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Individualising radiation therapy decisions in breast cancer patients based on tumour infiltrating lymphocytes and genomic biomarkers

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ABSTRACT

Radiation therapy (RT) has long been fundamental for the curative treatment of breast cancer. While substantial progress has been made in the anatomical and technological precision of RT delivery, and some approaches to de-escalate or omit RT based on clinicopathologic features have been successful, there remain substantial opportunities to refine individualised RT based on tumour biology.

A major area of clinical and research interest is to ascertain the individualised risk of loco-regional recurrence to direct treatment decisions regarding escalation and de-escalation of RT. Patient-tailored treatment with RT is considerably lagging behind compared with the massive progress made in the field of personalised medicine that currently mainly applies to decisions on the use of systemic therapy or targeted agents.

Herein we review select literature surrounding the use of tumour genomic biomarkers and biomarkers of the immune system, including tumour infiltrating lymphocytes (TILs), within the management of breast cancer, specifically as they relate to progress in moving toward analytically validated and clinically tested biomarkers utilized in RT.

1. Introduction

Reduction of treatment-related burden is becoming more and more important in modern oncology. Radiation therapy (RT) is a mainstay option in the curative setting for breast cancer patients. While substantial progress has been made in the anatomical and technological precision of RT delivery, and some approaches to de-escalate or omit RT based on clinicopathologic features have been successful, there remain substantial opportunities to refine individualised RT based on tumour biology. RT adds costs to health care systems and might induce late normal tissue effects including risk of ischemic heart disease [1] and secondary cancers [2] severely hampering quality of life. Absolute reduction in loco-regional recurrences (LRR) and improvement of overall survival (OS) is negligible in selected patient groups, there is a need to develop tools that identify patients who benefit from de-escalating RT without risking oncological safety. There are different options available to de-escalate RT such as the selective delivery of the boost dose to the lumpectomy cavity, the introduction of partial breast irradiation, the omission of treatment in appropriately selected patients with low-risk features, and the reduction of target volumes. All aiming to reduce the irradiated volume of the organs at risk and hence decreasing normal tissue effects.

Currently, there is a lack of reliable stratification tools to assist RT treatment decisions, and patients may be over-treated (e.g., when having an underlying low LRR risk) or under-treated. Within this framework, a major area of clinical and research interest is to ascertain the individualised risk of LRR to direct treatment decisions for postoperative RT, where both over- and under treatment of patients can be decreased

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by patient-tailored treatment and de-escalation of RT. Predicting the LRR risk for an individual patient is challenging. Several clinical and histopathological factors, such as age, tumour size, histological grade, lymphovascular invasion, estrogen-receptor status (ER), progesterone-receptor status (PR), human epidermal growth factor receptor-2 (HER2), resection margins and involvement of the axillary lymph nodes, are known predictors for local recurrence.

Gene expression profiling studies have identified prognostic gene expression profiles to predict outcome in breast cancer. For example, the 70-gene signature (MammaPrint®, Agendia NV, Amsterdam, the Netherlands) is a gene expression classifier used to predict the risk of distant recurrence and guide systemic therapy [3]. There has been a search *for gene expression profiles* focusing on benefit from RT. These could be either prognostic of LRR, predictive in terms of benefit of RT or associated with cellular radiosensitivity, but they only apply to standard RT, and only a few of them have been externally validated [4,5]. However, RT is considerably running behind compared with the massive progress made in the field of personalised medicine that currently mainly applies to decisions on the use of systemic therapy or targeted agents.

The *genomic-adjusted radiation dose* (GARD) is a non-breast cancer specific, tumour genomic biomarker introduced to personalise the radiation prescription dose. This model uses individual tumour genomics to calculate an individualised metric quantifying the biological effect of a given physical dose of radiation [6]. GARD might serve as the first approach to biology-based RT and might be a candidate genomic biomarker for personalised radiation oncology [7].

The role of immune infiltrates on breast cancer progression and its function in the tumour microenvironment have also been the focus of substantial research. *Tumour infiltrating lymphocytes (TILs)* are a strong prognostic biomarker in some breast cancer subtypes in the early-stage setting [8]. Furthermore, increased levels of TILs have been shown to be predictive biomarkers for response to immunotherapy in breast cancer [9,10], and TILs are increasingly being used as an integral biomarker in immunotherapy clinical trials [11]. A wide variation in TILs levels is seen among the different subtypes HER2, luminal and triple negative breast cancer (TNBC) [8]. RT may induce an immunogenic cell death and thereby attract cytotoxic lymphocytes to the tumour area, but the role of pre-existing TILs in mediating response to RT among patients with invasive breast cancer is not yet well-established.

Recently, the EORTC (European Organization for Research and Treatment of Cancer) made recommendations to establish research and clinical priorities for radiation oncology [12]. Included in these five recommendations were '*the discovery of radiobiology-based biomarkers*', and '*combination systemic and radiation therapy*', subsequently demonstrating the high relevance of studying biomarkers in breast cancer. In the present review, a critical appraisal is given of the available evidence with regard to gene expression profiles, the tumour genomic biomarker GARD and histopathological biomarker TILs in breast cancer RT. Further, the potential role of biomarker-guided patient-tailored treatment and de-escalation of RT for invasive breast cancer will be discussed.

