Review Article

Irradiation and Immunotherapy: From Concept to the Clinic

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In recent years, an increased understanding of T-cell–regulatory mechanisms has led to the development of a novel class of immune-checkpoint inhibitors that have robust clinical activity against a broad array of malignancies—even those that historically were not believed to be sensitive to immune therapy. With this, there has been renewed interest in the potential for synergy with more traditional forms of anticancer therapy like radiation therapy (RT). The role of RT in palliation or as definitive treatment for certain malignancies has been well established. Yet, in recent years, the concept has come to light that RT could be an attractive partner for use in combination with other immunotherapies. The effects of RT include not only control of an irradiated tumor but also multiple immunomodulatory effects on both the tumor and the microenvironment, priming tumors for an immune-mediated response. Herein, the authors summarize relevant preclinical data and rationale supporting the synergy of combined RT and immunotherapy and highlight recent clinical work on promising combination strategies. ***Cancer*** **2016;122:1659-71**. © *2016 American Cancer Society*.

KEYWORDS: cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), immunotherapy, ipilimumab, programmed cell death 1/programmed death-ligand 1 (PD-1/PD-L1), radiotherapy.

INTRODUCTION

The ability of radiotherapy (RT) to elicit an immunologic response against cancer has been recognized for over a century.[1,2] However, knowledge gained from a body of preclinical work that elucidates the breadth of RT effects on both the tumor and the microenvironment, coupled with advances in systemic immunotherapies, has paved the way for the rational design of clinical combination strategies.

RT is an integral cancer therapy, either as definitive treatment or as part of combination therapy, for a wide variety of malignancies.[3] In addition, it has an established role palliating a multitude of tumor-related symptoms.[4] Typically, RT is delivered conventionally, in small daily doses over 1 to 7 weeks. Recent advances in imaging, tumor motion assessment and management, and RT planning allow for extremely precise RT delivery. Application of these technologies now allows for the delivery of a few, large, intracranial and extracranial RT doses that directly target tumors while excluding surrounding nontumor tissues. Commonly termed stereotactic radiosurgery when delivered to intracranial targets and stereotactic body RT (SBRT) or stereotactic ablative radiation (SABR) when delivered to extracranial targets, these treatments have generally led to promising treated tumor control rates with limited toxicity,[5] potentially engaging additional antitumor pathways.[6-8]

Historically regarded as immunosuppressive, it is increasingly evident that RT has several immunomodulatory effects that potentially sensitize tumors, making them more responsive locally and systemically to therapy.[9] Concurrently, the classical thinking on immunotherapy has also undergone a radical transformation, as increased understanding of T-cell–regulatory processes has led to the development of novel checkpoint inhibitors with promising activity across multiple tumor types.[10,11] There is a tremendous opportunity to clinically evaluate the potential for synergy as these fields converge. Herein, we summarize the preclinical rationale behind combination strategies as well as the most recent clinical trials investigating this approach.

IMMUNOLOGIC EFFECTS OF RT

Radiation exerts its tumoricidal effects through the induction of nonrepairable, double-stranded DNA breaks, leading to mitotic catastrophe, apoptosis, and accelerated senescence.[12] It has long been believed that radiation is immunosuppressive, because normal hematopoietic cells and hematologic malignancies appear to be very sensitive to low radiation

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DOI: 10.1002/cncr.29889, **Received:** September 29, 2015; **Revised:** December 11, 2015; **Accepted:** December 16, 2015, **Published online** February 23, 2016 in Wiley Online Library (wileyonlinelibrary.com)

doses.[13] However, a collective body of work suggests that the effects of RT are multifaceted and may serve as an immune stimulus. Therefore, ultimate tumor control likely depends on a balance of immunosuppressive and immunostimulatory signals generated within the tumor.

The ability of radiation to create an "in-situ" vaccine has been validated over the past decade.[14,15] The effects of radiation on the tumor and microenvironment include increased natural killer (NK) cell activity, greater cluster of differentiation 8 (CD8)-positive T-cell infiltration,[16] enhanced antigen presentation to dendritic cells, and increased production of immunostimulatory cytokines.[17] Ablative RT (a 20-Gray [Gy] single dose) to a B16 melanoma model primary tumor resulted in marked tumor regression, increased T cells present in the microenvironment, and increased T-cell priming in draining lymph nodes.[7] These effects were T-cell–dependent, with CD8-positive T-cell depletion diminishing the antitumor effects of RT. It is noteworthy that these effects were not observed with fractionated RT, suggesting that conventional RT may inhibit the immune-mediated effects of RT.[7] It also has been demonstrated that RT modulates the available peptide repertoire and increases major histocompatibility complex (MHC) class I expression.[14] The RT effects on the tumor microenvironment also play a key role with regard to immune sensitivity and responsiveness.[18] Finally, RT may down-regulate inhibitory immune signals from myeloid-derived suppressor cells and regulatory T cells.[19,20]

