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Optimizing Radiation Therapy to Boost Systemic Immune Responses in Breast Cancer: A Critical Review for Breast Radiation Oncologists

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Abstract

Immunotherapy using immune checkpoint blockade has revolutionized the treatment of many types of cancer. Radiation therapy (RT)-particularly when delivered at high doses using newer techniques-may be capable of generating systemic antitumor effects when combined with immunotherapy in breast cancer. These systemic effects might be due to the local immune-priming effects of RT resulting in the expansion and circulation of effector immune cells to distant sites. Although this concept merits further exploration, several challenges need to be overcome. One is an understanding of how the heterogeneity of breast cancers may relate to tumor immunogenicity. Another concerns the need to develop knowledge and expertise in delivery, sequencing, and timing

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of RT with immunotherapy. Clinical trials addressing these issues are under way. We here review and discuss the particular opportunities and issues regarding this topic, including the design of informative clinical and translational studies.

Introduction

Advances in systemic therapy and molecular classification of breast cancer subsets have facilitated the identification of patients with breast cancer who may potentially benefit from therapy escalation or de-escalation. In breast radiation oncology, most modern clinical trials have focused on identifying the patients whose tumor characteristics are most appropriate for strategies to minimize overtreatment by exploring the omission of regional nodal irradiation, boost, or radiation therapy (RT) altogether in low-risk breast cancer.[1] A smaller set of trials in women at high risk of locoregional recurrence is combining radiosensitizing agents such as PARP inhibitors with RT in an attempt to improve outcomes (NCT03945721, NCT03542175, NCT03598257, and NCT01618357). A critical goal, however, is the accurate identification of patients with breast cancer who remain at high risk of disease or progression, despite aggressive standard-of-care therapies. Furthermore, with multiple systemic therapies being rapidly incorporated into breast cancer treatment, critical questions arise regarding the optimal leveraging of RT to effectively synergize with DNA repair-based therapies, kinase inhibitors, endocrine therapies, or immunotherapies.

Among these novel emerging therapies, immunotherapy in particular is a promising therapeutic strategy in breast cancer, given established clinical activity, growing recognition of the role of immunosuppression in the tumor microenvironment (TME), and the association of more robust immune responses with favorable prognosis across many tumor types.[2,3] A variety of local, ablative strategies are under investigation for their potential to overcome intrinsic resistance to immune checkpoint inhibitors (ICI), including cryotherapy and RT.[4–6] RT is a well-established method of inducing localized tumor cell death. However, RT can modulate the TME by both stimulation and suppression of antitumor immune responses.[5–7] Furthermore, the development of sophisticated technologies that enable precise delivery of high RT doses to the tumor may also make RT a practical partner for immune-based therapies; however, much remains to be understood regarding the impact of dose and scheduling of RT on immunotherapy efficacy.

Clinical Opportunities for Immunotherapy/Radiation Therapy Strategies in Breast Cancer

Breast cancer is a heterogenous disease that can be grouped into clinically relevant subtypes defined by expression patterns of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Each subtype displays distinct genomic alterations and clinical behaviors and is treated differently.[8,9] The majority of patients with stage II-III ER-negative, PR-negative, and HER2-negative breast cancer (referred to as triple negative breast cancer [TNBC]) and HER2-positive (HER2+) breast cancer receive preoperative (neoadjuvant) chemotherapy.[10] This preoperative administration of systemic therapy has several advantages, including improved vasculature for drug

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delivery, the potential to allow for less radical surgery, an ability to assess the tumor response in vivo before surgery, and avoiding delay in starting systemically active agents in patients at elevated risk of distant disease progression. Despite favorable pathologic complete response (pCR) rates, overall response rates (ORRs) ranging from 40% to 70% with standard taxane-based chemotherapy, and the addition of dual HER2-targeted agents for HER2+ breast cancer,[11–13] patients with TNBC and HER2+ breast cancer with residual disease in the breast and particularly in the lymph nodes after neoadjuvant chemotherapy remain at extremely high risk of recurrence.[14] Patients with hormone receptor-positive (ER- and/or PR-positive)/HER2-negative breast cancer have historically fared better, but select subsets, including those with locoregionally advanced disease at presentation and high-grade or high genomic assay scores, remain at elevated risk of recurrence, despite standard hormone therapy.[15]

The emergence of ICI over the past decade has transformed the therapeutic landscape for diseases such as melanoma and lung cancer. Higher tumor mutation burden in these tumor types has been associated with a greater likelihood of ICI response.[16] In contrast, the relatively lower tumor mutation burden of breast cancer has been perceived as a potential barrier to immune recognition.[17] Furthermore, although the majority of breast cancers contain tumor-infiltrating lymphocytes (TILs), only about 11% are considered lymphocyte predominant.[18] Breast cancer-associated TILs may also affect response and efficacy of not just ICI, but radiation as well. These properties may explain in part the modest ORRs of ICI monotherapy in unselected metastatic breast cancer, ranging from 5% to 23% across breast cancer subtypes.[19–22]

Biomarker analyses of the responding patients within these trials led to the identification of pretreatment PD-L1 expression as a candidate predictive biomarker of ICI response.[19,21,23] For example, in the Impassion130 trial, patients with untreated metastatic TNBC were randomized to receive atezolizumab plus nab-paclitaxel or placebo plus nab-paclitaxel. Among patients with PD-L1 immune cell-positive TNBC (41% of all patients), the median overall survival was 25 months and 18 months for the atezolizumab plus nab-paclitaxel and placebo plus nab-paclitaxel groups, respectively (hazard ratio, 0.71; 95% CI, 0.54–0.94).[24] The results of this study led to the accelerated approval of this combination in PD-L1-positive metastatic TNBC. To date, clinical benefit of ICI in the metastatic setting has been greatest when administered earlier in a patient's disease course. These results highlight the potential opportunity for improved outcomes by introducing immunotherapeutic combinations earlier in the disease course.

In the phase 3 KEYNOTE 522 trial, the addition of pembrolizumab to standard-of-care neoadjuvant chemotherapy and adjuvant treatment of operable stage II or III TNBC led to higher rates of pCR (51.2% vs 64%) representing an absolute difference of 13.6% (95% CI, 5.4%−21.8%; $P = .00055$).[25] The magnitude of pCR benefit with addition of pembrolizumab to chemotherapy was subsequently reported to be higher among patients with node-positive disease, compared with the node-negative group.[26] This raises the question of whether this differential effect could have been secondary to an immune-priming phase in the involved lymph nodes and whether the addition of RT or other novel agents could further augment responses (Table 1). An early read-out of improved event-free survival was also reported in

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the pembrolizumab arm. If the exciting results from KEYNOTE 522 are sustained with prolonged follow-up, neoadjuvant ICI will be incorporated into the routine management of early-stage TNBC. In turn, this will provide an efficient platform to test whether the addition of RT can further amplify immune responses in TNBC. Of note, PD-L1 has remained an imperfect biomarker; pCR rate was also higher in PD-L1-negative tumors in KEYNOTE 522, and some PD-L1-negative patients appeared to also benefit from treatment in the metastatic setting.[25] Moreover, controversy still exists in terms of the optimal assay to define PD-L1 status. The lack of validated predictive biomarkers of response to ICI in breast cancer remains a major gap in our understanding of how to best use these agents, and these findings highlight the importance of incorporating biomarker discovery correlative studies in clinical trials to guide patient selection and improve long-term cure rates.

