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REVIEW

Avoiding over- and undertreatment in patients with resected node-positive breast cancer with the use of gene expression signatures: are we there yet?

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Prediction of benefit from adjuvant chemotherapy following resection of early breast cancer and, as a result, proper selection of candidates remains an elusive goal since the relative magnitude of benefit is the same regardless of the presence of clinicopathologic factors. Multiple studies, including randomized trials, establish the role of certain gene expression signatures in node-negative disease since they predict the risk of breast cancer relapse being so low that adjuvant chemotherapy can be omitted. In contrast, more limited data are available in higher risk, node-positive breast cancer patients, making the exclusion of adjuvant chemotherapy potentially hazardous. 'Prospective–retrospective' studies and limited prospective data show that several signatures, namely Oncotype Dx, MammaPrint, Prosigna, EndoPredict and Breast Cancer Index, select with different levels of success node-positive patients at very low risk for distant recurrence despite not receiving chemotherapy, although the long-term follow-up is still awaited. Pending, however the publication of the results from ongoing randomized studies which enroll patients with node-positive disease, major caution is warranted. Improper use and misinterpretation of these transcriptomic profiles can lead to undertreatment and exposure of patients to unnecessary risks resulting in increased breast cancer mortality for patients with axillary node-positive disease. With this review we critically discuss the available data on gene expression signatures that are used in clinical practice and offer practical recommendations regarding the management of patients with ER-positive, human epidermal growth factor receptor 2 (HER2)-negative, node-positive breast cancer.

Key words: adjuvant chemotherapy, breast cancer, gene expression signature, node-positive, Oncotype Dx

Introduction

Substantial advances on the understanding of the underlying complexity and heterogeneity of breast cancer (BC) biology and clonal architecture [1–3] have allowed for the development, validation and regulatory approval of transcriptomic gene expression profiles (GEPs) that aim to select patients that could be spared from adjuvant chemotherapy [4]. Several of these GEPs, described in this review, are recommended for clinical use by the American Society of Clinical Oncology (ASCO) [5, 6], although not in combination due to discrepancies in patient classification. GEP impact on patterns of usage of adjuvant chemotherapy has been shown prospectively, with published studies reporting a 20%–35% reduction in chemotherapy administration with the use of Recurrence Score (RS) determined by the Oncotype Dx assay [7–9]. These results are mirrored by retrospective, real-world data which demonstrate a marked decline in adjuvant chemotherapy use especially in patients with node-negative disease. Notably, chemotherapy use in patients with node-positive disease has also decreased, despite the fact that the assay is not universally reimbursed for this indication [10, 11]. The latter is of particular interest, taking into account the relative paucity of available data regarding node-positive BC. As a result, pending the publication of ongoing randomized trials regarding node-positive disease, there is a real risk for undertreatment associated by extrapolation of the available GEP. In this article, we aim to critically discuss the available data from published clinical trials, with an emphasis on node-positive BC.

Benefit from adjuvant chemotherapy

Data regarding the effect of chemotherapy on patient outcomes following resection of early BC are derived from the Early Breast Cancer Trialists' Collaboration Group (EBCTCG) meta-analysis of 100 000 individual patients that were randomized in 123 trials [12]. The postoperative administration of an anthracycline, when compared with no chemotherapy, improves the absolute relapse free survival (RFS) at 10 years by 8% and breast cancer specific survival (BCSS) by 6.5%, which correspond to a relative risk reduction of ~25%. Importantly, the proportional risk reduction for BCSS is the same regardless of nodal spread, estrogen receptor (ER) status, age and used endocrine therapy. Further adding a taxane to an anthracycline compared with anthracyclines alone results in a 4.6% absolute improvement in RFS and 2.8% in BCSS at 8 years, a relative reduction of 15%. Again, the relative improvement is independent of clinicopathologic factors. Interestingly and in contrast with the effect of anthracyclines which concerns mainly the first 5 years following BC diagnosis, the relative risk reduction in all end points with the addition of a taxane was similar between years 0–4 and years 5+.