Preclinical Evidence for Combining RT With Immunotherapy

Robust preclinical data now support the rationale for combinations of systemic immunotherapies with radiation. When combined with adoptive cytotoxic T-lymphocyte (CTL) therapy, irradiation markedly inhibited tumor growth in an MC38 colon cancer model compared with either modality alone.[14] Radiation has also augmented tumor eradication by enhancing the immune response to the tumor stroma and microenvironment. Tumor antigens in the stroma can be successfully targeted with antigen-specific CTLs. In cancer cells that express high antigen levels, adoptive CTL transfer has been effective; however, those expressing low antigen levels eventually escape, presumably because of the creation of antigen loss variants.[21] In a murine model, irradiation of MC57 tumors followed by CTL transfer resulted in tumor eradication. In the absence of RT, tumors ultimately progressed.[22] In addition, the combination of RT and L19 (a small-immunoprotein with immunostimulatory effects[23,24] targeting extradomain-B [a marker of neoangiogenesis expressed on fibronectin with variable expression in different tumor types[25]) linked with interleukin 2 (IL-2) has demonstrated the potential for synergy. In preclinical models, the coadministration of L19-IL2 with RT in C51 colon carcinoma cells (with high extradomain-B expression) was synergistic, with lesser effects in tumor types that had lower extradomain-B expression. The synergy appeared to be immune-mediated, with increased CD8-positive T cells observed posttreatment, and CD8-positive depletion completely abrogated this effect.[25]

Preclinical Rationale for Synergy of RT With CTL-Associated Protein 4 Inhibition

With level I evidence supporting the use of ipilimumab in melanoma,[26] preclinical data relating to the potential synergy of ipilimumab and RT has provided a framework for clinical application (Fig. 1). By using the poorly immunogenic 4T1 breast cancer model, the combination of RT plus an anti-CTL–associated protein 4 (CTLA-4) antibody extended survival compared with either modality alone.[27] Furthermore, control of distant metastases was immune-mediated, requiring CD8-positive T cells. The specific RT dose and fractionation may be critical to maximize the potential for immunotherapeutic synergy. Although some data suggest that single-dose RT has preferential immunologic effects on primary tumors,[7] when combined with CTLA-4 inhibition, fractionated dosing resulted in improved control of the primary tumor compared with single-dose RT and also resulted in immune-mediated responses in disease outside the irradiated field.[28]

Additional preclinical work has further elucidated the mechanisms underlying this synergy. By using the 4T1 model, the combination of RT and a CTLA-4 blocking antibody restored MHC-1–dependent arrest, allowing the formation of stable interactions with tumor-infiltrating lymphocytes (TILs) and tumor cells, leading to improved antitumor activity.[29] It is noteworthy that the NK cell group 2D receptor (NKG2D), an important mediator of the immunologic synapse, appeared to be a critical pathway, because combination therapy was not active in the presence of an anti-NKG2D antibody. In addition, in a glioma model, the combination of RT plus CTLA-4 blockade significantly improved survival compared with untreated animals, whereas CTLA-4 blockade alone had no impact.[30] Furthermore, adding an agonist antibody to the costimulatory immune-checkpoint 4-1BB resulted in further improvement. The synergistic effect did not appear to depend on the timing of RT relative to drug therapy,

