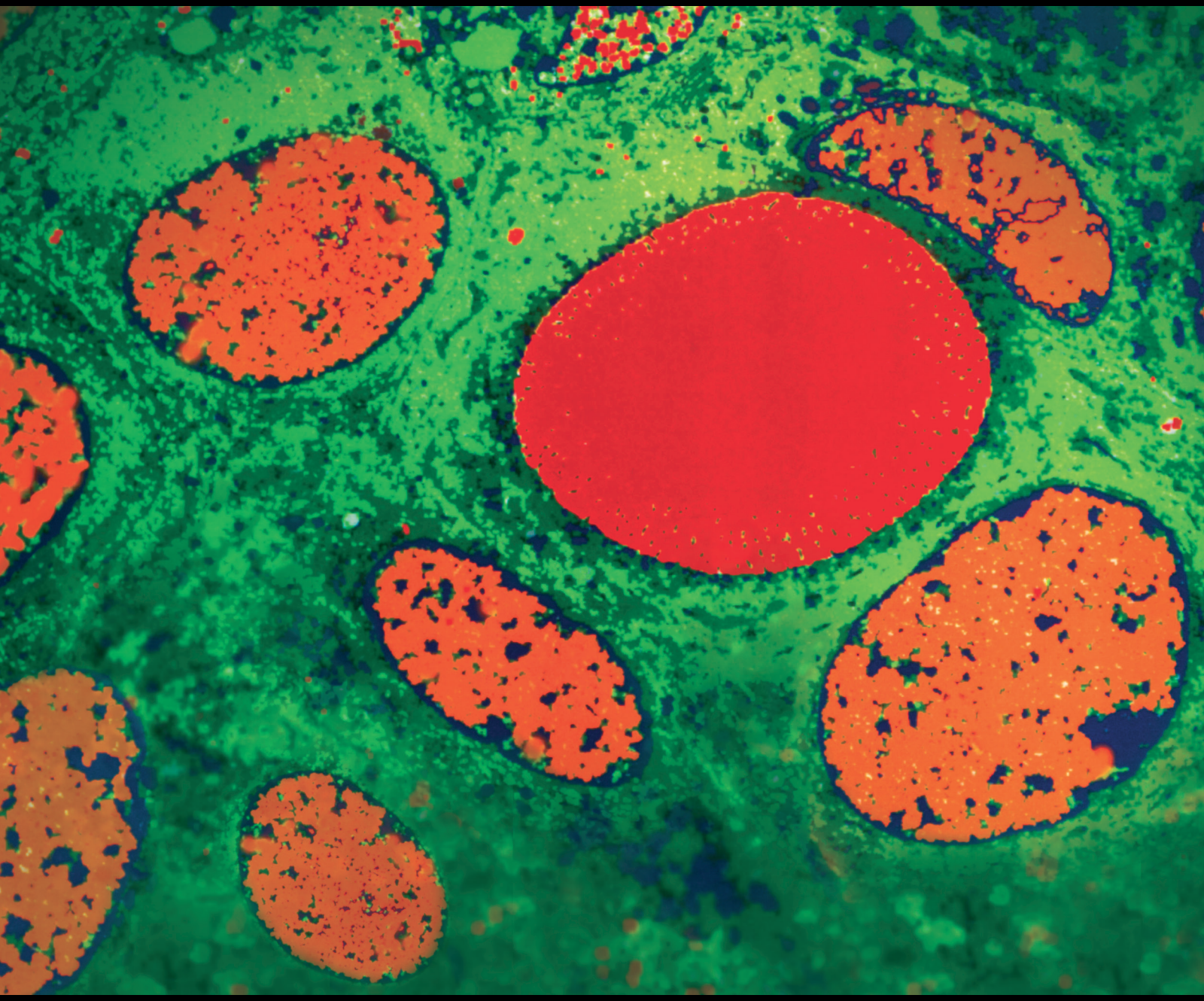
International Journal of Breast Cancer

Innovations in Personalized and Targeted Therapies for Breast Cancer

Lead Guest Editor: Eleanor E. R. Harris
Guest Editors: Stephen R. Grobmyer, Virginia F. Borges, and Jennifer De Los Santos



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Editorial Board

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Editorial

Innovations in Personalized and Targeted Therapies for Breast Cancer

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This special issue highlights many of the innovations and diverse methodologies for personalizing the treatment of breast cancer using novel assays and therapies targeting the molecular mechanisms driving individual tumor biology. In the current era of precision medicine, breast cancer treatment is a leading model for truly individualized diagnosis and treatment, as highlighted in our issue's reviews and original articles. This innovative approach to cancer therapy is occurring across the array of diagnostic and treatment modalities as our authors' contributions demonstrate. In particular this issue seeks to highlight the advances in precision medicine as it applies to the surgical and radiotherapy approaches to more individualized surgical and radiation treatments.

The quest to characterize breast cancers by molecular subtype has been pursued for decades, since the advent of estrogen and progesterone receptor expression as the simplest manifestation of subtyping. Molecular characterizations predict tumor behavior and prognosis and provide potential targets for systemic therapy and novel drug development. The breast develops under the influence of multiple endocrine pathways, and there are many endocrine therapies targeted to hormone receptor expressing breast cancers, with avid research ongoing to further define these molecular targets. Recent work on both androgen receptor and glucocorticoid receptor expression in the differentiation and tumorigenesis pathways of the breast provide potential new molecular targets for systemic therapies. At the other end of the biological spectrum are those breast cancers devoid of any established hormone receptors, the triple negative cancers, which are a heterogeneous group with a poorer prognosis and the subject of intense study to identify more effective systemic agents and delivery methods. One such approach is the use of continuous chemotherapy dosing, called metronomic delivery, designed to avoid any break in the systemic therapy that could allow tumor cell proliferation. Other investigations are looking at intensification of chemotherapy, new agents, and biologics including PARP and other DNA-damage repair inhibitors and immunotherapy drugs. Metastatic breast cancer is increasingly viewed as a chronic disease that can be managed and controlled rather than a terminal illness. An active research topic is the integration of radiotherapy to release tumor antigens and immunotherapy in the treatment of oligometastatic disease, with the goal of long term survival or even cure.

Local regional treatment of operable breast cancer over recent decades has evolved to embrace a "less is more" philosophy, from the adoption of breast conserving therapy, to the validation of sentinel node biopsy and more recently investigations into omission of surgery or radiation for biologically low risk cancers. The goal is to optimize the treatment for the individual patient based on the predicted capacity of their breast cancer to recur locally, regionally, or

systemically as predicted by multiple molecular markers and predictive gene panels. Without such information, patients are treated according to broad protocols and often receive more, or less, therapy than is indicated. One very innovative and promising area of active clinical research is the development of gene assays to predict tumor radiosensitivity. If an individual tumor can be tested for its response to radiation, the radiation dose and target volumes for treatment can be truly individualized to ensure optimal tumor control while minimizing toxicity and cost.

Patient centered value-based medicine is increasingly embraced as is patient preference and shared decision making. Patients are increasingly choosing more individualized approaches that meet their needs for not only treatment outcomes, but also quality of life, functionality, cost, and impact on their lives beyond the clinic. Clinicians are increasingly evolving from rigid adherence "cookie cutter" protocols to personalized treatment incorporating a vast array of integrated therapies. Many patients are opting for less extensive surgery, shorter courses of radiation, and optimized systemic therapies directed by predictive gene assays. Clinical trials are actively assessing ways to "right size" therapy, including omission of surgical, radiotherapy, and systemic treatments that are not therapeutic as well as abbreviating and accelerating radiotherapy regimens to improve efficacy and adherence.

Precision medicine in oncology seeks to individualize each patient's treatment regimen based on an accurate assessment of the risk of recurrence or progression of that person's cancer. Precision can be achieved at each phase of care, including detection, diagnosis, surgery, systemic therapy and radiation therapy, survivorship, and follow-up care. The precision arises from detailed knowledge of the inherent biological propensities of each tumor, rather than generalizing treatment approaches based on phenotypic or even genotypic categories. Extensive research is being conducted in multiple disciplines, including radiology, pathology, molecular biology, and surgical, medical, and radiation oncology. Clinical trial design is adapting to the new paradigms and moving away from grouping heterogenous patient populations into limited treatment comparison arms.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue

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Review Article

Personalizing Radiation Treatment Delivery in the Management of Breast Cancer

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Long-term data establishes the efficacy of radiotherapy in the adjuvant management of breast cancer. New dose and fractionation schemas have evolved and are available, each with unique risks and rewards. Current efforts are ongoing to tailor radiotherapy to the unique biology of breast cancer. In this review, we discuss our efforts to personalize radiotherapy dosing using genomic data and the implications for future clinical trials. We also explore immune mechanisms that may contribute to a tumor's unique radiation sensitivity or resistance.

1. Introduction

Radiotherapy is integral in the management of breast cancer. The 25-year results of NSABP B04 published in 2002 indicate radiation leads to less extensive surgeries, while maintaining relapse-free and overall survival [1]. Meta-analyses show that locoregional control as well as breast cancer mortality benefit from adjuvant radiation therapy following breast conservation surgery or following mastectomy with node-positive disease [2, 3].

Technical advances in radiation therapy treatment planning since these early studies including target motion management, image guidance, and conformal planning now result in improved ability to decrease dose to surrounding organs including the heart and lungs while accurately treating diseased tissue [4, 5]. Also a number of radiation fractionation strategies are now validated for both early and advanced stage breast cancer. Hypofractionation studies have revealed equivalent treatment outcomes with respect to in-breast local control, breast cosmesis, and toxicity while being more convenient and cost-effective with shortened treatment duration[6–8]. Partial breast irradiation via external beam, brachytherapy, or intraoperative techniques has been shown to limit the volume of irradiated tissue in select groups of women while preserving efficacy although data on long-term outcomes is limited [9–12].

Personalized medicine has been discussed in the medical oncology community; however, radiation oncologists have typically delivered uniform doses of radiation without consideration to biologic differences across tumors. As we enter a new era of genomic testing, personalized radiation therapy is becoming more feasible. Breast cancer is well suited to be a primary malignancy in which radiation oncologists' pilot efforts for personalization. The role of adjuvant radiation therapy is well established in breast cancer and gene expression data for breast malignances exists in large publicly available datasets. For this reason, our group has focused attention on personalizing radiation therapy in this malignancy.

In this review, we will discuss efforts to define the radiation sensitivity and resistance of genomically characterized breast tumor types as determined by our novel gene expression based radiosensitivity index (RSI). We also discuss

personalized radiotherapy dosing after interpretation of RSI as well as development of an actionable radiation metric, the genomically adjusted radiation dose (GARD). Initial validation studies in breast tumors using this model will be reviewed, and we will also discuss rational combinations of radiation therapy with immunotherapy utilizing RSI. Finally, we will close with thoughts on prospective trials to test these hypotheses.

2. Biologic Adaptation in Systemic Management of Breast Cancer

Genomic tests have become useful tools in predicting clinical outcomes and for guiding treatment decisions in breast cancer, particularly for systemic therapy. For example, the Mammaprint 70-gene signature test has been shown to be a powerful predictor of distant metastasis in node-negative breast cancer, and there is evidence it is a more accurate predictor of overall survival and metastasis-free survival than standard clinicopathological risk assessment methods [13, 14]. Traditionally, women considered to be at high clinical risk for distant metastasis were treated with adjuvant or neoadjuvant chemotherapy. However, approximately 46% of these women may not require chemotherapy if they are placed in the low genomic risk category by the Mammaprint signature as these women were found to gain no benefit in five-year metastasis-free survival with adjuvant chemotherapy [15].

The Oncotype Dx recurrence score was shown to be an accurate predictor for the risk of distant recurrence and overall survival at 10 years independent of age and tumor size in patients with lymph node-negative, ER+ tumors treated with tamoxifen [16]. In lymph node-positive patients treated with tamoxifen, adjuvant chemotherapy provided little benefit in terms of 10-year distant recurrence in tumors with Oncotype Dx recurrence scores of less than 18, while patients with recurrence scores greater than 31 benefited from chemotherapy [17, 18]. Furthermore, prospective studies showed patients with node-negative, ER+, HER2-negative tumors and recurrence scores <11 had less than a 1% risk of distant recurrence at five years and that such patients may be safely spared chemotherapy even if they are at high risk of distant failure by traditional clinicopathologic risk estimation [19, 20].

With growing acceptance of data supporting the use of tumor genomics in clinical practice, the newly released AJCC 8th edition staging guidelines will incorporate tumor genomic assays as staging modifiers. For example, in hormone receptor positive, HER2-negative, lymph node-negative tumors, a low risk score on the Mammaprint signature or a recurrence score less than 11 on the Oncotype Dx panel places a tumor into the same prognostic category as T1a-T1b N0 M0, regardless of tumor size [21]. While tumor genomic panels have seen an increased use in guiding systemic therapy decisions, similar tests have not gained widespread adoption in radiotherapy management of breast cancer.

3. Biologic Adaptation in Breast Radiotherapy

Efforts are currently underway in the breast radiation oncology community to tailor adjuvant treatment to a patient's biologic subtype. Although the role of radiation treatment is known in the advanced node-positive setting, in localized tumors with other favorable prognostic characteristics there is an understanding that as radiation oncologists we may be overtreating patients who may otherwise be eligible for systemic treatment alone. Trials omitting radiation in select favorable patients have shown higher rates of recurrences indicating improved techniques to select appropriate patients for treatment deescalation are needed. The 10-year results from the CALGB 9343 study have revealed in women ≥ 70 years with T1, node-negative tumors, that are ER+ and receiving hormonal therapy, radiation therapy can be eliminated after lumpectomy with fairly low recurrence rates [22, 23]. The CALGB study demonstrated a freedom from locoregional recurrence rate of 90% in the lumpectomy with tamoxifen alone treated arm and 98% in the arm treated with radiotherapy. The difference was significant; however, OS did not differ between groups. In addition, the PRIME II trial has demonstrated the feasibility of eliminating radiation in a cohort of women ≥ 65 years, pN0, up to 3 cm tumors and negative margins who received adjuvant endocrine therapy [24]. At 5 years, recurrence rates in the arm without radiation were significantly higher at 4.1% compared to 1.3%; however, the study requires continued long-term follow-up.

Given these trials, which have demonstrated the feasibility of excluding radiation therapy in the management of early breast tumors without decreasing overall survival, a number of studies are ongoing to assess which patients may be candidates for radiation therapy exclusion. The LUMINA study from the Ontario Clinical Oncology Group (Clinicaltrials.gov identifier NCT01791829) is a single arm study of women ≥ 55 years with T1 luminal A tumors receiving endocrine therapy. In addition, the University of Michigan has initiated the multi-institutional IDEA trial (NCT02400190) in which women between 50 and 69 years with ER+, PR+, and Her 2 negative early stage tumors with an Oncotype Dx score ≤ 18 will receive hormonal therapy alone. Finally, the Dana Farber Cancer Institute has initiated the precision trial for women between the ages of 50 and 75 years with luminal A tumors measuring ≤ 2 cm that receive hormonal therapy alone. Together, these efforts are targeting more specific populations of women utilizing biologic subtype to better characterize which women can be spared radiation therapy. Although there are ongoing efforts to personalize radiation therapy delivery based on a patient's unique clinical factors as well as receptor status, genomic data would be expected to vary amongst these tumors. Efforts from our group as well as others have suggested methods may exist to tailor radiation therapy based on a tumor's unique genomic profile.

Various groups have suggested utilization of gene signatures and biomarkers to predict the benefit of radiation therapy in both early and advanced stage breast cancer. The Danish Group published a gene signature, which predicted the benefit of postmastectomy RT in patients with high-risk breast cancer in the context of the Danish 82b and 82c trials [25]. A seven-gene signature was identified from 191 patients and then validated in 112 patients ultimately

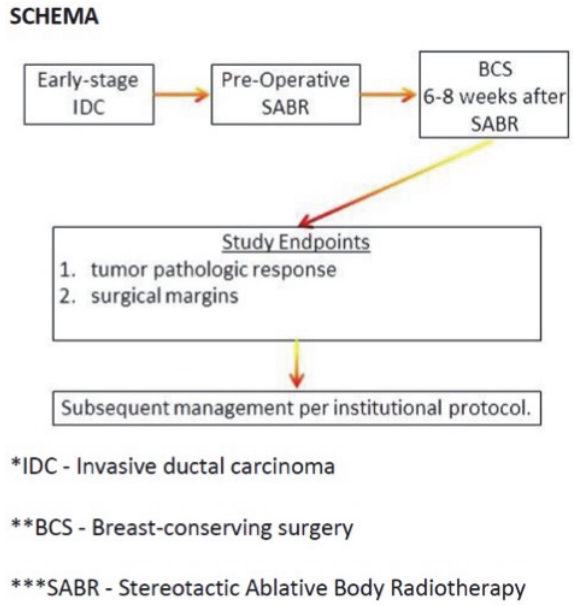

Figure 1: Trial schema for preoperative stereotactic radiation trial.

identifying a group of patients with sufficiently low risk of locoregional recurrence in whom there was no benefit from postmastectomy radiation therapy [26, 27]. Similarly, studies have revealed the Oncotype Dx score to be predictive of locoregional recurrence suggesting potential utility in radiotherapy treatment decision-making. The University of Michigan has also suggested a radiation sensitivity signature (RSS) to identify patients that would benefit from adjuvant radiotherapy [28]. The RSS was developed using clonogenic survival assays across breast cancer cell lines. The RSS was refined to 51 genes and validated in two independent datasets outperforming all clinical and pathologic features.

To better understand the effects of radiation therapy and the tumor microenvironment we have recently opened a phase 2 trial at our institution to assess a preoperative accelerated partial breast irradiation (APBI) regimen of 28.5 Gy in 3 fractions following 6 to 8 weeks later by surgical resection (Figure 1). Although several attempts at preoperative radiation have occurred in the phase I setting including 21 Gy in one fraction and 31.5 Gy in three fractions [29, 30], an important goal of our trial is to obtain pre- and postradiated tissue to assess the unique changes in the radiation sensitivity and to assess the immune landscape that may be important to the tumor's sensitivity to radiation.

4. RSI Development and Validation

Historically, radiation therapy dose delivery and fractionation schemes have been uniform with variation only in definition of target volume (i.e., +/- nodal volumes). A patient's unique tumor biology has not been taken into account. The development and validation of the radiosensitivity index (RSI) have taken place over the past decade as a means to help predict radiosensitivity of various tumor types and response to radiation treatment [31]. RSI was developed to predict differences in cellular radiosensitivity based on the surviving fraction of cells at 2 Gy (SF2) in cell lines. The process of developing the signature included two main steps. The first step included the identification of 10 genes using an algorithm correlating basal gene expression and other parameters (tissue of origin, Ras, and p53 mutation status) to SF2 in a panel of 48 human cancer cell lines. The top 500 genes were selected and interconnected in a biological network with a systems biology approach [31]. The 10 hub genes selected for the RSI algorithm were found to be the most connected genes in the network. Thus, the criteria for gene selection are based on the individual ability of the gene to predict SF2 and its biological importance within the network. The second step used the 10 genes to train a gene expression algorithm to predict SF2. This final algorithm is RSI and has been locked since the first validation studies in rectal cancer [32].

This model predicts RSI to be directly proportional to tumor radioresistance (RSI, high index = radioresistance). Prior work has shown RSI to be predictive for the benefit of radiotherapy in a number of different primary cancers, including esophageal, rectal, head and neck, breast, glioblastoma, pancreas, and prostate malignancies as well as colon and liver metastases [32–40]. RSI has correlated to outcomes of local control and overall survival in these disease sites. Since RSI is a radiation specific marker, we found RSI to be correlated to endpoints of local control and overall survival in these disease sites in patients treated with radiation but not in patients not receiving radiation therapy.

We have previously shown RSI to be prognostic in breast cancer patients treated in several independent datasets. In a dataset of patients treated at the Karolinska University Hospital (n=159), we noted patients predicted to be radiosensitive had an improved 5-year relapse-free survival when compared with radioresistant patients (95% versus 75%, $p = 0.02$) [33]. Since RSI is a radiation specific signature, there was no difference in radiosensitive/radioresistant patients treated without RT (71% versus 77%, $p = 0.67$). In addition, in a separate dataset of patients treated from the Erasmus Medical Center (n=344), radiation treated radiosensitive patients had an improved 5-year distant metastasis-free survival over radioresistant patients (77% versus 64%, $P= 0.0409$), but no difference was observed in patients treated without radiation (radiosensitive versus radioresistant, 80% versus 81%, $p = 0.94$) [33]. RSI has also been validated in a cohort of 343 patients treated at 4 Dutch Centers (Netherlands Cancer Institute, Radboud University Medical Center, Erasmus Medical Center, and Ziekenhuis Amstelland) with breast conserving therapy that included whole breast radiation with or without a tumor bed boost [37]. We noted that local recurrence was not predicted across the entire cohort. However, the combination of receptor type with radioresistance according to RSI identified a subpopulation of patients with an increased risk of local recurrence. In contrast, integrating RSI into the luminal subtypes did not identify additional risk groups with increased risk of local recurrence.

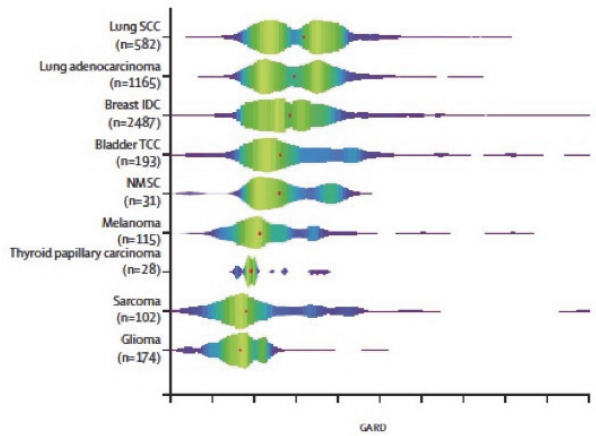

Figure 2: GARD score distribution and density within 60 Gy dose level, by disease site. The red dot represents the median GARD value for each disease site at assigned dose levels. Colors in the plot correlate with the sample density. GARD=genomic adjusted radiation dose. IDC=invasive ductal carcinoma. TCC=transitional cell carcinoma. NMSC=nonmelanoma skin cancer. Reprinted from *The Lancet Oncology*, Vol. 18, Scott JG, Berglund A, Schell MJ et al., A genome-based model for adjusting radiotherapy dose (GARD): a retrospective, cohort-based study, 202-211, 2017, with permission from Elsevier.

