an in situ vaccine effect. Additionally, in 1 patient, investigators were able to identify a tumor neoantigen recognized by a population of neoantigen-specific T cells that were not identified before radiation therapy.[68]

In a randomized trial specifically designed to measure abscopal response, McBride et al[69] randomized patients with metastatic head and neck squamous cell carcinoma to receive either nivolumab alone or nivolumab with stereotactic body radiation therapy to a single lesion (9 Gy × 3) between the first and second doses of nivolumab. The primary endpoint of objective response rate in nonirradiated lesions was not improved with combination therapy. In a phase 2 trial, Theelen et al[70] randomized patients with metastatic non-small cell lung cancer to receive pembrolizumab alone or in combination with radiation therapy (8 Gy × 3). There was a doubling in overall response rate between combination therapy and pembrolizumab monotherapy; however, this was not statistically significant ($P = .07$). Caution is warranted in interpreting this response rate, however, because PD-L1 status was not balanced between groups and may confound this outcome. In subgroup analysis, patients with PD-L1 negative tumors experienced significantly improved progression-free survival and overall survival.[70]

Ongoing clinical trials

Collectively, the current body of clinical data suggest that combining radiation with checkpoint blockade is safe and demonstrate that an in situ vaccine effect can be achieved with radiation. However, randomized, prospective studies have not yet shown a capacity for radiation to augment clinical response in the metastatic setting. With locally advanced disease, the PACIFIC trial provides strong evidence for upfront combination therapy. Combining radiation with immunotherapy is an active and growing area of investigation that extends beyond combination with immune checkpoint blockade to include preclinical and clinical testing of radiation with every class of immunotherapeutic.[71-74] The spectrum of immunotherapy agents currently being tested clinically is diverse and is summarized in Table 1.

Barriers to Effective Translation of Preclinical Findings to Patients

Preclinical barriers

One challenge that confronts the immuno-oncology research community is the difficulty of designing and choosing regimens with sufficient justification and likelihood of benefit to be prioritized for rapid initiation of clinical testing. The laboratory mouse can be studied in large numbers, in reproducible circumstances, with an immune system that is similar to that of humans, using clinically relevant therapeutics or their murine surrogates. However, implantable tumor models rely on murine cancer cell lines that are immortalized and generally more immunogenic than human tumors, which makes translation of immunotherapy regimens difficult. Actual human cancer cell lines or tumor fragments can be implanted in immunodeficient mice; however, these studies in mice lacking a functional immune system cannot be used to study the capacity of immunotherapy to act on these tumors via endogenous immune elements. Although grafting immunodeficient mice with human hematopoietic stem cells can create humanized mice, interpretation of data from such models is complicated by the xenogeneic mismatch between the immune cells and normal tissues of the host as well as allogeneic mismatch between the immune cell donor and the tumor cells.

To partially circumvent these challenges, we and others have begun using companion canines with cancer to validate observations made in immunocompetent murine tumor models. Each year approximately 6 million dogs will develop cancer; many owners are unable to afford treatment but are willing to participate in clinical trials. Canines develop a wide variety of spontaneously occurring cancers that share many characteristics with human cancers. In addition, many pets receive state-of-the-art medical care that can include image guided radiation therapy and experimental and proven immunotherapeutics, much like human patients, which provides a unique translational opportunity to test combination therapies in a heterogeneous population with spontaneously developing tumors.[75]

Several key cancer biology similarities exist between dogs and humans, including patterns of response or resistance to conventional therapy, as well as metastasis and recurrence.[76] Many specific cancers are functionally identical in dogs and humans at the histologic level, including osteosarcoma, mucosal melanoma, mammary tumors, soft tissue sarcomas, non-Hodgkin lymphoma, bladder cancer,

Table 1 Immunotherapy agents currently under clinical investigation in combination with radiation therapy