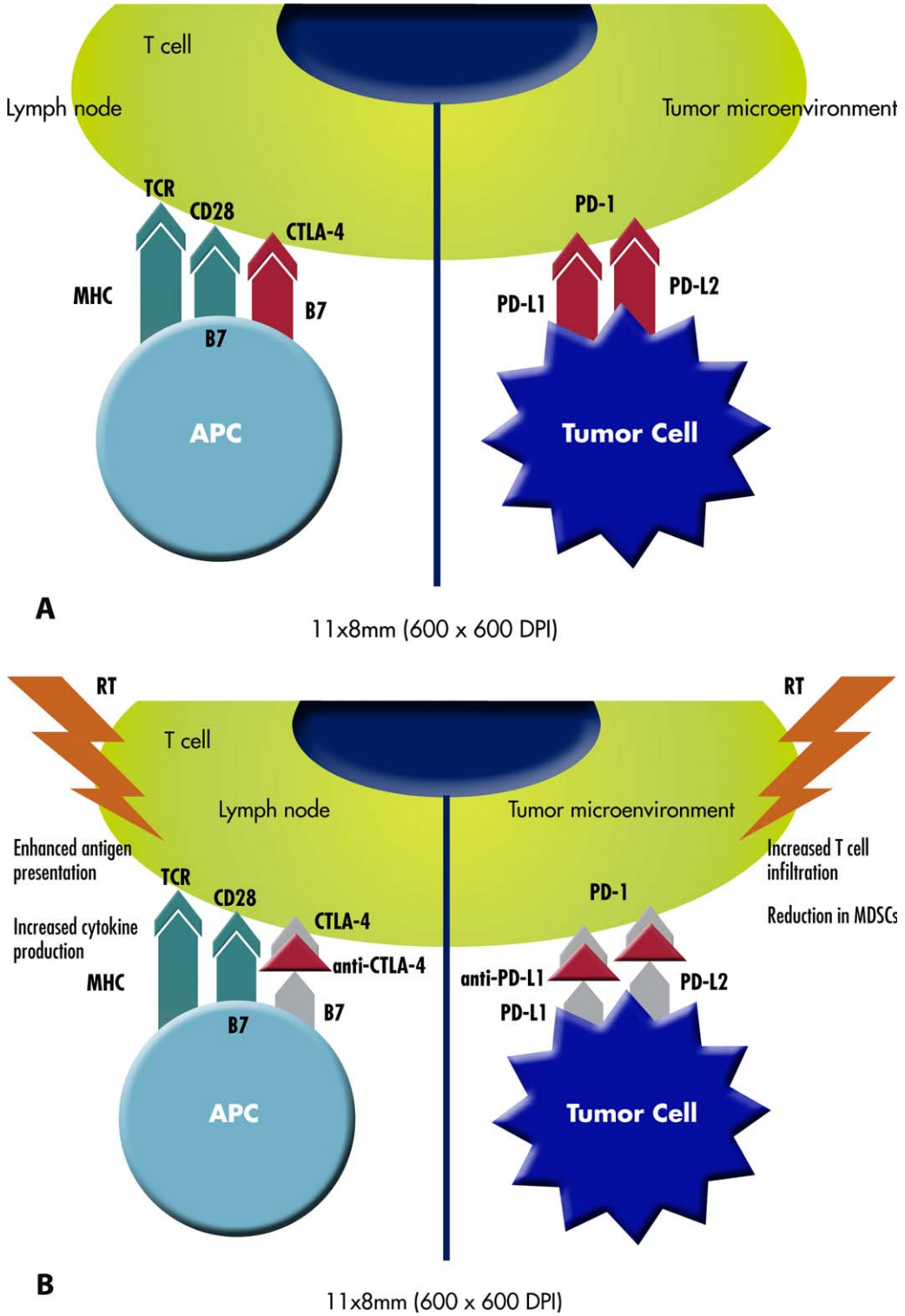

Figure 1. (A) The generation of a successful immune response requires costimulatory signals in addition to major histocompatibility complex (MHC)/T-cell receptor (TCR) binding, including engagement of cluster of differentiation 28 (CD28)/B7 (a type of peripheral membrane protein found on activated antigen-presenting cells [APCs]). Cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) regulates T-cell activation and expansion by competitively binding B7, limiting T-cell proliferation early in the immune response, primarily in the lymph nodes. In contrast, the programmed cell death 1 (PD-1) axis functions in the periphery in the tumor microenvironment to act as a negative regulator of T-cell function. (B) Radiation therapy (RT) has several immunomodulatory effects, including increased antigen presentation and greater tumor infiltration of CD8-positive T cells. It has been demonstrated that antibodies directed at CTLA-4 and PD-1 can restore T-cell activation and effector functions and that they have the potential to synergize with RT. MDSC indicates myeloid-derived suppressor cells; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2.

and it is noteworthy that it appeared to be primarily CD4-positive T-cell mediated.[30]

RT Synergy With Programmed Cell Death 1/Programmed Death-Ligand 1 Directed Therapy

The successful development of therapies targeting the programmed cell death 1/programmed death-ligand 1 (PD-1/PD-L1) axis have exhibited promising clinical activity in several malignancies.[10] As monotherapy, these agents have the ability to induce durable responses, although substantial proportions of patients ultimately progress. Combination strategies, either with other drugs or with RT, will likely play a key role in enhancing their effect. Preclinical work in this area has rapidly expanded in recent years, and several studies have suggested that these therapies have synergy similar to that observed with other immune-checkpoint inhibitors. Early data supporting the strategy of PD-1 inhibition with RT were reported in a triple-negative breast cancer model[31] in which PD-1 appeared to be a critical signal in mediating the antitumor response of RT in combination with an immunostimulatory antibody against CD137. In irradiated tumors, the addition of CD137 resulted in improved tumor control, whereas the addition of a PD-1 antibody resulted in a cure for all tumors. Furthermore, RT appeared to enrich the population of tumor-specific effector CD8-positive T cells.[31] Additional studies in a mouse glioma model demonstrated that the combination of stereotactic radiosurgery (SRS) with anti-PD-1 therapy improved survival over either modality alone and also resulted in an increase in tumor-infiltrating cytotoxic T cells.[32]

Recent data have suggested multiple potential underlying synergistic mechanisms, further supporting the rationale for this clinical combination. It has been demonstrated that RT results in an up-regulation of PD-L1 in the tumor microenvironment, and the combination of RT plus PD-L1 blockade results in the activation of cytotoxic T cells and a reduction in myeloid-derived suppressor cells[33] (Fig. 1). Additional data suggest that RT in combination with anti-PD-1 therapy induces an antigen-specific immune response in models of melanoma and breast cancer.[34] The interaction of the immunosuppressive cytokine transforming growth factor β (TGF-β) with the PD-1/PD-L1 axis appears to be a critical signal mediating the antitumor effects of RT. The combination of TGF-β blockade with RT resulted in tumor rejection in a poorly immunogenic breast cancer model, but up-regulation of PD-L1/L2 limited the durability of control. The addition of anti-PD-1 therapy subsequently improved survival, highlighting the complexity surrounding the generation of a successful immune-mediated antitumor response.[35] Collectively, these data provide a strong preclinical rationale for exploring the combination of PD-1/PDL-1 blockade with RT. To date, there are no published articles reporting the outcomes of patients who received treatment with PD-1/PDL-1 inhibitors and RT, although many trials are planned or are underway (National Clinical Trials NCT02407171, NCT02303366, and NCT02289209; clinicaltrials.gov).

