Advances in Combining Radiation and Immunotherapy in Breast Cancer

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Abstract

Breast irradiation has long been utilized in the adjuvant or metastatic setting to eliminate microscopic disease or to palliate existing disease, respectively. However, preclinical data have demonstrated that radiation can also alter the tumor microenvironment and induce antitumor immune responses. As a result, multiple clinical studies have been undertaken and have reported synergy between radiation and immune checkpoint blockade across various cancer types. Given recent clinical successes with immune checkpoint blockade in both early-stage and metastatic breast cancer, there has been substantial interest in combining radiation and immunotherapy to enhance local and systemic immune responses. Herein, we review the preclinical rationale for combining radiotherapy and immunotherapy, the early clinical trials that have adopted this strategy in breast cancer, and the landscape of ongoing relevant clinical trials. Finally, we propose future directions based on promising preclinical studies that integrate radiation, checkpoint blockade, and novel agents for the treatment of breast cancer.

Clinical Breast Cancer, Vol. 21, No.xxx, 143–152

Keywords: Cancer immunotherapy, Metastatic breast cancer, Triple-negative breast cancer, Tumor microenvironment, Radiation oncology

Introduction

Radiotherapy (RT) is a cornerstone of breast cancer treatment with multiple trials demonstrating the efficacy of RT in preventing local recurrence and improving survival.[1] This efficacy was predicated on the cell intrinsic cytotoxic activity of RT according to historical studies in radiobiology.[2] Prior studies examining the mechanistic underpinnings of RT largely focused on DNA-damaging properties and resultant cell death; however, preclinical and clinical evidence generated over the last decade suggest that RT-induced cell death can also alter the tumor microenvironment (TME) and trigger an antitumor inflammatory response.[3-6] In light of recent clinical successes with immune checkpoint blockade (ICB)-mediated immune modulation in breast cancer together with a growing body of preclinical and clinical data demonstrating synergy between ICB and RT,[7-9] the clinical application of ICB with RT is an active area of investigation. Herein, we review the preclinical and clinical rationale for combining RT and ICB in breast cancer, early data from recent clinical trials, and relevant future directions.

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Submitted: Jan 16, 2021; Revised: Mar 11, 2021; Accepted: Mar 14, 2021; Epub: 16 March 2021

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Preclinical Rationale for Combining RT and ICB

The immune system has long been recognized for its role in RT-mediated tumor responses. In a seminal 1979 article, Stone et al.[10] demonstrated in a murine model of fibrosarcoma that the response to RT is T cell-dependent. Since then, others have further elucidated the immune mechanisms that support the response to RT, demonstrating that the immune response elicited by RT is dependent on CD8+ cytotoxic T cells producing interferon gamma.[11-13] RT generates this response through induction of immunogenic cell death in which inflammatory molecules, such as high-mobility group box protein 1 (HMGB1),[14] calreticulin,[15] and cytosolic DNA,[16] are released from irradiated cells, generating a type I interferon response and subsequent antitumor CD8+ T cell responses.[17] Thus, strategies that augment specific T cell responses may produce more robust RT-mediated tumor responses as demonstrated in multiple preclinical models.[18] Moreover, it has been hypothesized that antitumor immune responses generated in the irradiated tumor may lead to systemic antitumor immunity, also known as the abscopal effect. In 1953, R.H. Mole first described the abscopal effect (from "ab scopus" meaning away from target) as the phenomenon by which RT induces the spontaneous regression of a distant, unirradiated lesion likely through immune-mediated mechanisms.[19] The abscopal effect of RT has since been reported in a variety of solid cancers, including papillary adenocarcinoma,[20] melanoma,[21] renal cell carcinoma,[22] and hepatocellular carcinoma[23]; however, cases from RT alone remain exceedingly rare.[24]

Multiple preclinical studies have demonstrated that targeting various aspects of the immune system can augment antitumor immunity following RT. Several investigators have shown preclinical synergy with RT and immunoadjuvants such as FMS-like tyrosine

1526-8209/$ - see front matter
https://doi.org/10.1016/j.clbc.2021.03.007

kinase receptor 3 ligand (Flt3-L), a growth factor for dendritic cells.[25-27] The combination of RT with an immunoadjuvant was tested in a proof-of-principle trial in which RT was combined with granulocyte-macrophage colony-stimulating factor and produced an abscopal response in nearly 30% of (11/41) patients.[28] Of note, 36% (5/14) of women with metastatic breast cancer in this trial demonstrated a partial response in an unirradiated lesion.

The identification of immune checkpoints such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-(ligand) 1 [PD-(L)1] and the subsequent successful development of drugs that target those ligands, created a novel opportunity to potentially augment the abscopal responses to RT. Early preclinical studies combining RT with ICB were performed in murine models of breast cancer and demonstrated synergy. For example, in 2005, Demaria et al.[29] demonstrated that combining RT with an anti-CTLA-4 antibody significantly delayed metastases and improved survival in a breast cancer model. Moreover, this synergy was augmented by administering short courses of fractionated RT and by administering anti-CTLA-4 prior to RT.[30,31]

Additional preclinical studies have demonstrated that RT further supports antitumor immunity by altering the TME. For example, in a murine model of breast cancer, PD-L1 was upregulated on tumor cells following irradiation.[18] Accordingly, PD-L1 blockade amplified the antitumor response to RT in a CD8+ T cell-dependent manner. As such, upregulation of PD-L1 in the TME following RT could potentially be overcome by ICB. Similar observations were made in a murine model of melanoma, in which tumors that were resistant to anti-CTLA-4 and RT were shown to upregulate PD-L1 in a nonredundant immune pathway of evasion.[32] In this study, PD-L1 blockade was shown to reinvigorate exhausted T cells and enhance response to RT and anti-CTLA-4.

