Original Article

Prediction of adjuvant chemotherapy benefit in endocrine responsive, early breast cancer using multigene assays

Kathy S. Albaina,b,*, Soonmyung Paikc,*, Laura van’t Veerd,*

a Loyola University Chicago Stritch School of Medicine, Division of Hematology/Oncology, b Breast Clinical Research and Thoracic Oncology Programs, Cardinal Bernardin Cancer Center, 2160 S. First Avenue, Maywood, IL 60153, USA

c Division of Pathology, National Surgical Adjuvant Breast and Bowel Project, 1307 Federal Street, Suite 303, Pittsburgh, PA 15212, USA

d Department of Pathology, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands

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SUMMARY

Background: Multigene assays performed on the primary tumors from women with non-metastatic breast cancer provide useful prognostic information and discriminate excellent versus poor outcome potential in diverse clinical scenarios. Recently, analyses were conducted to determine if these assays predict who benefits from adjuvant chemotherapy added to endocrine therapy and conversely, who might avoid chemotherapy because of lack of substantial benefit. This literature-based review summarizes these data and provides a perspective on the limitations and clinical utility of these assays.

Methods: The literature regarding multigene assays and signatures in early breast cancer was surveyed. Only two assays—the 21-gene recurrence score (RS) assay (OncoType DX) and the 70-gene signature (MammaPrint) – were analyzed in randomized or non-randomized clinical populations in order to determine the predictive utility of the test in the adjuvant chemotherapy setting in patients whose tumors were estrogen-receptor positive. These data are summarized by type of clinical analysis, with information on clinical utility and comparative studies with standard clinical-pathologic factors.

Results: From 2 independent analyses in phase III clinical trial settings with tamoxifen-alone control arms, the 21-gene RS assay defines a group of patients with low scores who do not appear to benefit from chemotherapy, and a second group with very high scores who derive major benefit from CMF or CAF chemotherapy. One study was conducted in node-negative disease, and the second in a node-positive population. Interaction terms were significant in both studies, and the effect of the assay remained upon adjustment for other standard factors. Utilizing a non-randomized clinical setting, the 70-gene signature could also predict chemotherapy benefit in the high risk group, versus no apparent benefit in the low risk group, an effect that remained after adjustment for standard factors. For both assays, the discordance rate between the assay prediction and clinical-pathologic risk category was approximately 30%. Clinical utility studies showed use of the assay results in a change in treatment decision in 25–30% of cases, most commonly from chemoendocrine therapy to endocrine therapy alone.

Summary: The prediction of adjuvant chemotherapy benefit over and above endocrine therapy using multigene assay-determined risk category differs greatly across risk level and challenges the previous adjuvant therapy paradigm that degree of benefit is the same regardless of risk. These data justify current clinical use of these assays, while ongoing prospective studies will refine their role in practice settings.
Despite a consensus regarding the clinical prognostic utility of many of these assays, data were limited on their role in predicting the degree of chemotherapy benefit. Only one provocative study showed that a 21-gene recurrence score (RS) assay (OncoType DX) defined a group of patients with node-negative disease that derived the greatest benefit from chemotherapy as well as a group that had no benefit over and above tamoxifen alone. With only this single study available, at that time the 2007 St. Gallen consensus panel voted against recommending clinical use of multigene assays for the prediction of chemotherapy benefit – that is – which patients with ER-positive disease might avoid chemotherapy. Since then, more data have emerged so that both the American Society of Clinical Oncology and the NCCN Guidelines currently recommend the use of the 21-gene RS assay as both a prognostic and a predictive tool in patients with node-negative disease, because of validation in a phase III randomized trial setting in an independent data set. Furthermore, very recently new analyses became available in the node-positive setting as well as additional supporting data in the node-negative setting, from both the 21-gene RS assay and a 70-gene assay, regarding prediction of chemotherapy efficacy.

This summary provides a review of all these prediction data, with justification for (1) the clinical use of multigene assays in adjuvant chemotherapy decision-making, and (2) a 2009 revision of the St. Gallen guideline on this subject. This review is restricted to the most rigorous analyses that were conducted in settings where two treatment groups could be compared: chemoendocrine therapy with endocrine therapy alone. No other assay summarized in Table 1 has prediction data (outcomes so far limited to prognosis).