EARLY CLINICAL OUTCOMES WITH IMMUNOTHERAPIES AND RT

Many studies seeking to harness the combination of the immune system and RT have used interferon-α-2b (INFα-2b),[36] β-interferon (β-INF),[37] IL-2,[38] intratumor dendritic cell injections,[39] and granulocyte-macrophage–colony-stimulating factor (GM-CSF).[40,41] Although the experience with interferons has been disappointing, the IL-2 and GM-CSF experiences are promising. Early results of intratumor dendritic cell injections are intriguing and awaiting final outcomes. Although the preclinical experience combining RT with immunomodulation has been positive, the early clinical results were underwhelming likely because of ineffectual immunotherapies, older nonablative RT techniques, and potentially the lack of advanced imaging techniques to detect comprehensive responses. A summary of selected studies is presented in Table 1.

Type 1 interferons have been combined with RT without improving tumor control and often with increased late toxicity. Because adjuvant INFα-2b[47-49] and RT[50,51] are commonly used after surgery for high-risk melanoma, the combination has been tested in patients at significant risk for lymph node and distant relapse. In one such phase 1/2 study, after induction IFNα-2b, 23 patients received 30 Gy in 5 twice-weekly fractions in addition to concurrent IFNα-2b. In that trial, 3-year disease outcomes were promising, with reported 78% locoregional control, 43% disease-free survival, and a 48% overall survival (OS).[36] However, others have reported unusual, significant acute, subacute, or late toxicities in a substantial number of patients (range, 17%-50%) who received the combination of RT and IFNα-2b, including brachial plexopathy, brain necrosis, wound dehiscence, and subcutaneous necrosis, without improved clinical outcomes.[42-45]

Based on promising early phase data,[52] the combination of β-INF administered before 60 Gy RT (2 Gy daily) was compared with RT alone in patients with stage III nonsmall cell lung cancer. Twenty-four percent of those randomized to the combination did not complete all β-INF doses because of toxicity, and combined β-INF plus

TABLE 1. Summary of Selected Studies That Included ≥10 Patients Who Received Interferon α-2b, β-Interferon, or Interleukin-2 Plus Radiotherapy

Institution (Reference)	Disease	No. of Patients	Median Follow-Up, mo	Immunotherapy	RT Dose	Toxicity Grade ≥3	Outcomes
INF α-2b							
Multicenter (Finkelstein 2012[36])	Melanoma	23	80	INF α-2b: induction, 20 MU/m^2/d IV 5 d every wk × 4 wk; concurrent/ consolidation, 10 MU/m^2 SQ 3 × per wk for 1 y	30 Gy (5 twice-weekly doses)	Acute, 9% (skin); late, 1 (brachial plexopathy)	3-y LRC, 78%; 3-y DFS, 43%; 3-y OS, 48%
University of Utah (Hazard 2002[42])	Melanoma	10	18	INF α-2b: NR	50 Gy (1.8-2.5 Gy daily)	Late, 50%	NR
University of Barcelona (Conill 2007[43])	Melanoma	18	28	INF α-2b: induction, 20 MU/m^2/d IV 5 d every wk × 4 wk; concurrent/ consolidation, 10 MU/m^2 SQ 3 × per wk for 1 y (15/18)	30 Gy (5-6 twice-weekly doses) or 50 Gy (2 Gy daily)	Late, 17%	3-y LRC, 88%
Peter MacCallum Cancer Center (Gyorki 2004[44])	Melanoma	18	29	INF α-2b: induction, 20 MU/m^2/d IV 5 d every wk × 4 wk; concurrent/ consolidation, 10 MU/m^2 SQ 3 × per week for 1 y (15/18)	40-50 Gy (15-25 daily doses)	Acute, 39% (7/18); late, 17% (3/18)	NR
Helsinki University (Maasilta 1992[45])	NSCLC	10	NR	INF α-2b: 3 MU IM and 1.5 MU inhaled 30 min before each RT dose	60 Gy (38 1.25-Gy BID doses)	Acute, 40% (severe esophagitis)	Median OS, 13 mo
β-INF							
RTOG 93-04 (Bradley 2002[37])		58	48	β-INF: 16 MU IV 3 times weekly 30 min before RT during wk 1, 3, and 5	60 Gy (2 Gy daily doses)	Acute, 31%; late, 14%	Median OS,10.3 mo; 1y OS, 42%
IL-2							
Providence Cancer Center (Seung 2012[38])	Melanoma and RCC	12	12	IL-2: 60,000 IU/kg IV bolus every 8 h × 14 doses for 2 cycles every 16 d	20 Gy; 40 Gy (20 Gy/ dose, twice weekly); 60 Gy (20 Gy/dose, 3 times weekly)	Not increased compared with IL-2 alone	Median OS, 12 mo; PET response, 100%
National Cancer Institute (Lange 1992[46])	Melanoma and RCC	28	NR	IL-2 with or without TILs: 720,000 IU/kg IL-2 or TILs plus 720,000 IU/kg IL-2	10-20 Gy, 5 Gy BID 2-24 h before IL-2		Systemic response, 7%
GM-CSF							
New York University (Golden 2015[41])	Multiple	41	NR	GM-CSF: 125 μg/m^2 SQ daily × 14 d	35 Gy (3.5 Gy daily doses)	NR	Median OS, 13 mo; 1-y OS, 51%

Abbreviations: BID, twice daily; DFS, disease-free survival; GM-CSF, granulocyte-macrophage–colony-stimulating factor; Gy, Gray; IM, intramuscular; INF, interferon; IV, intravenous; LRC, locoregional control; m, meter; MU, million units; NR, not reported; NSCLC, non-small cell lung cancer; OS, overall survival; PET, positron emission tomography; RT, radiotherapy; RTOG, Radiation Therapy Oncology Group; SQ, subcutaneous; TILs, tumor-infiltrating leukocytes.