In addition to the regulation of immune checkpoints, RT can also influence the diversity of T cell clones by increasing the number and diversity of tumor antigens. For example, RT was shown to increase the diversity of the T cell receptor repertoire of tumor-associated lymphocytes.[32] These findings were recapitulated on functional analysis of a phase II trial combining anti-CTLA-4 and RT in metastatic non–small-cell lung cancer (NSCLC).[33] Patients who responded to treatment had a significant T cell clonal expansion compared with nonresponders. In one patient with a complete response (CR), two new T cell clones were identified that suggested that RT enhanced the expression of new immunogenic mutations. Recent preclinical studies have further shown that neoantigens upregulated by RT are recognized by specific T cells whose activity can be augmented by immunization with RT-elicited epitopes.[34]

In total, these data indicate that the full efficacy of RT depends on eliciting an antitumor immune response. RT does this in part through upregulating the expression of immunogenic mutations that can be used for antigen cross-presentation and releasing proinflammatory molecules. Further, this process can be enhanced by adding ICB.

Clinical Rationale for ICB in Breast Cancer

Several trials have shown limited activity with anti-PD-1/PD-L1 monotherapy in metastatic breast cancer with objective response rates (ORRs) from 5% to 24% reported. The most robust responses have been observed in triple-negative breast cancer (TNBC), in PD-L1-positive (PD-L1$^+$) tumors, and in the first-line palliative setting.[35-40] In fact, two large phase III trials (IMpassion130 and KEYNOTE-355) have demonstrated clinical benefit with concurrent ICB and chemotherapy combinations in patients with PD-L1$^+$ advanced TNBC.[41-43] Notably, grade 3 or higher toxicities were observed in 48% to 68% of all patients. Ongoing clinical trials are focused on combining ICB with cytotoxic or molecularly targeted agents across all subtypes of breast cancer. In a recent overview of the immune-oncology landscape, ICB was most commonly coadministered with chemotherapy or HER2-directed therapy with these combinations comprising 35% (64 of 185) of all active ICB trials in breast cancer.[44]

Thus in the metastatic setting, there are several challenges to overcome if ICB regimens are to be more broadly relevant. First, there are limited ICB options for patients with PD-L1-negative (PD-L1-) tumors or combined positive score < 10 by the 22C3 assay. Second, novel ICB combinations are needed to minimize toxicity—in IMpassion130, approximately 16% of patients discontinued at least 1 agent owing to adverse events. Finally, it is unclear if patients should remain on therapy or transition to new ICB agents after progression on ICB.

In early-stage breast cancer, two clinical trials (I-SPY2 and KEYNOTE-522) have demonstrated promising activity with neoadjuvant ICB and chemotherapy. In the phase II I-SPY2 trial, estimated pathologic CR (pCR) rates more than doubled with the addition of ICB to standard neoadjuvant chemotherapy (NAC) among patients with early TNBC.[45] The estimated pCR rate also improved in the estrogen receptor–positive cohort of I-SPY2 from 13% to 34%. Similarly, the phase III KEYNOTE-522 trial demonstrated a significant improvement in pCR rates from 51.2% with NAC alone to 64.8% with the addition of ICB in patients with early TNBC.[46] Of note, the rate of grade 3 or higher adverse events was 78% in the ICB-chemotherapy arm. Although exploratory, a subgroup analysis demonstrated a pronounced benefit among node-positive patients, which suggests possible T cell priming in involved lymph nodes. Strategies exploring ICB combinations to optimize cure rates while minimizing treatment toxicity are both in development and underway.

Clinical Rationale for RT with ICB in Breast Cancer

Among current ICB trials in breast cancer, only 7% (13 of 185) of active trials combine RT and ICB.[44] In an early single-arm trial, 73 patients with metastatic cancer, including six women with breast cancer, were treated with concurrent stereotactic body radiotherapy (SBRT) and pembrolizumab.[47] In this study, the ORR of the entire cohort was 13.2%. Given that the response rate was not stratified by tumor type, it is unclear what is the true clinical benefit for metastatic breast cancer. Nonetheless, the trial demonstrated that the combination of SBRT and ICB was generally well tolerated and safe without significant side effects.