2. Gene expression profiles with benefit from RT as focus

Relatively few gene expression profiling studies focusing on benefit of RT has been published, and the majority has failed successful external validation. The published studies have been designed in different ways to identify either gene signatures with 1) **prognostic value** in terms of LRR, leading to possible identification of patients with either very low risk of LRR, where de-escalation of RT (or even omission) might safely be tested or high risk of LRR, where RT is indicated to improve patient prognosis, or signatures with 2) **predictive value** in terms of benefit from RT providing information on the tumours radiosensitivity, and e.g., which patients may have tumours likely to be radioresistant indicating a need for treatment intensification/other treatment options. In this scenario, the gene expression profile should ideally be tested in irradiated as well as non-irradiated patients, and a predictive profile should not show any impact in non-irradiated patients. Finally, some studies have been designed to describe 3) **cellular radiosensitivity** (Table 1).

It is also important to notice that the patient material used for the studies may include a mixture of patients treated both with and without RT and may encompass patients treated with breast conserving surgery (BCS) and/or mastectomy, and differ substantially in treatment given (± chemotherapy, ± boost, ± endocrine treatment) and included patient characteristics (e.g., age, ER/HER2 status, stage). The majority of the studies are based on tissue material from patients treated with BCS followed by RT. Consequently, these profiles provide, beside prognostic information on the risk of developing a LRR after RT, also an element of prediction of benefit from RT, but can obviously not provide predictive information on RT efficacy based upon comparison of irradiated/non-irradiated patients.

Some of the first attempts to find a classifier prognostic for LRR after BCS + RT was based on previously published gene profiles prognostic of distant metastasis or survival (70-gene profile, Hypoxia-induced profile, Wound signature, Intrinsic gene set) [13,14]. The studies did, however, either not succeed in identifying a robust gene signature or the classifiers failed external validation. Later attempts primarily examined gene expression of tumours from patients with or without known LRR using either supervised and/or unsupervised analysis. Though showing prognostic information in the training cohorts, the profiles have not been successful validation in independent cohorts [15–18]. Cheng et al. described initially two different gene signatures capable of predicting LRR in non-irradiated patients treated with mastectomy [19]. In 2016, the authors tested a minimized gene signature of 18 genes in an independent cohort identifying patients with very low risk of LRR and suggested that the 18-gene signature could assist in selecting patients in whom post-mastectomy RT (PMRT) could safely be spared [20].

Among signatures derived from breast cancer cell lines describing radiation sensitivity is the signature by Speers et al. [21], based on clonogenic assays assessing the surviving fraction of cells after exposure to a standard fraction of 2Gy in 16 breast cancer cell lines, and correlating the intrinsic radiosensitivity with gene expression levels for each gene. The identified 51 genes was hereafter validated in publicly available dataset (BCT or mast) and found to accurately identify patients with LRR.

Finally, the commercially available profiles capacity to predict LRR has also been tested. In Tamoxifen-treated, ER + patients treated with either BCT + RT or mastectomy (National Surgical Adjuvant Breast and Bowel Project, B14 and B20) a significant association between increasing 21-recurrence score (RS)/OncotypeDx score and risk of LRR was consistent for non-irradiated patients treated with mastectomy, but less clear for patients treated with BCS + RT [22]. A positive association between risk of LRR and increasing Risk-of Recurrence score (ROR) provided by the Prosigna analysis has also been shown in ER + patients treated with endocrine treatment after BCS (± RT) [23], whereas a predictive value of RT benefit could not be proven. Drukker et al. also showed a positive association between high risk 70-gene profile (Mammaprint) and LRR risk with the greatest effect within 5 years of follow-up, and with strongest association in irradiated patients treated either with BCS + RT or Mast + RT [24].

A few predictive profiles developed from patient cohorts randomised to ± RT have also been published. The DBCG-RT (Danish Breast Cancer Group) profile derived from a training set of high risk breast cancer patients randomised to ± PMRT identified two prognostically different groups with a 6-fold difference in LRR risk [25]. A predictive value of the profile was described, since PMRT significantly reduced the risk of LRR in the "High LRR risk" patients, whereas the "Low LRR risk" patients experienced no additional benefit from RT. The genes was, however, susceptible for formalin fixation, compromising validation of the profile.

In 2019, Sjöström et al. [26] tested a limited selection of the previously published profiles as well as the performance of OncotypeDx and

Table 1
Datasets on geneexpression profiles (GEP) prognostic of loco-regional recurrence and/or predictive of benefit from RT.

