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Combining Radiation Therapy with Immune Checkpoint Blockade in Breast Cancer

Shervin Tabrizi[1,2] · Susan McDuff[1,2] · Alice Y. Ho[1]

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Abstract

Purpose of Review Immune checkpoint blockade (ICB) is an emerging therapy in breast cancer. Its optimal integration with radiation therapy (RT) for breast cancer remains to be established. Herein, we review the current evidence on combining ICB and RT in breast cancer and discuss the challenges, open questions, ongoing trials, and future directions for use of this treatment combination.

Recent Findings Early trials of ICB in breast cancer show evidence of a modest response, limited due to the low baseline immunogenicity of most breast cancers. RT, as a potent stimulator of the immune system, has been used in combination with ICB with encouraging results. The optimal dose, fractionation, and timing of RT combined with ICB are active areas of investigation. Preclinical evidence suggests that moderate-dose, hypofractionated courses may be more effective at stimulating an immune response than high-dose, single-fraction courses.

Summary Recent studies suggest that ICB can be active in breast cancer, but optimizing the response rate remains a challenge. The immunostimulatory effects of RT have the potential to overcome this obstacle, with promising data from preclinical and early clinical trials. Future investigation on the optimal dosing and fractionation of RT in combination with ICB will be critical.

Keywords Immunotherapy · Breast cancer · Radiation · Abscopal effect · Checkpoint blockade

Introduction

Immunotherapy in the form of immune checkpoint blockade (ICB) has transformed oncology, with approvals now for management of melanoma, non-small cell lung, bladder, renal, head and neck, and breast cancers, and in tumors with microsatellite instability. The most widely investigated targets are immune inhibitory mechanisms mediated by cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) or its ligand, programmed death ligand 1 (PD-L1).

The evidence base for use of ICB in breast cancer is only now emerging, delayed in part due to the low immunogenicity of breast cancer compared to other tumors such as melanoma or non-small cell lung cancer. Atezolizumab, an inhibitor of PD-L1, was the first ICB agent approved by the United States Food and Drug Administration (FDA) in March 2019 for treatment of PD-L1-positive metastatic and locally advanced triple-negative breast cancer [1, 2••].

Radiation therapy (RT) is one of the pillars of therapy for definitive management of early-stage and locally advanced breast cancer and plays a critical role in palliation of metastatic disease. Furthermore, increasing evidence points to a role for ablative RT in the oligometastatic setting for breast cancer [3]. RT also has effects on the tumor microenvironment and immune system, which interact in complex ways with ICB. Here, we review the evidence on ICB in breast cancer to date and the ongoing work on clinical trials combining RT and ICB.

Breast Cancer and the Immune System

ICB seeks to release the breaks on the immune system that are induced by tumors, typically through inhibition of T cell activation, as a method of escape from immune surveillance. Inhibition of CTLA-4 and the PD-1 axis are the two best-elucidated mechanisms that have been described so far.

T cell activation occurs via binding of the T cell receptor with the major histocompatibility complex (MHC)—antigen

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✉ Alice Y. Ho
alice.ho@mgh.harvard.edu

[1] Department of Radiation Oncology, Massachusetts General Hospital, Cox Level 3, 55 Fruit St, Boston, MA 02114, USA

[2] Harvard Radiation Oncology Program, Boston, MA, USA

complex on antigen-presenting cells (APC). For T cell activation to occur, a co-stimulatory signal provided by binding of CD28 to CD80/CD86 on APCs is necessary. However, CTLA-4 expression on T cells, which usually occurs within 48–72 h of activation, competes with CD28 for binding to CD80/CD86 and attenuates T cell activation. This mechanism is hypothesized to play a role in dampening an exuberant and excessive immune response [4, 5].

The PD-1 axis, in contrast, operates in the peripheral tissue, where it attenuates the activity of effector T cells in response to infections and in autoimmunity. PD-1 is expressed on effector T cells, and when PD-1 binds to its ligands PD-L1 or PD-L2 that are expressed on tumor cells, it transduces an inhibitory signal that limits T cell proliferation and cytotoxic activity and promotes regulatory T cell (Treg) activity [6]. Solid tumors can co-opt this mechanism to evade immune surveillance and clearance through increased PD-L1 expression [7].

Most breast cancers are considered "immunologically cold," which has presented a challenge to the widespread adoption of ICB in the treatment of breast cancer. In this context, tumor-infiltrating lymphocytes (TILs) are increasingly recognized as an important marker for prognosis and response to therapy in breast cancer. Higher TIL levels have been associated with an increased likelihood of pathologic complete response (pCR) to neoadjuvant chemotherapy [8–10] and improved recurrence-free survival endpoints [11, 12]. There is significant variation in the presence of TILs among breast cancer subtypes. Lymphocyte-predominant tumors, defined as having at least 50% or 60% lymphocytic infiltrate, occur in 11% of breast cancers overall, 20% of triple-negative breast cancers (TNBC), 16% of human epidermal growth factor receptor 2 (HER2)-positive breast cancers (HER2+), and only 6% of hormone receptor-positive/HER2-negative (HR+/HER2−) breast cancers [13]. PD-L1 expression mirrors this pattern. In a study of PD-L1 expression across breast cancer subtypes, PD-L1 expression > 1% was observed in 84% of TNBCs, 73% of HER2+ breast cancers, and 53% of ER+/HER2− breast cancers [14]. A major caveat in interpreting these results is that the rate of PD-L1 positivity can vary significantly based on the antibody used for testing. In one study of TNBC, the prevalence of tumors with combined positive score (CPS) ≥ 1 after staining for PD-L1 was 64% with SP263 versus 60% with 22C3 and 35% with SP142 [15]. In IMpassion130, a phase III trial evaluating the efficacy of nab-paclitaxel +/− atezolizumab in metastatic TNBC, PD-L1 expression was measured via SP142, and tumors with ≥ 1% staining on tumor-infiltrating immune cells were considered PD-L1 positive [2••]. A 40.9% rate of PD-L1 positivity was reported in that trial. Despite these variabilities, given the overall enrichment of TNBC and HER2+ breast cancers for TILs and PD-L1 expression, most clinical trials in breast cancer to date have focused on exploring the efficacy of ICB in these subtypes. There is a growing realization that varying PD-L1 assays may result in discordant results, and further study is required to evaluate the association between PD-L1 and response to ICB therapies.