Despite the modest number of breast cancer dedicated RT-ICB trials, several key trials have been reported (Table 1). In the first study of RT with ICB for the treatment of breast cancer, the safety and efficacy of brain RT and concurrent CTLA4-mediated

Table 1 Published Trials Combining Radiotherapy and Immune Checkpoint Blockade in Metastatic Breast Cancer

Trial	Phase	N	Tumor Type	Intervention	ORR	Toxicity
McArthur et al.[48]	-	20	HER2$^+$ or HER2$^-$ mBC with brain metastases	Concurrent WBRT and tremelimumab	12-week non-CNS DCR 10% in HER2$^-$ mBC 33% in HER2$^+$ mBC	15 grade 3 AE (fatigue, diarrhea, colitis), 0 grade 4 or 5 AE
Voorwerk et al. (TONIC)[49]	2	12	mTNBC	Sequential RT (24 Gy in 3 fractions) and atezolizumab	12% (1 PR) 95% CI, 0.2%-38.5%	3 grade 3 AE, 0 grade 4 AE, 1 grade 5 AE (nivolumab-related)
Ho et al.[54]	2	17	mTNBC	Concurrent RT (24 Gy in 3 fractions) and pembrolizumab	17.6% (3 CR) 95% CI, 4.7%-44.2%	3 grade 3 AE (fatigue, infection, lymphopenia), 1 grade 4 AE (lymphopenia), 0 grade 5 AE
Barroso-Sousa et al.[55]	2	8	HR$^+$/HER2$^-$ mBC	Pembrolizumab prior (2-7 days) to RT (20 Gy in 5 fractions)	0%	1 grade 3 AE (AST elevation), 0 grade 4 or 5 AE

Abbreviations: AE = adverse event; AST = aspartate aminotransferase; CI = confidence interval; CNS = central nervous system; CR = complete response; DCR = disease control rate; HR$^+$/HER2$^-$ = hormone receptor-positive/human epidermal growth factor receptor 2-negative; mBC = metastatic breast cancer; mTNBC = metastatic triple-negative breast cancer; ORR = objective response rate; PR = partial response; RT = radiotherapy; WBRT = whole-brain radiotherapy.

immune modulation with tremelimumab, +/- trastuzumab, a HER2-directed antibody, were explored in patients with HER2-negative (HER2$^-$) or HER2-positive (HER2$^+$) brain metastases.[48] The primary endpoint was 12-week non-central nervous system (CNS) disease control rate (DCR). The 12-week non-CNS DCR was 10% (2/20 patients) in the HER2$^-$ cohort and 33% (2/6 patients) in the HER2$^+$ cohort. One patient with heavily pretreated, trastuzumab-resistant, HER2$^+$ disease experienced a durable partial response with evidence of peripheral T cell activation. Given the encouraging responses observed in the HER2$^+$ safety cohort, a clinical trial to determine efficacy of ICB with trastuzumab for HER2$^+$ breast cancer brain metastases is planned.

The phase II TONIC trial tested whether priming with RT or chemotherapy prior to ICB can alter the TME and increase tumor sensitivity to PD-1/PD-L1 blockade.[49] In this noncomparative study, 67 women with metastatic TNBC were randomized to nivolumab, a human monoclonal antibody to PD-1, without induction or with 2-week low-dose induction with RT (3 × 8 Gy), cyclophosphamide, cisplatin, or doxorubicin. Although the ORR of the entire cohort was 20%, the ORR for the RT arm was only 8% (1/12 patients), whereas the ORR for the "no induction" cohort was 17% (2/12 patients). Based on the preset criteria by Simon two-stage design, the RT arm was discontinued for further study.

There are several possible explanations for the poor response rate to sequential RT and ICB on TONIC, which was notably lower than the response rate in the "no induction" control arm. Because of the small patient cohorts, several patient characteristics were unbalanced, including decreased stromal tumor-infiltrating lymphocytes (TILs) at baseline in the RT cohort.[50] TILs have been shown to be important predictors for response to immunotherapy in breast cancer.[51] Moreover, the trial included a 2-week waiting period between RT and ICB to test whether induction could turn "cold" into "hot" tumors. Preclinical evidence suggests that concurrent RT or ICB induction prior to RT may be more effective in generating an immunologic response.[31,52,53] Thus sequential administration of RT prior to ICB may have hindered any potential synergy. Finally, irradiation of only a single index lesion was allowed to detect an abscopal response in any untreated lesion. However, treatment of multiple metastatic lesions may potentially increase systemic response rates to RT and ICB by both debulking metastatic disease and exposing additional neoantigens that may enhance T cell priming.

In a multi-institutional phase II trial of concurrent standard-of-care palliative RT with ICB in 17 women with heavily pretreated, metastatic TNBC, patients received SBRT (30 Gy in 5 fractions) with pembrolizumab, a humanized, monoclonal antibody to PD-1.[54] In contrast to the TONIC study, pembrolizumab was administered within 3 days after the first fraction of RT. The ORR of the entire cohort was 17.6% (3/17 patients), which compared favorably to the response rate of 5% with pembrolizumab monotherapy in a similar PD-L1 unselected population in cohort A of KEYNOTE-086.[39] Of the women who were radiographically evaluable at week 13, 33% (3/9 patients) demonstrated a CR with 100% reduction of tumor volume outside the irradiated field. The antitumor response was durable, ranging from 18 weeks to >108 weeks, consistent with a systemic immunologic response to the combination.