Dense lymphocytic infiltration has been observed in a significant proportion of TNBCs and HER2+ enriched tumors, indicating a complex interplay between the tumor and the immune system.[27,28] ER- and/or PR-positive breast cancers are less likely to be infiltrated by CD8+ cytotoxic TILs compared with TNBC and HER2+ tumors, and it has been suggested that these HR+ tumors are associated with decreased immunogenicity.[29,30] Consistently, response rates of ICI have been lowest in patients with HR+ disease.[20] That said, elevated mRNA expression of immune checkpoint molecules, including PD-L1, CTLA4, B7H-3, and IDO1, has been noted in the HR+ luminal population. Furthermore, a subset of HR+/HER2− tumors has increased lymphocytic infiltrate that is not as strongly driven by estrogen and belongs to the luminal B subtype.[31,32] Whether this subset will be more responsive to ICI and radiation combinations is currently being tested (NCT03875573) and highlights the potential for further optimization of ICI combinations to augment the antitumor immune response beyond TNBC.

The Abscopal Effect of RT in Breast Cancer

The goal of immunotherapy/RT (IO/RT) is to induce prolonged local and distant antitumor effects through the induction of systemic immunity. This is clinically manifested through the so-called abscopal effect, in which systemic regression of tumor occurs outside the irradiated portal, after focal irradiation of a single site.[6] Although rarely observed after RT alone,[33] it has been increasingly reported in IO/RT regimens in breast cancer (Fig. 1).

Little is understood about systemic modifiers of abscopal responses beyond the activities within the TME or regional lymph nodes, resulting in a critical need to understand the potential rate-limiting steps involved in the conversion of a localized immune response to a systemic immune response. Additionally, heterogeneity or lack of reporting of this abscopal response in trials using IO/RT in breast cancer further hinders our ability to define populations most likely to experience out-of-field responses. Progress in these areas of research and more careful and consistent reporting of out-of-field response rates will refine our selection of patients for IO/RT combination therapies, guide biomarkers of treatment response and efficacy, and help select additional therapeutic combinations to overcome resistance to IO/RT. A key barrier is the development and establishment of clinically relevant preclinical models for immunotherapy research and focused clinical translational studies to

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understand the biological mechanisms that may help develop predictive biomarkers of response and resistance and inform rational, immunotherapy-based combinations.

Early Evidence for IO/RT Combinations in Metastatic Breast Cancer

To date, IO/RT combinations constitute only 13 (7%) of 185 trials involving ICI, compared with 64 trials (35%) that combine chemotherapy with ICI.[34] An early experience with IO/RT in breast cancer was reported in the TONIC trial, an adaptive phase 2 trial of 67 women with metastatic TNBC that explored a variety of induction strategies in combination with nivolumab, an anti-PD-1 antibody.[35] In the cohort of patients (n = 12) who received palliative RT (24 Gy in 3 fractions) followed 3 weeks later by nivolumab, the ORR was disappointingly low (8%) relative to the ORR in the overall cohort (20%), precluding any further investigation into the combination. A limitation of this study is the relatively long interval between completion of RT and initiation of ICI, which may reduce the likelihood of therapeutic synergy.[36]

Subsequently, more encouraging experiences with IO/RT in breast cancer were reported in a multicenter, phase 2, single-arm study of pembrolizumab, a humanized, monoclonal anti-PD-1 antibody, and hypofractionated stereotactic body RT (SBRT) (30 Gy in 5 fractions) in 17 patients with metastatic TNBC who were unselected for PD-L1 status.[37] Pembrolizumab was administered within 3 days of the first fraction of RT and continued every 3 weeks until tumor progression. The definition of ORR was radiographic response outside of the irradiation portal as per Response Evaluation Criteria in Solid Tumors (RECIST). The ORR in the entire cohort was 18% (3 of 17); however, among the 9 women who were radiographically evaluable at week 17, 3 demonstrated a complete response, with 100% reduction in tumor volume outside the irradiated field. All 3 responders were PD-L1 positive, suggesting that PD-L1 status might serve as a biomarker of response in patients receiving IO/RT, as it has for other immunotherapy trials in breast cancer.[13,19,22,38] Although the results from this signal-seeking study were positive, the small sample size and inability to differentiate the effect of RT and ICI on the primary end point limit immediate application among metastatic TNBC patients. Nevertheless, this study demonstrated the tolerability of SBRT using a hypofractionated approach concurrently with ICI in heavily pretreated patients with metastatic TNBC, paving the path for clinical trials using other combinatorial approaches with IO/RT.

Finally, the combination of pembrolizumab and RT was also well tolerated in another phase 2 trial of metastatic HR+/HER2− breast cancer performed at the Dana Farber Cancer Institute.[39] In contrast to earlier trials, the RT dose was lower in this trial (20 Gy in 5 fractions) and directed to a bone metastatic lesion. Owing to the lack of any objective responses, the trial was closed after accruing 8 patients, leading to the speculation that the lower doses of RT used in the trial may have been insufficient to invigorate a response. Similar to the pembrolizumab/RT trial in metastatic TNBC, limitations were the inability to associate biomarkers with response owing to lack of evaluation and no responses.

Collectively, these early experiences signal the potential for IO/RT combinations to be effective in breast cancer. Further study is essential to elucidate the optimal dose,

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fractionation, and timing of RT delivery with respect to ICI. Accurate patient selection is critical to ensure benefit with IO/RT combinations and may potentially be informed by PD-L1 status, quantification of TILs, or a combination of biomarkers. Insights gained from these early trials have inevitably guided the next generation of IO/RT clinical trials in breast cancer, as will be further described.

Opportunity and Practical Challenges of Combining Breast Radiation and Immunotherapy in Preoperative Settings

The incorporation of immunotherapy agents into the preoperative (ie, neoadjuvant) setting presents unique advantages for priming antitumor immune responses and potential eradication of disseminated micrometastatic disease.[40] Additionally, the ability to accurately and definitely localize primary disease and target it with radiation is an advantage of preoperative administration of radiation. Despite these advantages, however, integration of RT with immunotherapy in the preoperative setting for patients with localized breast cancer poses several pragmatic challenges (Table 1). Foremost is the issue of identifying the optimal dose of RT required to elicit a productive immune response with the least risk of toxicity. It is possible that the dose can be escalated to elicit a productive immune response when administered preoperatively because much of the irradiated tissue will be resected. Nevertheless, many patients will require standard-of-care adjuvant RT after surgical resection, which could pose additional acute and late toxicity risks to reirradiated tissue that is not removed at surgery.

Vanpouille-Box et al have demonstrated that RT doses higher than 10 Gy per fraction can stimulate expression of the exonuclease Trex1, which abrogates the synergy between RT and ICI by suppressing activation of cytosolic DNA damage-sensing pathways.[41] When combined with anti-CTLA-4, hypofractionated RT (8 Gy × 3) led to increased accumulation of cytosolic DNA damage, activation of cGAS/STING, and increased type I interferon signaling that was necessary for CD8+ T-cell mediated antitumor immune responses and regression of nonirradiated lesions, compared with single-fraction high-dose RT. It is important to note that the widely used dose of 24 Gy in 3 fractions was developed in murine model systems and not yet validated as the preferred RT dose in human clinical trials.[42] Thus, the optimal immunostimulatory preoperative RT dose in patients poses a genuine dilemma when translating preclinical findings into a clinical setting. Insufficient dose might not induce an immune response, whereas excessive dose could suppress the immune response or lead to excess toxicity, inferior cosmesis, and diminished quality of life for patients.

