Has the time come for genomic tests to guide the use of adjuvant chemotherapy in node-positive breast cancer?

Several multigene assays have been developed over the past decade for risk stratification in early breast cancer [1]. They were primarily developed as prognostic tools, and have indeed shown to add significant prognostic information to classic clinico-pathological risk classifiers [2–7]. They are most useful in patients with estrogen receptor (ER)-positive HER2-negative tumors, as a consequence of identifying the low-proliferative luminal-A tumors at low risk of recurrence, whereas they are less informative in triple-negative and HER2-positive tumors, as most of these tumors are classified as high risk by virtually all these assays [1].

While refining prognostication is important, the oncology community was more eager to understand whether these genomic tests can identify patients who could be spared adjuvant chemotherapy. This is becoming more relevant in the current era given that ~40% of breast cancer patients are over 65 years of age at the time of diagnosis where morbidities other than cancer often contribute to patient mortality [8, 9]. Yet, whether modifying treatment strategies based on the result of a genomic test would lead to improved patient outcome, continue to be an area of debate [10].

Which genomic test is best is a tough question to answer. It is reasonable to say that they are highly comparable although few differences exist in their clinical development, and prognostic ability (Table 1). These tests are commercially available for clinical application, yet their use is largely restricted to the node-negative population, not only because we have more data, but also due to the dogma that node-positive patients should be anyways treated with adjuvant chemotherapy.

In the article that accompanies this editorial, Gnant et al. reported the largest study to date investigating the role of a genomic test; PAM50 ROR in determining prognosis of patients with node-positive (one to three) breast cancer who were treated with 5 years of adjuvant endocrine therapy but no chemotherapy [11]. The study included a total of 543 patients who were enrolled in two phase III adjuvant trials; ABCSG-8 and ATAC. The results indicated that in patients with one positive lymph node, 40% of patients were classified as ‘low risk’ by PAM50 ROR with a risk of distant recurrence of 6.6% at 10 years [95% confidence interval (CI) 3.3% to 12.8%]. In patients with two and three positive lymph nodes, such group had nearly double the risk of distant recurrence; 12% (95% CI 6.6% to 22.8%). So the legitimate question raised by the investigators of this study is whether it is reasonable to consider omitting chemotherapy in patients with nodal involvement who are classified as ‘low risk’?

The case for patients with one positive lymph node is perhaps less controversial. Assuming that chemotherapy could reduce the risk of recurrence by 30% [12], and given the low absolute risk of recurrence of 6%, the added benefit of any further therapy would be clinically negligible. The case is more challenging in patients with more than one positive lymph node where the risk of recurrence predicted by the genomic test is ~12% with a 95% CI reaching up to 22%. Assuming the relative risk reduction of 30% associated with chemotherapy, the absolute benefit could be clinically meaningful. It is worth noting though that figures provided for the two to three positive lymph node cohort are not only those of the PAM50 ROR low-risk category, but for the combined low and intermediate risk groups. The authors could not provide separate analysis for each group due to low number of events, but it is plausible that if a separate analysis for the low-risk category was provided, risk predictions could have been more favorable.

But irrespective of the risk prediction of the genomic test, one needs to question whether the higher risk of relapse per se that is associated with the high nodal burden would make the tumor more responsive to chemotherapy. The answer is probably not. Based on the latest Oxford overview [12], the relative risk reductions with adjuvant chemotherapy on relapse and death were similar irrespective of nodal status. Neoadjuvant trials also informed us that the pathological complete response after preoperative chemotherapy is modest for the low-proliferative ER-positive tumors irrespective of their nodal involvement [13]. Thus, we need to distinguish between a high risk of recurrence due to high nodal burden and the likelihood of benefit to chemotherapy.

Chemotherapy acts on rapidly dividing cells, and given that genomic tests like PAM50 ROR are mainly driven by proliferation signals, it comes as no surprise that patient subsets defined as ‘low risk’ or in other words have ‘low-proliferative’ tumors would benefit less of adjuvant chemotherapy. There is no reason to think that this would be different in node-negative or node-positive disease. Of note, patients with node-negative disease often have higher proportion of tumors classified as ‘low risk’ by a genomic test compared with patients with node-positive disease (Figure 1). This is in line with the current study in which patients with less nodal involvement (one positive node) had higher proportion of tumors classified as low risk compared with those with two to three positive nodes. Thus, it is possible that as tumors advance in stage, they become more proliferative and hence have higher risk scores. Yet, this does not negate that among patients with ‘higher stage’ tumors, we can still identify a subset with low-proliferative tumors (i.e. those defined as low risk by the genomic test) that could be potentially spared chemotherapy, as they are unlikely to benefit, even if their risk of recurrence is ‘relatively’ high due to their stage. The results of two previous studies using Oncotype Dx and Mammaprint reinforce this argument in which node-positive patients with low-risk scores derived no benefit of adding chemotherapy to adjuvant endocrine therapy with benefit only restricted to patients classified as high risk [14, 15].