5. The Genomic Adjusted Radiation Dose (GARD)

The linear quadratic model is a commonly used metric by radiation oncologists to quantify the biologic effect of radiation dose on various tumor types as well as normal tissue [41]. Since the model was developed as a molecular estimate of SF2, our group hypothesized RSI could be integrated into the model to represent a tumor's unique response to radiation therapy. Such a model, which integrates tumor specific biology into the response to radiation, is vital for progress towards precision radiation oncology. The result was the genomically adjusted radiation dose or GARD, which has since been published and validated in independent datasets of breast, glioblastoma, pancreas, and lung tumors [42]. We assessed GARD for 8,271 tumor samples in the Moffitt Total Cancer Care Database, our institution's tissue biorepository [43]. GARD values varied based on primary tumor histology (Figure 2). Median GARD values were lowest for tumor types traditionally thought to be more radioresistant including glioblastoma and sarcoma and higher for tumor types thought to be more radiosensitive including virally associated cervical cancer as well as oropharyngeal cancer.

Similarly, we have shown RSI to be correlated with the 12-Chemokine (12-CK) signature, a validated gene expression signature for immune-related and inflammation related genes[44–46]. We found the two signatures to be inversely correlated across tumor types indicating greater immune systemic activation to be correlated with radiosensitivity [47]. The 12-CK score has also been assessed in breast cancer samples at our institution [48]. Higher 12-CK scores (immune active) were noted in white patients, poorly differentiated, and basal and Her 2+ molecular subtypes. Higher 12-CK scores also demonstrated superior overall survival ($p=0.008$) and recurrence-free survival ($p<0.0001$) especially in basal and Her 2+ patients.

GARD predicted distant metastasis-free survival and relapse-free survival on multivariate analysis in two independent datasets of patients treated with adjuvant breast radiotherapy. In addition, in a separate analysis from our group presented in abstract form and currently in preparation for publication, GARD predicted for local recurrence in patients with ER negative tumors but not ER positive tumors [49]. In this analysis, we note a small subset of women who may benefit from dose escalation to the whole breast to 60 Gy reaching an optimal GARD level to compensate for an unfavorable genetic profile. These data suggest uniform dosing may lead to inferior outcomes in select populations of patients. A dosing optimization strategy, which takes into account a tumor's unique genetic profile, may allow for improved outcomes with dose escalation and potential sparing of toxicity with dose deescalation in more favorable profile tumors. Although further prospective validation is required, the model provides a framework to integrate a genomic component into the assessment of radiation effect to assess a tumor's unique sensitivity to radiation.

6. Immune Infiltrates and Radiation Response

It is evident the response to RT varies widely across tumors, which is partially driven by topographic biophysical variability in the microenvironment as well as the intrinsic mutational profile in individual tumor cells. Breast cancers are composed of tumor cells and a diverse mixture of stromal cells, including fibroblasts, endothelial, and innate or adaptive immune cells [50]. Each of these cellular constituents contributes to the dynamic framework of the tumor, which either promotes or antagonizes further cancer progression. Recently, immune cells have received considerable attention in oncology due to the sustainable clinical responses observed after altering the host immune response to various malignancies [51]. Whether breast cancer patients will benefit from immunomodulating strategies is an area of active investigation.

Breast cancer has traditionally been considered a more immunologically silent tumor relative to other malignancies (e.g., melanoma) due to lower observed mutational burden [52, 53], origination in a tissue that requires strict immune regulation during dynamic remodeling cycles [54], and the absence of a higher incidence in immunosuppressed individuals [55]. Though these features suggest an immunosuppressive microenvironment, decades of literature have qualitatively described tumor-infiltrating lymphocytes (TILs) in breast cancer specimens [56]. Immune cells appear to increase in density along the spectrum of breast atypia to overt malignancy [57] with the latter being further influenced by the specific breast cancer subtype [58]. As our knowledge has continued to progress, it has become more evident that not only the proportion, but also the type of TIL(s) present in breast cancer is important in determining clinical

outcomes. For instance, a high CD8-positive T cell infiltrate may predict for improved clinical outcome whereas high levels of T-regulatory cells may portend to worse tumor control [59]. Most studies have associated clinical outcomes with individual immune cells or small subsets, which does not fully recapitulate the diversity of immune cells orchestrating the immune response to a tumor [60]. More recently, advancements in molecular and computational biology have facilitated a higher resolution of the immune landscape in breast cancer. Ali et al. profiled 11,000 breast tumors for 22 different immune cell types by gene expression analysis and identified that there were baseline differences in TIL presence between ER+ and ER- tumors and that the prognostic value of a given TIL differed between hormone receptor statuses [61]. Similarly, analysis of 7,270 nonmetastatic breast tumor samples demonstrated different TIL types predict differences in clinical outcomes, which is strongly influenced by breast cancer subtypes [62]. In this study, increased presence of $\gamma\delta$ T cells and M1-polarized macrophages resulted in improved tumor control in ER+ tumors, whereas HER2-enriched tumors with elevated T-regulatory cells had worse tumor control. These results underscore the importance of stratifying breast tumors by subtype and immune cell composition when attempting to design rational therapeutic combinations that alter the immune response to a given tumor.

The relationship between radiation therapy and the immune system continues to evolve. Currently, it is believed radiation causes an immunogenic cell death, which is characterized by the release of various proinflammatory cytokines as well as cell autonomous 'danger-signals' that cause TIL infiltration and tumor remodeling [63]. Consequently, radiation-induced tumor antigen release can assist in generating an '*in situ*' tumor vaccine [64]. Furthermore, the effects of radiation therapy may extend beyond the targeted tumor by a phenomenon termed the abscopal effect [65–67]. During this process, immune cell cross-reactivity may occur between the targeted tumor and distant tumor deposits outside the radiation field, thus potentially amplifying the systemic antitumor response.

Clinical decision-making tools that incorporate the immune composition of individual breast tumors with RSI/GARD may reveal optimal therapeutic approaches for both primary and oligometastatic disease. For example, if a given tumor has a low proportion of antitumor TILs, then radiation delivery may enhance the repertoire of responding immune cells and can be delivered up to an optimal GARD. Alternatively, if the tumor has a high proportion of antitumor TILs, then systemic delivery of an immune-modulating drug may be more appropriate as delivery of radiation to an immune-primed tumor may inadvertently destroy any present immune effectors due to their intrinsic radiosensitivity [68].

7. Immunotherapy Trials

A number of clinical trials are currently underway to assess the utility of immune checkpoint inhibitors in the management of breast cancer. These immunomodulators may have particular promise in the management of triple negative tumors. These tumors have been shown to have higher levels of PD-L1 expression than other breast subtypes [69, 70]. KEYNOTE-012 provided initial clinical activity and feasibility data on the use of pembrolizumab in heavily pretreated recurrent or metastatic triple negative breast cancer [71]. Response rates of 18.5% in 27 evaluable patients were noted. The median time to response in this cohort was 17.5 weeks and the median duration of response has not yet been reached. Combining radiation therapy with immune checkpoint inhibitors may hold promise [72, 73]. There is strong preclinical rationale for synergy in a combined modality approach including upregulation of PD-L1 expression and enhancement of the immunogenicity of these tumor types [74]. In addition, there is clinical evidence to suggest oligometastatic breast cancer patients treated with high dose per fraction radiation have improved overall survival as well as duration of responses compared to other solid tumor histologies [75]. Fractionated and high dose per fraction radiation may also be an optimal regimen to stimulate the immune system based on preclinical evidence [76].

There are ongoing efforts to improve upon the response rate of KEYNOTE-012 by utilizing high dose per fraction radiation. A trial has demonstrated the feasibility of combined extracranial hypofractionated radiation with pembrolizumab with assessments of response rates to be reported (NCT02730130). There is the possibility that combining data gathered with RSI and tailoring radiation therapy dose could lead to an improved ability to stimulate the immune system and the immunogenicity of immune checkpoint inhibitors. Numerous efforts are ongoing in a number of malignancies to combine radiation therapy and immune checkpoint inhibitors. A variety of different dosing schedules are being used in these trials. Although data from these ongoing trials will be available in the near future, an informed genomic approach to rationally combine these modalities with optimal sequencing and radiation dosing would be highly informative for future trial design.

8. Conclusion

Radiation therapy has long played an integral role in the management of breast cancer. Although for many years radiation oncologists have delivered uniform doses of radiotherapy based on long-term prospective data, an improved understanding of tumor biology as well as access to genomic information may allow for greater personalization of radiotherapy dosing in the near future. Various fractionation schedules have now shown equivalence in data and we are now at a point that, through genomics, strides can be made towards the personalization of radiation treatment delivery for the management of breast cancer. This has the potential to not only decrease treatment burden and side effects of treatment but also decrease recurrence rates.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

The Evolution of Radiation Therapy in Metastatic Breast Cancer: From Local Therapy to Systemic Agent

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Radiation therapy is a mainstay of treatment in early and locally advanced breast cancer but is typically reserved for palliation of symptomatic lesions in patients with metastatic breast cancer. With new advances in the field of tumor biology and immunology, the role of radiation in the metastatic setting is evolving to harness its immune-enhancing properties. Through the release of tumor antigens, tumor DNA, and cytokines into the tumor microenvironment, radiation augments the antitumoral immune response to affect both the targeted lesion and distant sites of metastatic disease. The use of immunotherapeutics to promote antitumoral immunity has resulted in improved treatment responses in patients with metastatic disease and the combination of radiation therapy and immunotherapy has become an area of intense investigation. In this article, we will review the emerging role of radiation in the treatment of metastatic disease and discuss the current state of the science and clinical trials investigating the combination of radiation and immunotherapy.

1. Introduction

Radiation therapy is traditionally utilized in stages I–III breast cancer as a local therapy after surgical management to improve disease-free survival and in some cases overall survival. In the metastatic setting, it is used for effective palliation of symptomatic metastases. Advances in tumor biology and immunology have led some to suggest a role for radiotherapy in the metastatic setting to augment traditional systemic therapies such as chemotherapy or immune-modulating agents. While decades of research have demonstrated that a major component of local tumor control is mediated by irreparable damage to the DNA of malignant cells resulting in cell death [1], recent research has elucidated multiple radiation-induced effects on both tumor cells and the tumor microenvironment. Following ablative doses of radiotherapy, release of tumor antigens, tumor DNA, cytokines, and chemokines promote innate intratumoral immunity, leading in some cases to an adaptive response [2, 3]. This suggests that the immune response may play a part in high local control rates seen with radiation therapy. Immune-modulating therapeutics, such as checkpoint inhibitors, might therefore be incorporated with radiation to enhance the antitumor immune response with the intent of improving outcomes in patients with oligometastatic or polymetastatic disease [2]. Here we will review the rationale for the use of stereotactic body radiotherapy (SBRT) for the treatment of oligometastatic breast cancer and explore the data to suggest that incorporating immunotherapy may expand the use of SBRT to polymetastatic disease.

2. Oligometastases in Breast Cancer

Breast cancer has provided one of the earliest models in our understanding of cancer progression and metastasis. Pioneering work by Halsted resulted in the theory of orderly spread of cancer from the initial primary tumor location to the regional draining lymph nodes, followed by metastatic spread to distant organs [4]. When subsequent radical surgical interventions and en bloc resections did not eliminate the occurrence of distant metastases, competing hypotheses by Keynes and subsequently Fisher proposed that breast cancer

was likely already systemically disseminated at the time of diagnosis at the primary tumor [5, 6]. With this came a shift from aggressive surgery to neoadjuvant and adjuvant systemic therapies, with the goal of targeting disseminated microscopic disease.

A third hypothesis established by Hellman, the "spectrum theory," stated that breast cancer more likely existed as a spectrum of localized and widespread disease [7]. Indeed, in clinical practice, patients present within a spectrum of involvement, some with localized primary tumors, and others with disseminated metastases at time of initial presentation. Hellman and Weichselbaum later refined this theory with the description of an intermediate disease state between localized and widely metastatic cancer [8]. This disease state, known as "oligometastases," was defined as a clinical state of limited metastases, typically one to five lesions, in a single or limited number of distant sites.

The concept of oligometastases has resulted in a change in therapeutic goals for women with metastatic breast cancer and a limited burden of disease, as these patients may be able to achieve durable disease control with ablation of their metastases. In recently published first-line metastatic breast cancer trials, many patients were found to have fewer than 4 sites of disease and those with fewer metastases had improved outcomes. Similar findings have also been noted in large cohort studies of breast cancer patients, in which more than 25% of early-stage breast cancer patients had 1–5 metastases at time of disease progression [9]. These women were noted to have improved median survival compared to those with >5 metastases (108 versus 22 months). A meta-analysis of metastatic breast cancer patients who received anthracycline-based systemic therapy showed that those with 1–5 metastases had significantly improved outcomes compared to those with >5 metastases [10]. These studies demonstrate that a clinically significant population of oligometastatic breast cancer patients exists and may achieve long-term disease control with aggressive treatment of their metastatic disease and form the rationale for continued clinical investigations [2].

3. Ablative Treatment of Oligometastatic Breast Cancer

Standard of care for metastatic breast cancer includes systemic therapy in the form of hormonal, biologic, and cytotoxic agents. Ablative treatment for metastases using radiation or surgery is typically reserved for palliation of symptomatic lesions. Even in patients with good performance status and limited metastatic disease, few achieve complete and durable responses with systemic agents alone. In a study of over 1,500 women treated with doxorubicin and alkylating chemotherapy for metastatic breast cancer, only 1.6% achieved complete response and were free of relapse at 15 years [11]. Interestingly, 8 of those patients who achieved lasting response received metastasis-directed therapy with radiation, surgery, or chemoembolization, suggesting that local therapies could improve outcomes in selected patients.

The idea that certain patients with limited metastatic disease may derive lasting benefit from more aggressive treatment of their metastatic disease has resulted in multiple retrospective and single-arm studies evaluating outcomes with local therapy and/or metastasectomy. Several studies in various cancer types have shown that standard systemic therapy combined with the elimination of all clinically detected metastases via surgery or ablative radiotherapy results in superior disease control than systemic therapy alone [12–14]. Surgical resection of metastases in breast cancer patients has shown promising results with regard to progression-free survival and overall survival [14]. Pockaj et al. demonstrated favorable median progression-free survival (14–34 months) and overall survival (24–63 months) after surgical resection of liver metastases [15].

In metastatic breast cancer patients who are poor surgical candidates due to medical comorbidity or metastases that are unresectable due to location or invasion, radiotherapy can be utilized for the treatment of limited metastatic sites. A study of patients treated with ablative radiation therapy for limited metastatic disease on two sequential protocols from University of Rochester demonstrated an 89% local control of treated metastases, with 4-year actuarial overall survival of 59% and progression-free survival of 38% in those with metastatic breast cancer [16]. Multivariate analysis revealed that patients with primary breast cancer, single bony metastases, and stable or responsive disease prior to SBRT had the largest benefit from ablative radiation therapy. However, due to the lack of published randomized prospective trials evaluating the aggressive treatment of oligometastatic disease, it is uncertain whether these reported series pointing to a long-term survival after local therapeutic interventions in breast cancer patients with limited metastatic disease are generalizable. In carefully selected patients, aggressive local therapy may be beneficial. The mechanism behind this benefit remains unclear but may result from inhibiting progression of metastatic disease, preventing additional metastatic seeding, or through an immunostimulatory abscopal effect on micrometastatic disease.

4. Immunooncology and Enhancing Abscopal and Adscopal Effects

The immune system is a powerful network of cells providing surveillance to prevent the development and progression of malignancy. Avoiding immune destruction is critical in cancer progression and is an important hallmark of cancer pathogenesis [17]. Enhancing the immune system's ability to detect and destroy cancer cells and inhibiting immune escape mechanisms, both key elements of immunooncology (IO) therapy, represents a transformational approach to cancer care with a potential for long-term antitumor responses.

The effector T cell response to cancer is controlled by a balance of antigen presentation to the T cell receptor (TCR) and subsequent costimulatory and coinhibitory signals [18]. Initially, immunotherapies were developed to target negative regulators of the T cell receptor response, primarily cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Inhibition of these negative regulatory receptors, referred to as immune

checkpoint blockade, results in the enhanced activation of T cells and potent antitumor activity.

Early studies in mouse models of metastatic breast cancer, radiotherapy combined with immune-mediated inhibition of CTLA-4 demonstrated a significant survival advantage compared to groups treated with radiotherapy alone [19]. Subsequent human studies with CTLA-4 blockade have demonstrated improved overall survival in patients with metastatic melanoma and studies using anti-PD-1 antibodies such as pembrolizumab, nivolumab, and avelumab have shown disease response in patients with non-small cell lung cancer, melanoma, breast, and renal cell carcinoma [20–23]. The success of these agents has resulted in a multitude of new immunotherapy agents, which are now being investigated in an attempt to harness the natural immune response against malignant cells. In addition to enhancing the T cell response directly, further agents are being developed to target cell populations beyond effector T cells such as natural killer (NK) cells, T regulatory (Treg) cells, tumor-associated macrophages (TAMs), and dendritic cells (DCs).

Several of the key activation steps required for effective systemic immunotherapy are also impacted by radiation [2, 18]. As with any immune reaction, the initial response is triggered via innate immunity identifying the presence of an infection or tumor, leading to antigen capture/processing and presentation to the adaptive immune compartment, classically including T cells. Tumor cell death induced by radiation can result in DC cross-priming and subsequent activation of the stimulator of interferon genes (STING) pathway [1], a major driver of type I interferons [1, 23, 24]. This type I interferon (IFN) release acts as a bridge between innate and adaptive immunity, improving DC activation and recruitment to the tumor as well as inducing type II interferon and leading to cytotoxic T cell (CTL) activation [2, 25, 26]. In mouse models, induction of type I IFN by radiotherapy activates proapoptotic signaling cascades within the tumor cells, while also increasing activation of antigen-presenting cells (APCs) and T cells [27–29]. Additionally, this radiation-induced IFN secretion has been shown to increase the release of chemokines CXCL10 and CXCL16, which enhances cytotoxic and type 1 helper T cell migration into the tumor and increases cytotoxic and helper T cells' killing capability via upregulation of Fas/FasL [2, 3, 30].

Although radiation has been shown to have immunostimulatory effects, immunosuppressive effects can also be observed with IFN-γ associated recruitment of regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) [2, 18]. The increase in Treg cells in the tumor microenvironment results in increased IL-10 and TGF-beta production that stimulates MDSCs, which results in suppression of T cell activation and the promotion of tumor regrowth. Additionally, TGF-beta has been shown to inhibit T cell activation. Mouse models of breast carcinoma treated with TGF-beta blocking agents show enhanced priming of CD8+ T cells to endogenous tumor antigens released by radiation [31]. Low-dose radiation can also increase recruitment of tumor-promoting M2 type TAMs [32]. Arginase, an enzyme overexpressed in M2 type TAMs, results in depletion of arginine, which impairs expression of the T cell receptor (TCR) zeta chain that is necessary for T cell activation and proliferation [33, 34]. Preclinical studies of inflammatory breast cancer have demonstrated enhancement of angiogenesis, metastasis, and invasiveness due to IL-6 secretion by M2 TAMs, resulting in accelerated tumor growth [35].

By combining immunotherapy agents with radiation, the balance of these immunostimulatory and immunosuppressive effects could be shifted to improve local and possibly distant disease control in oligometastatic patients. Indeed, this effect was shown in mouse models, where concurrent administration of anti-PD-1 and anti-PD-L1 antibodies with fractionated radiotherapy generated CD8+ T cell responses that improved local tumor control [35].

A phenomenon known as the abscopal effect has been described in some patients with metastatic disease who received treatment with radiation therapy to one or more metastatic lesions and had nonirradiated tumors shrinking outside the radiation field. This effect was first described by Mole in the 1950s and is thought to be an immune-mediated tumor response [36]. Abscopal effects in untreated lesions have been demonstrated in mouse tumor models treated with radiation and immunotherapy, with augmented T cell responses in both primary and metastatic lesions [37, 38]. Radiation has been shown to increase PD-L1 expression on tumor cells, resulting in an impaired T cell response. The addition of anti-PD-L1 therapy after radiation in these studies resulted in a synergistic amplification of tumor killing mediated by TNF-alpha signaling, increased CD8+ T cells, and decreased MDSC within tumors [38]. In the clinical setting, metastatic melanoma patients being treated with ipilimumab who received palliative radiation with 28.5 Gy in three fractions had regression of the radiated lesion as well as other areas of metastatic disease [39]. One patient with metastatic NSCLC treated with ipilimumab and radiation not only showed a dramatic initial response in the treated and untreated sites of disease but also had a durable response with no evidence of disease 1 year later [40]. In addition to effects on distant lesions, local or "adscopal" responses within the irradiated target or an unirradiated portion of the contiguous tumor have been described in patients treated with radiation and PD-1 blockade [41, 42].