Limited knowledge of and expertise in developing consistent techniques of preoperative high-dose RT delivery in breast cancer IO/RT trials across centers also represents a practical barrier to developing large, multicenter trials of breast radiation and immunotherapy. Thus, there is a need for development of standardized contouring guidelines and dose constraints for normal tissues and tumor in the context of IO/RT. Furthermore, the optimal scheduling of RT with ICI continues to be hotly debated, with early clinical trials such as the aforementioned TONIC trial administering nivolumab sequentially, following

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hypofractionated RT (24 Gy in 3 fractions) and other data from the GEPARNuevo study suggesting that IO given before chemotherapy may prime T-cell responses.[43] In contrast, all current trials of preoperative RT and ICI (Table 2) are delivering RT concurrently with ICI, based on preclinical data suggesting concomitant RT/ICI is more effective for generating a robust antitumor immune response than sequential administration.[44,45]

Finally, establishing reliable end points and reproducible biomarkers to assess response to neoadjuvant IO/RT treatments is critical for uniform assessment of a systemic, immune-mediated response. Irradiation of an intact tumor may confound assessment by the classical definition of pCR, which includes response in the primary tumor and/or lymph nodes.[46] pCR rates of 25% to 67% after preoperative RT in breast cancer have been reported.[47,48] A phase 1 dose-escalation study examined the effect of 5 different dose levels of SBRT delivered concomitantly with neoadjuvant chemotherapy (NAC) in 25 evaluable patients with HER2-negative breast cancer.[39] The pCR rate was 36% in the entire cohort, with no pCR observed at the first 2 dose levels and the maximum response of 67% observed at dose level 3 (25.5 Gy delivered in 3 fractions). pCR rates did not increase with dose escalation beyond dose level 3, suggesting 25.5 Gy is the optimal dose for preoperative SBRT in the development of future phase 2 trials.

A key goal of current studies of IO/RT in development is to ultimately build a standardized approach for RT delivery and response assessment that balances all of the previously mentioned considerations. A treatment paradigm that permits systemic evaluation of preoperative RT by assessing response in the lymph nodes (rather than the primary irradiated breast tumor, which would confound interpretation of pCR) is illustrated in Figure 2. Adopting this preoperative RT paradigm in node-positive TNBC would obviate concerns about depriving patients of the potential benefit of adjuvant capecitabine for residual disease that could be obfuscated by a radiation-induced pCR.

The Biologic Complexity of Combining Radiation With Immunotherapy

The biological complexity that underlies immunotherapy response remains an active area of investigation. Increasingly, evidence points to a complex interplay of tumor genomics, TME, host germline genetics, host immune status, and host microbiome that determines the likelihood of eliciting an antitumor immune response to immunotherapy. Furthermore, understanding the unique immunobiology of RT response is critical for establishing when, where, and how RT should be applied to overcome immunotherapy resistance (Fig. 3). This knowledge will also provide a distinction between RT and other ablative modalities, which may be associated with distinct biological effects on the TME and systemic level, despite inducing similar levels of localized cell kill. We will summarize 4 key knowledge gaps in the fundamental biology of RT-induced immune modulation. Progress in these areas will help facilitate scientifically guided translation of IO/RT combinations for clinical investigation.

Radiation and innate immune signaling

A critical interplay has emerged between RT-induced damage and immune-sensing pathways that determine response to both RT and immunotherapy.[5,49,50] One such mechanism involves the transmission of nuclear DNA damage to the cytoplasm, where it can

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engage cytoplasmic DNA sensors (eg, cGas/STING) and stimulate innate immune signaling, such as the type I interferon gene response.[51–53] Abrogation of the cGas/STING pathway blunts abscopal responses induced by RT and ICI in preclinical cancer models.[51,54,55] Alternative immune-sensing pathways are also operative after RT-induced DNA damage. Recently, DNA-PK was shown to act in a STING-independent pathway for cytosolic DNA damage sensing.[56] RT also induces expression of endogenous retroviral genes and other immunogenic RNAs that can activate cytoplasmic RNA sensor pathways such as RIG-I.[57,58] RT-induced DNA damage responses (DDRs) also induce expression of ligands for the activating NK cell receptor, NKG2D, which can potentiate responses to RT.[59,60] Leveraging this RT effect in combination with emerging NK cell-based immunotherapy is a promising area for future research.[61]

The relative contributions of these RT-induced immune-sensing pathways likely depend on a variety of tumor-specific factors. Some tumors may silence or otherwise inactivate the cGas/STING pathway during tumorigenesis,[62] whereas others may co-opt the pathway in a chronically activated form to facilitate metastatic potential.[63] How functional perturbations in the STING pathway affect responsiveness to IO/RT combinations remains an outstanding question.[41,53] Chronic activation of interferon-mediated JAK/STAT signaling has been shown to induce broad resistance to ICI and may represent targets for combination therapy in some tumors.[64] Investigation of IO/RT response determinants will clarify how RT-induced innate immune signaling may be optimized to engender productive antitumor immune responses in patients.

Radiation and tumor neoantigen presentation

Another key step in the induction of a productive antitumor immune response is the processing and presentation of tumor-specific neoantigens to the adaptive immune system. RT has been shown to stimulate antigen presentation in a variety of tumor model systems, including breast cancer.[65] RT can facilitate cross-presentation of HLA class I-restricted tumor neoantigens by professional antigen presenting cells (APCs) in tumor-draining lymph nodes (TDLNs), which promotes CD8+ T cell-mediated cytotoxicity.[65] Conversely, irradiation of TLDNs can also negatively affect the balance between tumoricidal and immunosuppressive TILs and attenuate the combinatorial efficacy of IO/RT strategies.[66]

In breast cancer, there is a growing appreciation for the role of a more complex antigen-directed immune response, namely, one that entails follicular-type T helper cells and antibody-producing B cells that can infiltrate tumors and potentially generate tertiary lymphoid structures within the TME.[67–69] This phenomenon may explain why antibody genes and B cells are strongly associated with favorable outcomes in breast cancer data sets.[70] It is plausible that RT may also be able to stimulate the processing of HLA class II-restricted antigens by professional APCs-an activity that may be even more crucial in neoantigen-poor breast cancers. Improved research tools to predict, monitor, and assess HLA class II-restricted tumor neoantigens are in development.[71–73] Understanding the barriers to effective tumor neoantigen presentation in the TME and in TDLNs will help guide future, rational IO/RT strategies.

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Effects of RT on the immune tumor microenvironment

RT can also have direct effects on stromal and immune cells in the TME. For example, it is known that PD-1/PD-L1 expression by tumor-associated macrophages can inhibit their phagocytic activity and confer sensitivity to anti-PD-1/PD-L1 blockade.[74–76] Thus, RT-induced expression of PD-1/PD-L1 may counteract RT-induced stimulation of HLA class I genes and impede productive antigen cross-presentation. This may, in part, explain the observed synergy between RT and anti-PD1/L1 therapy in stimulating productive antitumor immune responses.[65] Additionally, RT induces expression of transforming growth factor β, which is a potent immunosuppressive growth factor[77] and thus a pathway of great interest in IO/RT combination trials.[78] Transforming growth factor β is a key upstream regulator of T-cell reprogramming and contributes to intratumoral T-cell radioresistance.[79] Recruitment of immunosuppressive myeloid cell types can also mediate radioresistance, including by inhibiting tumor neoantigen priming to the adaptive immune system.[80,81] Investigations into the impact of scheduling of different immunotherapeutic strategies with RT has shown that the best choice of sequence can depend on the exact mechanisms of action,[45] an important aspect to consider when translating current insight to more effective IO approaches. Elucidation of additional immunosuppressive effects of RT will help to identify novel therapeutic combinations to augment IO/RT therapy.