One exceptional responder treated on the pembrolizumab/RT metastatic TNBC trial, who previously received treatment with a colony-stimulating factor 1 receptor (CSF-1R) antibody, had no evaluable disease at time of last follow-up at 108 weeks. Although the three patients achieving a CR were PD-L1$^+$ at baseline, PD-L1 expression was not correlated with ORR and progression-free survival overall. Limitations of the trial include a modestly sized patient cohort and the single-arm, nonrandomized design. Nonetheless, the durability of some of the results are encouraging for this heavily pretreated population with a historically poor prognosis. Further clinical investigations are warranted to further delineate the interplay between RT and ICB in metastatic TNBC.

Finally, the efficacy of RT and ICB was tested in a phase II trial of women with heavily pretreated, hormone receptor-positive/human epidermal growth factor receptor 2-negative ($HR^+/HER2^-$) metastatic breast cancer.[55] Patients received pembrolizumab followed by palliative RT (20 Gy in 5 fractions) within 2 to 7 days. There were no objective responses observed in the first 8 patients who were enrolled, and the trial was consequently closed to accrual per the Simon two-stage design. The lack of clinical activity in this study may reflect the innate resistance of metastatic HR^+ disease to ICB as demonstrated in other studies,[36,37] the allowance for bone RT on study, which may not represent the optimal RT target to generate synergy, the low prevalence of PD-$L1^+$ tumors, and the fact that the RT dose was lower than in previously reported trials. Moreover, given that anti-PD-1 acts on both new and exhausted T cells,[56-58] RT after ICB may eradicate any newly infiltrated or activated T cells.

Insights for Future RT and ICB Trials

These early experiences with RT plus ICB have provided important insights that should directly inform future trials. First, the identification of predictive biomarkers is needed to optimize response to combination RT and ICB.[59] Several candidate pretreatment biomarkers include PD-L1 expression, tumor infiltrating lymphocytes, and tumor mutational burden. In several trials, PD-1/PD-L1 status has served as a critical biomarker for response to ICB monotherapy or in combination with chemotherapy. For example, response rates to pembrolizumab monotherapy varied drastically between the unselected cohort A and PD-$L1^+$ cohort B of KEYNOTE-086.[38,39] Similarly, in IMpassion130, the overall survival benefit to ICB-chemotherapy for metastatic TNBC was limited to patients with PD-$L1^+$ tumors.[41,42]

Unfortunately, there is limited prospective validation of PD-L1 expression as a predictive biomarker for response to RT and ICB. In a phase II trial of pembrolizumab and RT in metastatic TNBC, it was noted that all 3 patients with CRs were PD-$L1^+$; however, PD-L1 was not statistically correlated with response or survival due to the limited sample size. Moreover, in the metastatic NSCLC, patients with PD-L1-negative (PD-$L1^-$) tumors experienced the greatest clinical benefit with concurrent RT and ICB.[8] By contrast to ICB-chemotherapy trials, RT may induce the expression of PD-L1,[18,32] and therefore pretreatment PD-L1 or PD-1 status may fall short as a prognostic factor.

Another key question of combining RT and ICB is the question of which lesion is the optimal target for RT to induce an abscopal response. To date, it is unclear whether this would be visceral, lymph node, or bone metastases. In the phase II trial of pembrolizumab and RT in $HR^+/HER2^-$ breast cancer, all eight patients received palliative RT to the bone, and there were no objective responses observed. Although the palliative RT doses may have contributed to this futility, this also raises the possibility that bone lesions may not be an optimal target for RT-ICB combinations. Although bone is a highly vascular organ that contains high levels of multiple immune cells, a large retrospective trial of patients with metastatic NSCLC demonstrated that patients with bone metastases had decreased immunotherapy efficiency.[60] Given that these patients experienced early progression and death compared with patients without bone metastases, it is hypothesized that immunotherapy cannot overcome the negative prognostic factor of bone dissemination. Bone metastases may also promote the differentiation of myeloid-derived suppressor cells that may limit the efficacy of RT-ICB.

Similarly, the presence of liver metastases is a negative prognostic factor for survival and response to immunotherapy.[61] This is mediated in part through the apoptosis of CD8+ T cells on interaction with monocyte-derived macrophages within the liver. However, in preclinical models, liver-directed RT promotes T cell survival by eliminating the population of suppressive macrophages. Therefore liver-directed RT may improve systemic responses to ICB with the caveat that these patients are at increased mortality risk at baseline. Finally, although lymph node recurrences or metastases may be common, preclinical studies have demonstrated reduced synergy between RT and ICB with irradiation of the draining lymph nodes in breast cancer.[62] Given that PD-1 inhibition promotes T cell activation or reinvigoration, nodal RT may negate any benefit from ICB activation.

Finally, the question of whether RT should be delivered to multiple metastatic sites or a single lesion remains. The TONIC trial allowed RT to a single index lesion, which may have limited its synergy with ICB. However, the benefit of irradiating multiple metastatic lesions would be two-fold. First, the immune response generated by RT-ICB may be insufficient to handle the tumor burden associated with multiple metastases; therefore multisite irradiation would serve to debulk oligometastatic disease. Indeed, SBRT for oligometastatic disease has been shown to improve survival with minimal toxicity in both phase II trial and meta-analyses.[63,64] Second, multisite RT may help expose neoantigens that can prime a T cell response. Indeed, multisite SBRT and pembrolizumab was associated with improved survival, increased expression of innate and adaptive immune genes, and decreased expression of DNA repair genes.[65] Finally, preclinical models demonstrated a role for "high-dose" RT to the primary tumor site, and "low-dose" RT to secondary metastatic lesions to reprogram the inhibitory TME of secondary sites.[66] In total, multisite irradiation may improve response rates to RT-ICB and promote antitumor immunity.