Several new immunotherapeutics show promise for combination with radiation due to their parallel effects on immune cell function to amplify these abscopal and adscopal effects. Nivolumab and pembrolizumab are antibodies that target the negative regulatory molecule programmed death-1 receptor (PD-1; CD279). PD-1, a cell surface membrane receptor expressed by activated T and B lymphocytes, functions to downregulate lymphocyte activation upon interaction with its ligands PD-L1 and PD-L2. Inhibition of this interaction promotes antigen-specific T cell responses to both foreign and self-antigens as well as increased IFN-γ associated gene expression. As radiation induces T cell migration into the tumor, the addition of a checkpoint inhibitor may further enhance T cell-mediated killing of the tumor. The phase Ib KEYNOTE-012 pembrolizumab study evaluated 111 heavily pretreated patients with advanced triple negative breast cancer and showed an 18.5% response rate [43] and the results of a single-agent phase II study of

pembrolizumab in advanced triple negative breast cancer were recently reported. Six-month progression-free survival was 12% and overall survival was 69% in the 170 patients enrolled [44]. Similar results have been seen with PD-L1 inhibitors atezolizumab and avelumab. The results of a phase II study of atezolizumab showed overall response rates (ORR) of 26% in first-line patients and 7% in second-line (2L+) patients [45]. Overall survival was 41% at 1 year and 22% at 3 years. A phase III trial is now underway in triple negative breast cancer of atezolizumab with or without paclitaxel [46]. In the phase Ib JAVELIN solid tumor study, 168 patients with metastatic breast cancer showed a 16.7% ORR in PD-L1+ tumors [47]. Combining these agents with radiation could amplify the response rate seen with pembrolizumab alone by increasing activated T cell migration to the tumor through the radiation-induced release of chemokines.

Ipilimumab is a human monoclonal IgG1κ antibody against human CTLA-4 (CD152), a negative regulator of T cell activity expressed on a subset of activated T cells. Ipilimumab blocks the interaction of CTLA-4 and CD80/86, which allows for increased T cell activation and clonal expansion via an independent mechanism from PD-1 blockade [48]. Additionally, CTLA-4 blockade reduces Treg function, thereby preventing Treg-mediated inhibition of T cell activation and cytotoxic killing. Ipilimumab has shown a statistically significant survival benefit in advanced and metastatic melanoma in phase III trials [49]. In breast cancer, a pilot study evaluating tumor-infiltrating lymphocyte (TIL) profiles in 19 early-stage breast cancer patients treated with preoperatively with ipilimumab alone and in combination with cryoablation demonstrated increased intratumoral T cell density [50]. The ability of ipilimumab to decrease Treg function and increase T cell infiltration would increase the effectiveness of radiation therapy by reducing the immunosuppressive effects of radiation while simultaneously increasing the stimulatory power.

In the same vein as checkpoint inhibitors, 4-1BB agonists promote T cell activation. As members of the TNFR-TNFL family, 4-1BB (CD137) and 4-1BBL provide a costimulatory signal to CD4+ and CD8+ T cells which upregulate anti-apoptotic genes to promote T cell survival. In mouse models of metastatic breast cancer, treatment with 4-1BB agonists resulted in a regression of metastatic tumors and increased survival, which was further increased with the addition of radiation [51–53]. As the 4-1BB signal provides a positive costimulatory signal, combination with blockade of the PD-1/PD-L1 negative costimulatory signal could further enhance the radiation-induced antitumoral T cell response.

LAG-3 (CD223) is a type 1 transmembrane protein that promotes Treg activity and is required for the maximal suppression of T cell activation and proliferation [54]. High LAG-3 expression has been shown on exhausted CTLs, which results in limited antitumor CTL responses [55, 56]. A phase I study in ER+ metastatic breast cancer patients using a combination of a LAG-3 antagonist with paclitaxel showed an improved objective response rate of 50% compared to a historical rate of 25% with paclitaxel alone and a phase IIb study is underway to further investigate its effect in this patient population [57]. It is possible that this impressive response rate could be further improved in combination with SBRT to increase antigen presentation and enhance the antitumor immune response.

Cabiralizumab is a recombinant, humanized IgG4 monoclonal antibody that binds to the human colony-stimulating factor 1 receptor (CSF1R) and prevents binding of its ligands' colony-stimulating factor 1 (CSF1) and interleukin-34 (IL-34). CSF1 is required for survival of immunosuppressive TAMs that promote angiogenesis and tumor metastasis and studies in mouse models of breast cancer have shown TAM recruitment into the tumor due to CSF-1/CSF-1R signaling [58, 59]. Subsequent blocking of CSF-1 signaling results in reduced TAM migration and improved response to subsequent therapies [60]. Preclinical studies in inflammatory breast cancer demonstrate significant reduction in M2 TAM populations in mice treated with anti-CSF1 antibodies [61]. The reduction in TAM migration into the tumors resulted in decreased IL-6 signaling and delayed tumor growth and skin invasion. Using CSF1R inhibitors to effectively deplete the TAM population in breast cancer patients could allow for improved response of metastatic lesions subsequently treated with SBRT or fractionated radiation.

5. Harnessing the Abscopal and Adscopal Effects with SBRT and Immunotherapy

Mounting evidence suggests that, apart from its direct effects, radiotherapy, and particularly SBRT, can act as a trigger for the innate and adaptive immune system. However, despite these effects, many patients with limited metastatic disease treated with radiation alone will frequently experience disease progression leading to widely metastatic disease. Although radiation can induce activation of T cells and tumor infiltration by DCs and antigen-presenting cells, the accompanying Treg cells, MDSCs, and TAMs prevent a more robust immune response. The combination of radiotherapy with immune-modulating agents could enhance the effectiveness of radiation in treating oligometastatic breast cancer by shifting the balance away from the immunosuppressive effects using anti-LAG3 and anti-CSF1R agents and simultaneously enhancing the immunostimulatory effects via checkpoint inhibition with anti-PD-1/PD-L1 and increased T cell survival with 4-1BB agonists. Although radiotherapy alone does not provide a sufficient antitumor immune response to achieve a clinically meaningful effect at untreated sites, augmenting the immune response with the addition of immunotherapy to SBRT may increase the downstream effects of radiation-induced IFN release and induce abscopal and adscopal effects more frequently [62].

Many questions remain regarding the best method for combining radiation and immunotherapy. Currently, the most effective radiation dose fractionation for this combined modality treatment is not known. The available data on fractionation and its effect on the radiation-induced immune response are conflicting. Animal models combining anti-CTLA-4 agents with radiation showed an improved abscopal effect with 8 Gy × 3 dosing over a single ablative dose of 20 Gy [63]. Evaluation of TGF-beta blockade in concert with radiation in a mouse breast cancer model utilized 6 Gy × 5

and demonstrated enhanced T cell infiltration into radiated tumors [31]. Mouse studies evaluating MDSC and T cell infiltration showed increased T cell infiltration and lower MDSC numbers with a single 30 Gy dose compared to 3 Gy × 10 dosing [64]. Although optimal fractionation has not been determined, the ability of SBRT to target and treat metastatic lesions that are not amenable to surgery in a short time makes it an attractive option for combination with immunotherapy.

In addition to uncertainty of fractionation, there is also concern regarding the safety of combining immunotherapeutics with radiation. Multiple phase I and II studies are ongoing to investigate the safety of this approach, primarily with the use of single-fraction or hypofractionated radiation in conjunction with checkpoint inhibitors [2, 41, 64–66]. The University of Chicago recently published the results of a phase I trial of 73 participants combining multisite SBRT as per BR-001 followed by anti-PD-1 therapy and thus far has not observed any synergistic toxicity relative to either approach alone [41]. Objective overall response rate was 13.2% for all patients. Memorial Sloan Kettering and Cedars Sinai have completed a phase II trial investigating palliative radiation and pembrolizumab in metastatic triple negative breast cancer. Patients were treated with 30 Gy delivered in five fractions with pembrolizumab administered within three days of the first radiation treatment. Of 17 patients enrolled in the study, 3 had durable partial responses both in field and out of field, with 60%, 54%, and 34% decrease in tumor burden by RECIST, with no significant toxicities observed [65]. For patients with brain metastases, both stereotactic radiosurgery (SRS) and whole-brain radiotherapy with conventional fractionation which were investigated with tremelimumab were well tolerated, and 2 of 6 women with Her2+ disease had durable responses [66]. Two phase I studies at Weill Cornell Medical College are investigating anti-TGF-beta therapeutics in combination with radiation to induce abscopal responses. The first utilized an anti-TGF-beta antibody, fresolimumab, at two doses and radiation of 22.5 Gy in 3 fractions [67]. The higher fresolimumab dose was associated with high median overall survival compared to the low-dose arm, and seven grade 3 or 4 adverse events were noted out of 23 patients in the study. The second trial, which is currently enrolling, evaluates a small molecule inhibitor of TGF-beta, galunisertib, in combination with radiation (NCT02538471).

As the number of clinical trials investigating immunotherapy and radiation rapidly increases, several questions for optimal trial design are still unanswered. Radiation dosing and fractionation are widely varied between ongoing studies, although most are using hypofractionated or single-fraction regimens. Additionally, the ideal treatment field size is unknown. The out-of-field adscopal effects seen in partially irradiated tumors in the study by University of Chicago bring up the possibility that, for large tumors, coverage of the entire lesion may not be required [41]. While the ongoing trials will provide useful information regarding the safety of combining radiation and immunotherapy, additional trials to evaluate optimal sequencing, radiation dosing, and field size are needed to further advance this combination therapy.

6. Conclusions

Currently, there is evidence that radiation can alter the immune profile of the tumor microenvironment by increasing tumor-antigen presentation, altering cytokine and chemokine release, and recruiting T cells to the tumor, with both antitumor and protumor effects. The promising results in initial immunotherapy trials in metastatic breast cancer and animal data showing enhanced tumor killing and abscopal and adscopal effects when immunotherapeutics are combined with radiation provide an interesting hypothesis that enhancing the immune response could improve patient outcomes and overall survival. However, the science behind radioimmunotherapy is based primarily on data from animal models and has not been fully evaluated in patients. Further study of both the safety and effectiveness is needed before this combined modality therapy is widely implemented.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Precision Medicine for Breast Cancer: The Paths to Truly Individualized Diagnosis and Treatment

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Precision medicine in oncology seeks to individualize each patient's treatment regimen based on an accurate assessment of the risk of recurrence or progression of that person's cancer. Precision will be achieved at each phase of care, from detection to diagnosis to surgery, systemic therapy, and radiation therapy, to survivorship and follow-up care. The precision arises from detailed knowledge of the inherent biological propensities of each tumor, rather than generalizing treatment approaches based on phenotypic, or even genotypic, categories. Extensive research is being conducted in multiple disciplines, including radiology, pathology, molecular biology, and surgical, medical, and radiation oncology. Clinical trial design is adapting to the new paradigms and moving away from grouping heterogeneous patient populations into limited treatment comparison arms. This review touches on several areas invested in clinical research. This special issue highlights the specific work of a number of groups working on precision medicine for breast cancer.

1. Background

The National Research Council released a consensus study report in 2011 entitled "Toward Precision Medicine" [1]. The report proposes to define a "new taxonomy" of disease based on molecular and environmental determinants rather than signs and symptoms, harnessing the power of big data networks and expanding information about the molecular determinants of disease to first define these molecular subtypes and then apply that knowledge to personalized treatment approaches based on the individual patient's precise molecular pathology. The imprecise approach of "one size fits all" treatments to patients with general classes of disease, such as breast cancer, has been undergoing a paradigm shift for several years, with the identification of molecular pathways predicting both tumor biology as well as response to therapy. But the holy grail of truly individualized treatment based on each patient's specific tumor molecular and environmental subtype and application of a specific effective treatment regimen for that cancer subtype is the subject of ongoing research on many fronts. The "New Taxonomy" proposes to develop a more accurate disease classification system based on molecular biology [2]. A "Knowledge Network" will be created to incorporate genomic data from a large variety of sources, including DNA sequencing and other molecular technologies, basic science research, clinical trial data, observational studies, and electronic health records to analyze connections between the different sets of information to define disease classifications and to test potential targeted treatments. In January 2015, the Obama administration in the United States launched the Precision Medicine Initiative [3], specifically to improve the treatment and cure of diseases like cancer, which is the near-term disease focus of the initiative. This cancer focused component is designed to address existing challenges to achieving precision cures, including drug resistance, tumor genomic heterogeneity, reliable markers of tumor response, and optimal methods for combining multiple agents or modalities most effectively. Large scale data collection for over a million people will be collated, pilot studies of treatments and longitudinal studies of outcomes conducted. This massive federally sponsored initiative promises to accelerate precision medicine advancements more comprehensively, as data will be openly shared with investigators, physicians, and the public.

Precision medicine encompasses a very broad spectrum of clinical and basic science disciplines. True personalization

of treatment will account for the individual patient's genetics and genetic predispositions, the composition of their breast tissue, the "omic" profile of their cancer and consequent biologic propensities, tissue microenvironment, comorbid conditions, lifestyle, patient preference, and quality of life. Precision medicine enters the scene even before the cancer diagnosis, in the arenas of prevention and detection. After diagnosis, precision medicine requires a fundamental shift in the traditional approaches to clinical trial design, as ever smaller bins of molecularly staged patients receiving novel targeted agents will not provide the statistical power to detect significance for outcome endpoints such as local control or overall survival under traditional definitions.

Breast research cancer is well poised to make substantive advances in the precision medicine era. Much ground work has been laid. Some of the work being done to reach the goals of precision medicine in breast cancer detection, prevention, diagnosis, and treatment are highlighted throughout this special issue.

2. Precision Medicine Disciplines in Breast Cancer

2.1. Screening Diagnostics. Traditional screening guidelines have defined parameters such as age, risk level defined by mammogram appearance and breast biopsy findings, and genetic predisposition to guide the use of imaging studies (mammogram and ultrasound primarily) for population based screening. This approach is imprecise as it leads to overscreening of some and underscreening of other segments of the population. A precision medicine approach supports gene sequencing for profiling of individual genetic risk and determining the screening methodology and frequency based on individual risk level. An individualized risk score is based on factors including genetics, body mass index, family history, and imaging features such as breast density. This approach could allow less frequent use of unnecessary tests leading to overdiagnosis and overtreatment. It could also lessen underscreening, particularly in the younger population where early detection is more challenging yet arguably more impactful to outcomes. Low risk patients could have screening less often or not at all, saving cost and unnecessary tests due to false positive results. Very high risk patients could begin screening younger, have it performed more often, or include functional imaging such as MRI. Risk-based screening is being studied in clinical trials underway [4].

In the general screening population, revisions of risk stratification models are being adopted. Breast density has emerged as a significant risk factor for cancer incidence. The breast imaging and reporting data system (BIRADS) incorporates four categories of breast density to be included in the interpretation of screening mammograms: fatty; scattered fibroglandular densities; heterogeneously dense; and extremely dense [5]. In a large Swedish mammographic screening study of over 15,600 women ages 45 to 59 followed for 25 years, dense breast tissue was associated with a 1.57 adjusted relative risk of cancer incidence and 1.9 relative risk of breast cancer mortality [6]. The Breast Cancer Surveillance Consortium conducted a case control study from the SEER database and prospective risk factor collection from 1996 to 2012 from their registry of breast imaging facilities, reporting the BI-RADs breast density, among other risk factors [7]. Using an outcome of population attributable risk proportion of developing breast cancer, breast density was the most prevalent factor for all age groups. Body mass index was also highly contributory. Such data have led to studies assessing the timing, frequency, and imaging modalities optimally used for effective screening of women with dense breast tissue, potentially including functional imaging such as MRI. The Breast Cancer Surveillance Consortium is the first national organization to formally incorporate breast density into its risk calculation algorithm [8]. Interventions to reduce breast density using diet, dietary supplements, exercise, and pharmacologic agents have been investigated [9, 10]. The impact of lifestyle interventions such as healthy eating, weight reduction, and physical activity in reducing the incidence of breast cancer could be substantial.

2.2. Molecular Subtype and Systemic Therapy. Molecular subtypes of breast cancer defining phenotypic behaviors based on molecular determinants have been identified [11], although there is significant heterogeneity within and between these subtypes, which require further refinement [12]. Molecular subtyping using available immunohistochemical assays is the current standard for diagnostic characterization of all breast cancer patients and an integral component of treatment decision making. Perou et al. identified "molecular portraits" from 65 breast cancer specimens using DNA microarray technique for quantitative analysis of gene expression patterns in over 8000 genes [11]. These patterns were grouped into subtypes based on the differences between the expression profiles. Based on this seminal work and other studies, the most common general molecular subtypes characterized currently include the following classifications: Luminal A (estrogen receptor (ER) positive, Her2 negative AND Ki-67 low < 14%, OR Ki-67 intermediate 14–19%, and progesterone receptor (PG) high > 20%); Luminal B (ER positive, Her2 negative, AND Ki-67 intermediate 14–19%, and PG low/negative OR Ki-67 high > 20%, OR Her2+) [13]; Her2 enriched (ER negative, PR negative, and Her2 positive); and triple negative/basal-like (ER negative, PR negative, and Her2 negative). The use of these immunohistochemical assays to identify the expression profile (with FISH testing in equivocal Her2 cases) is the simplest and most widely available system. The molecular subtypes translate into phenotypes which correlate with tumor behavior, survival outcomes, and response to treatment. Overall, there is a worse prognosis for the triple negative and Her2+ groups, as well as a clear distinction between two ER+ groups [14]. Correlation between molecular subtype and local-regional recurrence risk has also been observed [15]. A meta-analysis of published breast cancer gene expression profiles and associated clinical data collated the various signatures into three main biologic pathways: proliferation; estrogen receptor (ER) expression, and Her2 expression [16]. The four basic intrinsic subtypes originally identified by Perou were validated, and proliferation pathways were noted to be the most highly prognostic.

These investigators conclude that different molecular signatures actually provide similar prognostic information due to identifying common pathways.

Personalization of breast cancer therapy over the past two decades has relied primarily on these subtypes. However, further refinement of these classifications is the focus of current basic science and clinical research, to achieve better outcomes in patients along the spectrum of risk within each broad subtype. A variety of multigene assays are in clinical use or under investigation, which further define the molecular characteristics of the cancers' dominant biologic pathways. These gene arrays are most commonly used to inform the decision making regarding systemic therapy and are being investigated for other prognostic uses, such as predicting locoregional recurrence to inform patient selection for surgery or radiation treatment. One of the first multigene assays was reported by Paik et al., using RT-PCR profiles of 21 preselected genes in a group of ER positive, node negative patients previously treated with tamoxifen alone [17]. This 21-gene assay categorized patients into groups of low, intermediate, or high risk for distant recurrence and resulted in the development of a predicative scoring system known commercially as Oncotype DX Recurrence Score (Genomic Health). This gene assay is commonly used in clinical practice to guide recommendations regarding the potential efficacy of systemic chemotherapy in addition to endocrine therapy in lower risk early stage ER positive patients. The 21-gene assay has also been shown to predict the risk of local-regional recurrence, suggesting a role in patient selection for adjuvant radiotherapy [18]. A 70-gene array of mRNA expression (commercially known as Mammaprint [19], Agendia) is also tested on tumor tissue, and its gene panel includes pathways of growth signaling, apoptosis, replication, metastasis, and angiogenesis [20]. The assay provides a good or poor prognostic signature that discriminates between risk of distant metastasis among a larger group of breast cancers, including those with Her2-positive and node positive cancers. It is similarly used clinically to guide use of systemic chemotherapy or sometimes to provide further prognostic information in women with intermediate recurrence scores after the 21-gene assay has been performed. A 50-gene mRNA expression array called Predication Analysis of Microarrays (PAM50, Prosigna, Nanostring Technologies) was designed to classify intrinsic molecular subtype [21]. The assay provides a prognostic score called risk of recurrence (ROR) score that was derived from a trial of early stage ER positive/Her2-negative postmenopausal patients treated with endocrine therapy related to their response to neoadjuvant or adjuvant chemotherapy [22]. Several other gene assays are used or undergoing validation. The latest American Society of Clinical Oncology (ASCO) guideline finds that the strongest level of evidence currently supports the use of either Oncotype DX Recurrence Score or PAM50 ROR [23].

Triple negative breast cancer comprises up to 20% of invasive breast cancers, and in itself it represents a heterogeneous group of subtypes further defined by additional molecular markers. Some of these subtypes are among the most aggressive poor prognosis phenotypes in breast cancer, while others have a relatively good prognosis. New biomarkers for association of triple negative cancers are under investigation. The term "basal-like" is not standardized but generally refers to breast cancers with certain gene expression profiles, including lack of ER, PR, and Her2 expression, expression of basal cytokeratins (CK5/6, 14, or 17), and/or EGFR. These tumors also tend to be high grade and have high mitotic indices, lymphocytic infiltrate, and necrotic or fibrotic areas [24]. While the majority of triple negative cancers are also basal-like, the two categories are not completely synonymous. There is an established link between basal-like and cancers arising in BRCA1 germline mutated carriers. In attempts to better characterize this high risk class of breast cancers, investigators are harnessing "omics" technologies, which have identified at least 6 triple negative subtypes based on gene expression, molecular pathways, and response to therapeutic agents [25]. Such complex diagnostics involve large volumes of data and require validation in clinical trials that are able to evaluate outcomes in such small subgroups of patients. One such novel clinical trial is I-SPY 2 [26], which uses pathologic response to neoadjuvant chemotherapy as the primary endpoint for assessing safety and efficacy of novel agents in stage II-III breast cancer. Results of combining the anti-PD1 antibody, pembrolizumab, with chemotherapy in Her2-negative patients was reported at the ASC0 2017 annual meeting and showed that triple negative patients had the highest pathologic complete response at 40% among all Her2-negative patients, representing an improvement in response when compared to chemotherapy alone [27]. While endpoints such as pathologic response must ultimately translate into overall survival benefit, this novel trial design has allowed more rapid assessment of both molecular data and response to therapy to inform future studies.