Author, year	Type of study	Number of patients	Surgical procedure and ± RT given	Endpoint	Question	Conclusion
Prognostic for local/locoregional recurrence						
Nuyten, 2006 [14]	Retrospective study of previous gene expression dataset	Training set: 81 Validation set: 80	BCS + RT	LRR after RT	Does established GEP with proven value in predicting metastasis-free and overall survival (wound-response signature, 70-gene prognosis profile and hypoxia-induced profile) hold prognostic value for LRR?	10 y: Modified Wound signature prognostic of LRR (29% vs 5%). Sensitivity 87.5%, specificity 75%.
Kreike, 2006 [13]	Gene expression profiling of tumour tissue	50	BCS + RT	LRR after RT	Does primary tumours associated with or without subsequent LRR differ by GEP patterns?	Inconclusive
Cheng, 2006 [19]	Gene expression profiling of tumour tissue	94	Mast (noRT)	LRR	Can a GEP prognostic of LRR after mastectomy be identified to guide decisions on PMRT?	Overall accuracy of the signatures to predict LRR around 75%
Niméus-Malmström, 2008 [16]	Gene expression profiling of tumour tissue	143	77 BCS + RT 66 BCS (NoRT)	LRR after RT	Can GEP identify patients who will develop LRR after RT?	In ER + subgroup, the gene expression profile distinguished patients with LRR after RT (n = 20) from patients with noLRR (n = 80 ± RT). ROC area = 0.91
Kreike, 2009 [17]	Gene expression profiling of tumour tissue	165	BCS + RT	LRR after RT	Does primary tumours that developed a local recurrence to their recurrences differ by GEP patterns?	The established GEP did not add independent value to established clinical risk factors for LRR
Mamounas, 2010 [22]	Retrospective study of prospective randomised trial	895 Tamoxifen (TAM) 355 placebo 424 TAM + placebo	TAM treated pts only: 390 BCS + RT 505 Mast (noRT)	LRR (after ± RT)	Does OncotypeDx hold prognostic value for LRR in Tamoxifen treated patients?	Significant association between risk of LRR and the 21-gene recurrence score observed in non-irradiated, patients treated with mast (and Tamoxifen), but less obvious association in patients treated with BCS + RT
Sabatier, 2011 [18]	Gene expression profiling of tumour tissue, followed by validation in publicly available dataset	81	BCS + RT	LRR after RT	Can gene expression profiling discover LR-associated transcriptional alterations?	Inconclusive
Servant, 2012 [15]	Gene expression profiling of tumour tissue	343	BCS + RT	LRR after RT	Can a GEP predictive for LRR after BCS + RT be validated on other platforms and in a different patient popualtion?	Inconclusive
Drukker, 2014 [24]	Retrospective analysis of non-randomised, non-consecutive cohort	1053	567 mast (52%+RT) 481 BCS + RT	LRR after RT	Does Mammaprint hold prognostic value for LRR?	MammaPrint is an independent prognostic factor for LRR with high risk profile associated with higher risk of LRR. 10y: Adj. HR of 1.73 (95% CI 1.02–2.93; p = 0.042)
Speers, 2015 [21]	In vitro study followed by validation in publicly available dataset	Breast cancer cell lines Training cohort: 342 Validation cohort 228	Training cohort: 342 BCS + RT Validation cohort: 228 BCS + RT/mast + RT	LRR + RT	Can information on intrinsic radiosensitivity of human breast cancer cell lines be used to develop a molecular signature capable of predicting LRR after RT?	The radiosensitivity signature predicted 10y LRR (HR = 5.3, p < 0.001) with a sensitivity of 84% and a negative predictive value of 89% in validation cohort
Cheng, 2016 [20]	Gene expression profiling of tumour tissue	Training set: 135 Validation set: 87	Training set: Mast (NoRT) Validation set: BCS (94% RT)	LRR	Can a gene signature prognostic of LRR identify patients with very low LRR in whom PNRT can be omitted?	18-gene signature prognostic of LRR in non-irradiated, N0–N1 pts. treated with mastectomy 5y rate of freedom from LRR based on 18-gene signature (30 vs. 99%), p < 0.0001
Cui, 2018 [28]	Retrospective analysis of microarray datasets followed by validation i publicly available dataset	3 training sets: 948 Validation set: 1439	Training sets: 343 BCS + RT 319 BCS + RT/mast + RT, 286 (87% +RT) Validation set: 80% mast/20% BCS 492 noRT 947 +RT	LRFS after RT	Can signatures of tumour radiosensitivity and immune respons provide information on clinical response to RT?	Radiosensitivity signature showed predictive value of RT in the validation cohort, but only in the immune effective group (HR = 0.46 (0.26–0.83), p = 0.0076)
Fitzal, 2021 [23]	Retrospective analysis of prospective randomised trial	1204	1034 BCS + RT 170 BCS (noRT)	LRR after RT	Does PAM50 hold prognostic value for LRR?	ROR score was an independent prognostic factor for risk of local recurrence. 10y, LRR: Subhazard ratio (ROR score) = 4.60 (1.99, 10.63),

(*continued on next page*)

Table 1 (*continued*)

Author, year	Type of study	Number of patients	Surgical procedure and ± RT given	Endpoint	Question	Conclusion
						$p < 0.001$ Predictive value of ROR score for efficacy of RT not proven
Predictive for RT						
Tramm, 2014 [25]	Gene expression profiling of tumour tissue from prospective, randomised trial	Training set: 191 Validation set: 146	Mast (randomised ± RT)	LRR	Can a GEP prognostic of LRR and/or predictive of RT efficacy be identified and validated from a cohort of patients randomised to ± RT?	*Prognostic value*: 20y LRR ("high risk" vs "low risk" profile, 57% vs 8%), adj. HR = 0.07 (0.02–0.30), $p < 0.0001$ *Predictive value:* 20y LRR. PMRT significantly reduced risk of LRR in "high LRR risk" patients (57% *vs.* 12%); adj.HR = 0.17 (0.08–0.34), $p < 0.0001$, but not in "low LRR risk" patients (8% *vs.* 9%), adj. HR = 1.13 (0.14–9.15), $p = 0.97$. Interaction test = 0.05
Sjöström, 2019 [26]	Gene expression profiling of tumour tissue from prospective, randomised trial	Gene profile derived from 3 publicly available data sets Validation set: 748	BCT (randomised ± RT)	LRR	Can 8 previously published genomic signatures developed for radiation sensitivity, for LRR or distant recurrence risk predict risk of LRR? Can a gene expression–based classifier predicting benefit from RT be developed from publicly available data sets?	*Prognostic* for LRR in both patients with RT (HR = 3.4 (2.0–5.9), $p = 0.001$) and without RT (HR, 1.6; (1–2.3), $p = 0.028$) *Predictive* for RT: low ARTIC -profile scores associated with larger benefit from RT (10y cumulative incidence of LRR, 6% v 21%), compared to high ARTIC scores (10-y cumulative incidence of LRR, 25% v 32%). Interaction test, $p = 0.005$
Sjöström, 2023 [27]	Gene expression profiling of tumour tissue from prospective, randomised trial	Training set: 243 Validation set: 354 External validation set: 132	BCT (randomised ± RT)	LRR	Can a GEP identify patients with very low risk of LRR and predictive value for benefit of RT?	*Prognostic* for LRR in non-irradiated patients at 10y (6% vs 19%) *Predictive* for RT: patients with high POLAR profile (HR 0.43 (0.24–0.78), $p = 0.0055$) showed benefit from RT in comparison to low risk profile patients showing no benefit from RT (HR 1.1 (0.39–3.40)