Ongoing and Future Trials of RT and ICB in Metastatic Breast Cancer

Given the observed synergy between RT and ICB in breast cancer, there are several ongoing trials exploring the combination in the metastatic, preoperative, and adjuvant settings. A recent search on clinicatrials.gov of breast cancer clinical trials with the terms "radiation" and "pembrolizumab or nivolumab or ipilimumab or atezolizumab or durvalumab or tremelimumab," yielded 46 clinical trials as of January 11, 2021. In the metastatic setting, we identified 18 clinical trials with 5 actively recruiting, 3 not yet recruiting, and 8 active but not recruiting (Table 2). For example, TROG 17.05 AZTEC (NCT03464942) is an ongoing phase II trial in women with metastatic TNBC without brain metastases who have received 1 or fewer prior lines of chemotherapy.[67] Patients are randomized to 1 of 2 SBRT dose fractionations (20 Gy in 1 fraction or 24 Gy in 3 fractions) followed by up to 24 months of atezolizumab, a human-

Table 2 Combination Radiotherapy and Immune Checkpoint Blockade Clinical Trials in Metastatic Breast Cancer

NCT Number	Phase	N	Status	Tumor Type	Intervention	Sponsor
NCT03004183	2	57	Recruiting	mTNBC or mNSCLC	ADV/HSV-tk + valacyclovir + pembrolizumab + SBRT (30 Gy in 5 fractions)	Houston Methodist Cancer Center
NCT03449238	1/2	41	Recruiting	mBC with brain metastases	Pembrolizumab + SRS	Weill Cornell College of Cornell University
NCT03464942	2	52	Recruiting	mTNBC	Atezolizumab + SBRT (20 Gy in 1 fraction or 24 Gy in 3 fractions)	Peter MacCallum Cancer Centre, Australia; Trans Tasman Radiation Oncology Group, TROG
NCT03524170	1	20	Recruiting	$HR^+/HER2^-$ mBC	M7824 (Anti-PD-L1/TGFβ Trap) + RT	MD Anderson Cancer Center
NCT03789097	1/2	56	Recruiting	Multiple tumor types, including mBC	Pembrolizumab + CDX-301 (Flt3 ligand) + RT + Poly-ICLC (TLR3 agonist)	Icahn School of Medicine at Mount Sinai
NCT03915678	2	247	Not yet recruiting	Multiple tumor types, including mTNBC	BDB001 (TLR7/8 agonist) + pembrolizumab + SBRT (27-60 Gy in 3-5 fractions)	Institut Bergonié
NCT04683679	2	56	Not yet recruiting	mTNBC	Olaparib + pembrolizumab + SBRT (24-27 Gy in 3 fractions)	Memorial Sloan Kettering Cancer Center
NCT04690855	2	23	Not yet recruiting	mTNBC	Talazoparib + atezolizumab + SBRT (24 Gy in 3 fractions)	Emory Winship Cancer Institute

Abbreviations: ADV/HSV-tk = adenovirus/herpes simplex-thymidine kinase; Flt3 = FMS-like tyrosine kinase 3; $HR^+/HER2^-$ = hormone receptor-positive/human epidermal growth factor receptor 2-negative; mBC = metastatic breast cancer; mNSCLC = metastatic non–small-cell lung cancer; mTNBC = metastatic triple-negative breast cancer; NCT = national clinical trial; PD-L1 = programmed cell death-(ligand) 1; RT = radiotherapy; SBRT = stereotactic body radiotherapy; SRS = stereotactic radiosurgery; TGFβ = transforming growth factor beta; TLR = Toll-like receptor.

ized, PD-L1-directed monoclonal antibody. It is hoped that this study will elucidate whether single fraction or multifraction SBRT is more efficacious in combination with ICB.

Given the promising response rates to RT-ICB in the metastatic TNBC setting to date, several ongoing and planned trials seek to combine additional therapeutic agents to further augment synergy. For example, the AGADIR trial (NCT03915678) is an upcoming trial from the Institut Bergonié that will combine atezolizumab, RT, and BDB001, a Toll-like receptor (TLR) 7/8 agonist.[68] Using a Simon two-stage design, this trial will enroll 247 patients over 6 independent cohorts, including an arm with women with anti-PD-1/PD-L1 refractory metastatic TNBC.

Three additional trials will examine the addition of a poly ADP ribose polymerase (PARP) inhibitor to RT and ICB. The phase II TARA trial (NCT04690855) will enroll 23 women who are germline BRCA1/2 (gBRCA1/2) pathogenic variant negative (eg, gBRCA1/2 wild-type or gBRCA1/2 variants of uncertain significance) with $PD\text{-}L1^+$ metastatic TNBC.[69] Patients will receive hypofractionated RT (24 Gy in 3 fractions) within 72 hours of receiving atezolizumab, plus concurrent talazoparib, which is an orally bioavailable PARP inhibitor. Another study (NCT04683679) will randomize women with metastatic TNBC to RT (8-9 Gy x 3 fractions), pembrolizumab, with or without olaparib, another orally bioavailable PARP inhibitor.[70] Finally, an upcoming phase II trial will test the combination of dostarlimab, a humanized anti-PD-1 monoclonal antibody, SBRT (24 Gy in 3 fractions), and niraparib, a PARP inhibitor, in metastatic TNBC (either $PD\text{-}L1^-$ or PD-L1+ with progression on ICB). Thus numerous studies of promising combinations of ICB/RT combinations are planned or ongoing for the treatment of metastatic TNBC.