RT resulted in significantly increased acute (primarily pulmonary and esophageal) grade 3 and 4 toxicities (31% vs 19%; $P = .02$). In addition, grade 4 and 5 pneumonitis was observed in 8% of patients who received β-INF but in no patients who received RT alone. There was no difference in median survival or OS between the 2 arms, and further study was not planned.[37]

High-dose IL-2 is an established therapy for patients with metastatic melanoma or renal cell carcinoma who have limited potential for deep, durable responses.[53] After surprising systemic responses were observed in patients who received ablative RT shortly before IL-2, the combination of SBRT and IL-2 was investigated in a phase 1 study of 12 patients with metastatic melanoma or renal cell carcinoma.[38] Patients received irradiation to 1 to 3 lesions dosed based on cohort (20 Gy, 40 Gy in 2 doses, or 60 Gy in 3 doses). Each IL-2 course started the week after RT and consisted of 2 cycles of 600,000 IU/kg administered according to a standard protocol. Up to 3 IL-2 courses were received by responding patients. No SBRT-related dose-limiting toxicities were reported. Although the study was small, 73% of patients had an objective response (1 complete response [CR] and 7 partial responses [PRs]), which was much greater than the 17% historic IL-2 response rate. All patients who had a positron emission tomography (PET) study available (6 of 6 patients) had a whole-body PET CR after protocol therapy. The responses were durable, and most lasted $\geq$12 months. All SBRT-treated lesions were controlled at last follow-up. These findings are the basis for an ongoing phase 2 randomized study comparing IL-2 and SBRT versus IL-2 alone in patients with metastatic renal cell carcinoma (NCT02306954). However, the results are in contrast to earlier attempts at integrating IL-2 and RT. A study that tested the efficacy of RT 5 Gy twice daily, delivering 10 to 20 Gy, followed by IL-2 or TILs plus IL-2 demonstrated the safety of the combination. However, because only 4 of 28 patients in that study had significant reductions of irradiated tumors, and only 2 had *both* irradiated and nonirradiated tumor responses, synergy was not detected.[46] The differing results between these 2 studies are likely because of the availability of PET-computed tomography surveillance and because the RT doses (5 Gy) and techniques used earlier may not have elicited a robust immune response.[7]

CLINICAL OUTCOMES

Concurrent CTLA-4 Inhibition and Extracranial RT

Great enthusiasm now exists for the combination of immune-checkpoint inhibitors and RT.[54] This is particularly true for ablative, stereotactic RT.[55] Despite this enthusiasm, published data are limited (selected series presented in Table 2), although they will likely increase exponentially. In general, series tend to focus on reporting results of intracranial and extracranial radiation treatments separately, given the force of morbidity and mortality of brain metastases.

The first and largest report of concurrent extracranial RT and ipilimumab described 29 patients, all with good performance status and most (n = 26) with visceral metastases.[56] The median ipilimumab and RT doses in this series were 10 mg/kg and 30 Gy in 3 Gy fractions, respectively. The combination of ipilimumab and RT did not increase the risk of immune-related adverse events (irAEs) compared with historic results. However, the rate of grade $\geq$3 toxicity in irradiated organs was 15%, and patients who received higher RT doses had a 44% chance of radiation-related adverse events (AEs) versus 0% in those who received lower doses ($P = .004$). The combination of concurrent anti–CTLA-4 therapy and RT did not improve survival compared with historic cohorts. However, in the group that received RT with 10 mg/kg ipilimumab, OS was 39 months. It is unknown whether this favorable survival rate is because of patient selection, given that this ipilimumab dose was available only on study, or because of a synergistic effect. Early reports of a prospective trial of palliative RT to 1 or 2 metastases within 5 days of ipilimumab also demonstrated no increased toxicity, with 1 CR and 10 patients who had PRs/stable disease (SD) at a median of 39 weeks.[62]

CTLA-4 Inhibition With or Without Single-Fraction, Palliative RT

One of the first prospective investigations of the combined RT and anti-CTLA-4 effects was a phase 1/2 study of dose-escalated ipilimumab (3 mg/kg or 10 mg/kg) and a single 8-Gy RT fraction directed at 1 to 3 metastases 24 to 48 hours before ipilimumab.[58] Seventy patients with metastatic, castrate-resistant prostate cancer enrolled on that trial, and 41 received RT. The delivery of palliative-dose RT before ipilimumab was identified as safe; and all AEs, including irAEs, were unchanged with the combination therapy at both 3 mg/kg and 10 mg/kg of ipilimumab. Of particular importance, diarrhea (41% vs 81%) rash (21% vs 56%), and colitis (12% vs 38%) were all less common in the RT patients, and there were no bowel perforations with or without ipilimumab. Furthermore, patients who received RT had less treatment-related grade 3 and 4 AEs (38% vs 63%) as well as irAEs (18% vs 63%), including colitis (6% vs 38%), hepatitis (6% vs 19%), and diarrhea (6% vs 13%).