**Chemotherapy benefit prediction from classical pathology factors**

Much can be gleaned regarding prediction of chemotherapy benefit from a good quality, standard pathology report. Level of estrogen receptor expression, tumor grading, ascertained of proliferation status by one of several methods, and immunohistochemical analyses of single gene protein levels are valuable in day-to-day practice. However, though they require a pathologist skilled in these techniques, they are not standardized across laboratories, and have not been used traditionally for prediction of chemotherapy benefit. These factors have been variably tested in randomized and non-randomized clinical trial settings, with no study yet done on prospective validation of the predictor or on whether use of information from the factor impacts survival (versus its non-use).

The most robust retrospective analyses were conducted for level of estrogen receptor as a predictive factor for chemotherapy efficacy. In exploratory, post-hoc analyses, high levels of ER protein expression measured centrally predicted lack of chemotherapy benefit. In these retrospective studies, conducted within phase III randomized trials, subsets with high levels of ER with either node-positive or node-negative disease appeared to derive no benefit from either CMF or CAF chemotherapy when added to tamoxifen. Based on these data, the St. Gallen 2007 panel stated that there were clear subsets of patients with endocrine responsive disease for whom the systemic therapy program need not contain chemotherapy.

**Predictive multigene assay data from the randomized phase III trial setting**

Three major studies have been conducted regarding the prediction of chemotherapy benefit prediction using multigene assays, two with the 21-gene RS assay and the other with the 70-gene assay (Table 2).

Only the RS assay (OncoType DX) has been studied with specimens obtained from phase III randomized clinical trials that employed an endocrine therapy-alone control arm. The RS assay, performed on paraffin-embedded tumor tissue, first entered clinical practice as a prognostic tool for women with node-negative, ER-positive breast cancer who were to be treated with five years of adjuvant tamoxifen, based on analyses in NSABP B14 and validated in a large community practice. The assay provides prognosis for distant relapse-free survival over 10 years. The initial analysis of the predictive utility of this assay was also in ER+, N0 disease, conducted by the NSABP on trial B20, which randomized patients between CMF or MF plus tamoxifen versus tamoxifen alone.

The trial population with available paraffin-embedded material was similar to the entire study population in demographics and outcome. The degree of chemotherapy benefit ranged from little or none in the lowest RS to an absolute benefit of 20% in the highest RS (Table 2), with a highly significant interaction p-value.

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**Table 1**

Multigene assays to determine clinical outcomes in primary breast cancer

<table>
<thead>
<tr>
<th>Name of assay</th>
<th>Reference(s)</th>
<th>Assay type or number of genes</th>
<th>Independent validation</th>
<th>Prognostic of outcome</th>
<th>Available for clinical use</th>
<th>Predictive of chemotherapy benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype DX</td>
<td>Paik et al. 2004⁹ Habel et al. 2006⁵</td>
<td>21 genes</td>
<td>Yes</td>
<td>Yes</td>
<td>US/Europe¹</td>
<td>Yes⁶</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>van de Vijver et al. 2002⁶ van’t Veer et al. 2002²</td>
<td>70 genes</td>
<td>Yes</td>
<td>Yes</td>
<td>Europe²</td>
<td>Yes⁴</td>
</tr>
<tr>
<td>MapQuant Dx</td>
<td>Sotiou et al. 2006⁵</td>
<td>Genomic grade</td>
<td>Yes</td>
<td>Yes</td>
<td>Europe³</td>
<td>N/A</td>
</tr>
<tr>
<td>Theros</td>
<td>Ma et al. 2006, 2009⁸, ¹⁰ HOXB13/IL17R and molecular grade index</td>
<td>Yes (as independent tests only)</td>
<td>Yes</td>
<td>Yes</td>
<td>US⁴</td>
<td>N/A</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>Wang et al. 2005¹¹</td>
<td>76</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Wound response</td>
<td>Chang et al. 2005¹²</td>
<td>512</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Invasiveness</td>
<td>Liu et al. 2007¹³</td>
<td>186</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>NCH</td>
<td>Naderi et al. 2007¹⁴</td>
<td>70</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Consensus</td>
<td>Teschendorf et al. 2006¹⁵</td>
<td>52</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>p53</td>
<td>Müller et al. 2005¹⁶</td>
<td>32</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Death–from-cancer</td>
<td>Glimský et al. 2005¹⁷</td>
<td>11</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

² Quality standards: ¹ CE mark, CLIA-registered; ² CE mark, ISO-accredited, CLIA-registered, FDA-cleared; ³ CE mark, ISO-accredited; ⁴ CLIA-registered.