Biological heterogeneity of RT-induced responses

As previously discussed, breast cancer is not a singular disease but rather a complex organ-specific malignancy with molecularly distinct subtypes.[8,9] Based on this understanding, molecular subtyping in a variety of forms has contributed to many of the clinical advances in personalized breast cancer therapy during the past 2 decades, including radiation response.[82,83] Emerging evidence indicates that breast cancers differ substantially in their biological response to DNA damage. Biomarkers of differential DDR in breast cancer are in development and may help guide the clinical implementation of RT in the standard-of-care management of breast cancer.[83–87] The observed heterogeneity in DDR across breast cancers will likely also affect tumor immunogenicity and the efficacy of IO/RT combinations. A more precise understanding of perturbed DDR in breast cancer may help to identify patients who are most likely to benefit from IO/RT combinations. Characterization of synthetic lethality vulnerabilities in the DDR pathway may also suggest targets for overcoming therapeutic resistance to IO/RT, such as PARP inhibitors, ATR inhibitors, or other DNA repair-directed therapies.[88,89] Thus, the development of biomarkers of DDR heterogeneity in breast cancer should remain a priority to facilitate the optimal clinical implementation of IO/RT combinations.

Exploiting Novel Breast Radiation Therapy Techniques With Immunotherapy

A pragmatic issue associated with the delivery of high doses of preoperative RT with immunotherapy is concern about the development of downstream toxicities, given that the vast majority of patients with breast cancer who are eligible will have high-risk disease characteristics requiring postoperative RT to the breast and lymph nodes. The impact of the

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sum of these therapies on local side effects such as treatment-induced fibrosis, breast cosmesis, lymphedema, and surgical and reconstruction outcomes is largely unknown. These concerns have led to the exploration of newer radiation techniques such as SBRT, particle therapy, and magnetic resonance imaging (MRI) guidance to improve coverage of the intact breast tumor while minimizing exposure of radiation to uninvolved breast tissue and surrounding organs.

This has also raised the intriguing question of whether irradiating the entire tumor is necessary in the IO/RT paradigm, in which the goals are to stimulate an antitumor response while minimizing toxicities in women who may receive further RT to the breast/chest wall and lymph nodes in the adjuvant setting. This concept has been compellingly illustrated in a preclinical study by Markovsky et al, in which 67NR murine orthotopic breast tumors received irradiation to either 50% (partial irradiation) or 100% (full irradiation) of the tumor volume. In immunocompetent mice, partial irradiation resulted in tumor responses similar to full-volume radiation, in contrast to immune-deficient nude mice, in which this effect was not observed.[90] Investigators at the National Cancer Institute demonstrated similar findings in an allogenic bilateral Lewis lung carcinoma in which they irradiated varying tumor volumes on one side.[91] These concepts were explored in a phase 1 trial of SBRT and pembrolizumab in multiple tumor types, in which safety and response to SBRT in combination with pembrolizumab was evaluated in 79 patients, including 6 patients with breast cancer. SBRT was delivered using 1 of 3 dose fractionation regimens, depending on the anatomic location of the tumor. The in-field ORR in the entire cohort was 13.2%. The out-of-field response rate was 13.5%, using the aggregate diameter of nonirradiated RECIST target metastases. Interestingly, the response rate was 26.9% using response defined as 30% reduction in any single nonirradiated RECIST target metastasis. Of note, in 25% of patients, metastases measuring >65 mL were partially irradiated. There was no difference in response reported between partially versus fully irradiated tumors.[92] Additional information on techniques for introducing intentional dose heterogeneity into tumors (GRID and lattice RT) and their potential mechanisms for local and distant responses can be found in a recent review.[93]

Although there are consensus guidelines on SBRT normal tissue dose constraints, these approaches have been little studied in the intact breast. Furthermore, it is uncertain whether these need to be adjusted in the setting of delivering immunotherapy and adjuvant RT. Similarly, as previously detailed, target volume coverage is also debated because the proportion of the target volume that needs to receive the prescription dose to achieve the desired immunostimulatory effect is unknown.

Alternative radiation modalities for IO/RT

Particle therapies, such as proton therapy and carbon ion therapy, are being investigated as part of multimodality therapy for breast cancer and as alternatives to photon therapy.[94–96] Particle therapies have distinct physical and biological properties that may be particularly advantageous for the delivery of IO/RT combinations, with the goal of augmenting the antitumor immune response (Table 1). Unlike photons, which slowly attenuate through tissue, charged particles deposit most of their energy at the Bragg peak, with reduced dose

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deposition proximal to the target volume and none distal to the target volume. Therefore, depending on target location and anatomy, particle therapy often enables less exposure to uninvolved normal tissue, such as the heart and lungs, which may reduce the risk of acute, subacute, and late adverse events of combination therapy.[97–99] Moreover, for partial breast irradiation, in which only the target area of the breast is treated, particle therapy would be expected to reduce the volume of irradiated breast tissue outside the target and thereby reduce the risk of adverse cosmetic outcomes.[100,101] This may lead to less exposure of infiltrating effector immune cells not only in the surrounding breast tissue but also the circulating blood cells, which could improve outcomes. The reduced risk of local lymphocyte depletion with proton therapy has been shown in large patient cohorts,[102–104] and lymphopenia is strongly associated with survival of metastatic patients treated with immunotherapy and IO/RT combinations.[105]

Initially, partial breast irradiation with proton therapy used aperture- and compensator-based double scattering delivery methods, which provided limited skin-sparing ability.[106,107] However, pencil-beam scanning has emerged in recent years and enables spot-by-spot intensity control within the proton field. This technology provides for the routine use of skin sparing when desired, which may lead to improved cosmetic outcomes.[100] Skin sparing may be particularly attractive in the setting of the large fraction sizes, which some preclinical data suggest may be most immunogenic.[41,108,109]

Recent findings have highlighted the interconnectedness of the cellular DNA damage and immune responses.[110] Photons and protons are both considered low linear energy transfer (LET) therapies. However, although a relative biological effectiveness (RBE) of 1.1 is used in the clinic for proton therapy, preclinical and clinical data have clearly established that the RBE of protons varies along the proton beam profile.[111] The RBE is highest at the Bragg peak and distal fall-off where the LET reaches its peak, and the DNA damage induced is more clustered and difficult to repair. These areas of high LET can be manipulated such that they localize to areas of gross disease during proton planning.[112,113] Therefore, proton therapy has recently attracted interest for IO/RT combination therapy, given the potentially distinct immunologic effects resulting from the unique spectra of DNA damage induced by photons and protons.[114] An important question facing the field is whether the more clustered and difficult-to-repair damage resulting from protons deposited at the distal track or from higher-LET heavy particle therapy (eg, carbon ions) may be more immunogenic than DNA damage induced by photon therapy, alone or in combination with ICI therapy.[114,115]

FLASH RT is another emerging technology that has generated significant interest with regard to combination with immunotherapy. FLASH RT consists of ultrahigh dose rates (>40 Gy/s), and preliminary data suggest markedly reduced radiation toxicity to normal healthy tissues while inhibiting tumor growth, with similar efficiency compared with conventional-dose-rate RT.[116,117] Data suggest that FLASH RT also modulates inflammatory cytokines (TGF-β and others) and differentially activates immunologic responses within tumor and normal tissues.[116–119] Development of a medical linear accelerator system that is capable of treating large-volume targets at FLASH dose rates is currently under way.[120] However, our understanding of FLASH RT is limited to preclinical studies, with no published studies reporting on human translation to date. Although the

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concept of FLASH RT holds promise, further development of the technology and a deeper understanding of the radiobiology underpinning FLASH RT is necessary before it is ready for primetime in patients receiving immunotherapy.