Several clinical trials have examined ICB monotherapy in breast cancer, generally with modest but promising results. The KEYNOTE-012 study examined the safety and activity of pembrolizumab, an anti-PD-1 antibody, in PD-L1-positive TNBC (defined as PD-L1 expression in stroma or ≥ 1% of tumor cells by immunohistochemistry using the 22C3 antibody), and reported an overall response rate (ORR) of 18.5% with a median time to response of 17.9 weeks [16]. KEYNOTE-028, in contrast, examined the response to pembrolizumab in PD-L1-positive metastatic HR+ breast cancer and reported a more modest response rate of 12% [17]. A study of the efficacy of atezolizumab in TNBC reported an overall response rate of 10%, with a 24% response rate when administered as first line versus 6% when administered as second- or later-stage therapy [18]. This study included both PD-L1-positive (defined as PD-L1 expression on at least 1% of tumor-infiltrating immune cells using the SP142 antibody) and PD-L1-negative tumors, and all responses were observed in the PD-L1-positive tumors (12%, 11 of 91). The JAVELIN study was a phase Ib study of the anti-PD-L1 antibody avelumab in all breast cancer subtypes. The trial reported variation in the objective response rate among subtypes, ranging from 2.8% in ER+/HER2− cancer, to 3.8% in HER2+ and 8.6% in TNBC subtypes respectively [19].

The relatively modest response rates to ICB monotherapy in breast cancer are thought to be driven, at least in part, by the low immunogenicity of breast cancer. Higher numbers of TILs and greater PD-L1 expression have been shown to correlate with improved outcomes. In the PANACEA trial of trastuzumab and pembrolizumab in metastatic HER2+ breast cancer that had progressed on previous HER2-directed therapy, patients with TIL levels greater than 5% had double the response rate. Furthermore, all observed responses were in PD-L1-positive tumors, and there were no responses in PD-L1-negative tumors, which were included in the phase II portion of this trial [20•]. Responses are most often seen in the TNBC subtype, which comprises only 15% of all breast cancers. These observations have led to attempts to boost the response rate through combination with other immunomodulatory therapies, including chemotherapy and radiation therapy.

Using Radiation Therapy to Enhance Response to Immune Checkpoint Blockade in Breast Cancer

RT and the Immune System

Ionizing radiation is a method of anti-cancer therapy which relies on DNA damage resulting in single- or double-strand

breaks to promote tumor cell death and inhibition of cellular proliferation. RT interacts with the immune system in complex ways. Early preclinical work demonstrated that a competent immune system is critical for tumor regression in response to radiation therapy in a mouse fibrosarcoma model [21], and established the role of T cells in facilitating tumor regression following ablative RT. Subsequent work demonstrated that cytotoxic T cells play a critical role in this process and has elucidated some of the mechanisms behind host immune response to RT.

RT is generally thought to augment the immune system response through promotion of inflammatory, pro-immunogenic cell death, characterized by cytokine release, release of tumor-associated antigens (TAA) and danger-associated molecular patterns (DAMPS), including HMGB and calreticulin, promotion of apoptosis and autophagy, and priming and activation of cytotoxic T cells [22–26]. Dendritic cell (DC) activation and T cell recruitment have been implicated in this process through a type I interferon (IFN)-dependent pathway [27–29]. The STING (stimulator of interferon genes) pathway has been implicated in this process, by promoting type I IFN production following activation via DNA release by dying tumor cells [30, 31].

DNA release into the cytoplasm appears to be critical to the initiation of an immune response following RT. Vanpouille-Box et al. compared responses to various RT regimens combined with anti-CTLA-4 therapy in TSA, a mouse mammary carcinoma model that is refractory to ICB [32••]. They demonstrated that RT with single fractions of 20–30 Gy did not produce a tumor response, whereas treatment with 8Gy × 3 fractions did. They also showed that higher RT doses increase expression of TREX1, a DNA exonuclease that degrades cytosolic DNA, thereby inhibiting DNA-induced STING activation, IFN production, and immune stimulation (Fig. 1).

RT additionally has effects on the tumor microenvironment (TME) that promote an immunogenic response by facilitating T cell infiltration. This includes remodeling of aberrant tumor vasculature [33] and upregulation of vascular cell adhesion molecule (VCAM)-1, leading to improved T cell infiltration [34, 35]. The effects of low-dose RT, which leads to inducible nitric oxide synthetase (iNOS) expression in macrophages, have also been shown to support this process [36], although the effect is reversed at high per-fraction RT doses [37]. RT can also lead to release of chemokines and cytokines that promote favorable TME changes. In a model of carcinoma with low immunogenicity, RT led to upregulated expression of C-X-C motif chemokine ligand 16 (CXCL16), which promoted the migration of C-X-C motif chemokine receptor 6 (CXCR-6)-positive T lymphocytes into the tumor with improved anti-tumor effect [38, 39]. These effects may be especially important in an otherwise immunologically cold tumor such as breast cancer. In a mouse model of metastatic breast cancer, a dense fibrotic stroma was found to exclude TILs. This fibrosis was decreased through pharmacologic inhibition of CXCR4/CXCL12 signaling, leading to doubling of response to ICB [40]. Modulation of TME will likely be critical to optimizing response to ICB in breast cancer.

Although RT can be a potent stimulator of the immune system, it can have inhibitory effects on the immune response to tumors as well. RT can promote tumor infiltration by myeloid-derived suppressor cells (MDSCs), which may occur through increased expression of macrophage colony-stimulating factor 1 (CSF1) [41]. Increased Treg infiltration, an important cell type for dampening and modulating immune response, has also been observed post-RT [42, 43]. In a mouse mammary carcinoma model, blockade of CSF1 and consequent macrophage depletion significantly delayed tumor growth following RT, with the effect observed approximately 8–12 days following RT. A similar effect was observed after selective depletion of CD4+ T cells or neutralization of IL-4 [44]. Finally, RT has been associated with increased expression of transforming growth factor β (TGFβ) and inhibition of TGFβ has been associated with improved response to RT and other therapeutics [45–47]. In mouse models of breast cancer and colorectal cancer, TGFβ blockade in addition to PD-1 and CD137 blockade improved the efficacy of RT [48].