Additional improvements in outcomes are conferred by the administration of every 2 weeks or dose-dense chemotherapy, as proposed by the Norton–Simon hypothesis and evaluated in a number of trials [13]. At 10 years, the absolute decrease in the risks for disease recurrence and for death due to BC was 4.3% and 2.8% in favor of dose-dense schedules respectively, a relative reduction of ~15% compared with the same treatment administered every 3 weeks [14]. The absolute benefit in ER-negative disease was 3.7% and in ER-positive disease 3.1%. The absolute risk is higher for patients with ER-negative disease so the decrease in recurrence and death is greater than for ER-positive disease though the proportional risk reduction is the same. These results confirm that dose-dense therapy should be considered as the standard of care for high-risk patients with node-positive disease and clearly do not support a generalized recommendation for de-escalation by omitting standard adjuvant chemotherapy.

Biomarkers in BC: prognostic, predictive or both?

In order to adequately evaluate the performance of a biomarker (clinical, protein-based or gene-based) one needs to grasp the difference between prediction and prognostication. The former implies benefit from administered treatment; in the presence of a positive predictive marker, the patient's outcome improves when treated with a specific therapy. The latter implies disease behavior; in the presence of a positive prognostic marker, the patient's outcome improves regardless of administered therapy.

The majority of evaluated biomarkers at the adjuvant setting in BC carry prognostic information: they select patients at very low absolute risk for recurrence, so that the presumed 1%–2% risk for death or serious long-term adverse events caused by chemotherapy is not justified. Assuming a stable benefit from chemotherapy at ~30% reduction in recurrences and a 10% risk for recurrence at 10 years that a patient with very early disease has, the absolute risk reduction is 3%—only a small difference from the toxicity threshold. In contrast, for a patient with

locoregionally advanced disease and an estimated 40% risk for recurrence at 10 years [15], the net benefit from chemotherapy would be 12%, significantly higher than the anticipated serious adverse events. Thus, chemotherapy administration is justified and the recommendation becomes even clearer when considering dose-dense chemotherapy and the further relative risk reduction it confers compared with standard interval treatment.

These observations however presume a constant reduction in BC recurrence of ~30% using standard every 3 weeks regimens. This hold true, at least regarding clinicopathologic factors [12]. Some evidence however indicates that the risk of relapse of Luminal A tumors might be less affected by adjuvant chemotherapy [16–18]. For example, in the Danish Breast Cancer Group (DBCG) 77B trial patients were randomized to no adjuvant therapy, levamisole, cyclophosphamide (C) or CMF (C, methotrexate, 5-fluorouracil); endocrine therapy, which could have diluted the effect of chemotherapy, was not administered. Luminal A patients, as determined by immunohistochemistry, did not derive any benefit from cyclophosphamide-based chemotherapy [19]. How these results translate to patients treated with contemporary, anthracycline and taxane-containing regimens are unclear. Similarly, GEP such as RS may distinguish patients with node-negative disease that seem to benefit less from adjuvant chemotherapy [hazard ratio (HR)=0.95, 95% CI 0.75–1.22 for RS = 11–15 in the TailorX trial and HR = 1.19, 95% CI 0.40–3.49 for RS < 18 in the NSABP-B20 trial) [20, 21].

Prediction and prognostication in early BC according to clinical factors

As clearly shown in the EBCTCG meta-analyses, traditional clinicopathologic factors such as tumor size, nodal stage, age and ER status are not predictive for benefit from adjuvant chemotherapy. Even within the ER-positive subgroup, the relative effect is same regardless of age and tumor grade, with a relative risk reduction for death due to BC of ~20% in the anthracycline versus no chemotherapy comparison [12]. In addition, although clearly prognostic, these routine factors alone may be inadequate to identify patients with sufficiently low absolute risk for recurrence so as the use of adjuvant chemotherapy is not justified, especially if the patients have node-positive disease. In another EBCTCG analysis of long-term data from 63 000 women treated with 5 years of endocrine therapy, distant recurrences occurred at a steady rate with the cumulative risk never reaching a plateau. Patients with node-negative disease had a 22% 20-year risk for distant recurrence (one-third had received chemotherapy) and patients with node-positive disease 31% (two thirds had received chemotherapy; however, chemotherapy allocation was not randomized and its effect cannot be evaluated). Even patients with T1N0 disease had a 13% 20-year risk for metastasis [22]. Although these results can be generalized regarding the long-term disease behavior of ER-positive, human epidermal growth factor receptor 2 (HER2)-negative BC, the risks may be overestimated due to the considerable recent therapeutic advances, as acknowledged by the authors of the study.