Mammaprint within the SweBCGRT cohort of stage I-II, breast cancer patients treated with BCS randomised to ± RT, but a predictive value of RT of the published profiles could not be proven. From three publicly available datasets, the authors succeeded in deriving a 27-gene classifier Adjuvant Radiotherapy Intensification Classifier (ARTIC) for LRR, which was validated the profile in the SweBCGRT trial. The ARCTIC showed predictive value in terms of RT with a significant benefit in patients with Low ARTIC scores (but not in patients with high ARTIC scores). However, patients with a high ARCTIC profile had a high 10-year LRR and the ARCTIC profile are not well-suited for identifying patients in whom RT can be omitted. The most recent publication by the Swedish group [27], however, show independent prognostic value of a 16-gene signature POLAR (Profile for the Omission of Local Adjuvant Radiation) with a low 10-year LRR of 6–7% among non-irradiated patients with a low risk POLAR profile (compared to 19% in high risk profile patients), as well as a predictive value in terms of efficacy of RT, since only patients with high POLAR profile (HR 0.43 (0.24–0.78), $p = 0.0055$) showed benefit from RT in comparison to low risk profile patients showing no benefit from RT (HR 1.1 (0.39–3.40), $p = 0.81$). The POLAR profile is promising and validated in a smaller patient group, but awaits larger external validation.

At least three of the published profiles show a connection between benefit from RT and the immune response [25,27,28]. Two recently published reviews dive deeper into this matter [29,30].

2.1. Genomic-adjusted radiation dose

The currently used radiation doses have commonly been identified through dose escalation/de-escalation trials based on standard clinico-pathological features. The clinical heterogeneity of radiation response, even within cancer types, is well established and might partly be driven by changes in the tumour genome [31]. Although the dose of radiation can be directly measured, the biological effect is empirically quantified using the linear quadratic (LQ) model. This LQ model provides a simple relationship between cell survival and delivered dose: $S = e^{-\alpha D-\beta D2}$, and has been used extensively to analyse and predict responses to ionising radiation both in vitro and in vivo. The biological effect varies across tissue models and patients, and no methods to measure it directly are currently available. The genomic-adjusted radiation dose (GARD) is a preclinically and clinically validated combination of a gene expression assay, and assumes pan-tissue biological networks of radiosensitivity and radioresistance to predict individual patient RT effect on the basis of the LQ model [32].

Initial in vitro work developed and validated a radiation sensitivity classifier that predicts the survival fraction of various cancer cell lines to 2 Gy dependent upon gene expression profiles of three novel genes (RbAp48, RGS19, and R5PIA) [33]. Follow-up work validated a tumour molecular signature based on the expression of ten genes that correlated with radiosensitivity (expressed as tumour cell survival fraction per 2 Gy fraction) in several cancer cell lines, called the radiosensitivity index (RSI) [34]. Using the gene expression based RSI and a LQ model, which is used to estimate biologic effective dose of varying radiation fractionation schemes, Scott et al. derived a genome-based model for adjusting RT dose (i.e. GARD) and calculated a GARD score for 8271 tumours across 20 disease sites [35]. In a recent pooled-analysis [36], the group validated GARD in 1615 patients with seven cancer types from 11 study cohorts (Table 2). GARD was associated with time to first recurrence (HR 0.98 [95% CI 0.97–0.99]; p = 0.0017) and overall

Table 2
Datasets on GARD and RT.