Identifying Biomarkers of Immune Response With IO/RT

Integration of immune and tumor biomarkers into clinical trials of IO/RT combinations is essential for the optimal development of this strategy. Evaluation of pretreatment tumor biopsies may help identify biomarkers that predict responses to IO/RT, which would be critical for optimal patient selection. Several baseline tumor and TME features are important to query before treatment. Morphologic analysis of TILs, although prognostic across many breast cancer subtypes,[121] may not provide adequate information regarding the molecular status of TILs. For example, quantification of TCF7-positive CD8+ T lymphocytes predicted clinical immunotherapy response more accurately than density of total CD8+ T cells.[122] In breast cancer, CD4+ follicular type T cells and antibody-producing B cell subsets are associated with favorable outcomes.[67,68,70] The co-occurrence of these proimmunogenic cell subsets is reminiscent of the recently identified correlation between immunotherapy response and tertiary lymphoid structures in the TME.[69] Tumor mutational burden and biomarkers of a T cell-inflamed TME represent independent features that correlate with ICI response rates.[123] Consideration of intrinsic biological subtype, as well as biomarkers of DDR status,[86,124] may also be critical for predicting responses to IO/RT combination therapy in breast cancer.

Whenever possible, serial posttreatment biopsies should also be obtained, within and outside the irradiation field. These samples enable assessment of treatment effects, which can inform mechanisms of action and resistance in responders and nonresponders, respectively. Establishing differences in biological response will be critical when comparing IO/RT versus IO monotherapy or when comparing different RT doses or fractionation regimens for immune-stimulatory effects. Methods to assess the immune response to radiation range from lymphocyte subsets, humoral markers, and cytokines to a variety of imaging methods based on magnetic resonance imaging and positron emission tomography.[125]

Blood-circulating biomarkers provide unique opportunities for monitoring treatment effects, systemic immune status, and disease response. Owing to their accessible nature, circulating biomarkers can be assessed at various timepoints, including pretreatment, during treatment, posttreatment, and at follow-up. These include analyses of peripheral blood mononuclear cells, plasma protein biomarkers, and circulating tumor nucleic acids. Microbiome representation has also been correlated with ICI response[126] and may provide useful information regarding diet, metabolic state, and host immune status that is not well represented by classical biomarkers. In addition to traditional biomarkers, artificial intelligence approaches based on imaging are being used to predict the response to IO, [127,128] demonstrating that standard diagnostic imaging may contain information that can help to improve patient stratification.

Technological advances have resulted in a wealth of opportunities when it comes to biomarkers that can potentially be integrated into clinical trials. A distinction should be

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made between pragmatic biomarkers that can be broadly applied to larger patient populations across many different institutions and discovery biomarkers that require specialized sampling procedures or complex molecular assays and thus can only be performed in a research setting. Pragmatic biomarkers should be selected to evaluate a prespecified hypothesis formulated on pre-existing clinical or preclinical data. In contrast, discovery biomarkers can be broad and exploratory in nature. Both types of biomarkers provide unique opportunities; however, pragmatic biomarkers have a greater potential for clinical utility in future settings.

Thus, careful design of research biospecimens and correlative biomarkers is essential for the success of IO/RT clinical trials. By stratifying patients based on the likelihood of response to IO monotherapy, response rates can be more meaningfully interpreted. Furthermore, biomarkers assessed both pre- and posttreatment may validate the suspected engagement of a productive immune response in responders and potentially identify mechanisms of treatment resistance in nonresponders to guide future combination therapy trials.

Framework for Progress: The P-RAD Study

To answer some of these key questions, a clinical trial of neoadjuvant “breast boost” RT, immunotherapy, and chemotherapy in biopsy-proven, node-positive TNBC or high-risk, HR +/HER− breast cancer has been proposed through the Translational Breast Cancer Research Consortium (TBCRC). The P-RAD trial (NCT04443348), a randomized study of preoperative chemotherapy, pembrolizumab, and no-, low-, or high-dose RT in node-positive, HER2− breast cancer, will randomize eligible patients in 1:1:1 fashion to 1 of 3 arms: no RT, conventional boost dose (9 Gy), or high-dose (24 Gy) RT, given concurrently with pembrolizumab (Fig. 4). The RT boost will be delivered in 3 consecutive, daily fractions preoperatively to the radiographically evident primary breast cancers. The immunologic effects induced by the different doses of RT will be assessed by quantifying peritumoral and stromal TILs analyzed from a biopsy of the primary tumor, which will be performed approximately 10 days after completion of the RT boost and first cycle of pembrolizumab. This approach is designed to reveal where and which immune cell subsets become activated after varying doses of RT.

Response in the lymph nodes, which were biopsy proven for malignancy and clipped before the initiation of neoadjuvant therapy, will serve as a surrogate for the abscopal effect. Response of the irradiated tumor will be assessed as a secondary end point but is specifically not included within the definition of the primary end point, given that focal irradiation of the tumor would confound the interpretation of pCR. Additional caveats are that the subsequent neoadjuvant administration of concurrent chemotherapy and pembrolizumab may also affect pathologic response in the nodes or disease-free survival. Toxicities, cosmesis, and patient-reported outcomes will also be assessed in all RT dose cohorts, adding important correlative data in relation to RT dose and immunotherapy.

To explore the hypothesis that targeting the intact breast tumor with proton therapy may result in better cosmesis compared with photon therapy, an exploratory, unrandomized cohort of patients with breast cancer receiving high-dose (24-Gy) RT has been incorporated

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into the trial, in which the RT boost will be delivered with proton therapy. Treatment, eligibility criteria, and end points remain identical to the randomized photon cohorts. Results will be compared descriptively to the photon cohort. Although the power of potential findings may be limited by the small sample size, this appears to be the most pragmatic approach to investigating the question of whether proton therapy may provide immunologic clinical advantages over conventional photon therapy when high doses of RT are used for the boost.