'b Level II predictive evidence in phase III randomized trial settings with tamoxifen alone control arms, both node-negative and node-positive disease.

c Predictive evidence in nonrandomized clinical settings only.
Table 2
Multigene assays and the prediction of adjuvant chemotherapy benefit in primary breast cancer from retrospective analyses

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Study population</th>
<th>Relative risk/hazard ratios&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Higher risk (endpoint)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogeneous</td>
<td>Phase III trial</td>
<td>Nodal status</td>
</tr>
<tr>
<td>21-gene&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Yes</td>
<td>Yes</td>
<td>Negative</td>
</tr>
<tr>
<td>21-gene&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
</tr>
<tr>
<td>70-gene&lt;sup&gt;d&lt;/sup&gt;</td>
<td>No</td>
<td>No</td>
<td>Negative and up to 3 positive combined</td>
</tr>
</tbody>
</table>

DRFS = distant relapse-free survival; DFS = disease-free survival; DDFS = distant disease-free survival.

<sup>a</sup> Benefit to chemotherapy plus endocrine therapy versus endocrine therapy alone if ratio < 1 with statistical significance.
<sup>b</sup> Paik et al. 2006<sup>18</sup>; interaction p-value: significant.
<sup>c</sup> Albain et al. 2007<sup>22</sup>; interaction p-value: significant; similar results for overall survival, breast cancer specific survival.
<sup>d</sup> Knauer et al. 2009<sup>24</sup>; similar results for breast cancer specific survival.

These provocative findings represented a striking shift of the traditional adjuvant chemotherapy paradigm – that the relative benefit from chemotherapy is the same across risk categories – to a new model in which chemotherapy is not beneficial in those with lower risk endocrine responsive disease, but is of great utility in patients at higher risk. Adjustment for other factors in multivariate models did not alter this significant interaction between RS as a continuous variable and treatment effect. Furthermore, the previous observation that CMF did not work as well in older patients on the B20 trial could be explained by the higher percentage of lower RS in the older population. This finding might in fact explain much of the discordance between chemotherapy response by ER status that was observed in many individual adjuvant trials, and is consistently reported by the Early Breast Cancer Trialsists’ Collaborative Group. While the B20 data were intriguing, the fact that the control arm of the B20 trial was originally used to develop the 21-gene RS model may have resulted in overfitting of the data. Therefore, independent validation of the predictive utility of the test was needed and was achieved in an unrelated phase III trial population.

This second predictive utility study with the 21-gene RS assay was conducted using specimens obtained from the Southwest Oncology Group study S8814 (INT0100) in postmenopausal women with ER-positive, node-positive breast cancer.<sup>22,27</sup> The parent trial randomized patients among tamoxifen alone, CAF chemotherapy followed by tamoxifen, and CAF chemotherapy concurrent with tamoxifen. The latter arm was inferior, with CAF followed by tamoxifen resulting in superior DFS and overall survival out to 10 years of follow-up. Using specimens banked in an optional ancillary substudy to the parent trial, the RS assay was assessed in the superior CAF followed by tamoxifen arm versus the tamoxifen alone control arm. This trial subset with available paraffin-embedded material had outcomes similar to the parent trial. As summarized in Table 2, there was no DFS benefit to CAF (despite the higher risk node-positive population) in the lowest RS, whereas the benefit was large in the highest RS. The interaction p-value was significant. Similar results were observed for overall survival.

The RS prediction was also significant after adjustment for other prognostic factors. Of note, there appeared to be no benefit to CAF in the low RS category even when the subset of patients with 4+ nodes was considered. Thus, even though risk of recurrence was high with increasing number of positive nodes, the tumor biology reflected by the low RS appeared resistant to anthracycline-based chemotherapy. A recent analysis of breast cancer-specific survival (BCSS) in this same study demonstrated a 90% 10-year outcome which was not impacted by CAF in the low RS, whereas the 10-year BCSS survival for patients with high RS was 73% vs 54% for CAF vs tamoxifen alone, respectively. (Albain K, personal communication, submitted for peer review).