Author, year	Type of study	Type of cancer	Number of patients	Endpoint	Question	Conclusion
Eschrich et al., 2009 [34]	Clinical trial	HNC – RC - EsC	118	LRC	Can RSI be a prognostic marker in locally advanced HNC?	2yLRC: yes (RS vs RR, 86% vs. 61%, p = 0.05)
Eschrich et al., 2012 [64]	Validation study	BC	503	RFS DMFS	Is RSI a predictor for clinical outcome in RT-treated BC patients?	5yRFS: yes (RS vs RR, 95% vs 75%, P = 0.0212) 5yDMFS: yes (RS vs RR: 95% vs 76.8%, P = 0.0343)
Torres-Roca et al., 2012 [65]	Multicenter study	BC	343	LRFS	Does RSI affect the ability to define LR risk in patients treated with BCT?	RSI didn't uniformly predict for LR across the entire cohort
Ahmed et al., 2015 [66]	Retrospective, cohort-based	GB	270	OS	Can RSI predict treatment outcomes in RT-treated GB patients?	1yOS: no (RS vs RR, 87% vs. 64.4%, p = 0.14)
Strom et al., 2015 [67]	Multicenter study	PC	73	OS	Association of RSI with OS	no difference in survival by RSI status
Scott et al., 2017 (35)	Retrospective, cohort-based	BC – NSCLC – PC – GB	538	DMFS	Can a patient-specific molecular signature of radiation sensitivity be used to identify the optimum radiotherapy dose?	GARD is associated with 5-year distant-metastasis-free survival (hazard ratio 2.11, 95% 1.13–3.94, p = 0.018)
Strom et al., 2017 [68]	Clinical trial	CM	410	RC OS	Can RSI identify patients who experience a survival benefit with regional RT?	5yOS: yes (RS vs RR, 75% vs 0%; HR, 10.68; 95% CI, 1.24–92.14)
Mohammadi et al., 2019 [69]	Clinical study	EnC	204	PC PFFS	Is RSI a predictor for PF in RT-treated EnC patients?	3yPC: yes (RR vs RS, 84% vs 100%; P = 0.02) 3yPFFS: yes (RR vs RS, 65% vs 89%; P = 0.04)
Ahmed et al., 2019 [70]	Retrospective, cohort-based	BC	113	LC	Is GARD associated with local recurrence?	GARD is associated with local control (HR: 2.5 95% CI 1–7.1; p = 0.05),
Scott et al., 2021 [6]	Cohort-based pooled analysis	BC – EnC – GB – HNC –NSCLC – CM – PC	1615	TFR OS	What is the association between the biological effect, quantified by GARD, and time to first recurrence and overall survival?	GARD is associated with time to first recurrence (HR ratio [HR] 0.98 [95% CI 0.97–0.99]; p = 0.0017) and overall survival (0.97 [0.95–0.99]; p = 0.0007)

Abbreviations: BC = breast cancer; BCT = breast-conserving therapy; CM = cutaneous Melanoma; DFS = disease-free survival; DMFS = distant metastasis-free survival; EnC = endometrial cancer; GARD = genomic-adjusted radiation dose; GB = glioblastoma; HNC = head-and-neck cancer; HR = hazard ratio; LC = local control; LR = local recurrence free survival; LRC = locoregional control; NCSLC = non-small-cell lung cancer; OS = overall survival; PC = pancreas cancer; PC = pelvic control; PF = pelvic failure; PFFS = pelvic failure free survival; RC = rectal cancer; EsC = esophageal cancer; RC = regional control; RFS = relapse-free survival; RR = radioresistant; RS = radiosensitive; RSI = radiosensitivity index; RT = radiotherapy; TFR = time to first recurrence.

survival (HR 0.97 [0.95–0.99]; p = 0.0007). The same analysis was performed in patients who were not treated with RT and these results showed no association between GARD and time to first recurrence (HR of 1.00 [95% CI 0.97–1.03; p = 1.00]) or OS (HR of 1.00 [0.98–1.02; p = 0.87]). So, the biological effect of RT, as quantified by GARD, is associated with improvements in time to first recurrence and OS in patients treated with RT, but physical dose is not. The interaction between GARD and RT was significant for OS, demonstrating that GARD is predictive of benefit from RT.

Nonetheless, customization of RT dose to match the individual genomic features of a given tumour using GARD does not take into account the genomic and microenvironmental heterogeneity that can occur even within tumours at the same site into account. As well, systemic therapy, given after the diagnostic biopsy or lumpectomy/mastectomy specimen used for genomic testing, might also alter the genome. In addition, because of the stochastic nature of radiation-induced cellular lethality, larger tumours, even with the same genomic characteristics, might have a lower chance of being controlled with RT. How GARD is affected by this heterogeneity is still unclear.

Further, GARD is only relevant when conventional fractionation is used (i.e., approximately 2 Gy per fraction) as it is benchmarked on the RSI, and with the current increase in use of (ultra)hypofractionated whole-breast radiation, the applicability of GARD in the coming future might become gradually more limited.

In addition, widespread adoption of tumour sequencing/expression profiling can be challenging in clinical practice, as demonstrated by the unhurried implementation of other gene expression-based assays which already have level 1 biomarker evidence after assessment in a randomised clinical trial (e.g., MammaPrint, OncotypeDX, DCISionRT). This might be due to practical reasons, such as cost of the assay and the logistics of performing the assay within different clinical constraints, all of which limit the uniform application of gene expression profiles for day-to-day clinical use. Another limitation of GARD is its focus on the genomic prediction of tumoural response to radiation without taking into account the difference between RT given to large elective nodes versus only to the primary tumour. SBRT with sparing of the draining nodes induces a different immunological tumoural response, as shown pre-clinically [37].

Several of the 10 hub genes in RSI have known functions in the immune microenvironment including STAT1 and IRF1 [38]. RSI is known to be strongly correlated with immunogenicity of different tumour types by using the previously developed 12-chemokine (12-CK) signature for immune activation and inflammation [39]. In this analysis of over 10, 000 unique solid tumour tissue samples, a significant correlation was demonstrated between RSI and 12-CK signatures suggesting links between radiation sensitivity and immunogenicity across tumour types including breast cancer. The question arises whether this might provide further insight into the biological basis of GARD. A similar relation between the immune system and benefit from RT was found for a gene expression profile that was developed in the DBCG82bc cohort of breast cancer patients randomised to ± postmastectomy RT [25].