Many classes of targeted agents are under investigation in response to actionable mutations being characterized. There are over 70 approved drugs for the treatment of breast cancer, used in many different sequences and combinations [28]. Among these, some of the greatest interests lie in immunotherapy agents. A current approach uses monoclonal antibody blockers of immune inhibitory proteins such as CTLA-4 and PD-1/PD-L1. Several clinical trials in advanced or metastatic breast cancer with immune agents including nivolumab, pembrolizumab, and atezolizumab have been completed and show promise [29], although complex interactions between the tumor immune environment, host immune system, and timing of therapy need extensive further study before routine clinical use. Immune modulators as currently designed are not effective as single agents and are often used in concert with other cytotoxic agents. An intriguing approach in limited, or oligometastatic, disease involves the use of ablative radiotherapy doses to an index lesion in order to activate host immunity through enhanced antigen presentation in combination with immunotherapeutic agents [30].

Although not in routine clinical use currently, next-generation DNA sequencing of tumor tissue can identify cancer related genomic changes in individual patients' tumors. Studies using this technology have identified the most commonly mutated genes in breast cancer, which include PIK3CA, p53, and Her2 amplification mutations

in 15% to 30% of breast cancers, although many other candidate genes are present in frequencies <5% [31]. These data provide information for development of targeted agents, including immune pathways. Genomic alterations implicated in several actionable pathways have been identified, including endocrine resistance, Her2 overexpression in apparent Her2-negative cancers and anti-Her2 therapy resistance, and characterization of circulating tumor cell DNA for monitoring treatment response or quantifying residual disease after therapy [32]. Using mutational status to test for response to specific agents has great promise to personalize systemic therapy based on the genomic pathways driving each cancer and to enhance survivorship surveillance.

2.3. Radiomics. The emerging field of radiomics involves the use of quantitative features from medical images in prognostic or predictive models as correlated with pathology, genomics, or clinical outcomes. In the breast cancer screening context, such features may be developed to personalize frequency or modality for screening dependent upon more individualized risk factors. A combination of functional imaging tests and molecular subtyping is anticipated to aide in differentiating aggressive or indolent phenotypes. Imaging modalities under investigation include tomosynthesis, contrast enhanced mammography, and MRI. High spatial resolution MRI may assess lesion characteristics to distinguish between DCIS and invasive lesions, potentially mitigating the need for further workup and treatment of the lower risk purely intraductal lesions. MRI can detect biologic features in situ such as cellularity, vascularity, and cell membrane integrity. Quantitative assessment of tissue vascularity can be obtained using dynamic contrast enhancement (DCE), while diffusion weighted MRI imaging (DWI) can measure cell density and membrane disruption [33]. In a prospective study of over 7300 women, breast MRI was performed in addition to screening mammograms [34]. MRI proved to detect a much higher percentage of DCIS and lack of enhancement was associated with lower grade lesions. The cooperative groups Eastern Cooperative Oncology Group (ECOG) and American College of Radiology Imaging Network (ACRIN) are currently accruing to protocol 4112, to study whether breast MRI and the Oncotype DX DCIS score can identify patients with low risk DCIS who may avoid radiation treatment [35].

MRI has been used in several series to characterize invasive cancers and correlate imaging findings with molecular subtype. One group of investigators used data in the National Cancer Institute's (NCI) The Cancer Imaging Archive (TCIA) to evaluate MRI findings with tumor subtype and found that features such as enhancement texture and heterogeneity were significantly correlated with molecular subtype [36]. Other groups found correlation of subtype and kinetic enhancement uptake patterns, suggesting overall that tumor enhancement kinetics are related to biologic characteristics in vivo [37, 38]. The recently activated NRG BR005 cooperative group study is assessing the accuracy of trimodality functional imaging to define the response in the breast and lymph nodes to neoadjuvant chemotherapy and is anticipated to roll into a randomized trial of surgery versus no surgery in women who have imaging defined complete pathologic response [39].

Radiomics may aide in screening, diagnosis, treatment planning for surgery or radiation treatment, evaluating response to therapy, and follow-up care. Radiomic characteristics properly validated might allow avoidance of biopsies or surgery, help define the tissue at risk for radiation target volume delineation, and distinguish posttreatment findings to customize workup and treatment for recurrence.

2.4. Surgical Management. In the Halstedian era, the belief was strongly held that more radical surgery was associated with a better outcome or survival rate. Since the seminal trials establishing breast conservation therapy as an equivalent treatment for early stage breast cancer with far less morbidity, the radical surgery concept has been abandoned. The focus of innovation in surgical management over the past two decades has been to reduce the extent of surgery performed [40]. It is already a standard of care to use neoadjuvant systemic therapy to increase the operability of breast cancers and convert women to breast conservation techniques. Once breast conserving surgery was established through several large randomized trials and further validated by the expansive Early Breast Cancer Trialists' Collaborative Group (EBCTCG) series of meta-analyses of these trials [41], the development and validation of sentinel node biopsy were undertaken. This innovation in axillary surgical technique has led to a significant reduction in morbidity with respect to lymphedema risk and arm function, improving quality of life for many breast cancer survivors. Clinicopathologic features such as number of positive nodes as determinants of adjuvant therapies are giving way to intrinsic subtyping and genomic profiling and are becoming much less important for guiding decision making for systemic therapy selection.

Current innovations are designed to further lessen the impact of surgical management, whether by refining the surgical techniques or by omission of surgery in selected cases. Studies have shown that axillary radiation is effective in controlling subclinical disease in clinically node negative patients [42], potentially obviating the need for axillary node biopsy and further reducing the risk of lymphedema. Intrinsic molecular subtype predicts both the risk or distant metastasis as well as the risk of local-regional recurrence after both mastectomy and breast conserving surgery [15]. Using such information in the design of clinical trials may allow refinement of the extent of surgery required as well as the use of radiotherapy and systemic therapies. For example, intrinsic luminal A cancers have an extremely low risk of nodal metastasis, distant metastasis, and local recurrence. With the use subtyping as well as additional predictive and prognostic gene assays, it may be possible to select low risk patients for omission of therapy and reduce overtreatment. An ongoing Patient Centered Outcomes Research Institute (PCORI) grant sponsoring a multi-institutional clinical trial is asking that very question in women with noninvasive ductal carcinoma in situ (DCIS), or stage 0, breast cancer. Known as Comparing Operative to Medical Endocrine Therapy (COMET) for low risk DCIS trial, the study randomizes women with DCIS (grade 1-2, ER or PR positive) to

surgery with or without radiation versus active surveillance consisting of mammograms every 6 months for 5 years, and endocrine therapy is allowed on both arms [43].

The use of breast and even axillary surgery will likely be needed for the foreseeable future, particularly for patients with more locally advanced disease. This patient population is the focus of optimizing the combination of surgery, systemic therapy, and radiation therapy. Neoadjuvant systemic therapy is widely used and often leads to downstaging, which challenges decision making regarding optimal postoperative treatment as defined by pathologic response as well as molecular subtype. Multiple innovative approaches in breast reconstruction techniques, including skin and nipple sparing mastectomy, techniques to preserve lymphatics and nerves, and oncoplastic rearrangements in women requiring larger volume resections to achieve breast preservation are enhancing quality of life and cosmetic outcomes for these women [44].

2.5. Radiation Therapy. Until recently, radiation for breast cancer was quite uniformly applied with respect to target volumes and dose. Radiotherapy treatment decisions revolved around clinical stage, especially the tumor size and the presence of positive nodes, and other pathologic features such as margin width. Target volumes were generally confined to whole breast or chest wall radiation, without or without nodal volumes, and were usually treated with conventional fractionation schemes that took 5 to 7 weeks to complete. About 15 years ago, alternate treatment approaches began to be reported. Hypofractionated regimens for whole breast radiation shortening overall treatment times from 6 to 7 weeks to 3 to 4 weeks have been well validated in several randomized trials and indeed appear to be as efficacious as well as potentially less toxic than conventional fractionation regimens [45]. Accelerated partial breast irradiation and intraoperative radiotherapy techniques have also been validated in several large randomized trials and multiple multi-institutional series as safe and efficacious for many women with early stage breast cancers, further reducing treatment times to 1 to 5 days [46]. These alternate techniques promise to increase access to breast conservation treatment among women who are socioeconomically or geographically challenged to participate in long courses of daily treatment and to reduce omission of treatment in women who may benefit from radiotherapy after lumpectomy. These techniques also reduce acute and late toxicity. Ongoing studies are defining alternate fractionations in the postmastectomy and node positive settings and after neoadjuvant chemotherapy. Current American Society for Radiation Oncology (ASTRO) [47, 48] and Groupe Européen de Curiethérapie (GEC) and the European Society for Radiotherapy and Oncology (GEC-ESTRO) [49] clinical guidelines encourage the adoption of these alternate techniques in properly selected patient populations to improve outcomes and reduce toxicity.

Clinical trial data are helping to refine personalization of target volumes: whole breast or partial breast; nodal volumes or not; which nodal levels. However, more refinement of target volumes is possible. For example, what is the correct volume of breast tissue to treat for whole breast or partial breast radiation? Exactly which nodal stations are at risk and need to be irradiated? Current techniques use anatomical landmarks such as the CT-defined breast tissue or the surgical bed plus a uniform expansion margin, and clinicopathologic features to define the risk of local-regional recurrence to determine target volumes. A pair of ongoing studies (NSABP B51 [50] and Alliance A011202 [51]) are defining the indications for local-regional radiation treatment and target volumes after neoadjuvant chemotherapy and surgery. Studies of intrinsic molecular subtypes have shown that local-regional recurrence is predicted by subtype [15]. As with distant recurrence risk, luminal A tumors have the lowest risk and triple negative or basal-like have the highest risk of local-regional recurrence. To date, there have been few prospective randomized trials to report local-regional recurrence outcomes based on molecular subtypes or to incorporate subtype into their study design.

There are several areas of interest in personalizing radiotherapy use in breast cancer. One major focus is to reduce overtreatment of low risk patients whose cancers fall below the 10–20% risk of local recurrence that defines a survival benefit for breast radiation [41]. There are several ongoing multi-institutional trials using gene arrays to select patients for observation (omission of radiation) after breast conserving surgery. The IDEA study [52] is using a low risk Oncotype DX Recurrence Score and the Precision study [53] is using a low risk PAM50 ROR for selection of stage I breast cancer patients for omission of radiation after lumpectomy. The premise of both studies is that tumor biology as defined by functional gene assays previously shown to predict local recurrence will be a better methodology for selection of patients for observation than previous unsuccessful trials which used clinicopathologic surrogates of tumor behavior.

Another important area of interest is the use of gene assays of radioresponsiveness to predict which cancers are more or less resistant to radiation. Such studies start to define not only who may benefit from radiation at all, but also what dose of radiation is needed to achieve optimal tumor control probability. The radiosensitivity index (RSI) developed by Torres-Roca and colleagues uses a systems biology approach and fraction of cells surviving 2 Gy (SF2) to define a clinically validated molecular signature that estimates radiosensitivity of multiple tumor types, including breast cancer [54]. In several breast cancer cohorts, RSI predicts for improved relapse free and distant metastasis free survival only in radiosensitive patients who were irradiated. RSI defined radioresistant and sensitive subpopulations especially among triple negative patients and distinguished outcomes by radiation dose in RSI subpopulations [55]. These investigators have proposed a genome-based model for adjusting radiotherapy dose (GARD) based on data from multiple clinical cohorts. The GARD shows a range of values across and within tumor types but generally agrees with clinical observations of known sensitive and resistant tumors and is associated with longer survival in irradiated breast cancer patients with higher (more sensitive) GARD values [56]. The heterogeneity within tumors indicates an opportunity to begin to use such assays to triage radiation dose to individual patients. A different gene assay has been

developed by Speers and colleagues using clonogenic survival assays to identify intrinsic radiosensitivity as correlated to gene expression in breast cancer cell lines, generating a radiation sensitivity signature (RSS) [57]. The RSS was cross validated and found to be the most significant predictor of local recurrence as compared to other clinicopathologic features.

The promise of precision medicine for radiation therapy in breast cancer is to develop an array of diagnostic, predictive, and prognostic tests including radiomics, molecular subtyping, gene panels predicting risks of local-regional recurrence, and distant metastases, as well as inherent tumor radioresponsiveness. Such a toolbox will allow for individualized treatment decisions based on likelihood of indolent versus aggressive disease, treatment of the appropriate volume of breast and nodal volumes to eradicate microscopic cells harbored in the highest risk tissues and to deliver the correct dose of radiation based on the individual tumor radiosensitivity.

3. Conclusion

Precision medicine holds the promise of truly personalized treatment which provides every individual breast cancer patient with the most appropriate diagnostics and targeted therapies based on the specific cancer's genetic profile as determined by a panel of gene assays and other predictive and prognostic tests. Intense research is being conducted in a wide array of disciplines relevant to breast cancer detection, diagnosis, treatment, and survivorship. This article has provided an overview of some of that research in the arenas of screening and diagnosis, molecular profiling, radiomics, and the major treatment modalities including systemic therapy, surgery, and radiation therapy. As these data emerge and coalesce, the next generation of clinical trials will likely combine panels of molecular assays to drive therapeutic selections. Novel endpoints that allow rapid assessment of these approaches are needed for validation, while traditional endpoints, especially survival and toxicity outcomes, will continue to need to be collected. This is one of the most dynamic periods of basic science and translational research in oncology, with the potential promise to accelerate the ultimate search for the cure for cancer.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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Review Article

Postmastectomy Radiation Therapy: Are We Ready to Individualize Radiation?

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Contemporary recommendations for postmastectomy radiation have undergone a shift in thinking away from simple stage based recommendations (one size fits all) to a system that considers both tumor biology and host factors. While surgical staging has traditionally dictated indications for postmastectomy radiation therapy (PMRT), our current understanding of tumor biology, host, immunoprofiles, and tumor microenvironment may direct a more personalized approach to radiation. Understanding the interaction of these variables may permit individualization of adjuvant therapy aimed at appropriate escalation and deescalation, including recommendations for PMRT. This article summarizes the current data regarding tumor and host molecular biomarkers *in vitro* and *in vivo* that support the individualization of PMRT and discusses open questions that may alter the future of breast cancer treatment.

1. Introduction

Breast cancer is the most commonly diagnosed malignancy and the second most frequent cause of cancer death in women [1]. Radiation therapy remains an important treatment modality for patients with locally advanced breast cancer, with randomized trials investigating postmastectomy radiation therapy (PMRT) continuing to demonstrate decreased locoregional recurrence (LRR) and improved overall survival (OS) in appropriately selected patients [2–4]. Historically, surgical staging provided risk-stratification that served as the basis for recommending PMRT; however, modern understanding of genetics, immunology, and molecular biology is shedding light on tumor and host biomarkers that may alter patients' rates of response, progression, or survival [5].

Precision radiation therapy currently refers to the use of advanced technology to improve target delineation and treatment delivery. However, to usher radiation oncology into the era of personalized medicine, we must consider individual patient and tumor parameters that influence treatment response and complications to provide appropriate treatment recommendations. Applying our understanding of molecular markers representing tumor and host biology may permit individualization of radiation therapy aimed at appropriate escalation and deescalation of PMRT based on biologic parameters. This article summarizes the current data regarding molecular markers, which support the individualization of radiation using tumor molecular profiles, stromal microenvironment data, and patient genetic and immune parameters.

2. Tumor Molecular Profile

2.1. Tumor Biology. In the past decade, great strides have been made in understanding tumor biology and using this knowledge to design tumor-directed targeted therapies. For example, the addition of targeted monoclonal antibody therapies against the HER2 protein (i.e., trastuzumab, pertuzumab) to chemotherapy regimens has significantly improved overall survival in patients with HER2 amplified breast cancers [6]. However, heterogeneity within individual tumors significantly limits targeted therapies potential to cure cancer, as we have recognized tumors developing evasion mechanisms and resistance to targeted treatment strategies.

In breast cancer, tumor heterogeneity, as represented by receptor status, has been recognized for decades. More recently, genomic assays of 12–70 or more genes, including Oncotype Dx (Genomic Health, Inc., USA, Redwood City, CA) and MammaPrint (Agendia, Inc., USA, Irvine, CA), have further elucidated the heterogeneity of breast tumors. Medical oncologists apply this data in making treatment recommendations regarding adjuvant chemotherapy; however, little data relates this genomic data to radiation oncology recommendations. While genomic models provide primarily information regarding distant failure rates, as opposed to local failure rates, several investigators have explored the applicability of existing recurrence scores to the issue of locoregional recurrence [7].

Some studies propose models accounting for intrinsic radiosensitivity of tumors to help guide radiation recommendations and dose schedules. Eschrich and colleagues validated a radiosensitivity molecular signature in a cohort of breast patients, which demonstrated that patients predicted to be radiosensitive versus radioresistant had improved 5-year relapse-free survival and 5-year distant metastasis-free survival when treated with radiation [8]. A similar model was used to predict local recurrence in breast cancer and identified that radiosensitive triple-negative phenotype breast cancers had similar local recurrence to those with luminal A or B subtypes [9]. While there is no defined role for these clinical models at this time, they potentially can provide clinicians with valuable information regarding intrinsic tumor radiosensitivity and biology that may shape clinical recommendations in the future.

2.2. Genomic Factors. While genetic mutations in tumors are widely recognized, epigenetic changes are now appreciated to influence response to radiation treatment across many cancer histologies, including breast cancer [10]. DNA methylation is a process involved in regulating gene transcription, and, therefore, gene expression and cellular function [11]. Data suggests a correlation between radiosensitivity and DNA methylation *in vitro* [12], and *in vivo* studies demonstrate DNA methylation levels of specific genes *before and after* exposure to radiation are significantly correlated with response to radiotherapy and with total dose [13]. In clinical context, using methylation of particular genes to predict treatment response may help physicians tailor radiation therapy using individual patients' genomic DNA methylation pattern.

3. Stromal Microenvironment

The tumor microenvironment is a known factor influencing radiation treatment and response but has proven difficult to study, given our limitations in reproducing its conditions *in vitro*. Several recent studies have demonstrated molecular influences of the stroma on irradiated tissues. When comparing tumor cells irradiated *in vitro* versus *in vivo*, gene expression profiles after radiation differed significantly, with authors concluding that the tumor microenvironment (accounting for hypoxia, nutrient availability, blood flow, etc.) plays a major role in gene expression after radiation [14]. Similarly, studies investigating DNA methylation of breast cancer show that methylation changes *in vitro* are significantly lower than the methylation seen in patient samples, suggesting a significant impact of the tumor microenvironment and host immune system [13].

There is also striking difference between gene expression after single fraction dose radiation versus multifraction dose radiation, with multifraction dose radiation demonstrating increased expression of IFN-related genes. IFN-related genes are known to contribute to inflammation and be associated with radiation resistance that is often associated with multifraction radiation schedules [14]. This data suggests that inflammation in the microenvironment around the time of radiation may influence the response to radiation therapy and could be a potential target to increase the therapeutic ratio of radiation in future studies. Other studies investigating the stromal microenvironment demonstrate that genetic changes, notably in the *c-Kit* and ERα proteins, are present and continually changing years after radiation therapy, likely contributing to fibrosis, telangiectasias, and other normal tissue complications [15]. While significant questions remain, there is now concrete data demonstrating that tumor microenvironment plays a substantial role in influencing the effect of radiotherapy and may play a part in individualizing radiation therapy in the future.

4. Host Parameters

4.1. Genetic Factors. Host genetic factors are known to play a role in development of cancer. Genetic mutations are of two varieties: germline genetic mutations, arising from the gamete and therefore affecting every cell in the offspring patient, and somatic mutations, occurring in a population of cells after conception and often found in tumor cells [16, 17]. Patients harboring germline mutations, such as BRCA1 or BRCA2 and TP53 (Li-Fraumeni syndrome) or ATM, are often at higher risk of developing cancers, since every cell in the body has an existing mutation.

In contrast, somatic mutations are not known to confer higher risks of developing future cancers, since the known defect is limited to a certain population of cells. Using known genetic risk information to assist in making treatment recommendations in breast cancer is crucial for optimizing patient outcomes. In general, adjuvant radiation therapy risks and benefits should be discussed in a multidisciplinary setting, with an understanding of treatment-related complications. Here we will briefly discuss two genetic syndromes that are relevant with regard to adjuvant radiation therapy.

4.1.1. Li-Fraumeni Syndrome. Patients with Li-Fraumeni syndrome have germline TP53 mutations, and significant risk of early onset breast cancer, the most common malignancy diagnosed in adults with this mutation [18]. With modern understanding of this genetic mutation, there is an appreciation for increased risk of second cancers, including sarcoma, leukemia, and second breast primaries (ipsilateral and contralateral) [19]. Radiation therapy is generally avoided in patients with Li-Fraumeni syndrome but should be discussed with a multidisciplinary team, considering the risks and

benefits of disease recurrence with complications including second malignancies.

4.1.2. ATM. Mutation in the ataxia telangiectasia mutated (ATM) gene is another one that confers increased risk of breast cancer. Homozygous ATM mutations also results in potentially increased toxicity with radiation therapy, due to defective DNA repair and genomic instability in normal tissues [20]. However, patients with heterozygous germline ATM mutations do not appear to have increased toxicity with radiation therapy [21]. Radiation therapy is generally contraindicated in patients with homozygous germline ATM gene mutations but appears to be safe in appropriately selected patients with heterozygous mutation [22].