Prospective randomized data of gene expression signatures as prognostic and predictive factors in early BC, regardless of nodal status

Oncotype Dx

Oncotype Dx (Genomic Health Inc., Redwood City, CA) is a GEP which consists of 16 genes and 5 reference genes, whose expression is used in order to calculate RS [23]. RS has been validated in 'prospective–retrospective' [20, 24], prospective non-randomized [25] and prospective randomized studies [21, 26]. In the TailorX trial, 10 273 women with ER-positive, HER2-negative, node-negative BC were enrolled. Those with low risk disease (RS ≤ 10, $n = 1626$) did not receive chemotherapy and had a 9-year disease-free survival (DFS) of 84%. Patients with intermediate risk disease (RS = 11–25, $n = 6711$) were randomized to chemotherapy versus no chemotherapy. Although there were no differences in the entire group in terms of DFS, subgroup analyses revealed that patients younger than 50 years had the following improvements in outcomes with chemotherapy and according to RS: RS = 11–15, 3.5% in DFS and 0.8% in distant disease free survival (DDFS); RS = 16–20, 9% in DFS and 1.6% in DDFS; RS = 21–25, 6.3% in DFS and 6.5% in DDFS [21, 26]. The relative contribution of chemotherapy-induced amenorrhea, if any, cannot be assessed since such information was not collected but may contribute to the benefit.

The results of the TailorX trial imply that the clinical utility of RS is somewhat limited since, as discussed above, patients younger than 50 and at intermediate risk of recurrence (at least with RS = 16–25) seem to benefit from the administration of chemotherapy. In addition, the identification of patients belonging to the low RS group that do not need chemotherapy may be feasible without the use of genomic assays as shown in a Swedish population-based registry study [27], therefore negating the necessity for Oncotype Dx testing.

MammaPrint

MammaPrint (Agendia Inc., Amsterdam, The Netherlands) is a 70-gene GEP that divides patients in low- and high-risk for BC recurrence [28] and has been validated in retrospective [29–31], prospective non-randomized [32] and randomized studies [33]. In the latter trial, 6693 women with early BC were enrolled; ~21% were node-positive. Patients were randomized to utilize the discordant clinical or genomic risk profile to allocate chemotherapy or not. The trial met its primary end point, since the lower boundary for DDFS at 5 years in the test population (high clinical and low genomic risk) was 92.5%, exceeding the prespecified threshold of 92%. With the use of MammaPrint, 46.2% of high clinical risk patients would be spared from chemotherapy, at the cost of an increased risk for distant recurrence by 1.5% in the intention-to-treat population (HR = 0.78, 95% CI 0.50–1.21) and 2.1% in the per-protocol analysis (HR = 0.65, 95% CI 0.38–1.10). Consistent trends in favor of chemotherapy were noted in discordant subgroups including a 2.5% absolute decrease in distant metastases in the node-negative, high clinical/low genomic risk group (HR = 0.69, 95% CI 0.39–1.21). The trial was not powered to exclude benefit from chemotherapy and also needs longer follow-up especially for patients with node-positive ER-positive disease [33].

Use of gene expression signatures in node-positive BC

Results from published prospective and 'prospective–retrospective' studies that have evaluated the prognostic and predictive power of the GEP in node-positive populations are discussed below and summarized in Table 1 [15, 33–38]. It should be noted that only one of these studies has a direct comparison of no chemotherapy to chemotherapy in the node-positive group [15].