The deluge of interest in the synergy of IO/RT in breast cancer is evidenced by the number of new trials being conducted both in the metastatic and preoperative settings. A search on clinicaltrials.gov with the terms “breast cancer,” “radiation,” and “immunotherapy” returned 38 clinical trials, 15 of which were not IO/RT trials and were subsequently excluded. As of April 1, 2020, there are 19 registered IO/RT trials in the metastatic setting with 10 actively recruiting clinical trials (Table 3) and 4 registered trials in the preoperative setting with 3 recruiting clinical trials (Table 2). Notable trials in metastatic breast cancer include the TROG AZTEC trial (n = 52), a randomized trial of SBRT doses (20 Gy in 1 fraction vs 24 Gy in 3 fractions) in patients with metastatic TNBC with brain metastases. Few trials include immunotherapy and RT in the post-neoadjuvant setting; however, trials in this category include SWOGS1418/NRGBr006, a phase 3 randomized trial of TNBC patients with ≥1 cm residual disease after NAC who will be randomized to pembrolizumab versus placebo. Adjuvant RT may be delivered either before or concurrently with pembrolizumab.[129] The BreastImmune03 trial is investigating adjuvant RT concurrent with ipilimumab and nivolumab versus RT concurrent with capecitabine.[130]

Future Directions/Conclusion

Understanding the heterogeneity of breast cancer is pivotal to designing studies that will effectively leverage RT to boost antitumor responses to immunotherapy. The paradigm of combining IO/RT is supported by compelling preclinical dose-response studies and clinical trial data demonstrating safety and tolerability in metastatic breast cancer. The risk-benefit ratio of IO/RT combinations may be most productive in high-risk patients with innate or acquired resistance to conventional therapies. In the non-metastatic setting, introducing IO/RT combinations early in the disease course will optimize responses by enabling treatment during the window in which tumor burden is lowest and the potential for eradicating micrometastatic disease is greatest. Alignment of expertise among breast radiation oncologists is essential for standardizing high-dose RT delivery with immunotherapy. Future success in conducting clinical trials with end points and biomarkers relevant to IO/RT calls for intensified collaboration with our medical oncology and surgeon colleagues. Ongoing awareness of long-term toxicities with IO/RT combinations continues to be critically important, particularly for patients with breast cancer with curable disease.

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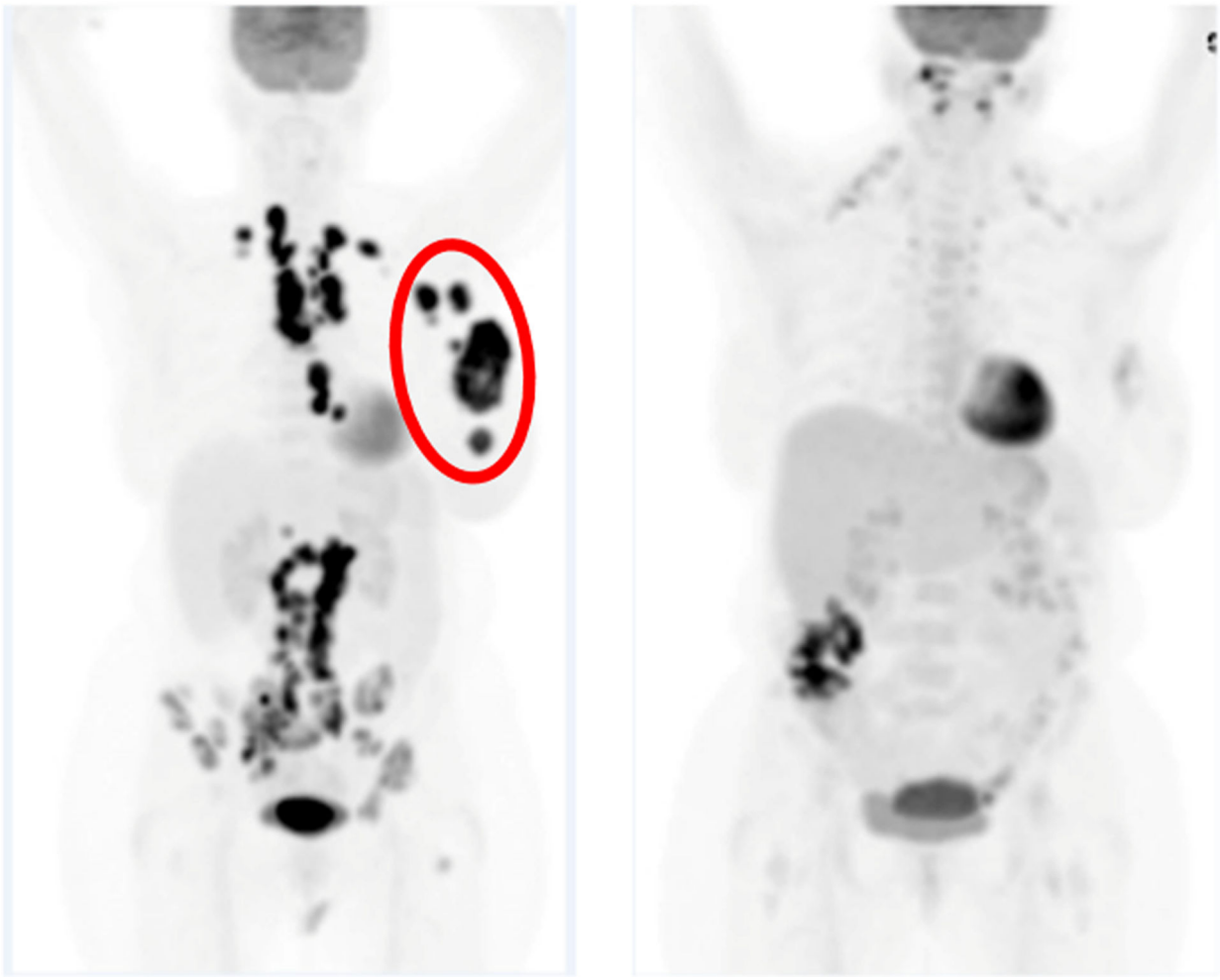

Fig. 1.

An example of an abscopal effect observed on PET/CT scanning in a patient with metastatic TNBC treated with pembrolizumab and hypofractionated RT (30 Gy/5 fractions) to a breast mass. The red circle denotes the RT field. At 19 weeks, regression of tumor in the mediastinal lymph nodes outside the RT portal is observed.[37] *Abbreviations:* CT = computed tomography; PET = positron emission tomography; RT = radiation therapy; TNBC = triple negative breast cancer.

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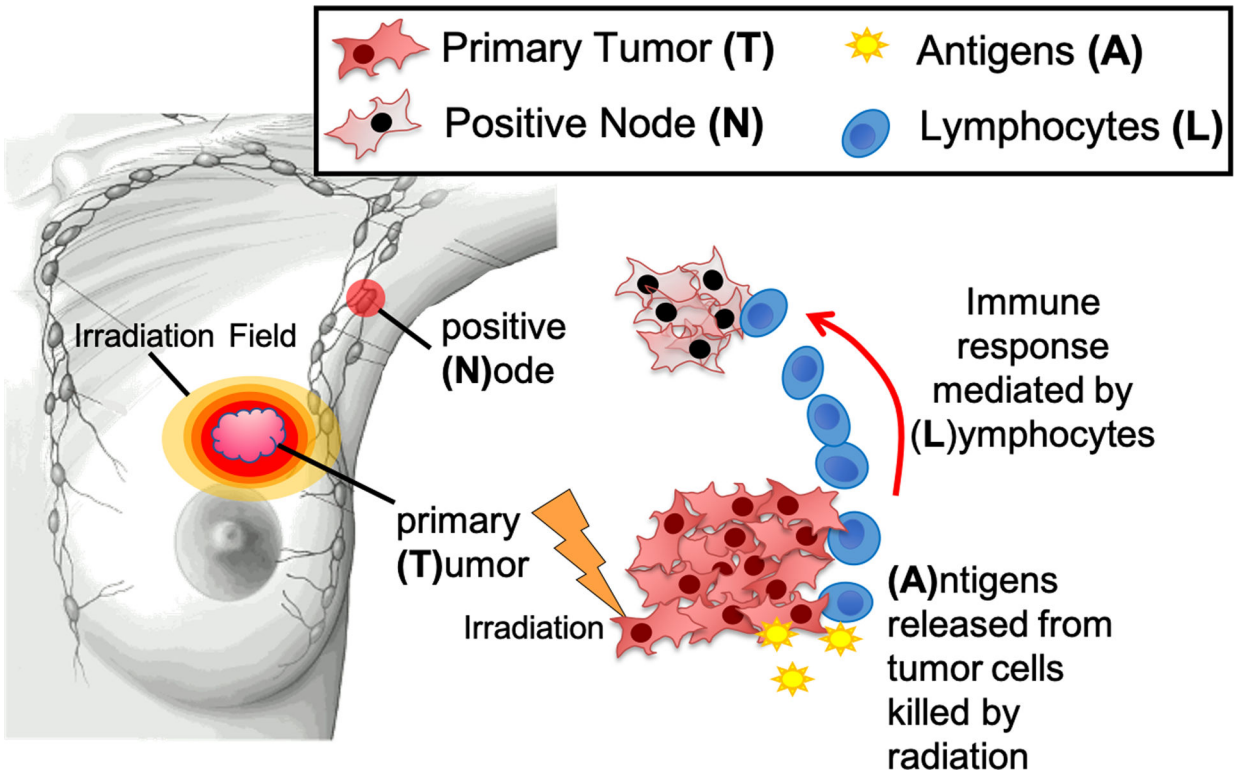