Category	Examples of immune Rx	Disease site	Phase	No. of current studies	Fold change in N from 2018[56] to present
Checkpoint inhibitors	Anti-CTLA-4	Cervix, melanoma, head and neck, pancreas, liver, lung	1/2/3	98	5
	PD-1/PD-L-1	Esophageal, NSCLC, malignant glioma, melanoma (brain metastases), invasive bladder, oligometastatic breast, head and neck, pancreas, gastric, colorectal, follicular lymphoma	1/2/3	451	5
Cytokines	IL-2, IFN, GM-CSF, and TGF-beta blockade	Metastatic breast, NSCLC, glioblastoma, follicular lymphoma, and pancreas	1/2	149	16
Cell therapy	CAR T cells (Anti BCMA, CD19, CD-30, TAI-meso, EGFRvIII, mesothelin, CD22)	B-cell lymphomas, pancreas, glioblastoma, follicular lymphoma, pancreas	1/2	18	0.67
Vaccines/ oncolytic viruses	AdV-tk, Sipluleucel-T, G207, ADV/HSV-tk, Oncolytic Adenovirus Ad5-yCD/ mutTKSR39rep-hIL12, and Ad5-yCD/ mutTKSR39rep-AD	Prostate, pancreas, malignant supratentorial neoplasms, NSCLC, triple negative breast, prostate, glioma, ovarian, sarcoma, glioblastoma, neuroblastoma	1/2/3	23	0.66
Other targeted immune Rx	OX40 antibody, CDX-301, GITR, and TLR-4,7,9 agonists	Melanoma, renal cell carcinoma, NSCLC, breast, sarcoma, cutaneous T-cell and recurrent lymphoma	1/2	22	0.76

Abbreviations: NSCLC = non-small cell lung cancer; Rx = prescription.

and others.[76-80] Genome-wide studies have identified similarities in gene dosage between corresponding cancers in dogs and humans, offering insights into potential conserved pathogenesis mechanisms involving key driver genes. Dog and human tumors also have many key similarities at the transcriptional level, and several tumor types are considered to be indistinguishable between species.[81]

In an effort to improve the translational drug development process, the National Cancer Institute created the Comparative Oncology program, which includes a clinical trial network of 20 academic veterinary teaching hospitals across the United States and Canada. Since its creation in 2004, the program has completed 12 multicenter clinical trials in pet dogs with spontaneous cancers, and its 24th trial concept is currently open for enrollment.[82] Several canine immunotherapies are available; these include cytokines such as IL-2, IL-12, and IL-15 as well as a US Department of Agriculture–approved cancer vaccine, Oncept, which targets the tyrosinase protein often expressed in melanoma. A canine anti-PD-1 has also been tested and is pending US Department of Agriculture approval.[81] Additionally, it is possible to administer clinical quality radiation therapy to dogs, and the spatial separation of tumors and normal tissues in canines more closely reflects that encountered in humans, compared with mice.[83]

Clinical barriers

A wealth of preclinical data describe inflammatory changes in both the tumor and surrounding microenvironment in response to varying doses and fractionation schedules of radiation. However, dynamic changes in murine tumors and immune systems are inherently different from what may occur in human patients. Therefore, it is imperative to confirm preclinical findings in humans to allow for effective translation of preclinical success to patients. Although it is a reasonable task to secure funding for phase 3 trials with survival as endpoints, it is very difficult to convince funding agencies to sponsor phase 1 trials designed to ask fundamental questions about dose and fractionation with endpoints of biologic correlates of immune activation in response to radiation. In contrast to pharmaceuticals, no

single private entity has ownership of radiation as an intervention. This limits the funding potential for mechanistic research in human subjects. Although difficult to undertake, the results of such mechanistic studies will prove invaluable when attempting to rationally combine radiation with immunotherapy.

Despite radiation being a critical component of treatment for a multitude of patients with cancer, there is a historical lack of clinical trials that formally explore combinations of radiation and other therapeutic agents such as immunotherapy. A search of currently registered clinical trials in the United States during the preparation of this review suggests that this disparity persists in the era of immunotherapy but has improved considerably compared with other therapeutics.[84] Among all current trials for cancer, 3516 (13%) are investigating an intervention with radiation therapy and 5240 (22%) are testing an immunotherapy, with 761 (4%) trials evaluating a combination of the 2 interventions (Table 1). Compared with a similar Boolean search conducted in 2018, this represents approximately a 4-fold increase in overall number of current combination therapy trials.[85] Focusing on phase 3 trials for cancer, a total of 543 (18%) are investigating an intervention with radiation therapy and 567 (19%) are evaluating an immunotherapy, and 78 (3%) are examining a combination of these 2 interventions (Fig. 3). This relatively strong uptake in the testing of radiation therapy in approximately 14% of phase 3 immunotherapy trials has no doubt been driven, at least in part, by the rationale arising from strong preclinical and early phase clinical studies, as