The distribution of RS scores has been shown to be similar regardless of nodal status, therefore implying that a subset of node-positive patients in the continuum might not benefit from adjuvant chemotherapy [39]. In the SWOG-8814 trial, the use of anthracycline-containing chemotherapy did not improve DFS in the 146 patients with RS < 18 (HR = 1.02, 95% CI 0.54–1.93). The improvement in DFS was not statistically significant in the intermediate risk group as well (RS = 18–30; HR = 0.72, 95% CI 0.39–1.31, $P = 0.48$), although the sample size was small (103 patients). Similar results were reported for other end points (BCSS, overall survival) [15]. These results, despite the lack of power, represent the only randomized evidence on a GEP predicting the lack of benefit from adjuvant chemotherapy in node-positive BC. Similarly, large retrospective analyses show excellent BCSS among patients with limited node involvement and RS < 18 [40, 41]. For example, a SEER data analysis of 3919 patients with 1–3 involved nodes and RS < 18 showed 5-year BCSS rates of 92.8%, 95.1%, 97.1%, 99.4% and 98.9% for patients with ≥4, 3, 2, 1 involved node and micrometastasis, respectively. It should be noted however that the follow-up is short, 18%–59% of patients were treated with chemotherapy and chemotherapy is commonly underreported to SEER [40]. Further support for RS is given by the reported long-term results from the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial [34] and prospectively from the planB trial [25, 35]; however, the relatively short follow-up of 5 years and low number of patients in the latter preclude any robust conclusions. The ongoing RxPONDER trial (Clinicaltrials.gov identifier NCT01272037) enrolls patients with ER-positive, HER2-negative BC with 1–3 positive lymph nodes and a RS ≤ 25, which are randomized to receive chemotherapy or not; the trial has completed accrual and the results are eagerly awaited. Pending their publication, no specific recommendations can be made. Notably, the inclusion criteria of RxPONDER seem to be utilized in clinical practice in order to exclude patients from chemotherapy before the publication of the results of the trial [42].

Limited support for the use of MammaPrint in node-positive disease is offered by the MINDACT trial [33]. In the node-positive subgroup of the primary study population of high clinical/low genomic risk patients, the absolute benefit from chemotherapy in terms of DDFS was 0.7% (HR = 0.88, 95% CI 0.42–1.82). As stated in the ASCO recommendations, this trial cannot exclude benefit from chemotherapy due to lack of power [6].

Prosigna (NanoString Technologies Inc., Seattle, WA) is an assay that can determine the tumor's intrinsic subtype by using

Table 1. Prospective and 'prospective–retrospective' studies on the use of gene expression signatures in node-positive breast cancer: distant metastasis-free survival events in low risk groups not treated with chemotherapy

Trial [references]	N (total node-positive)	Definition of low risk	N (low risk)	Distant recurrence rate in low risk group	Comparison with chemotherapy
Oncotype Dx					
SWOG S8814 [15]	367	RS < 18	146 (40%)	40% any disease recurrence at 10 years	HR = 1.02 (95% CI 0.54–1.93)
TransATAC [34]	306	RS < 18	160 (52%)	17% at 9 years	NA
PlanB [35]	905	RS < 12	170 (19%)	5.6% any disease recurrence at 5 years	NA
MammaPrint					
MINDACT [33]	1404	MammaPrint Index > 0.0	737 (high clinical, low genomic risk)	4.4% at 5 years	HR = 0.88 (95% CI 0.42–1.82)
Prosigna					
ABCSG-8 [36]	382	ROR < 16	15 (143 intermediate)	0% (6.4% intermediate) at 10 years	NA
TransATAC and ABCSG-8 [37]	557	ROR < 27	137 (24.6%)	3.3% (years 5–10)	NA
EndoPredict					
TransATAC [38]	183	EPclin < 3.3	43 (23.5%)	5.6% at 10 years	NA
Breast Cancer Index					
TransATAC [38]	183	BCI < 5.0825	95 (51.9%)	15.5% at 10 years	NA

BCI, Breast Cancer Index; EPclin, EndoPredict; HR, hazard ratio; ROR, risk of recurrence; RS, Recurrence Score.