Given the immunomodulatory effects of both RT and ICB, multiple preclinical studies have examined the potential synergistic effects of combining the two modalities [49–56]. Twyman-Saint Victor et al. examined the effect of combined RT and ICB in a phase I trial of patients with metastatic melanoma as well as B16-F10 mouse models of melanoma [55]. Initial therapy with RT and anti-CTLA-4 therapy resulted in response rates of 18% and 17% in non-irradiated tumors in the human and mouse studies. The investigators found that a poor response was associated with increased PD-L1 expression by the tumors, and with addition of PD-1 directed therapy, they were able to increase the response rate in mice to a remarkable 80%. RT was associated with increased diversity of the T cell receptor (TCR) pool. These findings suggest that combined ICB with inhibition of CTLA-4 and the PD1 axis may provide the best opportunity to elicit an anti-tumor immunogenic response but would likely come with the risk of increased side effects. They also suggest that RT-induced increase in expression of PD-L1 may be a potential avenue to inducing immunogenicity in otherwise immunologically cold tumors.

RT can also induce tumor response at distant, non-irradiated sites. Known as the "abscopal effect" and first described by Mole in 1953 [57], this phenomenon is characterized by systemic regression of tumors after focal irradiation of a single site. It is thought to be immunologically mediated through RT-induced release of tumor-associated antigens and priming of cytotoxic T cells. Several case reports have described the abscopal effect, but it remains relatively rare in clinical practice. With the advent of ICB, there has been interest in attempting to increase a systemic immune response

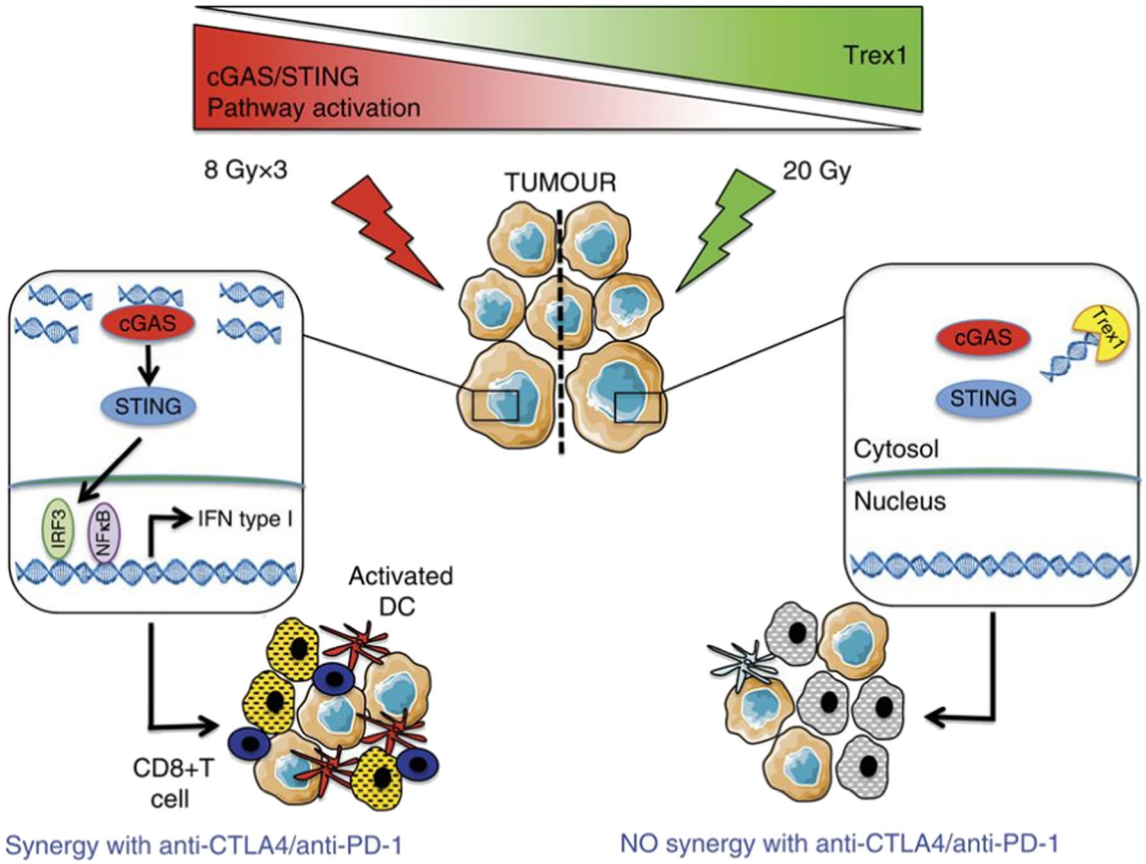

Fig. 1 Mechanism of variation in immune activation in low versus high per-fraction radiation therapy. At lower radiation doses, dsDNA release induces an immune response via activation of the type I interferon (IFN-I) pathway through cGAS/STING, leading to $CD8^+$ T cell activation and a synergistic response with ICB therapy. At higher radiation fraction sizes, Trex1 expression is increased, which degrades cytosolic DNA and dampens activation of this pathway, without synergy with ICB therapy. Figure taken from figure 9 of Vanpouille-Box et al. [32••] without modification, under the Creative Commons Attribution 4.0 International License

through a combination of ICB and RT [58]. Preclinical data provided early support for this approach. In breast cancer, treatment of the poorly immunogenic mouse mammary carcinoma 4T1 with anti-CTLA-4 therapy and RT led to improved control of distant metastases and overall survival following RT to the primary tumor [59].