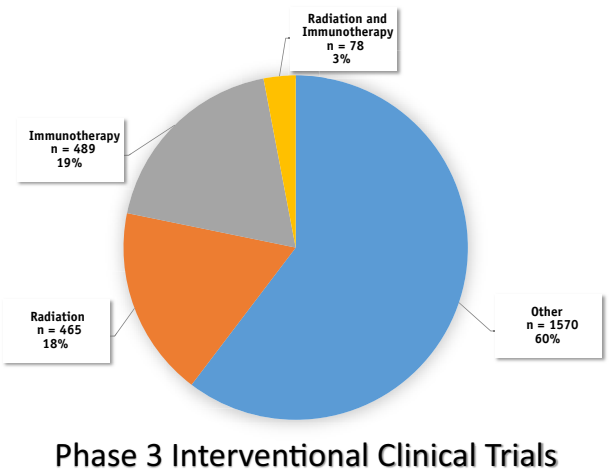

Fig. 3. Distribution of current phase 3 clinical trials in oncology. A search of www.clinicaltrials.gov for phase 3 clinical trials returned 2602 trials for condition = "cancer." When intervention = "radiation" was added to this search, 543 studies were identified. When the 2602 phase 3 cancer trials were sorted by intervention, 567 studies involved an immunotherapy agent as defined in this review. Of these, 78 studies examined a combination of an immunotherapy agent and radiation.

outlined. However, a disparity remains in testing combinations of radiation and immunotherapy in the nonmetastatic setting. Despite the success of the PACIFIC trial, <1% of early phase trials investigating a combination of radiation and immunotherapy are open to patients with nonmetastatic disease. Additionally, there are only 2 active phase 3 trials testing a combination of radiation and immunotherapy in patients with localized disease: 1 testing chemoradiation in combination with the PD-L1 inhibitor atezolizumab in muscle-invasive bladder cancer and the other investigating combining radiation with the cancer vaccine ProstAtak(AdV-tk) in localized prostate cancer. Effective collaboration between radiation oncology and industry investigators will be critical to redress this discrepancy, as will the attraction of proportionate federal funding, which has historically lagged in radiation oncology.

Future Directions

Radiation primarily has local effects that can be strongly immunogenic; however, in the context of metastatic disease, it is currently unclear whether all sites of disease need to be targeted by radiation to optimally synergize with immunotherapy. Radiating multiple tumor sites may increase risk of immunosuppression, and in such settings it may be critical to consider blood pool, draining lymph nodes, spleen, and/or bone marrow as organs at risk during treatment planning.[86] In patients with metastatic sites not amenable to external beam radiation or with occult disease, emerging targeted radionuclide therapies could offer an alternative approach to delivering radiation to all tumor sites.[87-90] Revisiting additional radiation therapy modalities such as brachytherapy or particle therapy may also prove useful in combination with immunotherapy.

Preclinical studies indicate that the immunogenic effects of radiation are sensitive to the radiation dose and field size. Because of its powerful conformality and dose heterogeneity, brachytherapy may confer meaningful advantages over external beam radiation when it comes to priming an in situ vaccine effect by simultaneously engaging multiple dose-dependent immunomodulatory mechanisms in a single tumor microenvironment.[85] As we gain further understanding of the complex interplay of tumor and immune signaling pathways, it may be beneficial to explore combinations of radiation with multiple classes of immunotherapies and molecularly targeted therapeutics simultaneously. Current combination strategies are not yet fully optimized, with many unknowns, including the appropriate sequence to administer immunotherapy in relation to radiation, appropriate route of delivery of immunotherapy (systemic or local), and appropriate choice of immunotherapy (or immunotherapies) to combine with radiation. Additionally, as described by preclinical and clinical studies there are likely to be considerable differences in clinical efficacy, with patient-specific tumor and

immune microenvironment characteristics underlying this observation. With additional breakthroughs in precision medicine, this may enable the logical design of personalized approaches to complex combined-modality treatment.[91]