Fig. 2.

Model overview presenting the relationship between the primary tumor (T), antigen released (A), circulating lymphocytes (L), and the tumor cells in the positive node (N). Image created by Clemens Grassberger, PhD and Corey Speers, MD, PhD.

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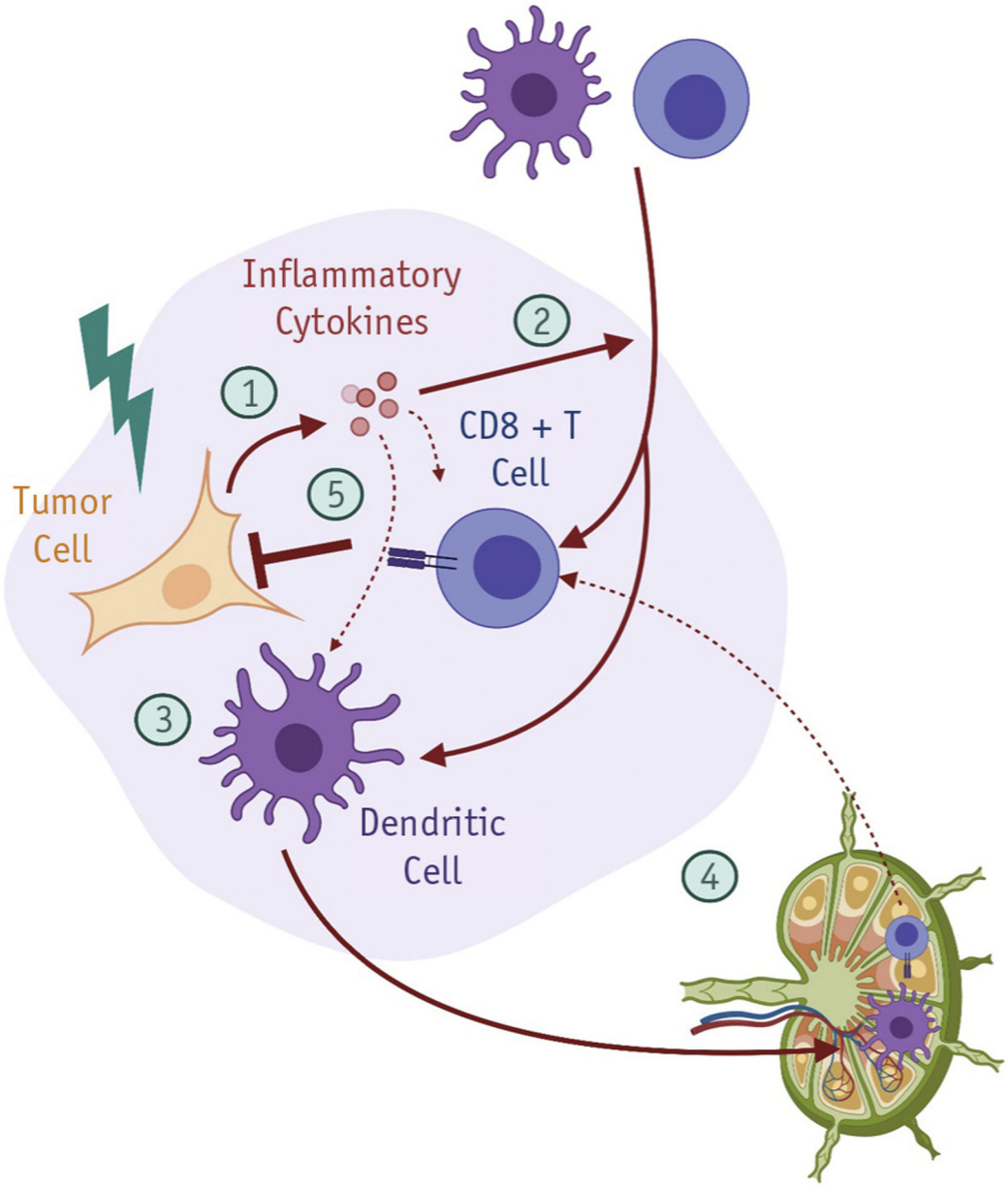

Fig. 3.

RT modulation of antitumor immune responses. RT exerts pleiotropic immunomodulatory effects on the tumor microenvironment. (1) RT-induced DNA damage in tumor cells stimulates innate immune signaling, in part through expression of type I interferon genes. (2) RT-induced chemokines promote infiltration of circulating lymphocytes and innate immune cells into the tumor microenvironment. (3) RT/IO can stimulate tumor cell phagocytosis by professional APCs, and (4) cross-presentation of tumor neoantigens in tumor-draining lymph nodes (TDLNs). (5) RT/IO can facilitate activation of primed tumor-reactive T cells to eradicate tumor cells at both the primary site and distant sites. Figure created with Biorender.com. *Abbreviations:* APC = antigen-presenting cells; RT = radiation therapy; RT/IO = radiation therapy/immunotherapy; TDLN = tumor-draining lymph nodes.