Shifting to a genome-based dose determination could provide a new direction for radiation oncology with multiple opportunities to improve clinical outcome, but prospective validation in randomised clinical trials remains necessary to drive change in clinical practice. Although GARD has not yet been utilized in a randomised clinical trial, it has been identified as trial ready by the European Organization for Research and Treatment of Cancer [12].

2.2. Tumour infiltrating lymphocytes (TILs)

Of particular interest is the immunomodulatory gene expression

profile (IM), indicating the presence of TILs, which can be found in all breast cancer subtypes [40]. TILs constitute a family of immunoregulatory cells, including cytotoxic and helper T cells, B cells, and natural killer (NK) cells. Increased levels of baseline TILs in patients receiving either primary systemic therapy or adjuvant chemotherapy are associated with improved recurrence-free and OS in HER2+ breast cancer and TNBC [9,10,41–46]. The prognostic value of TILs has triggered efforts to use TILs as an integral biomarker in clinical trial designs aiming to identify patients that may be able to substantially de-escalate the intensity and/or duration of cytotoxic systemic therapy if the baseline TIL-counts are high in their cancer. Furthermore, as the evidence for TILs as a predictive biomarker for immunotherapy response is becoming evident, TILs are increasingly being used as an integral biomarker in immunotherapy clinical trials [11]. Approximately 15% of all breast cancers show high levels of TILs, with a wide variation depending among the different subtypes, HER2, luminal and TNBC. The cut off for separating low and high TILs also remains ambigious. Nonetheless, the role of TILs in mediating response to RT among patients with invasive breast cancer remains largely unclear.

The published update of the SweBCG91 R T randomised trial studied 1178 patients with breast cancer stage I and II who were randomly assigned to BCS plus postoperative RT or BCS only (Table 3). Kovács et al. assessed the stromal TILs for 936 of these patients and used a dichotomized cutoff of 10% to distinguish between low and high TILs. Seventy-one percent of patients had TILs less than 10%. Looking at subtypes, the group with low TILs was dominated by luminal tumours (luminal A- and Luminal B-like tumours, respectively 79% and 73% had TILs less than 10%). In contrast, for HER2-positive tumours 49% had TILs less than 10% and for triple-negative tumours only 26% had TILs less than 10%. The primary endpoint of this study was the time to ipsilateral breast tumour recurrence (IBTR) as the first event within 10 years. It was demonstrated that RT was significantly beneficial in 71% of patients with low TILs tumours (HR, 0.37; 95% CI, 0.24 to 0.58; $p < 0.001$) but not significantly in the high TILs group (HR, 0.58; 95% CI, 0.28 to 1.19; $p = 0.138$) in terms of IBTR [47]. Regarding breast cancer survival, no significant difference in effect of RT could be noticed. For the multivariable regression analysis, RT (HR, 0.42; 95% CI, 0.29 to 0.61; $p < 0.001$) was predictive of IBTR, as were high TILs (HR, 0.61; 95% CI, 0.39 to 0.96, $p = 0.033$) grade (3 vs. 1; HR, 2.17; 95% CI, 1.08 to 4.34; $p = 0.029$), and age (≥50 *vs.* < 50 years; HR, 0.55; 95% CI, 0.38 to 0.80; $p = 0.002$). In the low TILs group, RT was significantly beneficial (HR, 0.37; 95% CI, 0.24 to 0.58; $p < 0.001$) compared to the high TILs group (HR, 0.58; 95% CI, 0.28 to 1.19; $p = 0.138$). In conclusion, the results show that the risk of an IBTR is reduced in the primary tumour with high TIL values regardless of treatment with RT. Furthermore, the benefit of RT is larger in patients with a low value of TILs with regards the risk of IBTR. However, there was no statistically significant interaction between RT and TILs. A possible explanation could be that high TILs are protective against IBTR and when they are not present (or less present), RT provides a greater reduction in IBTR. Another reason can be that RT has a greater beneficial interaction with the immune system when TILs are less than 10% present.

A recent Danish publication investigated impact of RT based on TILs using data from the DBCG82bc trial [48] (Table 3). In this trial patients diagnosed between 1982 and 1990 were included and treated with total mastectomy and axillary lymph node dissection followed by a randomization between PMRT or no PMRT. The authors evaluated TILs on haematoxylin and eosin (HE) stained sections-sections. TILs cutoff value for this study was 30%. Low TIL values were seen in 89.5% of the patients. The primary endpoint consisted of LRR, distant metastasis (DM) and OS. The multivariate regression analysis (with TILs as an independent factor) showed that high TILs was associated with lower risk of DM and improved OS, but without association with loco-regional control. The OS after PMRT at 20 years was significantly greater for high TILs compared to low TILs (8% improvement for low TILs (23%–31%) vs. 22% for high TILs (26%–48%), interaction-test: $p = 0.028$). The benefit of PMRT on the risk of LRR was not influenced by the level of TILs. Their results show that high TILs were associated with an improved OS and a trend for decreased risk of DM after PMRT. The reduced risk of LRR after PMRT was of similar size for patients with low and high TILs (24% *vs.* 26% difference at 20 years).