the 50-gene predictor analysis of microarray 50 (PAM50) gene signature and tumor size and therefore calculate a risk of recurrence (ROR) score [43]. 'Prospective–retrospective' data show that ROR can predict the long-term risk of distant recurrence in both node-negative and node-positive patients [36, 37, 44, 45]. Importantly, the prognostic capacity of ROR seems to outperform that of RS, adding long-term prognostic information in node-positive patients and identifying more precise low- and high-risk groups [38, 44]. Prosigna is being assessed in the on-going OPTIMA (Optimal Personalized Treatment of early breast cancer usIng Multi-parameter Analysis; registration number ISRCTN42400492) randomized trial which will inform on the management of clinically high-risk patients (ER-positive, HER2-negative BC with 1–9 positive nodes or tumor size >30 mm). Participants are randomized to either chemotherapy or to experimental biomarker-driven therapy (chemoendocrine therapy for patients with ROR > 25 and endocrine therapy alone for those with ROR ≤ 25).

EndoPredict (EP, Myriad Genetics Inc., Salt Lake City, UT) is a 12-gene prognostic assay [46] that can be combined with clinical factors (tumor size and nodal status) to form EPclin, a tool which integrates both genomic and anatomical prognostication. Both EP and EPclin identify patients at very low risk for late distant recurrence following adjuvant endocrine therapy [47], as well as patients with node-positive disease treated with [48] or without chemotherapy. This GEP also provides the most prognostic value for late distant recurrence compared with all tested prognostic signatures in the transATAC analysis [38, 49].

Finally, Breast Cancer Index (BCI) comprises two distinct GEPs. The first component is the HOXB13 (homeobox B13) to interleukin 17B receptor (IL17BR) ratio, which predicts shorter DFS, resistance to tamoxifen therapy [50, 51] and can identify patients who benefit from extended letrozole adjuvant therapy [52, 53]. The second component of BCI is the 5-gene molecular grade index and the full GEP has been shown to robustly identify node-negative patients with very low risk for distant recurrence [38, 52, 54–56].

Synthesis of the available data

Available 'prospective–retrospective' and population-based data indicate that GEP provide additional prognostic information when added to clinical factors [38, 57]. These data, in addition to the two published randomized trials whose shortcomings are well-known [58], support the role of GEP in therapy selection at least for node-negative patients; however, the interpretation of older trials where sub-optimal according to contemporary standard chemotherapy was administered has a number of caveats. In addition, contemporary clinical trials imply that the 1% treatment-related mortality and severe morbidity might be exaggerated: in two trials of dose-dense adjuvant chemotherapy enrolling 4108 patients, only one toxic death occurred and 7 hematologic malignancies (of which 5 in the dose-dense groups) were diagnosed after 5.3–7.0 years of follow-up [59, 60]. As a result, the necessary threshold that needs to be exceeded in order to classify an absolute risk reduction as clinically meaningful, which is a function of the treatment effect and treatment-related mortality, might be lower than previously thought. In contrast, advances in adjuvant endocrine therapy such as extended treatment and ovarian ablation in combination with tamoxifen or exemestane in premenopausal patients [61] improve the efficacy of chemotherapy-free adjuvant strategies and may increase the number of potential candidates without excess risk. The latter is of particular interest, since the absence of chemotherapy-induced ovarian suppression [62] should be mitigated by the administration of optimal endocrine therapy. This however should also be
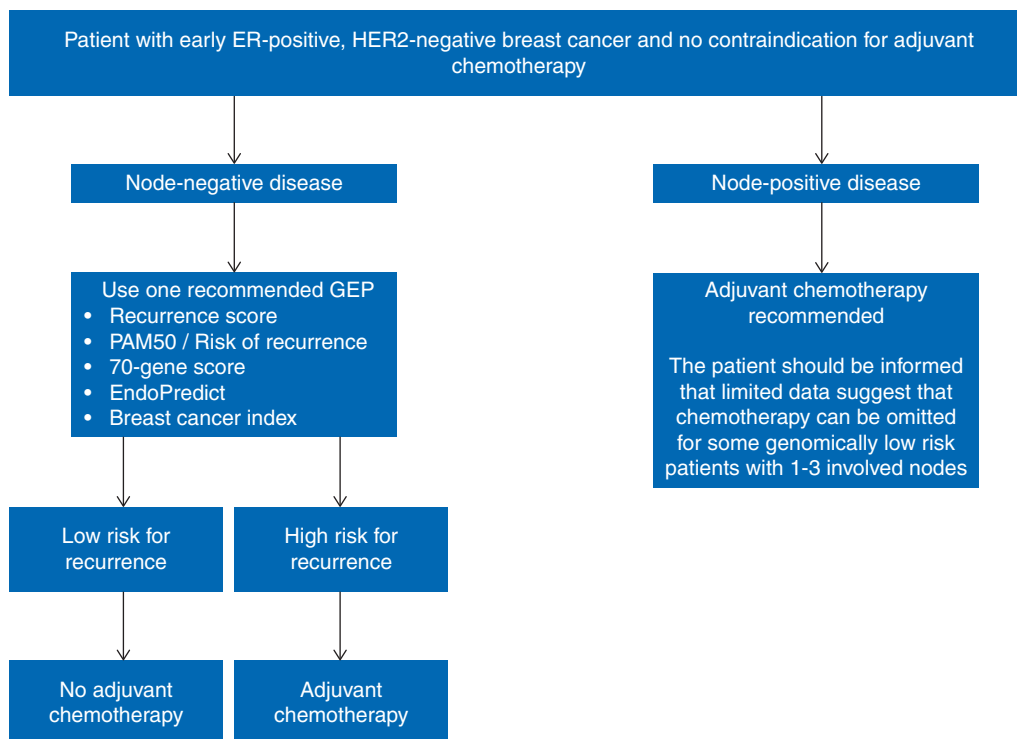
Figure 1. Approach to the clinical use of gene expression signatures following resection of early breast cancer. ER, estrogen receptor; GEP, gene expression profile; HER2, human epidermal growth factor receptor 2; PAM50, predictor analysis of microarray 50.