One of the first clinical case reports of an abscopal effect using combination RT and ICB therapy was in a patient with metastatic melanoma treated with palliative RT to a paraspinal mass to 9.5Gy ×3 while on ipiliumumab, an anti-CTLA-4 antibody [60]. The paraspinal mass as well as other lesions at untreated sites regressed following completion of RT, and at 10-month follow-up, the patient had stable, minimal disease. In breast cancer, the abscopal effect in response to therapy with RT alone has been reported infrequently. In a trial of RT in combination with granulocyte-macrophage colony-stimulating factor for solid tumors, Golden et al. observed abscopal responses in 11 of 41 patients (27%) and 5 of 14 breast cancer patients (36%) [61]. This phenomenon still remains relatively rare in the era of ICB therapy, and efforts to further understand and enhance the likelihood of an abscopal response are ongoing.

Dose, Fractionation, and Sequencing of RT in Combination with ICB

Dose, fractionation, and timing of RT are critical variables in combination therapy with ICB, where the goal, as opposed to typical ablative therapy, is to induce a systemic immune response [62]. The optimal dose and fractionation of RT when combined with ICB in breast cancer are unknown. Conventionally fractionated radiation courses, typically 10–30 fractions of 1.8-2 Gy, are commonly used both in definitive and palliative settings. However, these prolonged courses also expose blood lymphocytes to radiation that can increase the risk of lymphopenia, particularly when a large volume of tissue is irradiated. Radiation-associated lymphopenia has been associated with worse outcomes in patients receiving ICB [63–65]. In contrast, hypofractionated RT may be associated with lower rates of lymphopenia, as demonstrated in a cohort of pancreatic cancer patients [66].

Based on these findings, hypofractionated courses with stereotactic methods (SRS and stereotactic body radiotherapy; SBRT), which generally consist of 1–5 fractions, have been investigated. Preclinical data demonstrated the benefit of a single fraction regimen of 12Gy × 1 combined with PD-1 directed therapy in mouse models of glioma, MC38 colon cancer, and TUBO mammary carcinoma [51, 67]. Some studies report an effective response with a single fraction of 20–30 Gy [68–70], which would be the upper limit of doses that would be used clinically. Seung et al. examined response to RT delivered as one, two, or three fractions of 20 Gy in combination with interleukin-2 (IL2) in patients with metastatic melanoma and renal cell carcinoma (considered to be "radioresistant" cancers) and reported a complete or partial response in 8/12 (66%) patients [71].

Recently, preclinical studies suggest that single large fractions may have an inhibitory effect on the immune response [32••, 49, 72]. Increased TREX1 exonuclease expression and breakdown of cytosolic DNA is an important mechanism for the inhibitory effects on the immune response observed with higher dose per fraction [32••]. Cytosolic DNA stimulates the secretion of interferon-beta through activation of the DNA sensor cGAS, and its downstream effector, STING. TREX1 is an exonuclease that degrades DNA accumulating in the cytosol upon irradiation, leading to the attenuation of immunogenicity. Vanpouille-Box et al. reported that TREX1 was induced by radiation doses > 12–18 Gy in different cancer cells, but hypofractionated RT administered in doses < 12 Gy per fraction did not induce TREX1 amplification [32••]. An older study by Dewan et al. compared regimens of 20 Gy × 1, 8 Gy × 3, and 6 Gy × 5 in combination with anti-CTLA-4 therapy in mouse TSA breast and MC38 colon cancer models and demonstrated improved tumor response at distant, non-irradiated sites with fractionated regimens [49]. Collectively, these results suggest that compared to single fraction courses, hypofractionated RT may result in improved tumor control both at local and distant sites. The caveat to these studies is that as compelling as the results are, they have yet to be translated into human trials of breast cancer.

Another interesting concept is that irradiation of the entire tumor may not be necessary in the RT and ICB treatment paradigm, where the goals are not only to stimulate an anti-tumor immune response but also to minimize toxicities from pre-operative radiation in a setting where adjuvant RT to the whole breast and lymph nodes may also be indicated. This principle was demonstrated in a preclinical study by Markovsky et al., where 67NR murine orthotopic breast tumors received RT to either 50% or 100% of the tumor volume. In immunocompetent mice, partial irradiation resulted in similar tumor responses as full volume irradiation, whereas in nude mice, this effect was not observed [73]. The largest clinical study to investigate this concept was performed at the University of Chicago. In this phase I trial, safety and response to SBRT in combination with pembrolizumab was examined in 79 patients with 27 different types of metastatic solid tumors treated with SBRT to either 15 Gy × 3 (in peripheral lung, liver, and abdomen/pelvis), 10 Gy × 5 (in central lung/mediastinum), or 10 Gy × 3 (in bone, spinal, and paraspinal sites). The authors reported an overall objective response rate of 13.2% and a similar out-of-field response rate of 13.5%, with six grade 3+ toxicities, but no RT dose reductions. The authors reported no difference in response between partially irradiated tumors and completely irradiated tumors [74•]. Only six patients (8.2%) within the study were in the breast cancer cohort; an expansion study in breast cancer patients is currently ongoing.

Other questions regarding the optimal integration ICB and RT relate to timing and sequencing. In a preclinical study of combined RT and either anti-CTLA-4 or OX40 agonist therapy (OX40 is a potent co-stimulatory receptor on CD4+ and CD8+ T cells) in a CT26 murine colorectal carcinoma model, anti-CTLA-4 therapy was most effective when given before RT, whereas OX40 agonist therapy was most effective when given after RT [75]. In the aforementioned phase I trial of SBRT with pembrolizumab involving multiple tumor types, pembrolizumab was delivered within 7 days of RT and the RT dose varied by irradiated site (30–50 Gy in 3–5 fractions) [74•]. In the phase II, adaptively designed TONIC trial, nivolumab was administered 2 weeks after induction with RT (8 Gy × 3) in a cohort of 12 metastatic TNBC patients, with a relatively low response rate of 8% [76].