Predictive multigene assay data from the non-randomized trial setting

The 70-gene assay, performed on frozen tumor tissue in a large, non-randomized population untreated with systemic adjuvant therapy, was the first to show the prognostic utility of multigene assays over and above the best clinical prediction using standard St. Gallen risk discriminators.<sup>8,29</sup> This assay has performed well in follow-up validation analyses in other data sets, though in all reports adjuvant treatment was heterogeneous within the populations studied. (3,6,23,30)

To date, there have been no analyses of the 70-gene assay for prediction of chemotherapy benefit within phase III clinical trial data. However, at the 2009 St. Gallen Conference on Primary Breast Cancer, a new analysis of a large dataset using pooled patient data (n = 1,637, combined node-positive and node-negative) was reported.<sup>24</sup> The patients in this combined analysis were grouped by chemoendocrine therapy or endocrine therapy. As summarized in Table 2, patients in the 70-gene low risk group derived no significant DDFS benefit from chemotherapy added to the endocrine therapy. However, for those in the 70-gene high risk group, the 5-year DDFS was 88% in the chemoendocrine therapy group but only 69% in the endocrine therapy subset. This finding remained significant after adjustment for age, tumor size, nodes, grade, ER and HER2. Similar results were reported for BCSS.

Within the combined analysis just cited, a subgroup with 1–3 positive nodes was studied in more depth.<sup>25</sup> It appeared that those patients with the good 70-gene profile had excellent 8-year overall survival regardless of whether they received chemotherapy (95%) or not (94%). Although not a randomized population, this study is consistent with the evidence accrued to date that multigene assays identify a good risk/profile group whose tumors do not appear to respond to chemotherapy. This appears to be true regardless of risk of recurrence as identified by nodal status.

Additional predictive evidence from neoadjuvant trials

There have been at least 12 neoadjuvant studies that correlated treatment response outcomes with multigene assays, conducted across platforms and with a wide variety of drugs. In sum, these demonstrate that gene signatures are predictive of chemotherapy response.<sup>3</sup> These studies consistently show that gene-expression profiles of cancers highly sensitive to chemotherapy differ from those of less responsive tumors. It is not clear whether this is a “class effect” of the biology, or whether these signatures are truly specific for the drug combination tested.

Two studies in the neoadjuvant setting can be highlighted that provide further evidence that multigene assays identify a group of patients with tumors that are responsive as well as those that are quite non-responsive to chemotherapy. The first study was conducted with the 21-gene RS assay.<sup>26</sup> Within the group of patients with lower recurrence scores, pathologic complete remissions (pCR)
were not observed. The second study reached a similar conclusion with the 70-gene assay. A poor 70-gene signature predicted a high pCR rate, whereas the converse was true for the good profile.

Relevance of multigene assays in the clinical practice setting

Based on the studies reviewed above, clinical use of the 21-gene RS assay and the 70-gene signature increased in recent years. In order to address whether these assays impact on clinical decision-making over and above standard pathology factors, 5 trials with a variety of designs were conducted (Table 3). Four of these studies employed the RS assay and the other the 70-gene assay, 3 were retrospective in either academic or community settings or both, and 2 were prospectively conducted. Overall in the 2 prospective studies, a change in treatment decision after the use of the assay resulted about 30% of the time, most frequently a change from a pre-assay recommendation for chemotherapy plus endocrine therapy to a post-assay recommendation for endocrine therapy alone. The prospective study by Lo et al. also had a component for the patient’s side of the decision-making process. In this analysis, 95% of patients were glad they had the RS assay performed, 87% stated the assay influenced their choice of treatment, and 27% indicated that the results caused them to change their decision. In quality of life analyses, there was increased confidence in the treatment decision and increased satisfaction, with significant decrease in anxiety post-test.

