A 50-Gene Intrinsic Subtype Classifier for Prognosis and Prediction of Benefit from Adjuvant Tamoxifen


Abstract

Purpose: Gene expression profiling classifies breast cancer into intrinsic subtypes based on the biology of the underlying disease pathways. We have used material from a prospective randomized trial of tamoxifen versus placebo in premenopausal women with primary breast cancer (NCIC CTG MA.12) to evaluate the prognostic and predictive significance of intrinsic subtypes identified by both the PAM50 gene set and by immunohistochemistry.

Experimental Design: Total RNA from 398 of 672 (59%) patients was available for intrinsic subtyping with a quantitative reverse transcriptase PCR (qRT-PCR) 50-gene predictor (PAM50) for luminal A, luminal B, HER-2-enriched, and basal-like subtypes. A tissue microarray was also constructed from 492 of 672 (73%) of the study population to assess a panel of six immunohistochemical IHC antibodies to define the same intrinsic subtypes.

Results: Classification into intrinsic subtypes by the PAM50 assay was prognostic for both disease-free survival (DFS; \( P = 0.0003 \)) and overall survival (OS; \( P = 0.0002 \)), whereas classification by the IHC panel was not. Luminal subtype by PAM50 was predictive of tamoxifen benefit [DFS: HR, 0.52; 95% confidence interval (CI), 0.32–0.86 vs. HR, 0.80; 95% CI, 0.50–1.29 for nonluminal subtypes], although the interaction test was not significant \( (P = 0.24) \), whereas neither subtyping by central immunohistochemistry nor by local estrogen receptor (ER) or progesterone receptor (PR) status were predictive. Risk of relapse (ROR) modeling with the PAM50 assay produced a continuous risk score in both node-negative and node-positive disease.

Conclusions: In the MA.12 study, intrinsic subtype classification by qRT-PCR with the PAM50 assay was superior to IHC profiling for both prognosis and prediction of benefit from adjuvant tamoxifen. Clin Cancer Res; 18(16); 4465–72. ©2012 AACR.
underrepresented in biomarker investigations, leaving uncertainty to the performance of genomic assays in younger patient populations.

The intent of this study was to use both an established immunohistochemical (IHC) classifier and a gene expression classifier (PAM50) of the breast cancer intrinsic subtypes to assess for both prognostic and predictive value to adjuvant hormonal therapy in a study population of premenopausal women also treated with adjuvant chemotherapy (MA.12). The intrinsic subtypes were found to have independent prognostic value in a multivariate analysis that included standard clinical and pathologic variable. More importantly, the luminal subtypes, as discriminated by PAM50, had predictive ability to refine the population of patients who obtained a greater benefit from adjuvant tamoxifen.

Materials and Methods

The NCIC Clinical Trials Group (CTG) MA.12 study was a randomized trial of 672 premenopausal women with stage I–III breast cancer after adjuvant chemotherapy, of any hormone receptor status, comparing tamoxifen 20 mg per os daily with placebo for a planned 5 years. A ROR model, generated from the gene expression classifier data in addition to clinicopathologic data, was also applied to generate a continuous risk score for further investigation as a clinical test.

Statistical analyses

The detailed statistical plan and analyses for the MA.12 study are published elsewhere (11). The following are the definitions for the clinical endpoints of the study; disease-free survival (DFS): time from randomization to earliest date of recurrence or death or censored on the last date the patient was known to be alive; overall survival (OS): time from randomization to date of death or censored on the last date the patient was known to be alive. Biomarker subtype definitions and hypotheses to be tested were prespecified in writing, and statistical analyses were then conducted independently by the NCIC CTG statistical centre. The primary objectives of this translational study were 2-fold: (i) to determine whether the intrinsic subtypes, as categorized by PAM50, had prognostic
significance in the entire MA.12 cohort and (ii) to determine whether the luminal subtypes (A and B), as determined by PAM50, were predictors of greater efficacy to adjuvant tamoxifen than the hormone receptor–positive status in MA.12, as determined by biochemical or IHC methods. Both OS and DFS by ER status (determined locally), by subtypes based on the immunohistochemistry and by PAM50 classifiers, were described by Kaplan–Meier curves and compared with log-rank tests. A Cox model with an interaction term between treatment and subtype was used to assess for predictive significance based on differences between treatment arms and subtype classification. All statistical tests were 2 sided.