Conclusions

In the era of immunotherapy, radiation has the potential to become a critical component of systemic cancer therapy. Combined-modality approaches with immunotherapy may increase the curative capacity of radiation therapy in patients with locally advanced disease, as seen with the success of the PACIFIC trial. This therapeutic potential is supported by strong preclinical evidence providing abundant rationale for clinical testing of radiation and immunotherapy combinations. Mechanistic hypotheses originating from preclinical studies in murine models, such as the in situ vaccine effect of radiation, have been confirmed in human analyses, thus warranting further testing of next-generation strategies. Retrospective data and prospective clinical trials indicate that combinations of radiation and immunotherapies are generally safe. In contrast with prior molecular targeted agents, the uptake of radiation combined with immunotherapy in clinical studies has been more robust. Driven by strong preclinical rationale, further support for preclinical investigation is needed now to achieve successful translation to proof of clinical benefit. Investment now in clinical trials that combine radiation with immunotherapy is highly warranted in oncology.

References

1. Stone HB, Peters LJ, Milas L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. *J Natl Cancer Inst* 1979;63:1229-1235.
2. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* 2004;58:862-870.
3. Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol* 2009;10:718-726.
4. Abuodeh Y, Venkat P, Kim S. Systematic review of case reports on the abscopal effect. *Curr Probl Cancer* 2016;40:25-37.
5. Bach FH, Albertini RJ, Joo P, Anderson JL, Bortin MM. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. *Lancet* 1968;2:1364-1366.
6. Fyfe GA, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. *J Clin Oncol* 1996;14:2410-2411.
7. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol* 1999;17:2105-2116.
8. Hong TS, Ritter MA, Tome WA, Harari PM. Intensity-modulated radiation therapy: Emerging cancer treatment technology. *Br J Cancer* 2005;92:1819-1824.
9. Blomgren H, Lax I, Naslund I, Svanstrom R. Stereotactic high dose fraction radiation therapy of extracranial tumors using an accelerator. Clinical experience of the first thirty-one patients. *Acta Oncol* 1995;34:861-870.
10. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci USA* 1989;86:10024-10028.
11. Hamid O, Robert C, Daud A, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Ann Oncol* 2019;30:582-588.
12. Topalian SL, Hodi FS, Brahmer JR. Five-year survival and correlates among patients with advanced melanoma, renal cell carcinoma, or non-small cell lung cancer treated with nivolumab. *JAMA Oncol* 2019;5:1411-1420.
13. Steel GG. Terminology in the description of drug-radiation interactions. *Int J Radiat Oncol Biol Phys* 1979;5:1145-1150.
14. Bentzen SM, Harari PM, Bernier J. Exploitable mechanisms for combining drugs with radiation: Concepts, achievements and future directions. *Nat Clin Pract Oncol* 2007;4:172-180.
15. Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: A revived partnership. *Int J Radiat Oncol Biol Phys* 2005;63:655-666.
16. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015;520:373-377.
17. Vanpouille-Box C, Alard A, Aryankalayil MJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat Commun* 2017;8:15618.
18. Demaria S, Kawashima N, Yang AM, et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin Cancer Res* 2005;11:728-734.
19. Hodi FS, Ballinger M, Lyons B, et al. Immune-Modified Response Evaluation Criteria In Solid Tumors (imRECIST): Refining guidelines to assess the clinical benefit of cancer immunotherapy. *J Clin Oncol* 2018;36:850-858.
20. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013;31:51-72.
21. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology* 2014;3:e28518.
22. Rodriguez-Ruiz ME, Garasa S, Rodriguez I, et al. Intercellular adhesion molecule-1 and vascular cell adhesion molecule are induced by ionizing radiation on lymphatic endothelium. *Int J Radiat Oncol Biol Phys* 2017;97:389-400.
23. Waldmann TA. Cytokines in cancer immunotherapy. *Cold Spring Harb Perspect Biol* 2018;10:a028472.
24. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 2009;16:183-194.
25. Kachikwu EL, Iwamoto KS, Liao YP, et al. Radiation enhances regulatory T cell representation. *Int J Radiat Oncol Biol Phys* 2011;81:1128-1135.
26. Tanaka H, Shinto O, Yashiro M, et al. Transforming growth factor beta signaling inhibitor, SB-431542, induces maturation of dendritic cells and enhances anti-tumor activity. *Oncol Rep* 2010;24:1637-1643.
27. Tsai CS, Fang-Hsin C, Wang CC, et al. Macrophages from irradiated tumors express higher levels of iNOS, arginase-I and COX-2, and promote tumor growth. *Int J Radiat Oncol Biol Phys* 2007;68:499-507.
28. Xu J, Escamilla J, Mok S, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. *Cancer Res* 2013;73:2782-2794.
29. Liang H, Deng L, Hou Y, et al. Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. *Nat Commun* 2017;8:1736.