Limitation and caveats

There are several limitations and caveats regarding the use of multigene assays as predictive tools for adjuvant chemotherapy benefit. First, so far the 2 assays that have predictive data available (reviewed above, the RS and 70-gene assays) were studied with “older” chemotherapy regimens, in general based on clinical data in the pre-taxane era. Given the need to test the predictive performance of the assay in a setting where there is a non-chemotherapy control group, there will most likely be no other clinical trial set to test “third generation” taxane-containing chemotherapy regimens against a no-chemotherapy group. That said, there is a growing body of evidence in the neoadjuvant setting to support the predictive utility of multigene assays with a variety of current drug combinations, including those studied with the 21-gene RS and 70-gene assays. Two other frequently raised concerns about the multigene assays are that they do not control for host factors such as drug metabolism and inherited polymorphisms, nor do we know from a phase III prospective trial that using these assays will result in a survival benefit over and above the treatment chosen if no assay result is used in decision-making both of these are valid points, but it should be pointed out that the same concern exists for any of the single-factor markers such as ER, grade, HER2 or proliferation. Note that the predictive utility for chemotherapy benefit with these factors has been variable, with no prospective trial to determine survival benefit or not from use of the factor. Fortunately, prospective trials are ongoing with both the RS assay (TAILORx) and the 70-gene assay (MINDACT) that will address the survival impact from these assays. The most commonly debated point regarding multigene assays centers around strong contentions that a single factor or combination of factors from a standard pathology report may allow the treatment selection just as well as the assay. This statement would most likely be true if all the standard factors were favorable, or all were unfavorable. But, it is uncommon to get a composite profile from all these factors that has no uncertainty – that is, a patient whose tumor has all the factors pointing in one direction (eg: high ER level, lowest grade, low proliferation, HER2-negative). Furthermore, the multigene assay offers new information that changes the direction of the treatment decision for or against chemotherapy approximately 30% of the time (Table 3). The strongest rebuttal to this contention, however, pertains to a high rate of discordance between the risk category determined by clinical-pathologic factors versus the risk level determined by the multigene assay. The weight of the evidence suggests that the most accurate prediction model results when both genomic and clinical variables are used together. In multiple studies across several randomized and non-randomized data sets, it is clear that the 21-gene RS and 70-gene assays add independent prognostic information to clinical-pathologic risk assessed by the online program Adjuvant! Also, for the most part the 21-gene RS provides better discrimination of individual tumor behavior and a more reliable prediction of those who would benefit versus not compared to the traditional factors.

| Study                  | N     | Setting               | Type                | Change in treatment decision%
|------------------------|-------|-----------------------|---------------------|--------------------------
| Oratz et al.32         | 32    | Community, USA        | Retrospective, 21 gene | 25%                      |
| Asad et al.35          | 85    | Community, USA        | Retrospective, 21 gene | 44%                      |
| Kamal et al.33         | 80    | Academic, USA         | Retrospective, 21 gene | 18%                      |
| Lo et al.34            | 89    | Academic + Community, USA | Prospective, 21 gene | 32%                      |
| Bueno de Mesquita et al.36 | 427   | Community, Dutch      | Prospective, 70 gene | 26%                      |

* Most frequent decision change in all studies was from CHT HT.

Summary

Multigene assays allow better definition of which patients with ER-positive breast cancer should receive and will benefit from chemotherapy and greater confidence in the decision to avoid chemotherapy. The use of these assays significantly decreases number of patients whose tumor biology falls in an “uncertain risk” category based on standard clinical-pathologic factors. Unfortunately, there are no similar data of prognosis combined with prediction of chemotherapy benefit for ER-negative disease, but work in the neoadjuvant setting on treatment-selection signatures is ongoing. Completion of the MINDACT and TAILORx trials will reveal whether lowering the proportion of chemotherapy given will not jeopardize survival and determine the degree of chemotherapy for those patients whose risk levels are in the intermediate range. Until these trial results are known, it is reasonable to order multigene assays whenever feasible in the ER-positive setting to optimally inform systemic adjuvant therapy decision-making. This recommendation is suggested because it is difficult to guess the dominant biology of an individual tumor solely from expert
pathology reports in many cases, based on the 30% discordance data. The cost-effectiveness of these assays should be addressed to allow broader application of their utility world-wide, bearing in mind that avoiding otherwise-planned chemotherapy is an additional cost benefit both in terms of toxicity and economically. Based on the data reviewed herein, the 2009 St. Gallen consensus panel voted to recommend the clinical use of multigene assays for prognosis and prediction across many common clinical scenarios.

Competing interests: KSA has accepted two CME speaker honoraria and travel expense reimbursements from Genomic Health, Inc. 2007 and 2008. SP is a patent or trademark holder of the 21-gene recurrence score assay Oncotype DX, but the patent right is assigned to the NSABP. He has no financial or other interests. LvV is a shareholder and part-time employee of Agendia BV/Inc and is inventor but not owner of the patent for the 70-gene MammaPrint assay. The patent is owned by the Netherlands Cancer Institute.

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References