PAM50-derived ROR scores were calculated using formulas previously trained and described (19, 20). Harrell C-index was used to compare the prognostic and predictive capacity of clinical, IHC, and expression profile–derived models (21). A C-index higher than 0.5 implies a good prediction ability of the model, whereas a C-index equal to 0.5 implies no predictive ability (no better than random guessing). Bootstrap percentile method with 200 replications was used to calculate the 95% confidence interval (CI) for the predicted probability of disease at 10 years based on the ROR scores with the highest C-index. This study received research ethical board approval from the host institution (University of British Columbia, Vancouver, British Columbia, Canada).

Results

The NCIC MA.12 study enrolled 672 premenopausal women from 1993 to 2000: 338 randomized to tamoxifen and 334 to placebo (11). Baseline characteristics of all patients randomized (N = 672), patients within the TMA series for IHC classification (IHC cohort: n = 492), and the PAM50 cohort for intrinsic subtype classification (PAM50 cohort: n = 398) are outlined in Table 1. The standard clinicopathologic characteristics were similar among all 3 cohorts. Median age was approximately 45 years (range: 26–58 years), approximately 75% of the study cohorts were node-positive, and all patients received adjuvant chemotherapy (45% non-anthracycline and 55% anthracycline-based, as detailed in Table 1).

In the original MA.12 study population, the ER assay was done by a biochemical method (quantitative ligand-binding assay) in 60% of patients randomized to tamoxifen and in 51% of the placebo group and the remainder by immunohistochemistry. All clinical progesterone receptor (PR) assays were done by the biochemical method. The hormone receptor status was deemed positive (ER- and/or PR-positive) in 75% (n = 505) of the original study population, as determined by local laboratories. By the centrally assessed IHC classifier conducted for this study, 71% were classified as luminal breast cancers (40% luminal A and 31% luminal B) with the specific breakdown across all the intrinsic subtypes detailed in Table 2. Among the 398 patient

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All randomized (n = 672)</th>
<th>IHC Cohort (n = 492)</th>
<th>PAM50 cohort (n = 398)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median and range)</td>
<td>45.7 y (26–58)</td>
<td>45.6 y (27–58)</td>
<td>45.5 y (27–58)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>II</td>
<td>84%</td>
<td>85%</td>
<td>84%</td>
</tr>
<tr>
<td>III</td>
<td>6%</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25%</td>
<td>24%</td>
<td>25%</td>
</tr>
<tr>
<td>1–3</td>
<td>56%</td>
<td>56%</td>
<td>55%</td>
</tr>
<tr>
<td>≥ 4</td>
<td>19%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Clinical ER/PR status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+ and/or PR+</td>
<td>75%</td>
<td>74%</td>
<td>73%</td>
</tr>
<tr>
<td>ER−/PR unknown</td>
<td>14%</td>
<td>14%</td>
<td>15%</td>
</tr>
<tr>
<td>ER−/PR−</td>
<td>11%</td>
<td>12%</td>
<td>12%</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide, methotrexate and fluorouracil</td>
<td>45%</td>
<td>44%</td>
<td>43%</td>
</tr>
<tr>
<td>Doxorubicin and cyclophosphamide</td>
<td>33%</td>
<td>32%</td>
<td>32%</td>
</tr>
<tr>
<td>Cyclophosphamide, epirubicin and fluorouracil</td>
<td>22%</td>
<td>24%</td>
<td>25%</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>19%</td>
<td>26%</td>
<td>24%</td>
</tr>
<tr>
<td>II</td>
<td>30%</td>
<td>42%</td>
<td>42%</td>
</tr>
<tr>
<td>III</td>
<td>22%</td>
<td>31%</td>
<td>34%</td>
</tr>
<tr>
<td>Missing</td>
<td>29%</td>
<td>2%</td>
<td>0%</td>
</tr>
</tbody>
</table>
samples that had classification by PAM50, 53% were classified as luminal breast cancers (34% luminal A and 19% luminal B). The concordance for intrinsic subtypes among 348 patients who could be classified by both IHC and PAM50 classifiers (Table 2) was 82.2%, 81.0%, 87.6%, and 97.4% for luminal A, luminal B, HER-2–enriched (defined by PAM50)/luminal B HER-2
þ
/ER
−/PR
−/HER-2
−
(defined by immunohistochemistry), and basal-like breast cancers, respectively, with an overall
k
0.64 (95% CI, 0.58–0.71). Within these 348 patients: 209 (60%) were ER- and/or PR-positive by central immunohistochemistry, 66 (19%) were HER-2–positive by central immunohistochemistry, and 75 (21%) were core basal by IHC definition. Other than assessing for concordance in the 348 patients with available data across classifiers, no further assessment of prognosis or prediction was conducted because of the limited sample size.