4.2. Host Immune Profile. For over 40 years, the immune system has been implicated as crucial for optimal response to radiation therapy [23]. Modern studies show that DNA methylation of immune pathway genes is significantly altered after radiation therapy, consistent with an influence on response to radiation [13]. In addition, upregulated inflammatory signaling pathways are implicated in improving response to radiation and may be promising targets for enhancing radiosensitivity of tumors [24]. Current immunotherapies in use for other cancer types include ipilimumab, pembrolizumab, and nivolumab. Trials incorporating immunotherapies neoadjuvantly are currently underway in high risk breast cancer. One such study, the I-SPY 2 trial (http://www.ispy2.org), investigates the genomic profile of breast tumors using the MammaPrint Assay (Agendia, Inc. USA, Irvine, CA). Patients with high risk results on the MammaPrint are then randomized to various arms of neoadjuvant chemotherapy, with one arm including pembrolizumab (anti-PD-1 antibody). Preliminary results in the pembrolizumab arm demonstrate increased pCR rates (TNBC 20 versus 60%, HER2+ 13 versus 35%) compared to the standard chemotherapy arm [25]. These are promising results and will hopefully translate into improved outcomes with further follow-up and may open the door for incorporating immunotherapies into the standard chemotherapy regimen for locally advanced breast cancers.

5. Current Strategies for Individualizing Radiation

Researchers from Moffitt Cancer Center recently proposed one method of adjusting radiation dose using biological differences in tumors. The genome-based model for adjusting radiotherapy dose (GARD) incorporates gene-expression-based radiation sensitivity, standard of care radiation dose, and fractionation for a particular tumor and the linear quadratic model to produce a value which predicts for the therapeutic effect of radiotherapy. In short, a higher GARD value predicts a higher therapeutic effect of radiation, which could be extrapolated to clinical benefit. Retrospective cohort studies using the GARD model have stratified patients into low, middle, and high groups, demonstrating that GARD may be an independent predictor of clinical outcome in breast cancer patients. In multivariate analysis, distant metastasis-free survival and relapse-free survival in multiple cohorts were statistically associated with GARD values. Authors discussed that GARD may be used as a method to customize radiation dose to match the radiosensitivity of individual patient's tumors, paving the way for future clinical trial design. However, limitations of GARD include the fact that it does not account for host or microenvironmental factors, or normal tissue toxicity factors, which, if included, would further allow clinicians to optimize radiation dose [26].

6. Conclusion

In the past decade, we have seen a paradigm shift in the treatment of breast cancer, with a more individualized approach to understanding tumor biology and recommending adjuvant chemotherapy. Moving into the next decade of cancer care, it is imperative that personalized medicine moves beyond medical oncology and includes radiation therapy. This will present many challenges, requiring radical changes to the traditional model of delivering radiation—which has been to deliver the high dose radiation therapy within normal tissue tolerances.

Clinicians must synthesize our current understanding of tumor biology, stromal microenvironment, host genetic and immune factors, risk of recurrence and complications, and improved therapeutics to make up-to-date and appropriate recommendations for our patients. Ongoing research may enhance our understanding of factors that may be incorporated into future clinical trials, testing various dose levels based on the genetic, genomic, immune, stromal, and tumor molecular profiles discussed in the body of this paper. Future studies may include similar models to GARD, where combinations of tumor factors, tumor microenvironment, host immunity, and normal tissue complications and host genetics and immune profiles are implicated in designating radiation dose levels. Ultimately, precision radiation therapy must begin to incorporate personalized treatment recommendations, eliminating use of radiation in patients who do not benefit from it and preferentially treating those with highest benefit, to optimize breast cancer patient outcomes and improve our quality of care.

Additional Points

Key Points. (i) Current understanding of genetic, genomic, immune, stromal, and tumor molecular profiles may be useful in providing a strategy for bringing personalized medicine into the field of radiation oncology. (ii) Precision radiation therapy should eliminate the use of radiation in patients who do not benefit from it and preferentially treat those with highest benefit, to optimize breast cancer patient outcomes.

Conflicts of Interest

The authors have no commercial or financial conflicts of interest to disclose.

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Review Article

Establishing the Role of Stereotactic Ablative Body Radiotherapy in Early-Stage Breast Cancer

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Stereotactic ablative body radiotherapy (SABR) has a role as definitive therapy in many tumor sites; however, its role in the treatment of breast cancer is less well explored. Currently, SABR has been investigated in the neoadjuvant and adjuvant setting with a number of ongoing feasibility studies. However, its use comes with a number of radiobiological and technical challenges that require further evaluation. We have learned much from other extracranial disease sites such as lung, brain, and spine, where definitive treatment with SABR has shown encouraging outcomes. In women with breast cancer, SABR may eliminate the need for invasive surgery, reducing healthcare costs and hospital stays and providing an additional curative option for early-stage disease. This poses the following question: is there a role for SABR as a definitive therapy in breast cancer?

1. Introduction

In Canada, breast cancer is the third most common cancer after lung and colorectal cancer and is the most common cancer diagnosis in females. As our population continues to live longer, we will see an increase in those diagnosed with breast cancer over the age of 70. According to the 2017 Canadian Cancer Statistics [1], one-third of new breast cancer diagnoses are 70 years or older. The role of radiotherapy in the management of breast cancer is well established in the adjuvant setting [2]. However, it is increasingly recognized that short course partial breast radiation may be an option particularly in older low-risk women [3] and some may not require radiation at all [4]. Therefore, finding additional options for treatment reduction and improved efficiency within cancer care is important for both patients and our healthcare system.

There is little clinical data supporting the use of definitive conventionally fractionated radiotherapy [5, 6] and more specifically stereotactic ablative body radiotherapy (SABR) in breast cancer. Many sites, including lung, brain, spine, and prostate, have adopted SABR as a definitive treatment option in early-stage disease. Stereotactic ablative body radiotherapy has many advantages by delivering high doses of radiation over a short period and saving treatment days, overall time on the treatment machine, and overall cost. Patients, if treated definitively, may never require surgical intervention, eliminating the need for anesthetic and inpatient hospital stays, with a potential further reduction in health and hospital expenditure.

In this review, we discuss the current radiotherapy standards, where SABR has been studied and its potential role and challenges as a definitive treatment in early-stage breast cancer.

1.1. Current Standards of Treatment in Early-Stage Breast Cancer. Adjuvant radiotherapy following lumpectomy and sentinel lymph node biopsy is standard of care in early-stage breast cancer patients [2]. In recent times, breast cancer radiotherapy has evolved from conventional fractionated radiotherapy (1.8 Gy–2 Gy per fraction) to hypofractionated radiotherapy (>2 Gy per fraction), taking advantage of the perceived low alpha/beta ratio of breast cancer cells [7, 8].

Hypofractionation has been investigated in a number of prospective randomized studies [3, 8] and has been integrated worldwide into standard treatment protocols in early-stage

Table 1: Local recurrence risk and tumor dose.

Tumor dose (Gray)	Local recurrence risk	
	Arriagada et al. [5] % (number of patients)	Van Limbergen et al. [6] % (number of patients)
≤50	77% (99/128)	75% (3/4)
51–60	68.7% (55/81)	21% (4/19)
61–70	54.8% (17/31)	16.6% (2/12)
71–80	31.7% (33/104)	24.4% (12/49)
>80	24% (13/54)	12.5% (17/136)

breast cancer. These studies have demonstrated equivalent local control outcomes with minimal differences in side effects compared to standard fractionation schedules.

An additional option in early-stage breast cancer is the use of accelerated partial breast irradiation (APBI). As most local recurrences after breast conserving therapy occur in or close to the tumor bed [9], APBI aims to deliver high doses of radiation around the tumor bed to a limited volume of breast tissue over a short period. Options for APBI treatment delivery include conformal external beam radiation, interstitial brachytherapy, intracavitary brachytherapy, and intraoperative radiation. Two randomized trials comparing whole breast to partial breast irradiation [10, 11] showed partial breast irradiation to be a safe and effective treatment option with low recurrence rates in a well-selected cohort of early-stage breast cancer patients. The RAPID [12] study compared twice daily fractionated radiotherapy to standard whole breast radiotherapy with unfavorable cosmetic and late radiotherapy toxicity outcomes. Additionally, a recent Cochrane review [13] of patients receiving APBI versus whole breast irradiation found no difference in loco regional recurrence-free survival, cause-specific survival, distant metastasis-free survival, or mastectomy rates. Late subcutaneous fibrosis and telangiectasia were worse in the APBI cohort; however, longer follow-up results are still needed. The American Society for Radiation Oncology recently updated the APBI consensus statement to include patients above the age of 50 years, T1 disease, and DCIS less than 2.5 cm, as well as ER positive [14].

1.2. Radiotherapy Alone as Definitive Treatment in Breast Cancer. Radiotherapy's role as a definitive ablative therapy (i.e., without surgery) is less well considered and to the best of our knowledge there are no prospective comparative studies in the literature of the current standard (surgery +/− radiotherapy +/− chemotherapy/hormonal therapy) versus definitive radiotherapy. Age and medical comorbidities often deem patients inoperable and, in general, these patients are then treated with palliative intent using hormonal therapy alone or palliative radiotherapy if applicable.

A collaborative study between the Gustave-Roussy Institute and the Princess Margaret Hospital [5] reviewed the use of radiotherapy alone as definitive breast cancer treatment. Patients who had inoperable disease or who were unable to undergo general anesthesia received definitive hypofractionated radiotherapy (40 Gy in 16 fractions, 45 Gy in 20 fractions, or 45 Gy in 18 fractions). The retrospective study demonstrated tumor dose as being a highly significant factor in local disease control (Table 1) and on a subsequent review of the patient cohort 10 years later, the group described the incidence of disabling complications as low and as expected related to total dose.

Van Limbergen et al. [6] also performed a retrospective analysis of 221 patients with Tis-T3 N0-1 breast cancer treated with radiotherapy alone. The risk of local recurrence was significantly associated with the size of tumor, age, radiation dose (Table 1), and length of split course intervals. They concluded that doses needed to provide local control similar to a combination of surgery and radiation are 10 Gy higher for T1 tumors and 35 Gy higher for T2 tumors.

Unfortunately, higher doses may result in worsened cosmetic outcomes, as a separate adjuvant breast dose escalation paper by Van Limbergen et al. [15] reported. The authors reviewed 161 patients; those that received higher than 75 Gy in 37 fractions lead to very poor results in more than 30% of patients and only 15% of patients who received more than 80 Gy resulted in good cosmetic results. Additionally, in a paper by Calle et al. [16], who treated 394 patients with doses of up to 8500 rad (85 Gy) alone (no surgery), only 38% of patients reported a good-to-excellent 5-year cosmetic outcome. However, it is important to remember that both of these papers are more than 25 years old, using older radiotherapy techniques and larger treatment fields.

1.3. Clinical Application of SABR in Breast Cancer. Key advancements have been made in the delivery of hypofractionated radiotherapy, including advances in the delivery of more homogenous doses of radiation and radiobiological understanding of more definite dose equivalent estimation [17]. Stereotactic ablative body radiotherapy in breast cancer is appealing and has been shown to be a safe and effective definitive treatment option in many tumor sites, including lung, brain, and liver [18, 19]. Timmerman et al. [19] published one of the earlier extracranial SABR trials on its use in early-stage lung cancer, demonstrating excellent local control rates and side effect outcomes in patients deemed medically inoperable.

To date, SABR has been investigated in the setting of neoadjuvant and adjuvant therapy in breast cancer treatment.

1.3.1. Neoadjuvant SABR. Bondiau et al. [20] conducted a phase 1 study involving 25 patients to determine the maximum tolerable dose of SABR concomitant with neoadjuvant chemotherapy before surgery. One-third of patients had a pathological complete response, with the highest rate at dose

Table 2: Ongoing phase I/II studies exploring the use of SABR in the neoadjuvant setting.

Name of study	Estimated number to be enrolled	Inclusion	Primary endpoints	SABR dose
Feasibility Study of Stereotactic Body Radiotherapy for Early Breast Cancer (ARTEMIS) [21]	32	Women ≥ 70 yr with preoperative early-stage breast cancer, followed by lumpectomy at 8–12 weeks after SABR	Treatment feasibility	40 Gy in 5 fractions every other day
Single Dose Ablative Radiation Treatment for Early-Stage Breast Cancer (ABLATIVE) [22]	25	Core biopsy positive nonlobular carcinoma, with negative sentinel lymph node biopsy followed by lumpectomy 6 months after SABR	Pathological complete response	20 Gy in 1 fraction
Preoperative Single-Fraction Radiotherapy in Early Stage Breast Cancer [23]	100	Women ≥ 50 yr, biopsy proven, CT1N0, ER +ve, invasive ductal, or DCIS, followed by lumpectomy 8–12 weeks after SABR	Rate of pathological response at time of surgery	21 Gy in 1 fraction
Stereotactic Image-Guided Neoadjuvant Ablative Radiation Then Lumpectomy (SIGNAL) [24]	120	Postmenopausal women ≥ 55 yr, ≤3 cm, ER +ve, clinically node negative, invasive ductal carcinoma, followed by lumpectomy 6–8 weeks after SABR	Toxicity resulting from radiation	21 Gy in 1 fraction
Preoperative Stereotactic Ablative Body Radiotherapy (SABR) for Early-Stage Breast Cancer [25]	40	Women ≥ 50 yr, invasive adenocarcinoma, ≤2 cm, followed by lumpectomy 6 weeks after SABR	Rate of pathological complete response	3 fractions

level of 25.5 Gy in 3 consecutive fractions. However, the maximum tolerated dose was not reached as the group found that early SABR related toxicities were rare.

There are a number of ongoing phase I/II studies exploring the use of SABR in the neoadjuvant setting (Table 2). Currently, in Canada, the Juravinski Cancer Centre is conducting a phase I feasibility study of the role of SBRT for the treatment of early-stage breast cancer called ARTEMIS [21], which will deliver 40 Gy in 5 fractions every other day to the gross tumor, followed by breast conserving surgery. The primary outcome measure is feasibility and successful delivery of SABR.

Similarly, in Netherlands, a multicenter single-arm prospective study called the ABLATIVE study [22] is recruiting patients to undergo MR-guided single-dose APBI with an integrated boost, followed by breast conserving surgery at 6 months. The primary study point aim is to assess pathological complete response and secondarily to review radiological response and toxicity.

1.3.2. Adjuvant SABR. Stereotactic accelerated partial breast irradiation for early-stage breast cancer in the adjuvant setting has been studied in a small prospective trial [26] which looked at ten patients who received CyberKnife therapy. Each received a total dose of 30 Gy in five consecutive fractions. The group concluded that early findings show CyberKnife as a feasible, well-tolerated, and reliable platform for APBI.

1.4. Radiobiology, Toxicity, and Technical Challenges in Using SABR

1.4.1. Radiobiology and Toxicity. One of the many advantages of SABR is the ability to deliver large doses of radiation to the tumor while sparing surrounding normal tissue. Similar to prostate cancer, it has been hypothesized that breast tissue is sensitive to fraction size; that is, the larger the dose per fraction the higher the tumor cell kill; hence the application of hypofractionation in breast cancer is aiming to take advantage of this phenomenon [27].

Unfortunately, one of the drawbacks to delivering larger radiation doses is the increased risk of late normal tissue toxicity. Cosmetic breast outcomes from the hypofractionated trials have to date been acceptable, bearing in mind the fact that they refer to whole breast irradiation [3, 8], with poorer outcomes associated with large excision volume and delayed wound healing postoperatively. Also, the RAPID study trialists [12] reported significantly worse nurse, patient, and physician reported cosmetic outcomes compared to the standard fractionation group. So far, in the small SABR studies described in this review, cosmetic and breast outcomes have been acceptable. However, with these early results, where cosmetic outcome was not a primary endpoint, it is important to remember that potential adverse cosmetic outcomes may not be seen for many more years. Regardless, there is still need for clarity with respect to optimal dose fractionation schedules to avoid significant late effects.

1.4.2. Potential Technical Challenges. Safe delivery of large doses of radiation to any tumor site requires several rigorous quality assurance steps. The challenge, as with any radiotherapy plan, is location of disease and reproducibility. This is almost certainly even more relevant for patients receiving very high doses of radiation. Therefore, the use of breast molds and/or rigid immobilization may be required for patients being treated in the supine position.

Like lung and liver SABR, technical considerations such as 4DCT (4-dimensional computed tomography) and motion management using deep inspiration breath hold or active breath control may be useful in reviewing and managing the patients' breathing cycle, thus reducing or eliminating tumor motion and in turn reducing planning margins. These methods are currently widely used to reduce heart dose in patients receiving left-sided breast radiotherapy. Of note, Bondiau et al. [20] and Obayomi-Davies et al. [26] both used CyberKnife System (Accuracy Inc., Sunnyvale, CA) as primary delivery method by means of real-time respiratory tracking of implanted fiducial markers.

In addition to contrast-enhanced CT simulation, MRI as a simulation tool would be useful in providing superior soft tissue contrast and offering improved visualization of the breast tumor, with potential scope in the future for treatment delivery via an MR-guided linear accelerator. One of the challenges recognized is that breast cancer patients receiving a diagnostic MRI are in the prone position, while for reproducibility and consistency in radiation treatment, patients are treated in a supine position. A wide bore MRI scanner may then be required in order to acquire supine images in the radiotherapy position. Den Hartogh et al. [28] designed an MRI protocol incorporating a wide bore scanner and demonstrated high target volume delineation consistency among observers. Schmitz et al. [29] compared preoperative MRI-derived gross tumor volume (GTV) to histopathological measurements of the tumor in addition to any subclinical disease. There was good correlation between size of the visible MRI tumor and that measured at pathology (Pearson's correlation: 0.76). Only 10% of patients had invasive disease beyond 10 mm of the MRI-GTV, with the likelihood of subclinical disease greater in tumors with extensive intraductal components. In addition, preoperative delineation is shown to have less interobserver variability [30] compared with postoperative volume delineation and the expansion to a clinical target volume used to account for microscopic disease is not often employed during SABR treatment but may be considered in patients with potential adverse features, thus potentially reducing the volume of normal tissue in the treatment field. Fiducial markers were used in the neoadjuvant and adjuvant SABR studies [20, 26], which can help with tumor identification and volume delineation, in addition to tracking and locating treated disease sites on follow-up surveillance. An internal target volume is used to account for organ motion; this can be estimated by assessing the motion between the inhale and exhale phases on the 4DCT.

Defining organs at risk and dose constraints to normal tissues continues to be a challenge in the world of SABR. It is generally not recommended to extrapolate from dose constraints associated with conventional fractionation. Constraints may be extrapolated from prior lung and liver SABR trials, but this in turn is somewhat restricted.

1.4.3. Posttreatment Follow-Up. A challenge when delivering any type of definitive treatment is follow-up surveillance. We have learned from our lung colleagues that imaging changes can be confusing and often related to inflammation or fibrosis [31]. Definitive breast radiotherapy treatment we suspect will be no different and defining disease response and disease progression on what modality and frequency of follow-up imaging will require extensive review. Considerations to using CT, MRI, and functional imaging techniques such as PET-CT may be required to aid in this differentiation.

2. Conclusion

As we continue to strive to deliver the best of patient care with more patient convenience and less healthcare costs, it may only be a matter of time before SABR is integrated into the breast cancer treatment paradigm. With such rapid advancements in radiotherapy technology, it is imperative that well-constructed multi-institutional collaborative feasibility and randomized trials are developed. These should aim to explore the technique of SABR further, especially in early-stage elderly breast cancer patients and, in addition, to address the many radiobiological, technical, and toxicity issues that may arise through its use to deliver safe, quality-assured, evidence-based radiotherapy.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Review Article

Metronomic Chemotherapy in Triple-Negative Metastatic Breast Cancer: The Future Is Now?

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Triple-negative breast cancer (TNBC) shows a very bad prognosis, even in early stages of disease. Metronomic chemotherapy refers to the minimum biologically effective dose of a chemotherapy agent given as a continuous dosing regimen with no prolonged drug-free breaks that leads to antitumor activity. In the present article, we review preclinical and clinical data of metronomic administration of chemotherapy agents with or without biological agents in TNBC cell lines and patients, contextually reporting data from the VICTOR-2 study in the subgroup of patients with TNBC, in order to stimulate new ideas for the design of clinical trials in this subset of patients.

1. Introduction

Triple-negative breast cancer (TNBC), which accounts for 25% of the molecular subtypes, shows a very bad prognosis, even in early stages of disease [1]: after radical surgery, median time to relapse is approximately 18 months and from this point median overall survival is less than 24 months [2].

Different strategies have been studied to improve the prognosis of this subset of patients, and a lot of drugs are currently under evaluation. Despite the big efforts done to modify this clinical scenario, little or nothing has really changed in the last decades.

In elderly patients, the clinical scenario is, if possible, worse than expected: about 15% of breast cancers in older patients are of the triple-negative subtype [3] but only few of them receive adequate treatments, due to different reasons, mainly age-related factors such as comorbidities, deterioration of cognitive function, possible impairment in

organ function, and the concomitant use of other drugs. All these factors must be carefully assessed to avoid or minimize toxicity risks.

In this context, low-dose metronomic chemotherapy (mCHT) might represent a promising therapeutic option for elderly TNBC women [3].

mCHT refers to the minimum biologically effective dose of a chemotherapeutic agent given as a continuous dosing regimen, with no prolonged drug-free breaks that leads to antitumor activity [4]. Till now, few data are available regarding the use of mCHT in TNBC patients [5–7]; most of these studies have been conducted in HER2-negative breast cancer patients and results in the TNBC subset are generally reported as subgroups analyses.

In the present article, we review preclinical and clinical data of metronomic administration of chemotherapy agents with or without biological agents in TNBC cell lines and patients, contextually reporting data from the VICTOR-2 study in the subgroup of patients with TNBC.

Available literature on the subject was identified by using PubMed with three different keywords [metronomic chemotherapy], [triple negative] or [TNBC] and [breast cancer], without any custom range for year of publication, journal, or article type, with the exclusion of results only published as abstract reports. This search resulted in 26 articles published between 2008 and 2017: five articles reporting reviews on this topic were excluded; another article was excluded due to the subject not strictly related to the object of the online search.

Here we report available literature data grouped by setting of treatment.