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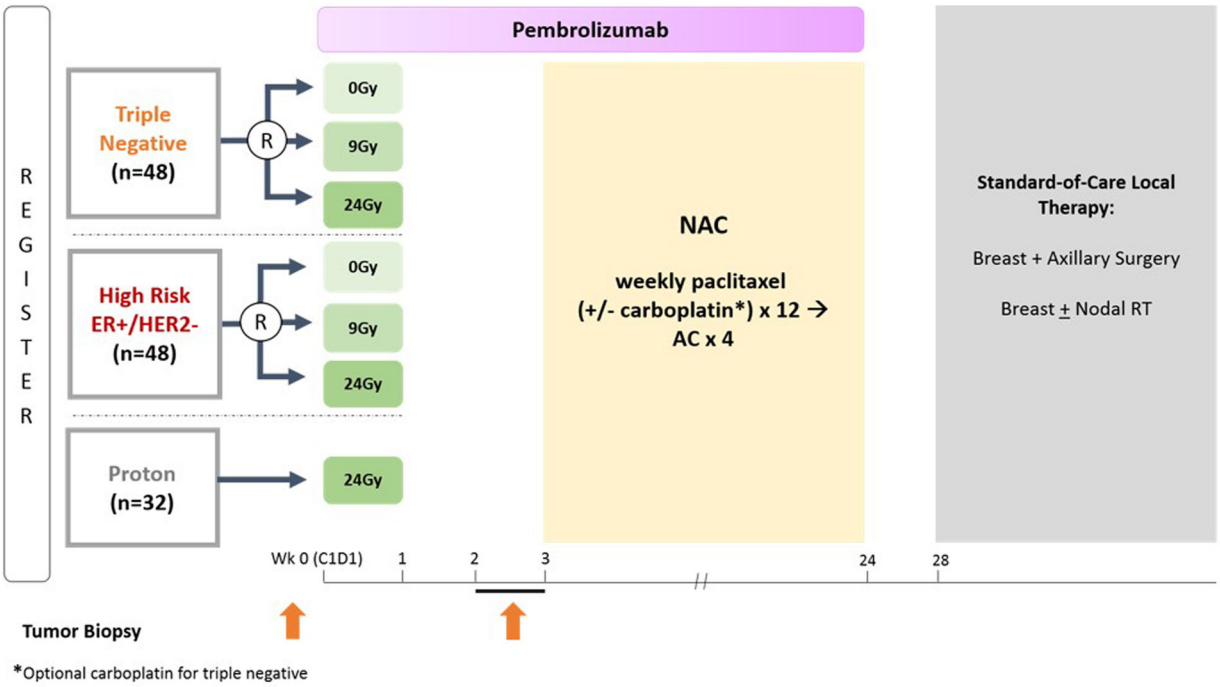

Fig. 4.

Trial schema for P-RAD, a randomized study of preoperative chemotherapy, pembrolizumab, and no-, low-, or high-dose radiation in node-positive, HER2− breast cancer. *Abbreviations:* AC = doxorubicin and cyclophosphamide; NAC = neoadjuvant chemotherapy; RT = radiation therapy.

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Table 1

Design elements of preoperative IO/RT clinical trials in breast cancer

Key elements	Pros	Cons
Inclusion of lymph node–positive disease only	• Pathologic response in the nodes can serve as surrogate for abscopal effect when lymph nodes undergo pathologic evaluation after NAC	• Reduced accrual by limiting eligible patients
Inclusion of TN and high-risk, HR+/HER2− breast cancer	• Evaluation of the efficacy of IO/RT combinations across spectrum of breast cancer subtypes • Addresses unmet clinical need for therapies that can potentially improve nodal response rates in high-risk HR+/HER2− disease	• Inconsistencies in defining triple negative and “high-risk” HR+/HER2− based on clinical definitions and genomic assays
Testing multiple dose levels including a control (no RT)	• Allows assessment of the independent contribution of RT in addition to IO	• Uncertainty that any dose levels proposed are the “correct” doses to compare • More complex than assuming a single, effective RT dose
Conducting trial within a cooperative group setting	• Far-reaching impact, development of practices and tools more broadly applicable to multiple practice settings • Often expedited accrual and shortened time to trial completion	• Enhanced complexity and cost, relative to single-institutional experiences
Inclusion of SBRT and proton radiation therapy	• Far-reaching impact, development of practices and tools more broadly applicable to multiple practice settings	• Lack of widespread availability of protons across United States, increased complexities in treatment planning with unknown benefits • Potential differences in immune system activation between protons and photons

Abbreviations: HR+/HER2− = hormone receptor positive/human estrogen receptor 2 negative; IO = immune oncology; NAC = neoadjuvant chemotherapy; RT = radiation therapy; SBRT = stereotactic body radiation therapy.

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Table 2

Preoperative IO/RT clinical trials* in breast cancer

Sponsor/study name	Phase	N	Tumor type	Intervention
Jules Bordet Institute, Institut Curie (NCT03875573)	2	147	HR+/HER2−	SBRT ± durvalumab and oleclumab (24 Gy in 3 fractions)
Weill Medical College of Cornell University (NCT03804944)	2	100	HR+/HER2−	HT + RT (24 Gy in 3 fractions) ± FLT-3, pembrolizumab or both
Cedars-Sinai Medical Center (NCT03366844)	1	60	HR+/HER2− or TNBC	Pembrolizumab + RT (24 Gy in 3 fractions)
Columbia University (NCT02977468)	1	15	TNBC	Pembrolizumab + IORT

Abbreviations: HR+/HER2− = hormone receptor positive/human estrogen receptor 2 negative; HT = hormone therapy; IO/RT = immunotherapy/radiation therapy; IORT = intraoperative radiation therapy; RT = radiation therapy; SBRT = stereotactic body radiation therapy; TNBC = triple negative breast cancer.

* Registered on clinicaltrials.gov as of April 1, 2020.

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Table 3

IO/RT clinical trials* in metastatic breast cancer

Sponsor	Phase	N	Tumor type	Intervention
Abramson Cancer Center of the University of Pennsylvania (NCT02639026)	1	30	Multiple tumor types, including BC	MEDI4736 and tremelimumab + hypofractionated RT (24 Gy in 3 fractions or 17 Gy in 1 fraction)
Kyoto University Hospital, Japan (NCT03430479)	1, 2	32	HR+/HER2− mBC	Nivolumab and hormone therapy + RT
RACHEL1: MD Anderson Cancer Center (NCT03524170)	1	20	HR+/HER2− mBC	M7824 (Anti-PDL1/TGF-beta trap) + RT
Houston Methodist Cancer Center (NCT03004183)	2	57	mTNBC or lung cancer	ADV/HSV-tk and valacyclovir and pembrolizumab + SBRT (30 Gy in 5 fractions)
Peter MacCallum Cancer Centre, Australia, Trans-Tasman Radiation Oncology Group (TROG) (NCT03464942)	2	52	mTNBC	Atezolizumab + SBRT (20 Gy in 1 fraction or 24 Gy in 3 fractions)
Institut Bergoni, Roche Pharma AG, National Cancer Institute, France (NCT03915678)	2	247	Multiple tumor types, including mTNBC	Atezolizumab and G100 + short-course RT (4 Gy in 2 fractions) or SBRT (27 Gy to 60 Gy in 3–5 fractions)
Memorial Sloan Kettering Cancer Center (NCT02563925)	1	28	mBC with CNS metastases	Tremelimumab and HER2-directed therapy and durvalumab + WBRT or SRS
Dana-Farber Cancer Institute (NCT03483012)	2	45	TNBC with CNS metastases	Atezolizumab + SRS
Weill Cornell (NCT03449238)	1, 2	41	mBC with CNS metastases	Pembrolizumab + SRS
H. Lee Moffitt Cancer Center and Research Institute (NCT03807765)	1	12	mBC with CNS metastases	Nivolumab + SRS

Abbreviations: CNS = central nervous system; HR+/HER2− = hormone receptor positive/human estrogen receptor 2 negative; IMRT = intensity modulated radiation therapy; mBC = metastatic breast cancer; mTNBC = metastatic triple negative breast cancer; RT = radiation therapy; SBRT = stereotactic body radiation therapy; SRS = stereotactic radiosurgery; WBRT = whole brain radiation therapy.

* Registered on clinicaltrials.gov of April 1, 2020.

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