Current Clinical Evidence on Combining RT and ICB in Breast Cancer

Although few clinical trials to date have examined the effects of combined RT and ICB in breast cancer, the number of clinical trials in this area is rapidly expanding. In one recent review of trials involving combination therapy with ICB, trials of RT or chemoradiotherapy comprised only 13 out of 185 such trials (7%), whereas 64 trials (35%) examined combination ICB with chemotherapy or HER2-directed therapy [77].

The first trial examining safety and efficacy of RT and ICB in a cohort comprised exclusively of breast cancer patients was a multi-center, phase II, single-arm study [78]. Key eligibility criteria included metastatic TNBC, any or unknown PD-L1 status, and at least 2 sites of metastatic disease, one site requiring RT. Subjects were treated with 30 Gy in 5 fractions, combined with pembrolizumab 200 mg administered within 3 days of the first fraction and subsequently every 3 weeks. The overall response rate in the entire cohort was 18% (3/17). Eight patients (47%) were not evaluable due to death or rapid disease progression. Among the 9 women who were radiographically evaluated at week 17, three demonstrated a

complete response with 100% reduction in tumor volume outside of the irradiated field. These results were modestly encouraging, compared to the 12–19% response rates reported with ICB monotherapy in KEYNOTE-012 and KEYNOTE-028 for metastatic TNBC. All three complete responders were PD-L1 positive (defined as PD-L1 expression in the stroma or in ≥ 1% of tumor cells using 22C3), suggesting that PD-L1 may be a biomarker of response in studies of RT and pembrolizumab. The small sample size and low number of patients evaluable for the primary endpoint (due to disease progression or death) limits broader applicability of the results.

The combination of ICB and RT has also been examined in HR+/HER2− metastatic breast cancer in a phase II study conducted at Dana-Farber Cancer Institute. In this study, palliative RT (20 Gy in 5 fractions) was delivered concurrently with pembrolizumab [79]. Eight women were enrolled in the first stage of the trial but given the lack of objective responses observed, the study was closed to further accrual. Similar to the pembrolizumab and RT trial in metastatic TNBC, the regimen was tolerated quite well, with only one grade 3+ adverse event.

The TONIC trial was an adaptive phase II trial of metastatic TNBC that investigated various induction strategies for combination with nivolumab, an anti-PD-1 antibody [76]. In the first stage of this trial, 67 patients were randomized to four induction strategies: (1) RT to 24 Gy in 3 fractions, (2) cyclophosphamide 50 mg daily, (3) cisplatin 2 × 40 mg/m^2, or (4) doxorubicin 2 × 15 mg, or a 2 week waiting period as a control. All patients then received three cycles of nivolumab. Response rates were highest with doxorubicin and cisplatin (35% and 23% respectively), but only 8% with RT and cyclophosphamide. The overall response rate for the study was 20%. Factors associated with improved response were higher TIL infiltration, higher levels of CD8+ cells, and higher PD-L1 expression. Furthermore, analysis of expression patterns of immune-related genes following induction therapy demonstrated upregulation of inflammatory pathways after cisplatin or doxorubicin, whereas downregulation of these pathways was observed after irradiation. This suggests that RT, as delivered in this study, may have had an inhibitory effect on the immune response, underscoring the critical importance of determining the optimal dose and fraction in these settings.

A study, reported in abstract form, examined the safety and efficacy of combined CNS RT (either whole brain RT or SRS) and CTLA-4 blockade (tremelilumab) in women with breast cancer metastatic to the brain. Among 20 HER2− patients, the best response was non-CNS stable disease in two (10%) patients. Among six HER2+ patients who received HER2-directed therapy in addition to RT and ICB, one had a non-CNS partial response and one had non-CNS stable disease (33%). The regimen was generally well-tolerated, with 15 grade 3 and no grade 4 toxicities [80]. Another study similarly examined the safety of tremelimumab 3 mg/kg combined with palliative RT to 4 Gy × 5 in six patients with locally advanced or metastatic breast cancer, one with TNBC and five with HR+ disease. The regimen was generally well-tolerated, with lymphopenia, fatigue and rash as common toxicities. One patient had stable disease for > 6 months [81]. Although these studies are small, they have nonetheless contributed to establishing the safety of combined RT and ICB in the metastatic breast cancer setting, in which patients received hypofractionated, palliative doses of RT. Further studies are required to evaluate the clinical efficacy of this strategy in metastatic breast cancer.

Safety of Combining RT and ICB

ICB can cause specific types of adverse events due to activation of the immune system, known as immune-related adverse events (irAEs). These have been well-documented based on data from large prospective randomized trials published over the past several years and differ slightly between anti-CTLA-4 therapy and PD-1 directed therapy [82]. Anti-CTLA-4 therapy is associated with higher rates of colitis and hypophysitis, whereas PD-1 directed therapy is associated with higher rates of hypothyroidism and pneumonitis. Combination ICB with both anti-CTLA-4 and PD-1 directed therapy is associated with a significant increase in the rates of irAEs [83]. Methods for reducing these effects while preserving the anti-tumor activity of combination therapy is an active area of investigation. In a preclinical study, Perez-Ruiz et al. demonstrated that prophylactic TNF blockade could improve colitis while preserving immunotherapeutic control of xenografted colon cancer on dual CTLA-4 and PD-1 immunotherapy in a mouse model [84].

Given the potent immunomodulatory effects of RT and the side effects associated with RT, the safety of combining RT and ICB must be taken into careful consideration. Available data generally support an acceptable toxicity profile for the combination of RT and ICB [85]. Of particular interest is the incidence of CNS toxicity in patients receiving SRS or other CNS-directed RT in combination with ICB, given the high prevalence of brain metastases in breast cancer. Several published retrospective series have suggested a slightly higher rate of CNS toxicity with combination therapy, although the majority of patients on these studies had metastatic melanoma [86–89]. To date, a small study of tremelimumab with brain RT reported in a HER2+ breast cancer patients has demonstrated an acceptable toxicity rate with the combination [80].