30. Benci JL, Xu B, Qiu Y, et al. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. *Cell* 2016;167:1540-1554.e12.
31. Kaur P, Asea A. Radiation-induced effects and the immune system in cancer. *Front Oncol* 2012;2:191.
32. Rosen EM, Fan S, Rockwell S, Goldberg ID. The molecular and cellular basis of radiosensitivity: Implications for understanding how normal tissues and tumors respond to therapeutic radiation. *Cancer Invest* 1999;17:56-72.
33. Ellsworth SG. Field size effects on the risk and severity of treatment-induced lymphopenia in patients undergoing radiation therapy for solid tumors. *Adv Radiat Oncol* 2018;3:512-519.
34. Diehl A, Yarchoan M, Hopkins A, Jaffee E, Grossman SA. Relationships between lymphocyte counts and treatment-related toxicities and clinical responses in patients with solid tumors treated with PD-1 checkpoint inhibitors. *Oncotarget* 2017;8:114268-114280.
35. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res* 2009; 15:5379-5388.
36. Grapin M, Richard C, Limagne E, et al. Optimized fractionated radiotherapy with anti-PD-L1 and anti-TIGIT: A promising new combination. *J Immunother Cancer* 2019;7:160.
37. Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. *Br J Radiol* 1989;62:679-694.
38. Werner LR, Kler JS, Gressett MM, et al. Transcriptional-mediated effects of radiation on the expression of immune susceptibility markers in melanoma. *Radiother Oncol* 2017;124:418-426.
39. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 2006;203:1259-1271.
40. Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature* 2017;548:466-470.
41. Deng L, Liang H, Xu M, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type i interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* 2014;41:843-852.
42. Diamond JM, Vanpouille-Box C, Spada S, et al. Exosomes shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to DCs. *Cancer Immunol Res* 2018;6:910-920.
43. Liu SZ. Nonlinear dose-response relationship in the immune system following exposure to ionizing radiation: Mechanisms and implications. *Nonlinearity Biol Toxicol Med* 2003;1:71-92.
44. Nakamura KYN, Akiyama M. Radiosensitivity of CD4 or CD8 positive human T-lymphocytes by an in vitro colony formation assay. *Radiat Res* 1990;123:224-227.
45. Liu R, Xiong S, Zhang L, Chu Y. Enhancement of antitumor immunity by low-dose total body irradiationis associated with selectively decreasing the proportion and number of T regulatory cells. *Cell Mol Immunol* 2010;7:157-162.
46. Liu S, Sun X, Luo J, et al. Effects of radiation on T regulatory cells in normal states and cancer: mechanisms and clinical implications. *Am J Cancer Res* 2015;5:3276-3285.
47. Balogh A, Persa E, Bogdandi EN, et al. The effect of ionizing radiation on the homeostasis and functional integrity of murine splenic regulatory T cells. *Inflamm Res* 2013;62:201-212.
48. Morris ZS, Guy EI, Francis DM, et al. In situ tumor vaccination by combining local radiation and tumor-specific antibody or immunocytokine treatments. *Cancer Res* 2016;76:3929-3941.
49. Marabelle A, Tselikas L, de Baere T, Houot R. Intratumoral immunotherapy: Using the tumor as the remedy. *Ann Oncol* 2017;28:xii33-xii43.
50. Pierce RH, Campbell JS, Pai SI, Brody JD, Kohrt HE. In-situ tumor vaccination: Bringing the fight to the tumor. *Hum Vaccin Immunother* 2015;11:1901-1909.
51. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. *Cell* 2017;171:1042-1056.e10.