Ongoing and Future Trials of RT and ICB in Early-Stage Breast Cancer

In the preoperative setting, we identified 7 clinical trials of RT and ICB in breast cancer with 3 actively recruiting, 3 not yet recruiting, and 1 active but not recruiting (Table 3). An active phase I/II trial of the preoperative combination of pembrolizumab and RT in 50 women with operable, early-stage TNBC recently completed enrollment (NCT03366844). Patients received 1 cycle of lead-in pembrolizumab, followed by concurrent pembrolizumab and RT (24 Gy in 3 fractions), and then standard of care NAC. In an interim analysis of the first 20 enrolled patients presented at the 2020 San Antonio Breast Cancer Symposium, the pCR rate with this combination was 60% (12 of 20; residual cancer burden [RCB] 0) and 15% (3 of 20) had a near pCR (RCB 1).[71] Notably, there were no observed grade 3 or 4 toxicities during RT plus ICB. Although we caution against cross-trial comparisons because of different treatment arms, the grade 3 or higher toxicity rate favorably compared with a 78% incidence of grade 3 or higher toxicities with concurrent pembrolizumab and NAC in KEYNOTE-522.[46] This preoperative

Table 3 Preoperative and Adjuvant Radiotherapy and Immune Checkpoint Blockade Clinical Trials in Breast Cancer

NCT Number	Phase	N	Status	Tumor Type	Intervention	Sponsor
Preoperative Trials						
NCT02977468	1	15	Recruiting	TNBC	Pembrolizumab + IORT	Columbia University
NCT03366844	1/2	60	Recruiting	TNBC or $HR^+/HER2^-$	Pembrolizumab + RT (24 Gy in 3 fractions)	Cedars-Sinai Medical Center
NCT03804944	2	100	Recruiting	$HR^+/HER2^-$	HT + pembrolizumab + CDX-301 (Flt3 ligand) + RT (24 Gy in 3 fractions)	Weill Medical College of Cornell University
NCT03872505	2	140	Not yet recruiting	TNBC	Durvalumab ± RT (24 Gy in 3 fractions) + chemotherapy	Cedars-Sinai Medical Center
NCT04443348	2	120	Not yet recruiting	TNBC or high-risk $HR^+/HER2^-$ (grade 2-3 or high genomic assay score)	No RT or 9 Gy in 3 fractions or 24 Gy in 3 fractions + chemotherapy + pembrolizumab with exploratory proton cohort	Massachusetts General Hospital (TBCRC)
NCT04454528	1/2	36	Not yet recruiting	TNBC or $HR^+/HER2^-$ or $HER2^+$ cT1	Surgery ± pembrolizumab ± RT	University of Pennsylvania
Adjuvant Trials						
NCT02954874	3	1,000	Recruiting	TNBC with residual disease after NAC	RT ± pembrolizumab	National Cancer Institute
NCT03818685	2	114	Recruiting	TNBC with residual disease after NAC	RT + ipilimumab and nivolumab OR capecitabine	Centre Léon Bérard

Abbreviations: cT1 = clinical T1 tumor (tumor size ≤ 2 cm); Flt3 = FMS-like tyrosine kinase 3; $HR^+/HER2^-$ = hormone receptor-positive/human epidermal growth factor receptor 2-negative breast cancer; HT = neoadjuvant hormone therapy; IORT = intraoperative radiotherapy; NAC = neoadjuvant chemotherapy; NCT = national clinical trial; RT = radiotherapy; TNBC = triple-negative breast cancer.

RT/ICB trial was recently expanded to include patients with high risk, $HR^+/HER2^-$ breast cancer.