A smaller retrospective study of breast cancer patients treated between January 2009 and December 2019 in Detroit, Michigan, United States investigated the effect of TILs on treatment outcomes [49]. The included patients had HER2+ or TNBC with a clinical T1 or T2 N0 stage and the locoregional treatment consisted of either mastectomy without RT or lumpectomy with RT. A total of 190 patients were included for this study. A dichotomized cutoff value of 50% was used to distinguish between low and high TILs. The primary endpoints were 5-year disease free survival (DFS) and 5-year OS. The results regarding recurrence in patients with high TILs, showed no recurrences in the lumpectomy with RT group, whereas 23% (n = 6) of patients in the mastectomy without RT group had a recurrence (mostly distant recurrence) and a 5-year DFS of 100% for the lumpectomy with RT group and 76% for the mastectomy without RT group ($p = 0.014$). In the low TILs group, the recurrence was 10% (n = 8) in the lumpectomy with RT group versus 14% (n = 9) recurrence in the mastectomy without RT group. For the group with low TILs, there was no difference in 5-year DFS (86% vs 87% in the mastectomy without RT and lumpectomy with RT groups, respectively; $p = 0.583$). The 5-year OS in the patients with high TILs was 100% in the lumpectomy with RT group whereas it was 86% in the mastectomy without RT group ($p = 0.028$). The 5-year OS difference between the two groups with low TILs was not statistically significant (86% vs 81% in the mastectomy without RT and lumpectomy with RT groups, respectively; $p = 0.241$). In conclusion, for patients with low TILs, lumpectomy with RT did not improve outcomes compared to mastectomy without RT. Moreover, patients with high TILs had a significant improvement of their DFS and OS with lumpectomy and RT when compared to mastectomy without RT.

Very recently, the prognostic and predictive capacity of tumour

Table 3
Effect of postoperative RT in breast cancer, as determined by low vs. high TILs.

Author, year	n	Stage	Randomization		PE	TILs cutoff	Low TILs	High TILs	Conclusion
Kovács et al., 2019 [47]	936	stage I or IIA N0	BCS 485	BCS + WBRT 451	Time to IBTR	10%	71%	29%	TILs <10% larger benefit from RT regarding the risk of IBTR
Tramm et al., 2021 [48]	1011	N+ or ≥ T2	ME 505	ME + PMRT 506	LRR, DM, OS	30%	90%	10%	High TILs associated with improved OS and decreased risk of DM after PMRT as compared to low TILs
Mouabbi et al., 2021 [49]	190	T1-T2N0 Her2+/TNBC	ME 90	L + RT 100	5y DFS, 5y OS	50%	74%	26%	L + RT associated with improvement of 5y DFS and 5y OS in high TILs as compared to ME

Abbreviations: BCS = breast conserving surgery; DM = distant metastasis; IBTR = ipsilateral breast tumour recurrence; L = lumpectomy; LRR = loco-regional recurrences; ME = mastectomy; n = number of patients included; OS = overall survival; PE = primary endpoint; PMRT = post-mastectomy radiotherapy; RT = radiotherapy; TIL = tumour infiltrating lymphocytes; WBRT = whole breast radiotherapy.

Infiltrating lymphocytes was investigated in the MA.20 regional RT trial [50], and demonstrated that $CD8^+$ stromal TILs benefit from regional nodal irradiation. This finding is in support with the findings on $CD8^+$ in DBCG82bc in terms of DFS, but in this trial (i.e., MA.20) all patients had whole breast RT and were randomised to ± regional nodal irradiation, subsequently it is not an ideal cohort for prediction, albeit it gives information on whether nodal RT plays a part in the systemic effect of RT.

The apparent different results from these studies might be explained by factors such as i) the different cut-points for low vs. high TILs used in the different studies, ii) the different patient groups (high-risk after mastectomy vs. low-risk after lumpectomy) included and hence the receipt of cytotoxic systemic therapy (CMF in DBCG82b vs. none in vast majority of SweBCG91 R T patients) and iii) whether the study was powered for interaction between TILs and outcome or response to RT.

These conflicting findings suggest that more data are needed and might hint to a different mechanism of interaction between RT and the immune system in different immunogenic environments throughout the different risk groups of breast cancer. It is suggested that RT through immunogenic cell death and release of tumour antigens triggers a local immune response that leads to a systemic effect outside the treatment field (i.e., abscopal effect), possibly with a more pronounced clinical effect in a higher risk group. An abscopal effect has, however, been found in several preclinical studies but has only been shown anecdotally in the clinical setting [51]. RT can also have negative effects on the immune system and treatment with RT alone has been associated with immunosuppressive effects [52].

Another explanation for the results could be differences in ER/HER status, since level of TILs in ER positive cancers have been shown to have an adverse clinical outcome than in ER negative cancers as shown e.g., in the neoadjuvant setting, due to differences in the immune cell composition that we know exists between ER+/ER-cancers [9].

The abovementioned studies have all evaluated TILs on HE stains. HE-sections do not allow for discriminating the various lymphocytes present in the tumour microenvironment, and it can be speculated if the composition of pre-existing immune cells influences the benefit of RT as was shown in by Stenmark Tullberg et al. demonstrating that patients with a favourable e.g. ($CD8^{High}$/$FOXP3^{Low}$) antitumoural immune infiltrate in the primary tumour have a reduced risk of any recurrence and may derive less benefit from adjuvant RT [53].