52. Deng L, Lian H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* 2014;124:687-695.
53. Dovedi SJ, Cheadle EJ, Popple Al, et al. Fractionated radiation therapy stimulates antitumor immunity mediated by both resident and infiltrating polyclonal T-cell populations when combined with PD-1 blockade. *Clin Cancer Res* 2017;23:5514-5526.
54. Gajewski TF. The next hurdle in cancer immunotherapy: Overcoming the non-T-cell-inflamed tumor microenvironment. *Semin Oncol* 2015; 42:663-671.
55. Wu CJ, Tsai Y-T, Lee I-J, et al. Combination of radiation and interleukin 12 eradicates large orthotopic hepatocellular carcinoma through immunomodulation of tumor microenvironment. *Oncoimmunology* 2018;7:e1477459.
56. Gillies SD, Reilly EB, Lo KM, Reisfeld RA. Antibody-targeted interleukin 2 stimulates T-cell killing of autologous tumor cells. *Proc Natl Acad Sci USA* 1992;89:1428-1432.
57. Rekers NH, Zegers CM, Germeraad WT, Dubois L, Lambin P. Long-lasting antitumor effects provided by radiotherapy combined with the immunocytokine L19-IL2. *Oncoimmunology* 2015;4:e1021541.
58. Niknam S, Barsoumian HB, Schoenhals JE, et al. Radiation followed by OX40 stimulation drives local and abscopal antitumor effects in an anti-PD1-resistant lung tumor model. *Clin Cancer Res* 2018;24:5735-5743.
59. Barker CA, Postow MA, Khan SA, et al. Concurrent radiotherapy and ipilimumab immunotherapy for patients with melanoma. *Cancer Immunol Res* 2013;1:92-98.
60. Ahmed KA, Stallworth DG, Kim Y, et al. Clinical outcomes of melanoma brain metastases treated with stereotactic radiation and anti-PD-1 therapy. *Ann Oncol* 2016;27:434-441.
61. Shaverdian N, Lisberg AE, Bornazyan K, et al. Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: A secondary analysis of the KEYNOTE-001 phase 1 trial. *Lancet Oncol* 2017;18: 895-903.
62. Martin AM, Cagney DN, Catalano PJ, et al. Immunotherapy and symptomatic radiation necrosis in patients with brain metastases treated with stereotactic radiation. *JAMA Oncol* 2018;4:1123-1124.
63. Harris TJ, Hipkiss EL, Borzillary S, et al. Radiotherapy augments the immune response to prostate cancer in a time-dependent manner. *Prostate* 2008;68:1319-1329.
64. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): A multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014;15:700-712.
65. Tang C, Welsh JW, de Groot P, et al. Ipilimumab with stereotactic ablative radiation therapy: Phase I results and immunologic correlates from peripheral T Cells. *Clin Cancer Res* 2017;23:1388-1396.
66. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *N Engl J Med* 2017;377:1919-1929.
67. Kwon ED, Foster BA, Hurwitz AA, et al. Elimination of residual metastatic prostate cancer after surgery and adjunctive cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade immunotherapy. *Proc Natl Acad Sci USA* 1999;96:15074-15079.
68. Formenti SC, Rudqvist NP, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. *Nat Med* 2018;24: 1845-1851.
69. McBride SM, Sherman EJ, Tsai CJ, et al. A phase II randomized trial of nivolumab with stereotactic body radiotherapy (SBRT) versus nivolumab alone in metastatic (M1) head and neck squamous cell carcinoma (HNSCC). *J Clin Oncol* 2018;36:6009.
70. Theelen W, Peulen HMU, Lalezari F, et al. Effect of pembrolizumab after stereotactic body radiotherapy vs pembrolizumab alone on tumor response in patients with advanced non-small cell lung cancer: Results of the PEMBRO-RT phase 2 randomized clinical Trial. *JAMA Oncol* 2019;5:1276-1282.