TABLE 2. Summary of Selected Studies of Patients Who Received Treatment With Extracranial Radiotherapy and Ipilimumab

Institution (Reference)	No. of Patients Receiving Combination RT and CTLA-4 Inhibition	Median Follow-Up, mo	Ipilimumab	RT Dose	Toxicity Grade ≥ 3	Outcomes
Memorial Sloan-Kettering (Barker 2013[56])	29[a]	11	3 mg/kg or 10 mg/kg every 3 wk up to 4 doses	Median, 30 Gy	irAEs: 10 mg/kg, 43%; 3 mg/kg, 20%	Induction ipilimumab: Median OS, 9 mo; maintenance ipilimumab: median OS, 39 mo
Duke University (Qin 2015[57])	44	23	3 mg/kg every 3 wk up to 4 doses	Ablative: median, 16 Gy; conventional, median, 35 Gy	Not increased with combination	Median OS, 18 mo; OS at 1 y, 64%; ablative: median OS, 20 mo; OS at 1 y, 80%
Multi-institutional phase 1/2 (Slovin 2013[58])	41	16	3 mg/kg or 10 mg/kg every 3 wk up to 4 doses	8 Gy	irAEs: 3 mg/kg, 43% (3/7); 10 mg/kg, 18% (6/34)	Median OS, 17.4 mo
Multi-institutional phase 3 (Kwon 2014[59])	399 (of 799 randomized)	10	10 mg/kg every 3 wk up to 4 doses	8 Gy (up to 5 metastases)	—	Median OS, 11.2 mo; OS at 1 y, 46.8%; PFS at 6 mo, 30.7%
University of Pennsylvania (Twyman-Saint Victor 2015[60])	22	21	3 mg/kg every 3 wk up to 4 doses	Lung/bone, 16-24 Gy (8 Gy × 2-3); liver/SQ, 12-18 Gy (6 Gy × 2-3)	18 at 24 Gy: 2 grade 3, 0 grade 4	Median PFS, 3.8 mo; median OS, 10.7 mo
New York University[61])	53	NR	NR	NR	NR	NR
Stanford University (Hiniker 2015[62])	20	NR	3 mg/kg every 3 wk up to 4 doses	NR	NR	NR

Abbreviations: CTLA-4, cytotoxic T-lymphocyte–associated protein 4; Gy, gray; irAEs, immune-related adverse events; NR, not reported; OS, overall survival; PFS, progression-free survival; RT, radiotherapy; SQ, subcutaneous.

[a] In total, 166 patients received RT before, during, or after ipilimumab administration.

Single-Fraction Palliative RT With or Without Sequential CTLA-4 Inhibition

The effect of sequential RT followed by CTLA-4 inhibition on prostate-specific antigen response and survival has been studied in patients with metastatic, castrate-resistant prostate cancer.[59] Within 2 days of a single 8-Gy RT dose delivered in up to 5 osseous metastases, 799 patients were randomized to receive either ipilimumab 10 mg/kg every 3 weeks up to 4 doses (n = 399) or placebo (n = 400). The patients who received ipilimumab after RT had improved median PFS (4.0 vs 3.1 months; $P < .0001$) and 6-month PFS (30.7% vs 18.1%) compared with the placebo group. OS at 1 and 2 years nonsignificantly favored the ipilimumab group (1-year OS: ipilimumab, 46.8%; placebo, 40.4%; 2-year OS: ipilimumab, 26.2%; placebo, 15%). In an unplanned subgroup analysis, favorable patients (those with hemoglobin levels >11 g/dL, alkaline phosphatase < 1.5 the upper limit of normal, and no visceral metastases) had a median OS of 22.7 months if they received ipilimumab compared with 15.8 months in the placebo group ($P = .0038$). In all others, the median OS was 6.5 months with ipilimumab, similar to the 7.3 months observed in those who received placebo. Like in the phase 1/2 study, pelvic RT did not increase the risk of colitis.

Comparison of CTLA-4 Alone Versus CTLA-4 and RT

Few reports have directly compared patients who received ipilimumab alone with a contemporaneous cohort that received ipilimumab plus RT.[57,61] One retrospective comparison included 44 patients with advanced melanoma who received ipilimumab alone versus an unmatched cohort of 44 patients who received ipilimumab plus RT.[57] Although there were more adverse clinicopathologic features in the RT group, OS was similar between patients who received ipilimumab alone and those who received ipilimumab combined with RT. There did not appear to be a clinically detectable increase in irAEs with the combination of RT and ipilimumab. It is noteworthy that patients who received ipilimumab before RT had improved 6-month and 12-month response durations (84.3% and 74.7%, respectively) compared with patients who received ipilimumab after RT (44.8% and 44.8%, respectively; $P = .01$). Those who received extracranial ablative radiation had a longer median OS (38 months) compared with those who received conventionally fractionated RT in another series (10.2 months).[56]

In a similar analysis, 48 patients who received CTLA-4 inhibitor alone at various doses were compared with a group of 21 patients who received CTLA-4 inhibition with RT; 35 patients who received CTLA-4 inhibition, steroids, and RT; and 40 patients who received CTLA-4 inhibition and steroids to determine whether there were differential immunostimulatory effects.[61] Interestingly, those receiving RT ($P = .02$) or steroids ($P = .02$) had better OS compared with those who received CTLA-4 inhibition alone. Patients who received RT had higher levels of IL-2, IL-17a, and TNFα compared with those who received CLTA-4 inhibition alone, suggesting the effects of T-helper cells.