Table 1 Clinical trials of combination radiation therapy and immunotherapy in metastatic breast cancer

Title (NCT number)	Status	Phase	Enrollment	Interventions	Sponsor	*N*
BOSTON II: Pilot Study of Stereotactic Ablation for Oligometastatic Breast Neoplasia in Combination With the Anti-PD-1 Antibody MK-3475 (NCT02303366)	Completed	1	Oligometastatic BC (up to 5 metastases)	Single arm: pembrolizumab and 20 Gy × 1 fx SBRT	Peter MacCallum Cancer Centre, Australia	15
OX40 Breast: Stereotactic Body Radiation and Monoclonal Antibody to OX40 (MEDI6469) in Breast Cancer Patients With Metastatic Lesions (NCT01862900)	Completed	1	CS IV BC with liver or lung mets	3 arms: (1) 15 Gy × 1 fx, (2) 20 Gy × 1 fx, (3) 25 Gy × 1 fx; all arms receive 3 doses of MEDI6469	Providence Health & Services, Robert W. Franz Cancer Center, Providence Cancer Center, Earle A. Chiles Research Institute, MedImmune LLC	14
Toll-like Receptor (TLR) 7 Agonist, Cyclophosphamide, and Radiotherapy for Breast Cancer With Skin Metastases (NCT01421017)	Completed	1, 2	BC with chest wall recurrence or skin mets	3 arms: (1) imiquimod/RT, (2) cyclophosphamide/imiquimod/RT, (3) cyclophosphamide/RT; RT given as 6 Gy × 5 fx	NYU Langone Health|National Cancer Institute	32
Study of PD1 Blockade by Pembrolizumab with SBRT in Advanced Solid Tumors (NCT02608385)	Recruiting	1	Metastatic solid tumors, 8% breast cancer	Pembrolizumab and SBRT (3–5 fx)	University of Chicago	76
Trial Of Hypofractionated Radiotherapy In Combination With MEDI4736 (Durvalumab)And Tremelimumab For Patients With Metastatic Melanoma And Lung, Breast And Pancreatic Cancers (NCT02639026)	Recruiting	1	Metastatic melanoma, lung cancer, BC, or pancreatic cancer	2 arms: (1) durvalumab and tremelimumab with 8 Gy × 3 fx RT, (2) durvalumab and tremelimumab with 17 Gy × 1 fx RT	Abramson Cancer Center of the University of Pennsylvania	30
Stereotactic Radiation and Nivolumab in the Management of Metastatic Breast Cancer Brain Metastases (NCT03807765)	Recruiting	1	Metastatic BC with CNS mets	Single arm: nivo followed by SRS 1 week after first cycle of nivo	H. Lee Moffitt Cancer Center and Research Institute, Bristol-Myers Squibb	12
Anti PD-1 Antibody With Radiation Therapy in Patients With HER2-negative Metastatic Breast Cancer (NCT03430479)	Recruiting	1, 2	Metastatic HR+/HER2− BC	Single arm: nivolumab, hormone therapy, and palliative radiotherapy	Kyoto University Hospital, Japan	32
STOMP: SBRT and Oncolytic Virus Therapy Before Pembrolizumab for Metastatic TNBC and NSCLC (NCT03004183)	Recruiting	2	Metastatic TNBC or lung cancer	Single arm: pts. receive SBRT and oncolytic virus therapy before pembrolizumab	Houston Methodist Cancer Center	57
AZTEC: Stereotactic Radiation and Immunotherapy in Patients With Advanced Triple Negative Breast Cancer (NCT03464942)	Recruiting	2	CS IV, TNBC	2 arms: (1) single fraction SABR, 20 Gy, (2) fractionated SABR, 24 Gy in 3 fx,; both arms treat 1–4 mets and receive 2y atezolizumab starting within 5 days of RT	Peter MacCallum Cancer Centre, Australia, Trans-Tasman Radiation Oncology Group (TROG)	52
Atezolizumab & SRS in Triple-negative Breast Cancer and Brain Metastasis (NCT03483012)	Recruiting	2	TNBC with CNS mets	Single arm: atezolizumab and brain SRS	Dana-Farber Cancer Institute	45
TONIC: Nivolumab After Induction Treatment in TNBC Patients (NCT02499367)	Recruiting	2	Metastatic TNBC	5 arms: RT arm consists of nivolumab given after induction 20 Gy × 1 fx SBRT; other arms use doxorubicin, cyclophosphamide, cisplatin, or no induction	The Netherlands Cancer Institute, Bristol-Myers Squibb	12
Immune Responses in Prostate, Lung, Melanoma and Breast Cancer Patients Following SBRT, IMRT, or Brachytherapy (NCT01777802)	Recruiting	Obs	Metastatic prostate cancer, BC, lung cancer, or melanoma	Observational, measuring immune biomarkers before and after RT (as SBRT, IMRT, or brachytherapy)	Mayo Clinic	130
Brain Irradiation and Tremelimumab in Metastatic Breast Cancer (NCT02563925)	Active, not recruiting	1	Metastatic BC with CNS mets	Pts receive either WBRT or SRS with tremelimumab; protocol expansion to tremelimumab and durvalumab with CNS RT	Memorial Sloan Kettering Cancer Center, MedImmune LLC	28
Study to Assess the Efficacy of Pembrolizumab Plus Radiotherapy in Metastatic Triple Negative Breast Cancer Patients (NCT02730130)	Active, not recruiting	2	Metastatic or locally recurrent TNBC, unselected for PD-L1	Pembrolizumab and palliative RT (given as 6 Gy × 5 fractions)	Memorial Sloan Kettering Cancer Center	17
Pembrolizumab and Palliative Radiotherapy in Breast Cancer (NCT03051672)	Active, not recruiting	2	CS IV, HR+/HER2− BC	Single arm: pembro with 5 fraction RT	Dana-Farber Cancer Institute	8
	Not yet recruiting	1, 2	CS IV or unresectable TNBC	NANT TNBC vaccine with either stereotactic body radiation therapy, chemotherapy, or biologic therapy	NantKwest, Inc.	79

Table 1 (continued)