2. Preclinical Data

Di Desidero et al. [14] evaluated the potential therapeutic impact and molecular mechanisms of topotecan administered in continuous low-dose metronomic (LDM) manner, alone or in concurrent combination with pazopanib in a triple-negative, primary, and metastatic breast cancer orthotopic model; potential molecular mechanisms of efficacy were also studied, especially the impact of hypoxic conditions. The combination of metronomic topotecan and pazopanib significantly enhanced antitumor activity compared to monotherapy with either drug and prolonged survival, even in the advanced metastatic survival setting, with a marked decrease in tumor vascularity, proliferative index, and the induction of apoptosis. Significant changes in tumor angiogenesis, cancer cell proliferation, apoptosis, HIF1α levels, HIF-1 target genes, and ABCG2 were found both *in vitro* and in tumor tissue. The authors concluded that the combination of metronomic topotecan and pazopanib warrants further investigations being a potential treatment option for this poor prognosis group of breast cancer patients.

3. Clinical Data

3.1. Neo/Adjuvant Setting. Different authors reported trials which have included metronomic chemotherapy in the regimens studied.

The largest randomized Phase III trial by Colleoni et al. [5] randomized 724 TNBC patients as part of a larger study (IBCSG 22-00) to receive the metronomic combination of CM (CTX 50 mg/day orally continuously for 1 year and MTX 2,5 m/twice a day orally, days 1 and 2 of every week for 1 year) or placebo after a standard adjuvant treatment. The reduction in DFS events was not statistically significant for maintenance CM versus no maintenance (HR = 0.84; 95% CI 0.66–1.06); however, in the TNBC/N+ group (n = 340), the estimated 5-year DFS was 72.5% for the CM maintenance group versus 64.6% for the non-CM group (HR = 0.72; 95% CI 0.49–1.05). Patients with TNBC and node-positive disease had a nonstatistically significant reduced HR (n = 340; HR, 0.72; 95% CI, 0.49 to 1.05).

The right selection of patients and the right choice of drugs, both with regard to doses as well as schedules, are crucial factors for determining the success or the failure of metronomic chemotherapy in the adjuvant setting: Pruneri et al. [6], by analyzing the prognostic and predictive value of tumor-infiltrating lymphocytes (TILs) in the TNBC cohort of the IBCSG trial 22-00, identified a subgroup of tumors, the so-called lymphocyte-predominant breast cancer (LPBC), for which metronomic CM confers a greater, even not statistically significant, clinical benefit.

Nasr et al. [7] investigated the role of oral methotrexate plus Cyclophosphamide given in a metronomic schedule for 1 year after finishing the adjuvant treatment for patients with TNBC in an attempt to prolong their disease free interval. The primary study objectives were to compare the disease free survival (DFS) and OS for TNBC patients after adjuvant chemotherapy, who underwent maintenance metronomic chemotherapy versus no maintenance therapy. The secondary end point was toxicity. Patients were randomly assigned to receive FEC-100 [FEC-100 was given in the form of 5-fluorouracil 500 mg/m2, epirubicin 100 mg/m2, and Cyclophosphamide 500 mg/m2 (day 1)] for 3 cycles, followed by Docetaxel 80 mg/m2 plus Carboplatin AUC 5 for 3 cycles then metronomic chemotherapy or the same FEC part followed by Docetaxel 100 mg/m2 for 3 cycles without any maintenance metronomic chemotherapy. Metronomic maintenance chemotherapy consisted of oral Cyclophosphamide (50 mg PO daily) and methotrexate (2.5 mg PO BID on days 1 and 2 of each week).

The median DFS for the two groups were 28 and 24 months, respectively. The median OS for the two groups were 37 and 29 months, respectively. Additionally, during the follow-up period, the overall distant metastasis recurrence rates for the two cohorts were 26% and 37%, respectively.

The authors concluded that extended adjuvant metronomic chemotherapy achieved significant improvement in the survival and was well-tolerated.

In another study, Masuda et al. [8] studied the effects of preoperative metronomic combination of paclitaxel, Cyclophosphamide, and capecitabine (mPCX) followed by 5-fluorouracil (FU), epirubicin, and Cyclophosphamide (FEC) as preoperative chemotherapy in 40 TNBC patients. The primary end point of the study was the pathological complete response (pCR) rate. The pCR rate was 47.5% (19/40) whereas the clinical response rate was 90.0%. The authors reported a

Table 1: Summary of data in the (neo)adjuvant setting.

Author (year)	Setting/type of trial	Drug(s)	Number of patients	Results
Pruneri et al. [6]	Adj/Phase III	CTX 50 mg/day orally continuously for 1 year and MTX 2.5 mg/twice a day orally, days 1 and 2 of every week for 1 year versus no maintenance chemotherapy	647	BCFI risk reduction: 13% DFS risk reduction: 11% DFRI risk reduction: 16% OS risk reduction: 17% (for every 10% increase of TILs)
Nasr et al. [7]	Adj/Phase II	*Group 1* FEC-100 × 3 cycles Docetaxel 80 mg/m^2 + Carboplatin AUC 5 *Followed by* CTX 50 mg/day + MTX 2,5 mg bid, days 1 and 2 every week	158 (whole population) 78	mDFS 28 versus 24 months mOS 37 versus 29 Distant mets recurrence rate 26% versus 37%
Masuda et al. [8]	(Neo)adj/Phase II	mPTX 80 mg/m^2 days 1, 8, and 15 CTX 50 mg/day CAPE 1200 mg/m^2, daily *Followed by* FEC100	40	pCR 47.5% cORR 90%
Shawky and Galal [9]	Adj/Phase II	mCAPE 650 mg/m^2, twice every day, after standard adjuvant chemotherapy for 1 year	19	2 ys-DFS rate 88.8% 3 ys-DFS rate 82.05%
Alagizy et al. [10]	Adj/Phase II	CAPE 500 mg twice daily continuously for 6 months after finishing six cycles of adjuvant FEC100	41	Mean DFS 42.4 months

EPI = epirubicin; CDDP = cisplatin; 5FU = 5-fluorouracil; PTX = paclitaxel; CTX = Cyclophosphamide; CAPE = capecitabine; mCAPE = metronomic capecitabine; FEC100 = 5FU + EPI 100 mg/m^2 + CTX; MTX = methotrexate; cCR = clinical complete response; cPR = clinical partial response; pCR = pathologic complete response; DFS = disease free survival; BCFI = breast cancer free interval; DFRI = distant recurrence free interval; OS = overall survival.

high incidence of severe adverse events, namely, neutropenia (35%), leukopenia (25%), and hand-foot syndrome (8%): these data are very different from the those reported by the vast majority of trials involving metronomic chemotherapy.

Different studies have also explored the role of metronomic chemotherapy in the adjuvant setting of treatment, mainly as prolonged therapy after a "standard" regimen.

Considering that there is no universally accepted standard chemotherapy regimen for adjuvant treatment of TNBC and classical regimens are currently reasonable choices, different authors tested alternative strategies with the aim of improving relapse free survival.

Shawky and Galal [9] investigated the tolerability of 1-year of metronomic capecitabine (650 mg/m2, twice every day) preceded by standard adjuvant therapy and overall survival in 19 patients with operable TNBC. The authors concluded that one year of capecitabine metronomic therapy preceded by standard adjuvant chemotherapy is active and well-tolerated in TNBC patients previously treated with standard adjuvant chemotherapy. With all the limits given by the small sample size and the single-arm design, the findings coming from this paper open important scenario for the future. In another Phase II trial, Alagizy et al. [10] evaluated the tolerability and efficacy of metronomic capecitabine as extended adjuvant treatment for women with triple-negative breast cancer. Forty-one patients received capecitabine 500 mg per os twice daily continuously for six months after finishing six cycles of adjuvant FEC100 ± postoperative radiotherapy. Even if this trial was not sufficiently powered to address the question regarding the role of angiogenesis bloc at the source by using metronomic chemotherapy, it was pioneer for subsequent trials investigating the same question, such as the BEATRICE trial and the IBCSG 22-00 one, published some years later.

It is our opinion that the use of metronomic chemotherapy, without strong preclinical data indicating which drugs should be used, how long, and at which doses, should not be adopted in the adjuvant setting.

Results of metronomic CHT in (neo)adjuvant setting are reported in Table 1.

3.2. Metastatic Setting. The literature only occasionally reports trials conducted with metronomic chemotherapy exclusively in TNBC patients being the majority of them case reports or analyses of subgroups of patients enrolled as part of trials conducted in HER2-negative patients.

Yoshimoto et al. [11] treated 45 patients, of whom only 9 were TNBC, with capecitabine 828 mg/m(2) twice daily with Cyclophosphamide 33 mg/m(2) twice daily, days 1–14 every 3 weeks. The primary endpoint was overall response rate (ORR). Secondary endpoints included progression-free survival (PFS), overall survival (OS), and safety. The median follow-up was 18.1 months. The authors reported an ORR

Table 2: Summary of data in the metastatic setting.

Author (year)	Line of treatment Type of trial	Drug(s)	Number of patients evaluable for end points	Results
Yoshimoto et al. [11]	1st-2nd line Phase II	CAPE 828 mg/m^2 twice daily + CTX 33 mg/m^2 twice daily, dd 1 → 14, every 21 days	9	ORR 44.4% CBR 57.8% Median PFS 10.7 months
Wang et al. [12]	2nd line or further (maintenance) Phase II	CTX 50 mg daily (after CTX 3 g/m^2 for the preparation of CD34+ and CTX 3 g/m^2, thiotepa 150 mg/m^2, and CBDCA AUC = 6, every 28 dd for 2 courses)	23	NA
Kummar et al. [13]	2nd line or further Phase II	CTX 50 mg/day versus CTX 50 mg/day + Veliparib 60 mg once daily throughout a 21-day cycle	39	ORR 5.6% versus 9.5% (NS)

ORR = overall response rate; CBR = clinical benefit rate; PFS = progression-free survival; CBDCA = carboplatin.

of 44.4% and a clinical benefit rate (CBR) of 57.8% in the TNBC population. Median PFS was 12.3 months in the whole population and 10.7 months in triple-negative disease. Grade 3 adverse events comprised leukopenia (26%), neutropenia (16%), and decreased hemoglobin (2%). There was no grade 3 hand-foot syndrome. The authors concluded that oral XC is an effective first- or second-line therapy for MBC, demonstrating high activity in both luminal A and triple-negative disease with few severe side effects, but the small sample size of TNBC group strongly affected transposition of these results.

Wang et al. [12] explored the combination of chemotherapy with immunotherapy, followed by maintenance metronomic Cyclophosphamide as a potential alternative option for the treatment of patients with metastatic TNBC. Results reported were strongly influenced by the induction phase of the trial and the authors do not report data for the metronomic maintenance part of the trial.

Kummar et al. [13] conducted a Phase II randomized trial in order to assess the role of PARP inhibition in the treatment of TNBC patients; Veliparib, a small molecule PARP inhibitor, was administered with the Cyclophosphamide 50 mg once daily and compared with Cyclophosphamide same dose alone in patients with refractory TNBC. The authors demonstrated that the addition of Veliparib to Cyclophosphamide did not improve the response rate (CR + PR) over Cyclophosphamide treatment alone.

Kontani et al. [15] analyzed 80 patients with MBC who received chemotherapy in the metastatic setting, comparing clinic-pathological factors and clinical outcomes between 52 patients who received metronomic regimens and 28 patients who received other cytotoxic regimens. The median time-to-treatment failure (TTF) and overall survival (OS) were significantly longer in the metronomic group compared with those in the nonmetronomic group; however, none of the 18 patients who responded to the regimen had triple-negative (TN) cancer. TTF and OS were significantly longer in patients with non-TN cancer compared with those in patients with TN cancer in the metronomic group (TTF, 16 versus 7 months, $P = 0.0014$; OS, 108 versus 20 months, $P = 0.000007$, resp.).

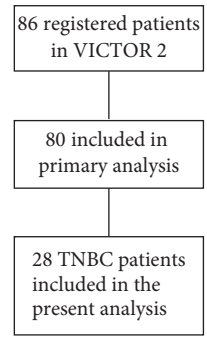

Figure 1: Consort diagram VICTOR 2 trial.

The authors concluded that metronomic chemotherapy could be a viable option for luminal-type MBC but at the same time an alternative treatment is required for TN cancer.

Results of the main studies regarding the role of metronomic CHT in the metastatic setting are reported in Table 2.

The VICTOR-2 is a Phase II, single-arm study evaluating the efficacy and safety of the metronomic combination of Vinorelbine (VNR), 40 mg three times per week, and capecitabine (CAPE) 500 mg three times per day, continuously, in 80 HER2-negative breast cancer patients. Twenty-eight out of 80 (35%) were TNBC (Figure 1). Patients and methods, as well as results regarding the whole population, have been reported elsewhere [16]. Median age in the TNBC group was 69 years (47–85), 23 patients (82.1%) had visceral involvement at the time of enrolment, and only 4 (14.8%) had less than 2 sites of disease. Fifteen patients received mCHT as second or further line of treatment. The clinical benefit rate (CBR) was 35.7% (95% CI: 18.6–55.9) and the median duration of CB was 11.3 months. Disease control rate (DCR; CR + PR + SD) was 53.7% and median duration of disease control was 7.4 months. Progression-Free Survival (PFS) was 4.7 months. Table 3 summarizes efficacy results. Severe toxicity did not exceed 8% and was mainly hematologic.

To our knowledge, these are the first prospective data ever published regarding the activity of mCHT in a population of metastatic TNBC patients.

Table 3: Efficacy results of VICTOR trial in the TNBC population.

Variable	Overall $N = 28$
Clinical benefit rate (CR + PR + SD ≥ 24 weeks) n/N (%)	10/28 (35.7)
[95% CI]	[18.6–55.9]
Kaplan-Meier estimate of median duration of clinical benefit (months)	11.3
Disease control rate (CR + PR + SD) n/N	15/28 (53.6%)
Kaplan-Meier estimate of median duration of response in disease control (months)	7.4
Kaplan-Meier estimate of median PFS (months)	4.7

Different drugs and regimens have been tested in TNBC patients, with the aim of disease control and survival prolongation.

Platinum salts, including carboplatin and cisplatin, lead to DNA cross-link strand breaks, which may be especially important in cells that are deficient in homologous recombination repair mechanisms such as *BRCA1/2*-associated tumors and TNBCs. The Phase III TNT (Triple-Negative Breast Cancer Trial) study compared carboplatin area under the curve (AUC) 6 every 3 weeks with docetaxel 100 mg/m2 every 3 weeks as first-line treatment for advanced stage disease. In the overall population, at a median follow-up of 11 months, PFS was 4.5 and 3.1 months, respectively, not so different from what we observed in our trial, while taking into account the differences in terms of study design between the two trials.

Our results do not significantly differ from those obtained by other authors with Eribulin, even in first-line setting: the ORR was 16.7%, the CBR was 25.0%, and median PFS was 3.4 months in 12 patients with TNBC treated with Eribulin in a Phase II study [17].

Few data are available regarding the use of nab-paclitaxel, a novel formulation of exclusively TNBC patients: in a Phase II study [18] first-line treatment with nab-paclitaxel, carboplatin, and Bevacizumab was associated with an ORR of 85%, a CBR of 94%, and a median PFS of 9.2 months. The study enrolled 34 patients and reported grade 3/4 adverse events in 53% and 18% of the patients (neutropenia and thrombocytopenia, resp.). In the VICTOR-2 study grade 3-4 leucopenia was observed in 7 patients (8.8%) and grade 3-4 thrombocytopenia in 2 patients (2.5%).

Our results suggest that metronomic combination of VNR and CAPE could represent a further treatment option for TNBC patients and for this reason could be considered in special populations, such as the elderly ones.

3.3. Toxicity. The evaluation of toxicity clearly related to metronomic chemotherapy is difficult to be done due to the fact that most trials describe general toxicity and not those specifically related to metronomic regimens, thus putting together those related to the nonmetronomic part of the regimen studies. The second issue is that, in trials specifically addressed to evaluate metronomic chemotherapy, there is often nondistinction between toxicities reported in TNBC patients and non-TRBC ones.

However, there could be no reason to split toxicities according to biological subtype, considering that specifically toxicities are mainly related to the regimen although to the type of disease.

We briefly summarize hereafter the toxicities described in trials specifically addressed to metronomic chemotherapy.

In the study by Colleoni et al. [5], the authors reported that, of 473 patients who received at least one CM maintenance dose (including two patients assigned to no CM), 64 (14%) experienced a grade 3 or 4 treatment-related adverse event; elevated serum transaminases were the most frequently reported (7%), followed by leukopenia (2%), but they did not distinguish toxicities occurring in TNBC patients from those observed in non-TNBC ones.

Nasr et al. [7] detailed the toxicity occurring in the metronomic part of the treatment, reporting grade 3 neutropenia in 2.8% of the patients, grade 3 anemia in 1.5%, and vomiting in 11% of the patients, respectively.

Shawky and Galal [9] reported that treatment-related adverse events were manageable with only 1 patient (5.3%) suffering from grade 3/4 hand-foot syndrome and another 1 patient (5.3%) suffering from grade 3 diarrhea. No grade 3/4 hematologic toxicity was recorded.

Yoshimoto et al. [11] reported grade 3 adverse events in their patients treated with capecitabine 828 mg/m^2 twice daily with Cyclophosphamide 33 mg/m^2 twice daily, days 1–14 every 3 weeks, mainly leukopenia (26%), neutropenia (16%), and decreased hemoglobin (2%). There was no grade 3 hand-foot syndrome.

However, considering the doses used and the schedule, this regimen cannot be considered, according to the widely accepted definition of metronomic chemotherapy, a truly metronomic regimen, and this could explain the high rates of toxicity observed in these patients, which are almost different from those reported by the vast majority of metronomic trials, even in non-TNBC patients [5, 16].

4. Conclusion

At the moment, few data, mainly obtained by Phase II trials, are available regarding the use of metronomic chemotherapy in TNBC patients; however, others are on the way, exploring different settings.

An international, randomized Phase II study (VICTOR-3) is currently ongoing to investigate the role, as maintenance therapy, of metronomic VNR, either single agent or in combination with metronomic CTX, in TNBC patients, after an induction chemotherapy with standard-dose regimens.

The CAMELLIA (NCT01917279) trial was designed to explore the efficacy and safety of metronomic CTX versus intermittent standard-dose CTX as maintenance therapy after first-line therapy with CTX plus docetaxel in HER2-negative metastatic breast cancer.

Even if the future is probably not now for the routine use of metronomic chemotherapy in TNBC patients, some promising results are ready to consider this regimen in

particular subgroups, such as the elderly ones, for whom few therapeutic options exist.

Conflicts of Interest

The authors declare no conflicts of interest.

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Review Article

Molecular Signatures of Radiation Response in Breast Cancer: Towards Personalized Decision-Making in Radiation Treatment

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Recent advances in gene expression profiling have allowed for a more sophisticated understanding of the biology of breast cancers. These advances led to the development of molecular signatures that now allow clinicians to more individually tailor recommendations regarding the utility and necessity of systemic therapies for women with breast cancer. Indeed, these molecularly based tests have been incorporated into national and international best practice guidelines and are now part of routine practice. Similar, though slower, progress is being made in the development of molecular signatures predictive of radiation response and necessity for women with breast cancer. This article will discuss the history of radiation response signature development, the current state of these signatures under ongoing clinical development, the barriers to their clinical adoption, and upcoming changes and opportunities that may allow for the personalized radiation treatment recommendations enabled by the development of these signatures.

1. The History of Radiation Response Signature Development

The idea that genes (either DNA sequence or subsequent expression) may function as surrogate biomarkers of disease response forms the rationale for the development of molecularly based signatures to predict response to radiation treatment in breast cancer. Prior to the genomics era and for over half a century, the field relied upon the response of *in vitro* cultured normal and malignant cells to radiation as the basis for models describing the radiation response of *in vivo* malignancies [1–7]. While beyond the scope of this review, these experiments provided the foundational data upon which the linear-quadratic model was derived, but the data derived from these experiments was incomplete in its ability to adequately model the heterogeneous response of tumors and normal tissue to ionizing radiation. Given these limitations, investigators have broadened their interrogation to try to develop more effective ways of both describing and predicting the response of tumors to radiation treatment. This search, coupled with technological advances that allow for the more complete evaluation of DNA, RNA, protein, and cellular metabolism, has led to the development of "-omic" based approaches for the prediction of response of tumors to treatment [8–15].

Pioneering work in the area of genomic-based signature development focused on prognostication and response to systemic therapy [16–22]. These early investigations focused on using genomic-based approaches to predict response to chemotherapy and/or hormone therapy, or to determine prognosis if adjuvant therapies were altogether omitted after surgery. Indeed, several genomic assays are currently in clinical practice that function either as prognostic biomarkers to aid in determining prognosis independent of treatment or as predictive biomarkers that are useful in directing appropriate clinical management. Initially these genomic tests were restricted to the expression or mutation of a single gene. More recently, as sequencing and high-throughput assaying techniques have improved, these genomic tests have become more sophisticated and their indications for

utilization have increased. With respect to breast cancer prognostic and predictive biomarkers, OncotypeDx®, Prosigna™, MammaPrint®, EndoPredict, Mammostrat®, and Breast Cancer Index all represent genomic tests with either prognostic or predictive capability that aid in risk stratification beyond standard clinicopathologic parameters in breast cancer [20, 21, 23–28]. While each test varies in its clinical indication, utility, and genomic make-up, many have been successfully validated as having clinical utility and have been adopted, to varying degrees, into clinical practice. Indeed, some of these tests are now part of recommended work-up by the national and international "best practice" guidelines as set forth by the National Comprehensive Cancer Network (NCCN) and other professional societies.