71. Golden EB, Chhabra A, Chachoua A, et al. Local radiotherapy and granulocyte-macrophage colony-stimulating factor to generate abscopal responses in patients with metastatic solid tumours: A proof-of-principle trial. *Lancet Oncol* 2015;16:795-803.
72. Nemunaitis J. Vaccines in cancer: GVAX, a GM-CSF gene vaccine. *Expert Rev Vaccines* 2005;4:259-274.
73. Bentebibel SE, Hurwitz ME, Bernatchez C, et al. A first-in-human study and biomarker analysis of NKTR-214, a novel IL2Rbetagamma-biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov* 2019;9:711-721.
74. Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. *J Immunother Cancer* 2016;4:51.
75. Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Invest* 2000;18:781-792.
76. Baioni E, Scanziani E, Vincenti MC, et al. Estimating canine cancer incidence: Findings from a population-based tumour registry in northwestern Italy. *BMC Vet Res* 2017;13:203.
77. Schiffman JD, Breen M. Comparative oncology: What dogs and other species can teach us about humans with cancer. *Philos Trans R Soc Lond B Biol Sci* 2015;370:20140231.
78. Breen M, Modiano JF. Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans—man and his best friend share more than companionship. *Chromosome Res* 2008;16:145-154.
79. Decker B, Parker HG, Dhawan D, et al. Homologous mutation to human BRAF V600E Is common in naturally occurring canine bladder cancer—evidence for a relevant model system and urine-based diagnostic test. *Mol Cancer Res* 2015;13:993-1002.
80. Paoloni M, Davis S, Lana S, et al. Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. *BMC Genomics* 2009;10:625.
81. LeBlanc AK, Breen M, Choyke P, et al. Perspectives from man's best friend: National Academy of Medicine's Workshop on Comparative Oncology. *Sci Transl Med* 2016;8:324ps5.
82. Gordon I, Paoloni M, Mazcko C, Khanna C. The Comparative Oncology Trials Consortium: Using spontaneously occurring cancers in dogs to inform the cancer drug development pathway. *PLoS Med* 2009;6:e1000161.
83. Vail DM, LeBlanc AK, Jeraj R. Advanced cancer imaging applied in the comparative setting. *Front Oncol* 2020;10:84.
84. Morris ZS, Harari PM. Interaction of radiation therapy with molecular targeted agents. *J Clin Oncol* 2014;32:2886-2893.
85. Patel RB, Baniel CC, Sriramaneni RN, Bradley K, Markovina S, Morris ZS. Combining brachytherapy and immunotherapy to achieve in situ tumor vaccination: A review of cooperative mechanisms and clinical opportunities. *Brachytherapy* 2018;17:995-1003.
86. Marciscano AE, Ghasemzadeh A, Nirschl TR, et al. Elective nodal irradiation attenuates the combinatorial efficacy of stereotactic radiation therapy and immunotherapy. *Clin Cancer Res* 2018;24:5058-5071.
87. Sze WC, Grossman AB, Goddard I, et al. Sequelae and survivorship in patients treated with (131)I-MIBG therapy. *Br J Cancer* 2013;109:565-572.
88. Morschhauser F, Radford J, Van Hoof A, et al. 90Yttrium-ibritumomab tiuxetan consolidation of first remission in advanced-stage follicular non-Hodgkin lymphoma: Updated results after a median follow-up of 7.3 years from the International, Randomized, Phase III First-LineIndolent trial. *J Clin Oncol* 2013;31:1977-1983.
89. Weichert JP, Clark PA, Kandela IK, et al. Alkylphosphocholine analogs for broad-spectrum cancer imaging and therapy. *Sci Transl Med* 2014;6:240ra75.
90. Hernandez R, Walker KL, Grudzinski J, et al. (90)Y-NM600 targeted radionuclide therapy induces immunologic memory in syngeneic models of T-cell Non-Hodgkin's Lymphoma. *Commun Biol* 2019;2:79.
91. Bristow RG, Alexander B, Baumann M, et al. Combining precision radiotherapy with molecular targeting and immunomodulatory agents: A guideline by the American Society for Radiation Oncology. *Lancet Oncol* 2018;19:e240-e251.