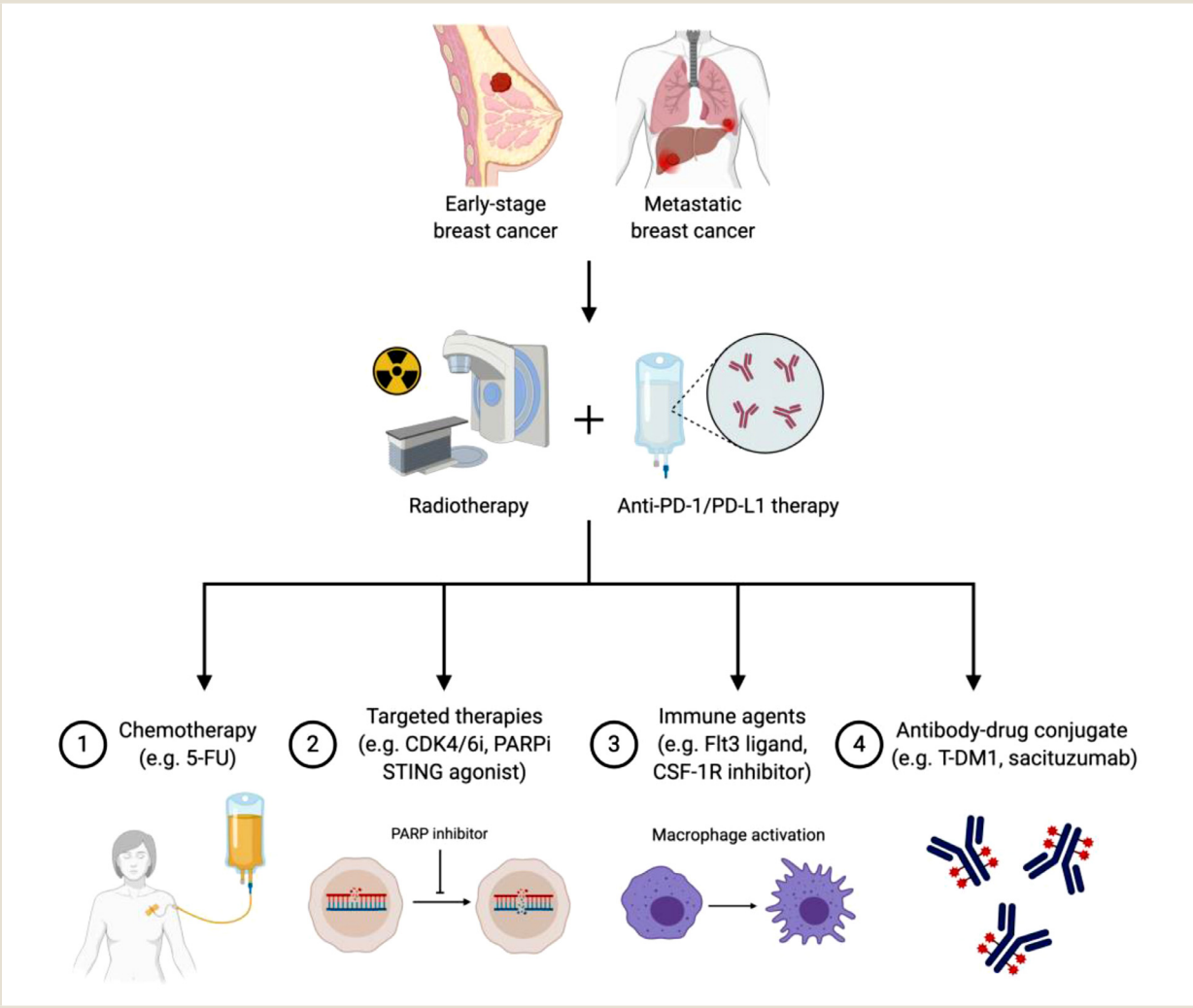
In a phase I preoperative "window of opportunity study" of pembrolizumab with intraoperative RT (IORT) in women with newly diagnosed early stage TNBC, patients will receive 1 to 2 cycles of preoperative pembrolizumab followed by IORT at time of surgery, with change in tumor infiltrating lymphocytes as the primary endpoint (NCT02977468). Finally, the "Converting HR^+ Breast Cancer into an Individualized Vaccine (CBCV)" study is a multi-institutional phase II trial in women with operable $HR^+/HER2^-$ breast cancer (NCT03804944). Patients will receive 4 months of neoadjuvant hormone therapy followed by randomization to RT alone (8 Gy x 3 fractions) or RT with various immunotherapy combinations (pembrolizumab alone, CDX-301 [Flt-3 ligand] alone, or both).

To address the critical question of RT dose in the preoperative space, the Translational Breast Cancer Research Consortium (TBCRC) is currently undertaking the P-RAD trial (NCT04443348) in women with node-positive TNBC or high-risk $HR^+/HER2^-$ early-stage breast cancer (either histologic grade II-III or high-risk genomic assay score [Oncotype RS > 25, high-risk MammaPrint, PAM50, EndoPredict, or Prosigna score]).[72] This phase II trial will randomize patients to no RT, conventional RT boost (9 Gy in 3 fractions), or high-dose RT (24 Gy in 3 fractions), concurrently with pembrolizumab, and followed by chemotherapy. Response will be assessed in a biopsy-proven metastatic lymph node as a measure of abscopal response outside of the field of RT. A third, unrandomized cohort will receive high-dose proton-based RT with an exploratory endpoint of cosmesis.

Ongoing Trials of RT and ICB in the Adjuvant Setting

Finally, several trials are addressing the question of whether there is a role for RT plus ICB in the adjuvant setting for breast cancer. Two large trials are currently exploring this combination in patients with TNBC with residual disease following NAC (Table 2). SWOG S1418/NRG BR006 (NCT02954874) is a phase III randomized trial that is randomizing women with ≥ 1 cm residual tumor or positive lymph nodes after NAC to either observation or pembrolizumab before or concurrent with RT.[73] The accrual goal is 1000 patients with 2 planned interim analyses. Similarly, given the poor outcomes associated with residual

Figure 1 Future strategies for augmenting synergism between radiation and immunotherapy in breast cancer. Abbreviations: 5-FU = fluorouracil; CDK4/6i = cyclin-dependent kinase 4/6 inhibitor; CSF-1R = colony-stimulating factor receptor 1; Flt3 = FMS-like tyrosine kinase 3; PARPi = poly ADP ribose polymerase inhibitor; PD-1 = programmed death 1; PD-L1 = programmed death-ligand 1; STING = stimulator of interferon genes; T-DM1 = ado-trastuzumab emtansine. Created with BioRender.com.



disease in TNBC,[74] the BreastImmune03 trial (NCT03818685) is a phase II trial that randomized women with residual disease to either adjuvant capecitabine per CREATE-X[75] or nivolumab and ipilimumab. RT is administered 1 week prior to C1D1 of immunotherapy/capecitabine.