weighed against the potential adverse events, their impact on patients' quality of life and overall modest treatment compliance [63].

Choosing the 'right' GEP can be a challenge and even more so in node-positive BC. While more data—including the only two randomized trials—are available on RS and 70-gene assay compared with other tools, limited head-to-head comparisons indicate that ROR and EP/EPclin might better select patients at very low risk for distant recurrence, despite the presence of nodal spread and lack of adjuvant chemotherapy. Of particular concern is the fact that the comparative analysis by Sestak et al. demonstrated the limited long-term prognostic information that RS adds to clinical factors, further underscoring the need for long-term, mature data from the TailorX and RxPONDER trials in order to properly utilize RS in routine clinical practice [38].

Definitive and proper recommendations regarding the clinical use of GEP among patients with node-positive disease can only be given when the results of randomized trials are available (Figure 1). Until then, any of three strategies can be applied [64]: treating everyone with chemotherapy, potentially overtreating some causing excess toxicity in an effort to minimize risk for recurrence; applying any one GEP to all patients with 1–3 positive lymph nodes, regardless of nodal burden and the presence of extracapsular invasion, and extrapolating the cut-offs from the available evidence, therefore sparing patients from chemotherapy but potentially exposing some to undertreatment; finally, employing a hybrid approach with GEP testing in those with low nodal burden (for example, only one positive lymph node with no extracapsular invasion, or selecting only patients with micro-metastases in a single lymph node), in an effort to minimize both over- and undertreatment; however, no data support the latter

and the definition of 'low nodal burden' is rather arbitrary. A frank discussion with the patient is highly encouraged in order to reach an informed and shared decision regarding the optimal management strategy. Until high quality data are available however, we recommend against the generalized usage of GEP in all patients with node-positive disease and patients should be informed that present standard of care still includes chemotherapy for most.

Discussion

Conclusion

Whether the use of GEP demonstrates improved clinical utility among node-positive patients compared with (or, added to) anatomic grouping is clearly an unresolved issue. Caution is warranted, since overinterpreting the scant evidence that is currently available can expose women to unnecessarily increased risks for disease recurrence, incurable metastatic disease and, ultimately, death due to BC. Ongoing and future biomarker-driven studies that integrate clinical, pathologic, genomic and transcriptomic components will help optimize the science of adjuvant BC therapy.

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Disclosure

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