Association with outcomes and prognostic values

Among all randomized patients in both treatment groups, there was no significant difference between patients with positive and negative ER by clinical assay in either DFS or OS. Intrinsic subtype classification by the 6 antibody IHC panel created a larger discriminatory effect for DFS and OS with the worst prognostic group being the HER-2–positive nonluminal breast cancers for DFS and basal-like breast cancers for OS compared with the best prognostic group being luminal A for both DFS and OS, but none of these differences were statistically significant. However, with the PAM50 classification, both DFS and OS were statistically significantly different across intrinsic subtypes (Fig. 1A and B) with the HER-2–enriched subtype having the lowest and the luminal A breast cancers the highest 5-year survival values. In multivariate analysis (Table 3) adjusting for tumor grade, tamoxifen treatment, and clinical variables including ER status, nodal status, and size of tumor, intrinsic subtyping, as classified by PAM50, was still independently prognostic from the standard clinicopathologic variables and treatment for both DFS (P = 0.02) and OS (P = 0.02). The multivariable Cox models that included the subtype and clinicopathologic variables listed earlier were significantly better than either clinicopathologic alone or subtype alone.

We also assessed the performance of ROR scores generated from PAM50 subtypes with and without clinical information based on algorithms developed previously (18, 19). The C-index for ROR-S (subtype only), ROR-P (subtype with proliferation signature index), ROR-T (subtype with tumor size), and for ROR-PT (subtype with both proliferation signature index and tumor size) are summarized in Supplementary Table S2. ROR-T had highest numerical prognostic capacity in both DFS and OS with a C-index of 59.8% (54.8%–64.2%) and 63.1% (57.9%–68.2%), respectively. Figure 2 shows that the ROR-T scores of tamoxifen-treated patients have a linear relationship with probability of 10-year relapse in both the node-negative and the node-positive cohorts.

Table 2. Intrinsic subtype distribution by immunohistochemistry and PAM50 (n = 348)

<table>
<thead>
<tr>
<th>Intrinsic subtypes by IHC/FISH</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Luminal/HER-2*</th>
<th>HER-2*/ER− PR−</th>
<th>Core basal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>101 (78%)</td>
<td>20 (15%)</td>
<td>8 (6%)</td>
<td>0</td>
<td>1 (1%)</td>
<td>130</td>
</tr>
<tr>
<td>Luminal B</td>
<td>23 (33%)</td>
<td>39 (57%)</td>
<td>7 (10%)</td>
<td>0</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>HER-2-E</td>
<td>9 (12%)</td>
<td>14 (19%)</td>
<td>26 (35%)</td>
<td>23 (31%)</td>
<td>3 (4%)</td>
<td>75</td>
</tr>
<tr>
<td>Basal-like</td>
<td>1 (1%)</td>
<td>2 (3%)</td>
<td>0</td>
<td>2 (3%)</td>
<td>69 (93%)</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>75</td>
<td>41</td>
<td>25</td>
<td>73</td>
<td>348</td>
</tr>
</tbody>
</table>