Future Directions

Given the success of the anti-PD-1/PD-L1 therapies, future trials will likely all include one of these PD-1/PD-L1 directed therapies. Combinations with RT will likely combine RT and an anti-PD-1/PD-L1 with other agents that can augment the antitumor immune response elicited by the combination. Preclinical studies looking at augmenting RT-induced antitumor immunity have focused on 2 strategies: increasing the initial inflammatory response or preventing suppression of the immune response. Aside from the addition of further chemotherapy or radiosensitizers as detailed earlier, preclinical studies demonstrate that potential immunostimulatory triple combinations targeting the type I interferon pathway, such as STING (stimulator of interferon genes) agonists,[17,76,77] TLR ligands, or T cell cytokines such as IL-15,[78] may hold the key to increasing antitumor immunity following RT.

Preclinical studies have demonstrated that the immune-mediate effects of RT are dependent on the STING-cGAS pathway.[17,77] Specifically, RT generates cytosolic DNA, which is sensed by cGAS and leads to the activation of STING in tumor cells and dendritic cells in the TME. This results in the induction of type I interferons (interferon-β) and promotes antigen uptake by the innate immune system and cross-priming of T cells. In preclinical models, intratumor STING agonists were shown to potentiate tumor response

to RT. Given that type I interferon signaling can induce PD-L1 expression, trials combining anti-PD-L1, STING agonists, and RT are currently underway. Potential toxicities of STING agonists include cytokine release storm and inflammatory- and immune-related toxicities. Similarly, TLR ligands, which are immunomodulatory and commonly used as vaccine adjuvants, may be used in combination with RT and ICB to potentiate an antitumor immune response.[79]

Additional targeted therapies include PARP inhibitors, which are currently being proposed in combination with RT and ICB. In tumors that are deficient in homologous recombination, PARP inhibitors can induce synthetic lethality by preventing DNA repair and replication.[80] As such, PARP inhibition can act as a radiosensitizer by delaying single-strand break repair and causing double-stranded breaks. The rationale for combining RT and PARP inhibition would be to potentiate RT-induced cell death, which can be recognized by reinvigorated T cells following ICB.

Other groups have also found that preventing RT-induced immune suppression by targeting inhibitory pathways such as TGF-β[81] or suppressive immune cells such as macrophages[82] or myeloid-derived suppressor cells[83] can enhance the antitumor immune response elicited by RT. The next generation of trials will hopefully test some of these preclinical findings to help fully realize the immunomodulatory potential of RT (Figure 1).

Conclusion

There is increasing preclinical and clinical evidence of potential synergy between RT and ICB in breast cancer. Early trials that have adopted this strategy have provided critical insight into the design of future clinical trials combining RT and ICB. These lessons are reflected in the diverse landscape of ongoing relevant trials in the metastatic and curative intent setting. Moving forward, critical questions remain including how to augment the immunogenic response to RT plus ICB through immunomodulatory agents or DNA damage repair mechanisms. Moreover, given increasing use of ICB in the neoadjuvant setting across solid tumors, ongoing trials will elucidate the role of ICB and RT in the preoperative setting for early-stage breast cancer. Finally, trials of adjuvant ICB with standard-of-care RT will attempt to improve oncologic outcomes in patients with residual disease following neoadjuvant treatment. To this end, there are several promising avenues for the integration of RT and ICB into the breast cancer treatment paradigm.

Clinical Practice Points

-Several preclinical studies have demonstrated synergistic activity between radiotherapy and immune checkpoint blockade -Although recent trials have demonstrated promising activity with immune checkpoint blockade in both early stage and metastatic breast cancer, there are several challenges to overcome if these regimens are to be more broadly relevant-Early trials combining radiotherapy and immunotherapy in breast cancer have demonstrated safety and potential clinical activity in triple negative breast cancer-These early trials have offered critical insight into patient selection, biomarkers, sequencing, and radiotherapy targets and doses -Ongoing and planned trials integrating radiotherapy and immunotherapy in breast cancer cover a broad landscape in the curative intent and metastatic setting.

Disclosure

The review described was supported in part by Cedars-Sinai Cancer, which had no role in the preparation, review, or approval of the manuscript, or the decision to submit the manuscript for publication.

HLM reports grants and personal fees from Bristol-Myers Squibb, Merck, AstraZeneca, and MedImmune; personal fees from Daiichi-Sankyo, Eli Lilly and Company, Pfizer, Genentech, Immunomedics, Puma Biotech, Amgen, Seattle Genetics, Genomic Health, and Spectrum Pharmaceuticals outside the submitted work.

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