CTLA-4 Inhibition and Intracranial Radiosurgery

The combination of immune-checkpoint inhibitors with intracranial radiosurgery (SRS) is being increasingly reported. In contrast to extracranial RT, for which reports are sparse, growing numbers of institutions have reported their experiences combining ipilimumab and SRS.[63-67] Some general principles have come to light. First, the optimal timing of SRS and ipilimumab is unknown. Furthermore, given the possibility of pseudoprogression from both radiosurgery[68,69] and ipilimumab,[70] care must be taken when determining progression on surveillance imaging after the combination of SRS and ipilimumab.

A summary of selected series of patients who underwent SRS and received ipilimumab is provided in Table 3. In total, < 150 patients have been reported. However, most have not reported increased intracranial hemorrhage[65] or other "unexpected toxicities."[67] Although the grade 3 and 4 toxicity rate reported by some appears to be high, this is consistent with some series of SRS alone that included long-term follow-up. One group has continued to report that increasing numbers of patients with melanoma, now totaling 7, who received treatment with ipilimumab and radiosurgery have experienced symptomatic brain necrosis, both pathologically proven[72] and radiographically diagnosed.[73] In patients with pathologically proven brain necrosis, the median time to symptomatic brain necrosis was 11 months after SRS. Because the number of patients who have received combination CTLA-4 inhibition and SRS is relatively small, further reports are needed to address the potential toxicities of these therapies in combination.

Some series that compared patients who received combined ipilimumab and SRS with those who underwent SRS alone[64,67] have reported significant improvements in OS with combination therapy (approximately 20 months) versus those who underwent SRS alone (approximately 4-5 months), whereas others have not.[65] It is unknown whether improved survival in those reports

TABLE 3. Summary of Selected Studies of Ipilimumab and Stereotactic Radiosurgery for Melanoma Brain Metastases

Institution (Reference)	No. of Patients Receiving Combination SRS and CTLA-4 Inhibition	Median Follow-Up, mo	Results	Toxicity	Comments
Hofstra University (Knisely 2012[64])	27	12.2	Median OS, 21.3 mo	NR	No difference in OS if SRS received before or after Ipi
New York University (Mathew 2013[65])	25	NR	LC, 65% vs 63%; FFBM, 35% vs 47%; OS, 56% vs 46%; Ipi + SRS vs SRS alone, NSSD	Intracranial hemorrhage 7% vs 10% (NSSD)	
Memorial Sloan-Kettering (Kiess 2015[63])	46	22	OS at 1 y: 56% pre-Ipli; 65% during-Ipi; 40% post-Ipi ($P = .008$)	15% CNS grade 3-4 toxicity	150% increase in BM diameter, 50%; for SRS before or during Ipi vs 13% for SRS after Ipi
University of Michigan (Silk 2013[67])	17	NR	Median OS, 19.9 mo	No unexpected toxicities	
Duke University (Olson 2015[66])	27	7.1	Median OS, 10.4 mo	No unexpected toxicities	
Emory University (Patel 2015[71])	20	7.3	OS at 1 y: 37.1%	No unexpected toxicities	

Abbreviations: CNS, central nervous system; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; FFBM, freedom from new brain metastases; GK, gamma knife; Ipi, ipilimumab; LC, local control; NR, not reported; NSSD, no statistically significant difference; OS, overall survival; RC, regional control; SRS, stereotactic radiosurgery.

is because of the combination or is simply a reflection of the potential survival of patients who were treated in an era in which active systemic medications were available for the treatment of melanoma.

An important finding from these studies is the potential for patients to have significant tumor enlargement after a course of SRS and ipilimumab.[63] This effect adds another layer of complexity to the post-SRS imaging evaluation, which already is challenging with the various methods used to determine local progression postradiosurgery.[74] Thus, as the use of immunomodulatory systemic therapies increases, a consensus recommendation based on clinical outcomes of those treated in combination with SRS will be needed to standardize outcomes reporting and clinical decision making.[75]

REPORTS OF THE ABSCOPAL EFFECT

One of the greatest hopes for the combination of RT and immune-checkpoint therapy is the ability of radiation to sensitize the immune system, enabling systemic regression of *all disease*, not just irradiated sites, commonly referred to as the abscopal (ab, off; scopus, target) effect, which was described first in 1953.[76] Although a true abscopal effect does not include the effect of systemic therapies, many recent reports have described *new* or *enhanced* systemic responses after RT in patients progressing through immunotherapy. Radiation augmentation of systemic therapy is not new.[77] However, reported dramatic responses[78] as well as the understanding that the abscopal effect is mediated by the immune system have escalated the interest in RT enhancement of systemic therapies.