Currently, Oncotype Dx is the only genomic test to demonstrate an ability to predict benefit to adjuvant chemotherapy based on retrospective evaluation of two randomized trials.
However, it should be noted that it is not unlikely that other genomic tests would have produced similar results if they had the opportunity to be evaluated in a similar context. The same applies for this study in which other assays would have possibly produced comparable results to PAM 50 ROR in refining prognosis of node-positive disease. Nevertheless, as clinicians, confidence is built with a given test when consistent results using robust methodology are obtained. This was the case in the study by Gnant et al. [11], which provide to the literature an important contribution on the potential added value of using a genomic test like PAM50 ROR in managing patients with node-positive disease. Currently, four prospective phase III randomized trials are ongoing investigating the role of different genomic tests in defining the need for adjuvant chemotherapy (Table 1). These trials included patients with node-positive disease (up to three positive lymph nodes), and will provide level I evidence on the clinical utility of these tests in daily practice. Nevertheless, until then and based on current evidence including the study by Gnant et al., it is not unreasonable to consider the use of genomic tests to guide adjuvant treatment decisions in node-positive disease.

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HAA Jr served on an advisory board for PAM50 ROR (Prosigna®) and conducted clinical research using the genomic grade.

references

Table 1. Different genomic tests that are currently available to refine prognosis of patients with ER-positive HER2-negative primary breast cancer

<table>
<thead>
<tr>
<th>Test</th>
<th>Oncotype Dx</th>
<th>MammaPrint</th>
<th>PAM50 ROR</th>
<th>EndoPredict</th>
<th>Breast Cancer Index (BCI)</th>
<th>Genomic grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has it been retrospectively validated on prospective phase III trials?</td>
<td>B-20</td>
<td>x</td>
<td>ATAC</td>
<td>ABCSG6</td>
<td>ATAC</td>
<td>BIG 1–98</td>
</tr>
<tr>
<td>Can it predict early recurrence (0–5 years)?</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
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<tr>
<td>Can it predict late recurrence (after 5 years)?</td>
<td>x</td>
<td>?</td>
<td>(superior to Oncotype Dx)</td>
<td>√</td>
<td>(superior to Oncotype Dx)</td>
<td></td>
</tr>
<tr>
<td>Can it be tested on FFPE tissue?</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Can the test be decentralized with established reproducibility data?</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

*Test subjected to prospective validation is on frozen tissue. FFPE, formalin-fixed paraffin-embedded.
Heterogeneity of driver genes and therapeutic implications in colorectal cancer

Next-generation sequencing (NGS) of patient tumors has been rapidly incorporated into both prescreening programs and clinical trials over the last couple of years, with the goal of identifying gene alterations that can guide personalized decisions. In the report accompanying this editorial, Normanno et al. describe the intratumor heterogeneity for known driver mutations in colorectal cancers (CRC) [1]. KRAS exon 2 wild-type CRC patients were treated with FOLFIRI in combination with cetuximab in the first-line setting, followed by FOLFOX therapy with or without cetuximab upon progression. Investigators tracked mutant alleles to determine clonality in a sensitive and quantitative manner using NGS, and correlated KRAS, NRAS, BRAF and PIK3CA mutations in primary tumor samples of eligible patients with benefit to anti-EGFR therapy in the advanced setting. When examining the sequencing results, they noted that mutant allele frequencies (MAFs) did not clearly correlate with the fraction of neoplastic cells in the sample. In addition, in tumors with more than one mutation, MAFs were often different among the affected genes. These discrepancies prompted a more detailed analysis of the genomic complexity in CRC.

MAF is the number of mutant reads divided by the total number of reads—coverage—at the specific genomic position of interest. It is largely influenced by tumor purity (fraction of neoplastic cells in the sample) and copy number alterations (gene amplifications and deletions). Therefore, authors normalized MAFs for the neoplastic cell content and calculated what can be named ‘MAF in neoplastic cells’ (MAFnc). For example, if one gene mutation has a MAF of 30%, the MAFnc increases to 60% when tumor purity of the sample is 50%, and remains at 30% when 100% of the cells in the sample are neoplastic. As mutations in oncogenes frequently affect one allele, investigators decided to multiply MAFnc by 2 and calculated the ‘Heterogeneity Score’ (HS), which was used in all downstream correlative analyses. Since the MAFnc should be no more than 50% in a pure tumor sample with a heterozygous mutation, an HS larger than 100 suggests PCR amplification bias of mutant allele, genomic amplification of the mutant allele or loss of the wild-type allele. Additionally, recent studies with single-cell analysis demonstrate that mutations of known driver genes occur in both homozygous and heterozygous states [2]. Consequently, readers should be careful when assessing the HS, as the assumptions made by the authors may be wrong. Furthermore, most of the published literature on quantitative assessment of somatic cancer mutations and their potential predictive value describes MAF or MAFnc counts of NGS data, including studies described below.

first insights on the genomic structure of CRC

The results reported by Normanno et al. suggest that in most CRC the majority of neoplastic cells carry mutant KRAS or NRAS (higher MAFnc on average). In contrast, in BRAF and PIK3CA mutant cases only a fraction of neoplastic cells carried the mutant allele (lower MAFnc on average). It is important to emphasize that patients were eligible to the trial if no mutations were identified on exon 2 of KRAS as per standard sequencing platforms. Therefore, the distribution of KRAS MAFnc reported in the study may not reflect the overall KRAS-mutant CRC population, and one can expect that on average, it may be even higher considering the lower sensitivity of conventional mutation detection assays compared with NGS platforms.

Other studies have also described clonal–subclonal frequencies of driver alterations in cancer. In a comprehensive analysis of TCGA data in nine solid tumors, McGranahan et al. found a clear tendency for mutations in driver genes to be clonal compared with mutations in non-cancer genes [3]. Interestingly,