Several questions arise: Can the immune system, of which the TILs are an important metric, help define which patients derive the most benefit from RT? Can TILs identify those who can be offered omission of RT? Does the immune system have a role in predicting impact of RT in patients with different breast cancer subtypes and with a different risk for recurrence? Can TILs predict the need for regional RT in node-positive or high-risk node-negative patients? Is there an impact on the immune system when using different RT fractionation schemes, as dose-dependency of the immune response pathways are shown in preclinical studies, showing no or less immune activation due to inhibition of the cGAS/STING pathway after activation of TREX1 with high doses of RT [54].

If an association between TILs and response to RT could be demonstrated, it would establish the predictive value of TILs as marker of response to RT and open the way to biomarker-guided RT, plus, this would also add to evidence that the effect of RT might be partially mediated by the immune system [55]. In contrast to expensive genomic assays, TILs can be scored on routine diagnostic H&E sections, making it very attractive for clinical application.

3. Future Perspectives

1. Biomarker-guided patient-tailored treatment

Prospective biomarker-driven randomised RT trial data utilizing genomic biomarkers or immune biomarkers are currently lacking, and due to the paucity of this evidence it is currently too early for biomarker-guided RT in the clinic. But despite not directly changing patient management, TILs remain to be a proven prognostic biomarker in breast cancer. It is also notable that we routinely report on other prognostic markers like histological grade and lymphovascular invasion that, while not explicitly validated, are indeed considered when making nuanced decisions about RT for individual patients. Regardless, the TILs has now been included in several international guidelines for early-stage disease. This includes the 2019 St Gallen consensus conference for early-stage breast cancer and the European Society of Medical Oncology (ESMO) Guidelines for early-stage breast cancer [56,57].

Integration of tumour genomics and immune biomarkers into current and future clinical trials will allow for a more robust identification and utilization of predictive biomarkers and ultimately allow for personalised RT in the management of patients across the entire spectrum of breast cancer.

Moreover, the division between GARD being a tumour genomic biomarker and the TIL biomarker might be arbitrary as the biological basis of GARD is unknown. It is demonstrated that there is a significant correlation between RSI and 12-CK signatures indicating radiation sensitivity to be correlated to immunogenicity across tumour types including breast cancer [39]. This suggests that tumours with increased radiosensitivity also have increased immune activation and visa-versa. This observed association between radiosensitivity and immune-activation may have some clinical implications. It might be possible that the immune system primes the tumour for improved response to radiation. Alternatively, it could be that RT primes the tumour for increased immune activation, re-activates a pre-existing immune response, overcomes anergy in an already inflamed (but exhausted) tumour microenvironment or potentially all processes take place in a synergistic manner. Understanding the clinical impact of this association is critical given the emerging immunotherapy options that could be utilized in combination with RT.

2. Combination of immunotherapy and breast RT

The potential of synergistic therapeutic strategies with modern RT, through modulation of the tumour microenvironment using RT is beyond the scope of this review but emerging data suggest that radiation can convert immune desert tumours into an inflamed immunological hub, potentially increasing sensitivity to immunotherapy. Different groups are currently investigating radiation-induced “immune priming” to activate an immune response and enhance tumour-killing potential [58]. Pre-operative RT for luminal B breast cancer might be used to prime the immune response and increase the efficacy of immune checkpoint inhibitors (ICI), because luminal B breast cancer is generally less inflamed in comparison to TNBC [59]. In future trials, TILs could potentially play a key role to select patients that would receive pre-operative RT in combination with ICI to activate an immune response.

The use of RT alone might not be sufficient to start the immune response, consequently supplementary agents are being investigated to assist RT in the immune response stimulation. For example, CD73 blockade can be added to RT and ICI to prevent adenosine-mediated immunosuppression and improve overall response (e.g. NCT03875573) [60]. These and other strategies are being explored to shift the balance from immunosuppressive to pro-immunogenic effect of RT [61]. Analysis and recruitment from these studies are still ongoing. In principle, RT may have additive effects to immune modulating agents since they share similar immune induction pathways (i.e., RT triggers apoptotic and necrotic cell death, upregulates inflammatory signals and triggers T-cell response, based on evidence from animal models) [62]. When translating immune modulation with RT to the clinic the optimal treatment timing and sequencing, radiation dose, target volume, and toxicity profiles must be considered [63]. Identifying response biomarkers to guide immunotherapy and RT would accelerate personalised treatments for high-risk disease, which could be achieved by measuring

TILs.

4. Conclusion

Radiation oncology has thus far employed an empiric uniform approach to prescribing RT that is based on models developed and published over 70 years ago. Current evidence suggests that this one-size fits all approach might be biologically inaccurate, thereby disadvantaging a subset of breast cancer patients.

Growing evidence continues to demonstrate the prognostic implications of genomic biomarkers and biomarkers of the immune system across the spectrum of breast cancer. But less is understood about how biomarkers may be predictive of various radiation techniques and dosing, and remains an active area of interest.

Prospective randomised integral-biomarker RT trial data utilizing gene expression profiles, GARD or TILs are currently lacking, however, such trials will become active in the coming years. Integration of tumour genomics and immune system-related biomarkers into these current and future clinical trials will allow for a more robust identification and utilization of predictive biomarkers and ultimately allow for precision RT in the management of patients across the entire spectrum of breast cancer.

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