The interest in the abscopal effect belies the rarity with which this has been reported.[79] A recent systematic review that included studies published between 1960 and 2014 identified 51 patients (23 case reports, 1 retrospective study; n = 28) who reportedly had an abscopal effect.[80] Abscopal effects were observed a median of 5 months after RT (range, 1-24 months), with a median response duration of 13 months (range, 3-39 months). The median RT dose that elicited the abscopal effect was 32 Gy. It is noteworthy that 5 abscopal effects were achieved with a combined immunotherapy-RT approach.[80] One of the largest reports of RT-induced immune stimulation describes 21 patients with advanced melanoma who received RT after progressing through ipilimumab.[81] After intracranial (62%) and extracranial RT, 52% of patients demonstrated an abscopal effect, defined as an immune-related and tumor-inhibitory effect observed outside of the irradiated field. Most patients had PRs (n = 9), whereas 2 patients had SD for at least 4

months. It is noteworthy that the patients who exhibited an abscopal effect had a longer median survival (22.4 months vs 8.3 months) than those who did not. These results are similar to a prospective study of subcutaneous GM-CSF 125 $\mu g/m^2$ daily for 14 days plus RT in 41 patients who did not respond to systemic therapy. In that study, improved survival was observed in the 27% of patients who exhibited an abscopal effect,[41] all whom had ≤ 6 metastases. Because the optimal radiation dose schedule and timing required to induce an abscopal effect is unknown, many studies are underway hoping to answer this question.

Proposed Immunologic Mechanisms of the Abscopal Effect

In a report of a true abscopal effect, palliative RT to a primary scalp melanoma induced a CR in unirradiated dermal metastases. It is interesting to note that, at the time of relapse, serology demonstrated increased circulating melanoma antigen A3 (MAGE3) at titers of 1:300. After SRS and ipilimumab, the MAGE3 titers increased to 1:700, and there were new antibodies to the cancer antigen PAS domain-containing 1 (PASD-1), and the patient achieved complete remission.[82]

Further reports of the abscopal effect in multiple histologies have described enhanced systemic responses based on biomarkers as well as standard imaging. In 1 report, radiation was used to resensitize a patient with metastatic melanoma to CTLA-4 inhibition after symptomatic progression of a paraspinal mass. Immune monitoring indicated renewed systemic disease control after RT with continued ipilimumab.[83] Specifically, serologic testing detailed increased antibody reactivity to new antigens and a decline in myeloid-derived suppressor cells. Similarly, focal hepatic radiation was used to sensitize a patient with metastatic nonsmall cell lung cancer to ipilimumab.[84]

Mechanisms of Enhanced Immunologic Checkpoint Inhibitor Responses and Resistance After Radiation

The development of novel immunotherapies, coupled with the potential for synergy with RT, has garnered much attention recently; yet, for many patients, the current reality is that their tumors are refractory or ultimately resistant to primary therapy. It remains critical to gain a better understanding of the immunologic underpinnings to ultimately identify predictors of response and resistance.

The potential mechanisms of resistance to combined RT and immunomodulatory agents are not well understood. In a mouse B16-F10 melanoma model, the ratio of CD8-positive T cells to T-regulatory (Treg) cells appeared to be the most important predictor of response. In sensitive tumors, concurrent administration of anti CTLA-4 therapy with RT resulted in an increase in the CD8-positive/Treg ratio; yet, in resistant tumors, this was not observed, because an expansion of CD8-positive cells was not detected.[60] It is noteworthy that genomic profiling of resistant cells identified up-regulation of PD-L1, and blockade of PD-L1 restored sensitivity. Furthermore, triple-combination therapy (CTLA-4, PD-L1, and RT) appeared to be superior compared with dual-checkpoint blockade in terms of response rates, with each contributing a discrete component to the immunologic response. Whereas CTLA-4 appeared to decrease Treg cells, PD-L1–directed therapy increased the number of CD8-positive TILs, and RT resulted in an increased diversity of the T-cell receptor repertoire. Clinically, tumors from patients who progressed on a clinical trial of SBRT plus ipilimumab also appeared to have high expression of PD-L1, highlighting this pathway as a likely key mediator of resistance.

CHALLENGES AHEAD

Many issues related to the combination of immune modulators and RT need to be addressed as the field of immuno-oncology continues to advance. An inherent challenge will be keeping pace with rapidly evolving systemic therapy standards. Although data on combination with RT using single-agent CTLA-4 inhibition are only now emerging, PD-1/PDL-1 inhibition alone or in combination is becoming preferred therapy for many malignancies.[85-87] The safety and efficacy of integrating RT with combined CTLA-4/PD-1/PDL-1 inhibition are unknown, although it is likely that these data will be reported in the next few years.

Detecting the additional benefit of RT to augment systemic response rates may be a challenge in malignancies like melanoma that have high response rates, but it will likely be detectable in nonsmall cell lung cancer, in which response rates approach 20%. Beyond augmenting response rates, the addition of RT may augment the depth and duration of responses and also may provide another means of measuring synergistic effects. Prospective (preferably randomized) studies are needed to elucidate the preferred timing, dosing, and volume of RT required to maximize the effects of combination immunotherapy and RT.[88]

CONCLUSIONS

The field of immuno-oncology continues to rapidly expand. Radiation, a longtime cornerstone of cancer therapy, is developing a new role as an immune stimulus. Decades of preclinical work have created the foundation for a better understanding of the mechanisms that underlie the potential for synergy with these modalities. Now, with the development of advanced radiation planning techniques, along with more tolerable, clinically active immunotherapies, the translational aspect of this work can be fully realized.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

April K. S. Salama reports personal fees from Bristol-Myers Squibb and grants to her institution from Merck, Bristol-Myers Squibb, Genentech, and Reata Pharmaceuticals outside the submitted work. Michael A. Postow reports grants and personal fees from Bristol-Myers Squibb, personal fees from Caladrius, and personal fees from Amgen outside the submitted work.

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