Title (NCT number)	Status	Phase	Enrollment	Interventions	Sponsor	*N*
NANT TNBC Vaccine: Combination Immunotherapy in Subjects With TNBC Who Have Progressed on or After Anthracycline-based Chemotherapy (NCT03175666)						
Pembrolizumab And Stereotactic Radiosurgery (SRS) Of Selected Brain Metastases In Breast Cancer Patients (NCT03449238)	Not yet recruiting	1, 2	Metastatic BC with at least 2 CNS mets	Single arm: pembrolizumab infused the day before select brain SRS	Weill Cornell	41
AGADIR: Atezolizumab Combined With Intra-tumoral G100 AnD Immunogenic Radiotherapy in Patients With Advanced Solid Tumors (NCT03915678)	Not yet recruiting	2	Metastatic TNBC, pancreatic cancer, virus-associated tumors, lung cancer, melanoma, bladder cancer	TNBC cohort: atezolizumab and intra-tumoral injection G100 with RT to start 1 week after first atezo administration; RT given as either short-course (2 Gy × 2 fx) to the injected mets, or SBRT to non-injected mets (27–60 Gy in 3–5 fx)	Institut Bergoni, Roche Pharma AG, National Cancer Institute, France, Immune Design	247

N, number; *BC*, breast cancer; *CS*, clinical stage; *HR+*, hormone receptor positive, *HER2−*, human epidermal growth factor receptor 2 negative; *HER2+*, human epidermal growth factor receptor 2 positive; *RT*, radiation therapy; *HT*, hormone therapy; *pembro*, pembrolizumab; *NED*, no evidence of disease; *Gy*, gray; *fx*, fractions; *TNBC*, triple-negative breast cancer; *met*, metastasis; *mets*, metastases; *y*, year; *SABR*, stereotactic ablative body radiotherapy; *SBRT*, stereotactic body radiation therapy; *obs*, observational; *IMRT*, intensity modulated radiation therapy; *pts*, patients; *WBRT*, whole brain radiation therapy; *SRS*, stereotactic radiosurgery; *IORT*, intraoperative radiation therapy

Clinical Trials of RT and ICB in Breast Cancer: Present and Future

Multiple trials are currently investigating the combination of ICB with RT in breast cancer, both in the metastatic and non-metastatic setting.

In metastatic breast cancer, a search on ClinicalTrials.gov returned 18 registered trials, 9 of which are currently recruiting and 6 of which are not yet recruiting (Table 1). Thirteen include only breast cancer patients, whereas 5 include patients with other tumor types. All of the trials in the metastatic setting are either phase I or phase II, the largest of which are highlighted here. The TROG AZTEC trial ($n = 52$) will randomize patients with metastatic TNBC without active brain metastases to receive SBRT with either 20 Gy × 1 or 8 Gy × 3, followed by atezolizumab for up to 24 months. This trial will help define the optimal dose and fractionation regimen to be used with RT in the metastatic setting [90]. A phase II trial at Dana-Farber Cancer Institute is similarly evaluating the combination of SRS and atezolizumab in patients with TNBC and brain metastases [91], and a trial at Weill Cornell Medical College is evaluating the combination of pembrolizumab and SRS in patients with brain metastases from breast cancer, irrespective of breast cancer subtype [92].

Several trials are also evaluating the addition of ICB to standard-of-care RT in the non-metastatic setting (Table 2). As of June 2019, 11 trials were registered on ClinicalTrials.gov, 6 of which are currently recruiting, including 2 phase III trials, and 4 that have not started recruitment. KEYNOTE-756 is a randomized, double-blind phase III trial of patients with ER+/HER2− breast cancer with localized high-risk disease randomized to pembrolizumab versus placebo with neoadjuvant chemotherapy and with adjuvant endocrine therapy, with RT as indicated. The primary endpoints are pCR rate and event free survival [93]. SWOG S1418/NRG-BR006 is a phase III randomized trial with planned enrollment of 1000 participants that randomizes patients with TNBC and ≥ 1 cm residual disease after neoadjuvant chemotherapy to pembrolizumab versus placebo [94]. RT is delivered adjuvantly per standard of care, and where possible, initiated after randomization and delivered concurrently with pembrolizumab. If RT is initiated prior to randomization, pembrolizumab can still be added to ongoing RT or after completion of RT. The BreastImmune03 trial is investigating post-operative RT with nivolumab and ipilimumab versus RT with capecitabine, with a primary outcome of disease-free survival [95].

Pre-operative ICB and SBRT are being evaluated in two phase II trials that are not yet recruiting. The Neo-CheckRay trial is exploring RT with 8 Gy × 3 with or without durvalumab and oleclumab (an anti-CD73 antibody) in luminal B breast cancer [96]. Similarly, the PANDORA study is evaluating 8 Gy × 3 with paclitaxel, carboplatin, and

Table 2 Clinical trials of combination radiation therapy and immunotherapy in non-metastatic breast cancer