The concept of using genomic-based approaches for prediction of systemic therapy response is not unique. Although similar genomic tests for the prediction of tumor response after radiation have been slower in development, within the last several years an increasing number of tests with varying clinical indications and utility have been reported [9, 11, 14, 15, 29, 30]. Numerous groups have described gene expression-based genomic tests that predict the likelihood of breast cancer response to radiation treatment (or lack thereof) in an effort to identify the patients either (A) likely to respond (or not respond) to adjuvant radiation treatment or (B) not needing adjuvant radiation treatment at all. Most of these groups utilized high-throughput RNA expression technologies to develop gene expression signatures prognostic of low local recurrence risk (for the prognostic signatures) or predictive of response to radiation treatment in the adjuvant setting for patients with early-stage disease.

2. Radiation-Induced Gene Signatures

The concept of utilizing gene expression changes induced by radiation exposure as the basis for molecular signature development was foundational to the development of a number of signatures, either predictive or prognostic, for outcomes of patients with breast cancer. These signatures vary widely in the methods employed for their development, as well as their specificity for breast cancer outcomes and relationship to radiation treatment. Given this heterogeneity in development and validation, it is not surprising, then, that there is not significant overlap in the genes associated with these signatures. Additionally, the external validation of these signatures has been challenging for a number of reasons that will be discussed later in this article.

In 2009, Piening et al. described the derivation of signature that was developed based on the change of gene expression in human lymphoblast cells from 12 persons' cells exposed to 5 Gy of Cesium radiation [31]. When compared to unirradiated cell controls, the subsequent significantly induced (160 genes) and repressed (59 genes) genes were used as the basis of a prognostic signature that was applied to 2 independent breast cancer cohorts. While the datasets were not derived from breast cancer patients, the authors found that the application of, and supervised hierarchical clustering in, these two breast cancer datasets did indeed have prognostic import. Unsupervised hierarchical clustering broadly identified two clusters of patients and when Kaplan-Meier survival analysis was employed, the radiation-induced and radiation-repressed gene signatures were significantly associated with local recurrence. In addition, the authors identified biological pathways associated with radiation response, including p53 responsive and proliferative genes.

Another early predictive signature was an interferon-based signature that was developed to predict response not only to ionizing radiation, but also to DNA-damaging chemotherapies [13]. This group developed the interferon-related DNA damage resistance signature based on genes associated with radiation resistance in a single squamous cell carcinoma cell line model and subsequently demonstrated that this signature was predictive of response to chemotherapy and ionizing radiation in women with breast cancer. They further refined the signature to include a 7-gene-pair classifier (from the original panel of 49 differentially expressed genes) that predicted the efficacy of adjuvant chemotherapy as well as local control after radiation treatment. Despite this early development, subsequent translation into a clinically relevant test has not been detailed.

Another group simply applied previously developed molecular signatures which were either prognostic of outcomes for metastasis-free or overall survival endpoints or predictive of response to systemic chemotherapy in breast cancer and evaluated the utility of these signatures to predict locoregional disease control. Although many of these previously developed signatures proved to be able to predict local control after radiation treatment, a previously developed "wound response" signature was found to be most prognostic of locoregional disease control in women treated with postoperative radiation therapy [32].

In addition to radiation-induced gene response signatures, other groups have looked at signatures that may predict response to radiation treatment, though not necessarily for breast cancer. These signatures include hypoxia-related signatures [33–35], cell cycle and DNA damage gene related signatures [36, 37], and signatures predictive of response to radiosensitizing drugs [38–40]. As with many of the other *in vitro* derived signatures, none of these signatures has thus far withstood the rigors of external validation and thus has not yet been translated into the clinic.

3. Radiation Intensification Signatures

While intriguing at the basic biology level, as noted previously these radiation-gene induced signatures have not been readily translated into clinically useful tests for women with breast cancer. Other groups have taken an alternative approach to identify patients who need treatment intensification. Indeed, a potential additional use of molecularly based signatures is to identify women for whom standard trimodality therapy (surgery, chemotherapy, and/or radiation) is insufficient. If these signatures were significantly robust in identifying women likely to fail despite radiation treatment (because of, e.g., radioresistance inherent in the tumor), more aggressive or alternative treatment strategies

might be employed. One of the first potentially useful signatures was developed by investigators in Sweden. They analyzed gene expression differences between 143 early-stage breast cancer patients all of whom were treated with radiation after surgery to identify gene signatures prognostic of local recurrence in patients treated with radiation [41]. This group identified an 81-gene signature (the 81 genes being selected as the "most strongly associated" with recurrence) and found that this signature could outperform clinical and pathologic characteristics alone for prognostication of local recurrence events. Another similarly designed study from Dutch investigators performed gene expression microarray profiling to determine differently expressed genes between tumors from women who developed local recurrence after breast conserving surgery and radiation and women who did not [25]. In this institutional study, 56 primary invasive breast carcinomas from patients with a local recurrence were profiled and compared to 109 tumors from patients who did not experience a local recurrence. This study identified over 7,000 significantly regulated genes and performed supervised and unsupervised hierarchical clustering to further refine the list to 104 genes associated with recurrence [25]. This list was significantly enriched for genes involved in cell proliferation, and then a subsequent classifier of local recurrence was built using a 111-gene list that was shown to be independently associated with local recurrence using a multivariate Cox regression analysis. Despite early potential and enthusiasm, both the Swedish and the Dutch signatures failed external validation in datasets in which they were not trained, and other investigators have reported a similar inability to derive a radiation response signature using gene expression from patient tumors as the basis of their signature development [10, 42]. Other investigators have focused on the development of prognostic signatures in breast cancer that are associated with local recurrence after surgery alone and which are independent of radiation treatment, but, like the Swedish and Dutch counterparts, these signatures have not performed well in external validation [10]. A different approach was employed by investigators at the University of Michigan who used breast cancer cell line expression and radiation response data as the basis for the development of a radiation response signature [11]. This radiation response signature was found to be independent of breast cancer intrinsic subtype, and when applied to two independent breast cancer cohorts of patients with breast cancer treated with breast conserving surgery and radiation treatment, the signature was able to identify patients likely to recur despite surgery and radiation and may also have the potential to identify women for whom radiation is altogether unnecessary. The resulting signature, termed RadiotypeDx®, to connote potentially similar application for radiation as OncotypeDx is currently used for systemic therapy, has been externally validated in additional datasets, and now is being studied in previously completed phase III randomized trials in low-risk, early-stage breast cancer patients treated with breast conserving surgery randomized to +/− radiation treatment. While encouraging, this signature also will require true external validation either as part of a prospective randomized trial or with "prospective retrospective" analyses from previous phase III trials before it can be adopted into clinical practice.

4. Radiation Omission Signatures

While many signatures have been developed aimed at predicting the sensitivity to, or efficacy of, radiation in breast cancer, data from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis suggests that the majority of women with early-stage breast cancer treated with breast conserving surgery are cured of their disease with surgery and endocrine therapy alone [43]. Thus, the search for a signature that may identify these extremely low-risk women for whom radiation treatment is unnecessary has been an area of active interest. While there are no true "radiation omission" molecular signatures currently reported for women with invasive breast cancer, numerous groups are working to validate previously derived molecular classifiers as sufficient surrogates for appropriate radiation treatment omission. Indeed, there are four ongoing observational or investigational trials looking at OncotypeDx (IDEA study), Prosigna (PRECISION trial), IHC (LUMINA trial), and IHC4 (PRIMETIME trial) scores as sufficient stratification methods for radiation omission. The Individualized Decisions for Endocrine Therapy Alone (IDEA) study is an investigational trial testing whether an OncotypeDX recurrence score ≤ 18 is sufficient to identify women who may safely omit radiation treatment. In this study with a target accrual of 200 patients, postmenopausal women between the ages of 50 and 69 with early-stage disease will enroll on a trial that omits radiation treatment for women with the low (≤18) OncotypeDx score. These women will receive adjuvant endocrine therapy, and rates of locoregional recurrence, metastasis, and overall survival as well as type of salvage therapy will be tracked, with follow-up planned for 10 years and the primary endpoint being 5-year locoregional recurrence-free survival. In a similarly designed phase II trial, the Profiling Early Breast Cancer for Radiotherapy Omission (PRECISION) trial is looking at whether women 50–75 years of age with a low-risk score based on Prosigna testing are safely able to omit adjuvant radiation after breast conserving surgery. This trial will study the outcomes of 1380 patients with low-risk scores treated with lumpectomy and endocrine therapy without radiation; 5-year rates of locoregional recurrence will be tracked as the primary endpoint of the study. The LUMINA trial, being run by investigators in Canada, is a prospective cohort study evaluating the risk of local recurrence after breast conserving surgery and adjuvant endocrine therapy for women with Luminal A subtyped breast cancer. With a target accrual of 500 women, patients with Luminal A subtype breast cancer (as defined by ER, PR, HER2, and Ki-67 staining, not PAM50 assessment) will be treated with endocrine therapy (tamoxifen or aromatase inhibitor) for five years and will not be treated with breast irradiation. The primary endpoint is local recurrence at 5 years and patients will be followed up for 10 years with other endpoints, including new primary cancers and event-free and overall

survival, also being measured. Finally, investigators in the UK are looking at whether IHC4 testing (which combines protein expression of estrogen receptor, progesterone receptor, HER2, and Ki67) in addition to clinical factors can be used to identify patients at low risk for local recurrence in the absence of breast radiotherapy such that radiation can be safely omitted. This trial, called the PRIMETIME trial, is a prospective biomarker-directed case-cohort study in which the IHC4 + C score determines whether radiotherapy is recommended. In this trial, 2,400 patients will be recruited to yield 1550 patients to be treated without radiotherapy. As with the IDEA trial, 5-year local control is the primary endpoint with plans to follow patient outcomes for 10 years [44]. In addition to these trials, various groups are working to develop radiation omission signatures specific to breast cancer patients, and in the coming 5–10 years the results of these and other planned studies will determine whether molecularly based "risk stratification" signatures are able to identify patients for whom adjuvant radiation treatment is unnecessary.

5. "Pan-Cancer" Signatures

Rather than developing a breast cancer-specific signature of radiation response, some groups have focused on the development of a "pan-cancer" genomic signature of radiation response. One of the first reports was from a group at the Moffitt Cancer Center. In these initial studies, cell line sensitivity to ionizing radiation was evaluated across 35 and then 48 cell lines in the NCI-60 panel of cancer cell lines [12, 45]. Genes associated with intrinsic radiation response were then identified and formed the basis of the subsequently developed radiation sensitivity index (RSI), which has been refined to a 10-gene, RNA expression based signature. After additional modifications of the signature, the group has assessed the utility of RSI in various disease types with varying levels of utility identified [8, 29, 30, 46]. A similar approach was utilized by investigators at Columbia University that utilized the same NCI-60 cell line panel to identify radiation response-associated genes [47]. Like the Moffitt group, they identified genes whose basal expression was different between the radiosensitive and radioresistant cell lines and identified a total of 36 genes differentially expressed between these two groups. Unlike the Moffitt group, however, they further identified genes whose expression changed after radiation treatment and were associated with survival after varying doses of radiation treatment. Interestingly, they found that the genes induced (by RNA expression profiling) by radiation were remarkably consistent between tumor types and were a function of p53 status, suggesting an underlying conserved set of genes responsible for responding to genotoxic stress. Comparing the gene lists identified by the Moffitt and Columbia groups, there was surprisingly no overlap between the genes identified in these studies, suggesting that the response to radiation treatment (at least in terms of RNA expression) may be complicated and differs under basal and genotoxic conditions.

Additional genomic classifiers and signatures predictive of response to radiation are currently being developed for prostate, lung, rectal, anal, glioblastoma, and head and neck cancers. As with signatures for breast cancer, these classifiers will need to be externally validated using phase III trials prior to adoption into clinical practice, and it remains to be seen whether a more global "pan-radiation" response signature (discussed previously) can be developed and validated for use in women with breast cancer.

6. Tailored Radiation Signatures

In an effort to move towards truly personalized radiation treatment, efforts are also underway to utilize the genomic and transcriptomic information from a woman's own tumor to determine, on an individual patient level, whether radiation is likely to be effective and, if so, what dose may be sufficient for this patient. A recent provocative publication suggests that there may be methods available to achieve this level of personalization. As was discussed earlier, the Moffitt group developed a radiation sensitivity index (RSI) to determine the intrinsic sensitivity of tumors to ionizing radiation [12]. Expanding upon that initial work and to determine ways in which to tailor radiation doses across differing cancer histologies (including invasive ductal carcinoma), Scott et al. recently reported results of their effort to develop a genomic-adjusted radiation dose (GARD) framework as the basis for future radiation trial design [9]. To develop GARD, the investigators incorporated their previously developed radiation sensitivity index (RSI) with the linear-quadratic model to derive the genomic-adjusted radiation dose and used this to calculate the GARD-value for over 8000 tumor samples. In this retrospective analysis, GARD appeared to predict clinical outcome in several cancer types. While limited by an incomplete evaluation in patients who have not received radiation treatment (to evaluate whether GARD has more than just prognostic value) and lack of external validation, this approach does hold promise and is worthy of continued investigation. It also underscores the potential utility of a genomic-based radiation response signature that may be used to assess, prior to the initiation of treatment, the likelihood of tumor response and control of disease with radiation treatment.

In addition to the development of GARD, a more comprehensive assessment was done by investigators at the Cleveland Clinic and the Broad Institute who sought to understand the genetic basis of DNA damage response after radiotherapy [14]. In this study, investigators profiled the radiation response of over 500 cell lines to ionizing radiation and showed that sensitivity to radiation is characterized by significant genetic variation across and within lineages (in contrast to the Moffitt group, whose work suggests that a 10-gene signature may be sufficient to describe radiation response across all disease types and tumor lineages). In addition to identifying genes whose expression was associated with response, they also identify somatic copy number alterations and gene mutations that correlate with the radiation survival. This work offers arguably a more comprehensive

(and a more complicated) picture of the genomic basis of radiation response and may serve as a foundation for future signature development to predict response of various tumors to radiation treatment across all cancers.

7. Barriers to Clinical Adoption

Although numerous in development, molecular signatures predictive of radiation response have been slow to gain clinical traction. Reasons for their slow uptake include a lack of external validation, feasibility concerns in a clinical setting, and nonspecificity for the clinical situation at hand. As was noted previously, most of the radiation response signatures generated to date have failed external validation in cohorts in which they were not trained. This may reflect factors such as variation in the clinic-pathologic characteristics of the patients represented in these cohorts, differences in radiation treatment techniques and doses, and imbalances between the treated and untreated patients. It may also reflect the heterogeneous biological underpinnings of radiation response in breast cancer, suggesting that these signatures are unable to account for the complexity of the radiation response. Additionally, variation in gene expression measurements and normalization between fresh frozen, formalin fixed, and paraffin embedded tissues has proved challenging as previously developed tests have transitioned from research endeavor to clinical implantation. This same challenge has limited the scalability of these molecular signatures. Furthermore, translating research-lab derived molecular signatures into a clinically available test is not a trivial task. Aside from barriers with cross-platforming of molecular tests, developing standard operating procedures and certification in accordance with Clinical Laboratory Improvement Amendments (CLIA) is often costly and fraught with regulation meant to safeguard patients. Taken collectively, these reasons help explain why so many tests have been reported in the academic literature but so few have successfully been translated into clinical practice.

8. Future Directions

Much as the development of molecularly based signatures (OncotypeDx, MammaPrint, Prosigna, etc.) has revolutionized the decision-making process surrounding the need for adjuvant chemotherapy in women with early-stage breast cancer, the development of prognostic and predictive signatures to determine the need and efficacy of radiation for women with breast cancer holds similar promise. While preliminary efforts to develop these signatures has been encouraging, much work remains in order to successfully translate these signatures into the clinic. Whether RSI, OncotypeDX for Breast DCIS, GARD, RadiotypeDx, or other as-yet developed signatures will gain similar traction remains to be seen. Ultimately, for any of these tests to be translated into the clinic it will require demonstration of their accuracy and reproducibility as a test and perhaps more importantly demonstration within the context of clinical trials of the utility of these tests at improving outcomes for women with breast cancer. While not yet realized, the ongoing development of these signatures holds much promise as the field seeks to finally realize "personalized medicine" as it relates to radiation treatment for women with breast cancer.

Disclosure

Corey Speers and Lori J. Pierce hold patents and are cofounders of PFS Genomics.

Conflicts of Interest

The authors declare no conflicts of interest related to this paper.

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Research Article

The Immunoexpression of Glucocorticoid Receptors in Breast Carcinomas, Lactational Change, and Normal Breast Epithelium and Its Possible Role in Mammary Carcinogenesis

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The role of estrogen and progesterone receptors in breast cancer biology is well established. In contrast, other steroid hormones are less well studied. Glucocorticoids (GCs) are known to play a role in mammary development and differentiation; thus, it is of interest to attempt to delineate their immunoexpression across a spectrum of mammary epithelia. *Aim*. To delineate the distribution pattern of glucocorticoid receptors (GRs) in malignant versus nonmalignant epithelium with particular emphasis on lactational epithelium. *Materials and Methods*. Immunohistochemistry (IHC) for GRs was performed on archival formalin-fixed paraffin-embedded tissue blocks of 96 cases comprising 52 invasive carcinomas, 21 cases with lactational change, and 23 cases showing normal mammary tissue histology. *Results*. Results reveal an overexpression of GRs in mammary malignant epithelium as compared to both normal and lactational groups individually and combined. GR overexpression is significantly more pronounced in HER-2-negative cancers. *Discussion*. This is the first study to compare GR expression in human lactating epithelium versus malignant and normal epithelium. The article discusses the literature related to the pathobiology of GCs in the breast with special emphasis on breast cancer. *Conclusion*. The lactational epithelium did not show overexpression of GR, while GR was overexpressed in mammary NST (ductal) carcinoma, particularly HER-2-negative cancers.

1. Introduction

Glucocorticoid receptors (GRs) are expressed in about 50% of invasive breast cancers and many breast cancer cell lines [1, 2]. Glucocorticoids (GCs) are known to play a role in mammary development and differentiation as well as an essential role in embryonic development and tissue homeostasis. They possess important anti-inflammatory and immunosuppressive properties [1, 3, 4].

The endocrine system coordinates the development of the mammary gland with reproductive development and the demand of the offspring for milk. Reproductive hormones—estrogen and progesterone—act directly on the mammary gland to bring about developmental changes.

Massive tissue remodeling occurs within the mammary gland during pregnancy. This results in the formation of the secretory lobuloalveolar units in preparation for lactation. Prolactin and progesterone are implicated in the initial proliferative phase of alveolar morphogenesis. Other hormones such as growth hormone and placental lactogen can influence alveolar morphogenesis [5]. The secretory activation stage of mammary gland development occurs after parturition and

converts inactive lobuloalveoli to active milk secretion. This process is triggered by progestin withdrawal and depends upon augmented prolactin signaling [6]. Prolactin induces mammary gland development and lactogenesis. Binding of prolactin to its receptor leads to the phosphorylation and activation of Signal Transducers and Activators of Transcription (STAT) proteins, which in turn promote the expression of specific genes and are essential for mammopoietic and lactogenic signaling [7].

Metabolic hormones such as GCs, whose main role is to regulate metabolic responses to nutrient intake or stress, often have direct effects on the mammary gland as well. An understanding of the mechanisms by which hormones such as GCs bring about secretory and lactational differentiation may offer clues to the prevention of breast cancer as studies have linked full-term pregnancies in early life with reduction of the likelihood of breast carcinogenesis [8].

Mammary epithelial cells do not attain full differentiation until the advent of pregnancy. With the establishment of lactation, mammary epithelial cells undergo further differentiation [9, 10]. Hence, a mammary cell differentiation spectrum includes lactational change epithelium representing the most differentiated cell on the one hand and malignant epithelium representing the least differentiated cell on the other hand, with normal "resting" epithelium in between.

It is therefore of interest to answer the following questions: Since GCs are one of the hormones involved in the process of lactogenesis resulting eventually in terminal differentiation of the cell (lactational change), is the opposite true? Do they protect the cell from moving towards the least differentiated end of the spectrum (malignant change)?

Our aim is to study the immunoexpression of GRs along the aforementioned differentiation spectrum which may shed some light on the influence of GCs on carcinogenesis, if any.

In addition, we aim to study the relationship of GR expression with grade, estrogen receptor (ER), progesteron receptor (PR), and human epidermal growth factor receptor-2 (HER-2) expression status and axillary lymph node (ALN) status within the malignant group.

2. Materials and Methods

A retrospective study was performed on archival formalin-fixed paraffin-embedded tissue blocks retrieved from files of the Histopathology Department, Salmaniya Medical Complex, Kingdom of Bahrain, between 2001 and 2007.

A total of 96 cases were included in this study: 52 in the malignant group (invasive no special type (NST) ductal carcinomas) and 44 in the nonmalignant group (21 cases with lactational change and 23 cases with normal mammary tissue histology). The lactational change cases were compiled over time in our department. The method of compilation was that a representative block was kept from cases that show lactational change during routine practice. These were kept aside for future studies including this study. No accompanying clinical information was available. The reason for this approach was that lactational change is not routinely coded by Systematized Nomenclature of Medicine (SNOMED) by practicing pathologists, and it would have been difficult to retrieve retrospectively from the archives of the department by SNOMED diagnosis code.

Both normal and lactational groups comprised the nonmalignant group (44 cases) and were all taken from excisions performed for reduction mammoplasties or benign pathology. The latter comprised fibrocystic changes of the nonproliferative type, fibroadenomata, sclerosing adenoses, and inflammatory conditions such as organizing inflammation or organizing abscesses. Cases that harbored usual type hyperplasia (UTP), atypical ductal hyperplasia (ADH), or any grade of lobular neoplasia were excluded from the study.

In the malignant group, carcinomas other than (NST) ductal carcinomas, cases with incomplete hormonal immunohistochemical status, and cases for which representative tumor blocks were not available in the files of the department were all excluded from the study. Carcinomas other than (NST) ductal carcinomas were excluded from the study in order to reduce the bias of variability attributed to tumor type and hence to have as uniform an immunoexpression result as possible within the malignant group.