Title (NCT number)	Status	Phase	Enrollment	Interventions	Sponsor	*N*
Non-metastatic setting						
Combination Immunotherapy With Herceptin and the HER2 Vaccine NeuVax (NCT01570036)	Completed	2	HER2+ BC treated with standard therapy and NED	2 arms, both after completion of standard of care chemotherapy/radiotherapy: (1) Herceptin/NeuVax vaccine, (2) Herceptin alone	Genentech, Inc.; Cancer Insight, LLC	275
Breast Cancer Study of Pre-operative Pembrolizumab & Radiation (NCT03366844)	Recruiting	1	Operable high-risk HR+/HER2− BC or TNBC	Single arm: 2 cycles of neoadjuvant pembro with second pembro cycle given in conjunction with RT boost (8 Gy × 3 fx)	Cedars-Sinai Medical Center	30
Neoantigen DNA Vaccine Alone vs. Neoantigen DNA Vaccine Plus Durvalumab in Triple Negative Breast Cancer Patients Following Standard of Care Therapy (NCT03199040)	Recruiting	1	CS II-III TNBC	2 arms, after completing standard therapy (including chemo, surgery, and RT as clinically indicated), (1) neoantigen DNA vaccine alone, (2) neoantigen DNA vaccine and Durvalumab	Washington University School of Medicine, MedImmune LLC	24
Effects of MK-3475 (Pembrolizumab) on the Breast Tumor Microenvironment in Triple Negative Breast Cancer (NCT02977468)	Recruiting	1	Early-stage, node-negative TNBC	Single arm: pts. receive pembro 2 weeks before IORT	Columbia University Medical Center	15
Pembrolizumab in Treating Patients With Hormone Receptor Positive, Localized Inflammatory Breast Cancer Who Are Receiving Hormone Therapy and Did Not Achieve a Pathological Complete Response to Chemotherapy (NCT02971748)	Recruiting	2	Inflammatory breast cancer and did not achieve a pathologic complete response to chemotherapy	Single arm: following RT or with plans to start RT within 28 days, pembro for 2y	M.D. Anderson Cancer Center, National Cancer Institute	37
Study of Pembrolizumab (MK-3475) Versus Placebo in Combination With Neoadjuvant Chemotherapy & Adjuvant Endocrine Therapy in the Treatment of Early-Stage Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative (ER+/HER2-) Breast Cancer (MK-3475-756/KEYNOTE-756) (NCT03725059)	Recruiting	3	Non-metastatic BC	2 arms: (1) standard neoadjuvant chemotherapy, surgery, and RT as clinically indicated; (2) neoadjuvant and adjuvant pembro with standard chemotherapy, surgery, and RT as clinically indicated	Merck Sharp & Dohme Corp.	1140
Pembrolizumab in Treating Patients With Triple-Negative Breast Cancer (NCT02954874)	Recruiting	3	Non-metastatic TNBC pts. with 1 cm or greater residual invasive cancer or positive lymph nodes after neoadjuvant chemotherapy	2 arms: (1) observation, (2) pembro; pts. in both arms may receive standard of care radiation as clinically indicated to start within 12 weeks from surgery	National Cancer Institute	1000
Neo-CheckRay: Neo-adjuvant Chemotherapy Combined With Stereotactic Body Radiotherapy to the Primary Tumour +/− Durvalumab, +/− Oleclumab in Luminal B Breast Cancer (NCT03875573)	Not yet recruiting	2	Non-metastatic HR+/HER2− BC	3 neoadjuvant arms: (1) chemotherapy and RT, (2) chemotherapy, RT, and durvalumab, (3) chemotherapy, RT, durvalumab, and oleclumab; in all arms, RT given as 8 Gy × 3 fx	Jules Bordet Institute, Institut Curie, AstraZeneca	147
PANDoRA: Pre-operAtive Non-Anthracycline Chemotherapy, Durvalumab +/− RAdiation Therapy in Triple Negative Breast Cancer (NCT03872505)	Not yet recruiting	2	CS II-III TNBC	2 arms: (1) neoadjuvant durvalumab, carboplatin, paclitaxel (DCP), (2) neoadjuvant DCP with RT given with the second dose of durvalumab as 8 Gy × 3 fractions	Cedars-Sinai Medical Center, AstraZeneca	140
CBCV: Converting HR+ Breast Cancer Into an Individualized Vaccine (NCT03804944)	Not yet recruiting	2	Post-menopausal, CS II-III, HR+/HER2− BC	Neoadjuvant, 4 arms: (1) RT/HT alone, (2) RT/HT with pembro; (3) RT/HT with CDX-301; (4) RT/HT with pembro & CDX-301; RT given as 8Gy × 3 fx	Weill Medical College of Cornell University	100

Table 2 (continued)

Title (NCT number)	Status	Phase	Enrollment	Interventions	Sponsor	*N*
BreastImmune03: Evaluate the Clinical Benefit of a Post-operative Treatment Associating Radiotherapy + Nivolumab + Ipilimumab Versus Radiotherapy + Capecitabine for Triple Negative Breast Cancer Patients With Residual Disease (NCT03818685)	Not yet recruiting	2	TNBC with residual cancer burden III residual disease after neoadjuvant chemotherapy	2 arms: (1) adjuvant ipilimumab/nivolumab, (2) adjuvant capecitabine; both arms receive adjuvant RT	Centre Leon Berard	98

N, number; *BC*, breast cancer; *CS*, clinical stage; *HR+*, hormone receptor positive, *HER2−*, human epidermal growth factor receptor 2 negative; *HER2+*, human epidermal growth factor receptor 2 positive; *RT*, radiation therapy; *HT*, hormone therapy; *pembro*, pembrolizumab; *NED*, no evidence of disease; *Gy*, gray; *fx*, fractions; *TNBC*, triple-negative breast cancer; *met*, metastasis; *mets*, metastases; *y*, year; *SABR*, stereotactic ablative body radiotherapy; *SBRT*, stereotactic body radiation therapy; *obs*, observational; *IMRT*, intensity modulated radiation therapy; *pts*, patients; *WBRT*, whole brain radiation therapy; *SRS*, stereotactic radiosurgery; *IORT*, intraoperative radiation therapy

durvalumab (an anti-PD-L1 antibody) in TNBC [97]. The primary endpoints of these trials are pCR.

Conclusions

The synergy between RT and ICB offers significant potential for improving both local and systemic control in breast cancer. Heightened interest in this combination is evidenced by the recent surge in clinical trials utilizing RT and ICB reviewed above. Early trials of hypofractionated RT + ICB including breast cancer patients suggest that the combination is safe and tolerable. Modest responses with pembrolizumab and RT have been observed in heavily pre-treated, metastatic TNBC. However, further study is needed to elucidate the ideal dose, fractionation, and timing of RT to achieve immunostimulatory and clinical responses when combined with ICB. Careful patient selection is critical and can be informed by PD-L1 status or extent of TILs. Future phase II-III trials will focus on studying the efficacy of RT and ICB in triple-negative and high-risk breast cancer patients receiving treatment in the neoadjuvant, adjuvant, and metastatic settings.

Compliance with Ethical Standards

Conflict of Interest Alice Ho reports grants from Tesaro, Inc., and Merck & Co., Inc., outside the submitted work. Shervin Tabrizi and Susan McDuff declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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