2.1. Immunohistochemistry. 5 μm sections were cut and mounted on saline-coated slides, dried, deparaffinized in xylene, and rehydrated in alcohol. Endogenous peroxidase was quenched by 3% hydrogen peroxidase for 10 minutes followed by heat antigen retrieval in 0.01 M citrate buffer, pH 6.0, in a microwave oven at a medium oven setting. Slides were incubated overnight at 4°C with mouse monoclonal [3D5] to glucocorticoid receptor (ab9568) (Abcam, UK) in 1 : 100 dilution. Immunoreaction was detected and visualized by using a DakoCytomation LSAB2 System (HRP code K0673). Positive cases were determined by exhibiting a focal or diffuse moderate to dark brown nuclear staining pattern in more than 10% of the cells. The percentage of cases showing positive GR expression was calculated for various categories of mammary epithelium.

Other parameters were extracted from the pathology reports of the cases such as tumor grade, ER, PR, and HER-2 expression, and axillary lymph node (ALN) status. IHC for ER and PR were determined by the Histoscore method (H score). Positivity ranged from 20/300 to 300/300. HER-2 was determined by scoring membranous staining as negative (0/1+), equivocal (2+), or positive (3+).

GR expression was compared between the malignant and the lactational groups and the malignant and the nonmalignant groups. Within the malignant group, GR expression was further analyzed according to grade, ER, PR, HER-2, or axillary lymph node (ALN) status. Analysis of GR expression according to HER-2 status excluded cases that showed HER-2 equivocal results by IHC.

Data was analyzed by using the Statistical Package for the Social Sciences (SPSS), version 15. Data was grouped into categories and analyzed for correlations using Pearson's Chi-Square test.

3. Results

The immunoexpression of GR among the malignant group was significantly higher than that of the normal and

Table 1: Immunoexpression of GR in various categories of mammary epithelium.

Category	GR status		Total	% positivity	p value
	Positive	Negative			
Malignant	51	1	52	98.0%	<0.001
Normal	10	13	23	43.5%	
Lactational	12	9	21	57.1%	

Table 2: Immunoexpression of GR in malignant versus nonmalignant mammary epithelium.

Category	GR status		Total	% positivity	p value
	Positive	Negative			
Malignant	51	1	52	98.0%	<0.001
Nonmalignant	22	22	44	50.0%	

Table 3: Immunoexpression of GR in the malignant mammary epithelium according to HER-2 status.

Category	GR status		Total	p value
	Positive	Negative		
Positive HER-2	3	1	4	0.002
Negative HER-2	36	0	36	
Total	39	1	40	

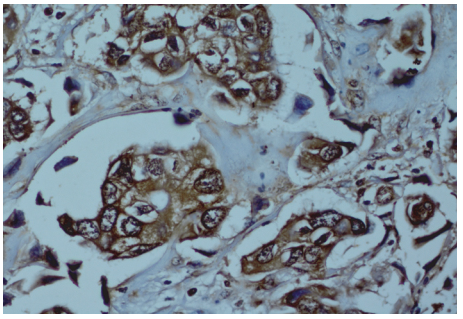

Figure 1: Infiltrating NST (ductal) carcinoma cells with granular brown staining of the nuclei. Background brown staining of the cytoplasm is also noted. IHC of GR (×400).

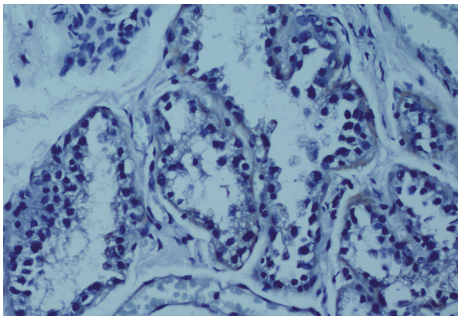

Figure 2: Negative staining for GR in lactational epithelium. IHC of GR (×400).

lactational groups ($p < 0.001$) (Figures 1 and 2 and Table 1). When both the normal and the lactational groups were combined and compared together with the malignant group, statistical significance of GR expression was maintained ($p < 0.001$) (Table 2). In addition, a statistically significant correlation was noted between the GR expression and the HER-2 status as 92% of the GR-positive samples were HER-2-negative and the remaining 8% were HER-2-positive. GR expression appears to be higher in the HER-2-negative compared to the HER-2-positive tumors (p value: 0.002) (Table 3). Of the HER-2-negative tumors, 25 cases (69.44%) were also negative for both ER and PR (triple negative). No statistically significant difference was noted for the expression of GR in tumors categorized according to grade, ER, PR, or ALN status (p values: 0.331, 0.322, 0.246, and 0.517, resp.).

4. Discussion

Glucocorticoid receptor (GR) is a steroid hormone receptor known to influence many metabolic processes in the body including mammary development and differentiation. It is one of many factors that influences proliferation and differentiation of mammary epithelium during pregnancy and lactation. Its exact role however in carcinogenesis needs further delineation [3].

The results of this study reveal an overexpression of GR receptors in mammary malignant epithelium compared to nonmalignant tissue. This is in line with other studies [1, 2]. The action of GCs on the prepartum proliferative stage, rather than the late postpartum lactogenic stage [8], could explain the lack of overexpression of GRs in lactating epithelium in this study.

Our study is unique in that it is the first study to compare GR expression in lactating epithelium versus malignant and benign epithelium. The proliferative influence of GCs on mammary epithelial cells might explain GR overexpression in malignant mammary epithelium (the least differentiated tissue) as compared to the lack of overexpression in both lactating epithelium (most differentiated) and normal epithelium ("resting" stage).

Previous studies on mammary epithelium have shown that lobules in the lactation period of the mammary gland represent the maximal expression of development and differentiation [11, 12], hence with the least susceptibility to the influence of proliferative hormones such as GC. This further explains our results.

Other studies have implicated an antiapoptotic effect as a possible mechanism of the influence of GCs on breast cancer [13–15], affecting its initiation and progression [16, 17]. Others suggested that GCs can attenuate estrogen responses, but the mechanism by which GCs inhibit estrogenic activity is unknown. It was suggested that activation of GR by dexamethasone induces the expression and activity of estrogen sulfotransferase, an enzyme important for the metabolic deactivation of estrogen. This may have implications in therapeutic development for breast cancer [18].

Studies in the literature point to the implication of GCs in cancer progression, particularly breast cancer. An operational glucocorticoid receptor system in breast tissue was found to influence breast cancer development [19–21]. This lends additional support to the results of our study.

Other studies detailing the relationship between GCs and breast cancer included those that explored the importance of stress and its association with cancer progression, particularly breast cancer. These studies indicated that stress enhances glucocorticoid synthesis, which alters inflammation and immune responses, as well as cellular proliferation and apoptosis in a number of tissues [19–21]. In addition, activating GR-mediated tumor cell survival pathways may occur following the administration of synthetic glucocorticoids as part of chemotherapy treatment premedication, and this has the potential to diminish chemotherapy's effectiveness. Hence, the study of potential selective GR modulators may be of benefit in preventing chemotherapy associated side effects without promoting cell survival [1, 4, 13, 22–25]. In our study, we did not attempt to explore the association of GR-positive breast cancer and the response of those patients to steroid-based prechemotherapy medications, as such data was not readily available. This could be the subject of future studies to determine the level of caution that is needed, if any, in the use of steroid therapy in such category of patients.

Our study also showed GR overexpression in HER-2-negative as compared to HER-2-positive breast cancers, of which 69.44% were triple negative. This is supported by other studies which showed GR overexpression in triple negative breast cancer (TNBC) [26, 27]. This might explain chemoresistance in this group, thus opening a potential window for a different therapeutic strategy such as GR antagonists in a specific subgroup of patients such as chemotherapy-resistant GR-positive TNBC [27, 28]. GC was also found to possess a potent survival pathway in the immortalized human mammary epithelial cell line MCF10A. The mechanism through which GC inhibits apoptosis is independent of phosphatidylinositol 3-kinase activity and its downstream target Akt, thus establishing the existence of a novel epithelial cell survival pathway mediated by GCs [14].

GR immunoexpression in tumors categorized according to grade, ER, PR, or ALN status showed no statistical difference in our study. A similar study by Buxant et al. however showed a significant correlation between the histologic grade and the GR immunoexpression where the latter decreased significantly with increasing tumor histologic grade [29].

In ER-positive breast cancer, it seems that GR positivity imparts a tumor suppressor effect [30] with resultant better prognosis [31, 32]. Further immunohistochemical studies are needed to explore these relationships as studies available currently deploy other methodologies such as those studies carried out by Smith et al. where they found that GR significantly increases mRNA levels in the stroma of estrogen receptor negative tumors and an inverse relationship between sex steroid hormone receptor and GR gene expression in human breast cancer cell lines, respectively [33, 34]. Furthermore, Kinyamu and Archer showed that cross talk between the GR and ER involves multiple signaling pathways indicative of the mechanistic diversity within steroid receptor-regulated transcription [35].

Studies also showed that estrogen increases the expression of protein phosphatase 5 (PP5), which mediates the dephosphorylation of GR at Ser-211. After PP5 knockdown, estrogen-promoted cell proliferation was significantly suppressed by glucocorticoids. Thus, PP5 inhibition may antagonize estrogen-promoted events in response to corticosteroid therapy. This is supported by the fact that the course of some inflammatory diseases tends to be more severe and less responsive to corticosteroid treatment in females [36].

In our study, no attempt was made to correlate lactational changes with history of pregnancy, lactation, or duration of lactation as this study concentrated merely on cellular level changes in lactational epithelia versus other types of epithelia as explained above. This is one of the limitations of this study; however, since the results of this study show lack of overexpression of GRs in lactational change epithelium, further studies concentrating on mammary carcinoma rather than lactational change are recommended. Another limitation of this study is that the malignant group comprised NST (ductal) carcinoma cases only. Subsequent studies could concentrate on delineating GR immunoexpression in a range of breast cancer types.

Further studies are also required to support the value of including GR expression in the algorithm of breast cancer testing, to test whether ER+ GR+ tumors have better prognosis and to test whether ER– GR+ tumors are more likely to develop chemoresistance and hence might benefit from anti-GR therapy.

5. Conclusion

Lactational epithelium did not show overexpression of GR, while GR was overexpressed in mammary NST (ductal) carcinoma, particularly HER-2-negative cancers. Further studies

are required to explore the possibility of using such receptors as targets for the development of therapeutic interventions.

Disclosure

The abstract of this article was accepted as an oral presentation at the 12th International Conference on Laboratory Medicine & Pediatric Pathology, 15-16 March 2017, London, UK, and was chosen as a keynote speech.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Research Article

Are Patients Traveling for Intraoperative Radiation Therapy?

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Purpose. One benefit of intraoperative radiation therapy (IORT) is that it usually requires a single treatment, thus potentially eliminating distance as a barrier to receipt of whole breast irradiation. The aim of this study was to evaluate the distance traveled by IORT patients at our institution. *Methods*. Our institutional prospective registry was used to identify IORT patients from 10/2011 to 2/2017. Patient's home zip code was compared to institution zip code to determine travel distance. Characteristics of local (<50 miles), regional (50–100 miles), and faraway (>100 miles) patients were compared. *Results*. 150 were patients included with a median travel distance of 27 miles and mean travel distance of 121 miles. Most were local (68.7%), with the second largest group living faraway (20.0%). Subset analysis of local patients demonstrated 20.4% traveled <10 miles, 34.0% traveled 10–20 miles, and 45.6% traveled 20–50 miles. Six patients traveled >1000 miles. The local, regional, and faraway patients did not differ with respect to age, race, tumor characteristics, or whole breast irradiation. *Conclusions*. Breast cancer patients are traveling for IORT, with 63% traveling >20 miles for care. IORT is an excellent strategy to promote breast conservation in selected patients, particularly those who live remote from a radiation facility.

1. Introduction

Over the last 10 years, an increasing number of breast cancer patients are receiving intraoperative radiation therapy (IORT) [1]. One of the major benefits of IORT is the delivery of radiation in a single setting rather than multiple visits over several weeks as required for whole breast irradiation (WBI). This time consideration is very important for patients as traveling for daily WBI has been shown to have a significant impact on psychological, financial, work, and social aspects of their lives [2, 3].

In addition, prior studies have demonstrated that rates of breast conservation and radiation therapy compliance are inversely related to patients' proximity to a radiation facility. These associations have been shown at state and national levels, as well as on an international scale [4–9]. Travel distance continues to be a barrier to care which is not improving with time, despite increasing numbers of hospitals and radiation centers across the United States [10]. The shortened radiation course offered by IORT has the potential to decrease this barrier to care and promote breast conservation in individuals who live far from a radiation facility and cannot travel for daily radiation treatments.

To the author's knowledge, the distance traveled by patients who undergo IORT in the United States has not previously been assessed. The aim of this study was to evaluate the average travel distance for patients treated with IORT at our institution, to assess patient and tumor factors associated with increased travel distance, and to determine if the distance traveled by patients changed over time.

2. Materials and Methods

Patients who undergo IORT at our institution are enrolled in an IRB-approved prospective data registry. Patient information is entered and maintained by the surgeon, radiation

oncologist, and research coordinators. Candidates for IORT are identified after multidisciplinary consultation and treated with the Zeiss Intrabeam system at the time of their initial breast cancer operation. Preplanned IORT boost is not routinely offered. The addition of WBI following surgery is determined after multidisciplinary discussion upon review of the final surgical pathology.

The institution registry was used to identify patients who underwent IORT from 10/2011 to 2/2017. Data obtained included patient demographics, tumor characteristics, and adjuvant therapy (chemotherapy and WBI). Patient income, education, employment, marital status, and insurance status have been associated with receipt of radiation in prior studies; however, these factors are not included in the registry and thus were not available for analysis. To determine travel distance, the patient's home zip code was compared to the institutional zip code using Google Maps. The shortest route was recorded as the patient's travel distance, in miles.

When designing the study, we purposefully elected to not include a comparison group of WBI patients. Our institution has numerous excellent WBI treatment centers throughout the region such that when WBI patients are seen at our tertiary facility for initial consultation, their actual WBI care is often coordinated purposefully at the closest WBI location to their home or work. Thus the travel distance for WBI patients at our institution is potentially confounded by bias with this purposeful redistribution of treatment locations, which could skew the results of a side-by-side comparison of IORT and WBI patients. With this in mind, we felt it was most sound to instead provide a detailed analysis of the IORT patients' travel distance and avoid potential for bias that would be introduced with a WBI comparison group.

For analysis, patients were subdivided into three groups: local (<50 miles), regional (50–100 miles), and faraway (>100 miles). The groups were compared to determine any differences. Patient characteristics in each group were reported as counts and percentages or mean with standard deviation, where appropriate. After comparing patient groups by travel distance, the median and average distance traveled by treatment year were compared to determine any trends over time. For analysis, two-tailed Chi-squared and Fisher's exact tests [R software (v3.31, 2016-0-21)] were used with p value < 0.05 being considered statistically significant.

3. Results

Registry review identified 150 women for study inclusion. The average age was 70.8 years, and most were Caucasian (89.3%). The average tumor size was 1.0 cm, with most patients having ER positive (99.3%), PR positive (87.3%), HER2 negative (99.3%), invasive ductal cancers (60.6%), and N0 disease (99.3%). Only 3% of patients received adjuvant chemotherapy. Three individuals (2%) had adjuvant WBI due to a close margin ($n = 1$), positive sentinel node on final pathology ($n = 1$), and multifocal disease in the surgical specimen ($n = 1$).

The median travel distance for all patients from home to the treatment facility was 27 miles. The mean travel distance was 121 ± 284 miles. Most patients were local ($n = 103$, 68.7%), with the second largest group living >100 miles away ($n = 30$, 20.0%). The remaining 17 patients (11.3%) traveled from a regional distance for treatment. Subset analysis of the local group indicates that 21 patients (20.4%) traveled <10 miles, 35 patients (34.0%) traveled 10–20 miles, and 47 patients (45.6%) traveled 20–50 miles. Thus, the majority of IORT patients (94/150, 63%) traveled at least 20 miles for treatment. In the faraway group, six patients (4%) traveled more than 1000 miles.

The three distance groups did not differ with respect to age, race, tumor characteristics, or adjuvant WBI (Table 1). However, the groups did differ with respect to adjuvant chemotherapy with patients living faraway receiving adjuvant chemotherapy more frequently despite similar tumor profiles between the groups (Table 1).

When comparing the travel distances by treatment year, there was no difference in median or mean distance traveled for patients treated across the years (Table 2).

4. Discussion

Breast cancer patients who undergo IORT are commonly traveling for treatment, with 63% of patients in this series traveling more than 20 miles for care. The national average distance that breast cancer patients travel to the nearest radiation facility is 4.8 miles [4, 11], and drop-off in breast conservation rates and radiation treatment use have been documented once patients must travel more than 9–25 miles [4, 10]. The median distance traveled by IORT patients in this study (27 miles) is more than 5x the previously reported average distance for individuals receiving WBI, indicating patients are willing to travel for the benefits offered by IORT.

The majority of patients in this study passed multiple other breast cancer centers along the way to our institution, including six National Accreditation Program for Breast Centers (NAPBC) sites within a 20-mile radius [12]. This documented increased travel distance is in striking contrast to a recent publication which showed that patients frequently choose lower-volume institutions for care in order to shorten travel distance, despite worse outcomes [13]. As our institution is the only center to offer IORT within this radius, we suspect the availability of this technology may in part attribute to the large travel distances documented in this study. There were no patient or tumor factors identified in our study which were linked to travel distance so further research would be required to delineate socioeconomic or psychologic factors which may impact patient decision regarding travel for IORT.

Patients presenting to our institution commonly inquire about IORT and its potential benefits, including eliminating recurrent visits for radiation treatment. Coombs et al. documented significantly reduced travel time as well as significantly improved environmental impact for patients who undergo IORT in the UK [2]. In their study, the average travel time for WBI was 14 hours versus 3 hours for IORT patients. In TARGIT-R, the largest study to evaluate IORT use in North America, most patients (65.4%) underwent IORT as single-dose treatment at the time of their breast cancer operation [1], potentially saving 4–6 weeks of travel for WBI

Table 1: Comparison of patients who live local (<50 miles), regional (50–100 miles), and faraway from the treating institution.

	Local patients (<50 miles) $n = 103$ (68.7%)	Regional patients (50–100 miles) $n = 17$ (11.3%)	Faraway patients (>100 miles) $n = 30$ (20.0%)	p value
Mean travel distance (miles)	19.8	70.0	500.6	<0.001*
Age (years)	71.3	71.8	68.6	0.28
Race				
Caucasian	90 (90%)	17 (100%)	27 (93.1%)	0.34
African American	8 (8%)	0	0 (0%)	
Asian	1 (1%)	0	1 (3.5%)	
Other	1 (1%)	0	1 (3.5%)	
Tumor type				
IDC	63 (61.2%)	11 (64.7%)	17 (56.7%)	0.41
ILC	4 (3.8%)	0	1 (3.3%)	
Mixed	35 (34.0%)	5 (29.4%)	10 (33.3%)	
Other	1 (1%)	1 (5.9%)	2 (6.6%)	
ER positive	103 (100%)	17 (100%)	29 (96.7%)	0.31
PR positive	88 (85.4%)	15 (88.2%)	27 (93.1%)	0.59
HER2 positive	1 (1%)	0	0	0.34
Adjuvant chemotherapy	0	0	4 (14.3%)	0.002*
Whole breast radiation	2 (2%)	0	1 (3.6%)	0.67

* <0.05 = significant.

Table 2: Travel distances (in miles) by year.

	2014 $N = 31$	2015 $N = 42$	2016 $N = 45$	p value
Median travel distance	23	33	27	0.17
Mean ± standard deviation travel distance	99 ± 281	102 ± 216	99 ± 220	0.99

per patient. Relatively fewer individuals received IORT as a boost (27.4%) [1], potentially saving these patients one week of travel for WBI boost. In our study, an even higher rate of patients (147/150, 98.0%) required only single-dose IORT during their operation, thus reflecting an even greater relative time and travel distance savings per patient, with the lower rates of WBI likely due to careful patient selection.

Across time, the geographic distribution of IORT patients remained stable as reflected in the median and mean travel distance (Table 2). For this analysis, only 2014–2016 data was included to represent established years for the IORT program at our institution. The remaining years of patients were excluded due to low patient numbers as the IORT program was starting (2011–2013) and only partial year of data available based on the timing of analysis (2017). Going forward, the authors suspect that distance traveled by IORT patients will increase as more patients and providers learn about this technology and seek it out for individualized breast cancer care.

Complete financial analysis from patient and institution levels was outside the scope of this manuscript but would be a potential area of interest in future studies. Financial considerations for the patient may include cost of travel, cost of missing work or school for multiple radiation visits, or potential copayment/insurance costs for single versus multiple visit treatment protocols. Future studies may also help to clarify what role, if any, financial considerations played for patients who had increased travel distance as documented in this study.

In summary, our study is the first to assess travel distance for IORT patients in the United States and demonstrates that patients are traveling for the benefits offered by IORT. It is important for clinicians to recognize the potential for IORT to decrease travel burden, promote breast conservation and radiation compliance, and eliminate a major barrier to care for appropriately selected breast cancer patients.

5. Conclusion

Breast cancer patients are traveling for the benefits offered by IORT, with 63% of patients traveling more than 20 miles for treatment. IORT is an excellent strategy to help decrease a barrier to care (distance), thus promoting breast conservation in selected patients who live remote to a radiation facility or those who cannot commit to daily radiation treatments.

Conflicts of Interest

Dr. Stephen Grobmyer has received travel support and research support from Zeiss Meditec. Dr. Chirag Shah is

a scientific consultant for Impedimed Inc. Dr. Stephanie Valente has received speaker and travel support from Zeiss Meditec.

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