Table 3. Multivariate analysis of prognostic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>DFS HR (95% CI)</th>
<th>DFS P</th>
<th>OS HR (95% CI)</th>
<th>OS P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype</td>
<td>Basal vs. Luminal A</td>
<td>1.57 (0.75–3.29)</td>
<td>0.02</td>
<td>2.19 (0.91–5.26)</td>
<td>0.02</td>
</tr>
<tr>
<td>Subtype</td>
<td>Her2− vs. Luminal A</td>
<td>2.25 (1.29–3.90)</td>
<td></td>
<td>2.74 (1.39–5.40)</td>
<td></td>
</tr>
<tr>
<td>Subtype</td>
<td>Luminal B vs. Luminal A</td>
<td>1.98 (1.16–3.39)</td>
<td></td>
<td>2.40 (1.25–4.63)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Tamoxifen vs. placebo</td>
<td>0.58 (0.41–0.82)</td>
<td>0.002</td>
<td>0.68 (0.45–1.02)</td>
<td>0.06</td>
</tr>
<tr>
<td>ER</td>
<td>Positive vs. negative</td>
<td>0.82 (0.53–1.28)</td>
<td>0.38</td>
<td>0.58 (0.35–0.97)</td>
<td>0.04</td>
</tr>
<tr>
<td>Nodal status</td>
<td>1–3 vs. negative</td>
<td>1.97 (1.20–3.32)</td>
<td>0.02</td>
<td>1.61 (0.92–2.81)</td>
<td>0.17</td>
</tr>
<tr>
<td>Nodal status</td>
<td>&gt;3 vs. negative</td>
<td>1.97 (1.12–3.48)</td>
<td></td>
<td>1.78 (0.94–3.37)</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td>T stage 1 vs. &gt;1</td>
<td>0.64 (0.44–0.92)</td>
<td>0.02</td>
<td>0.62 (0.40–0.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>Tumor size</td>
<td>1 vs. 2 vs. 3</td>
<td>0.96 (0.71–1.31)</td>
<td>0.81</td>
<td>0.83 (0.58–1.19)</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Prediction of tamoxifen benefits

With all randomized patients included in the analysis, the original MA.12 study showed a trend for improved DFS for tamoxifen (HR, 0.77; 95% CI, 0.59–1.01; \( P = 0.06 \); ref. 11). Differences between tamoxifen and placebo were not statistically significant in OS for the trial as a whole (HR 0.78 with 95% CI 0.57–1.06 and \( P = 0.12 \)) and, therefore, the following analyses were restricted to DFS only. Being ER-positive by a clinical test could not identify a group with statistically significant better outcomes on tamoxifen (Fig. 3A). Within the luminal cohorts (luminal A + B) based on the IHC classifier, there appeared a greater trend for benefit of adjuvant tamoxifen, though this was again not statistically significant (HR, 0.75; 95% CI, 0.51–1.09; \( P = 0.13 \); Fig. 3B). However, using the PAM50 classifier to identify luminal subtypes, there was a statistically significant benefit in DFS for tamoxifen (HR 0.52; 95% CI, 0.32–0.86; \( P = 0.009 \); Fig. 3C), whereas no statistically significant benefit was found for patients with nonluminal subtypes (HR, 0.80; 95% CI, 0.50–1.29). However, the interaction test between luminal type by PAM50 and tamoxifen treatment was not statistically significant (\( P = 0.24 \)). The corresponding absolute improvement in 5-year DFS rate was 11.8% for tamoxifen versus placebo in the hormone-sensitive cohort (luminal A and B), as defined by PAM 50 (5-year DFS rate = 86.1% vs. 74.3%, respectively; Fig. 3C). A forest plot comparing the efficacy of tamoxifen versus placebo across the various predictive classifiers is presented in Supplementary Fig. S2.

Discussion

The NCIC Clinical Trials Group MA.12 study was one of several randomized trials to assess the benefit of adjuvant tamoxifen for premenopausal early-stage breast cancer (11). The results of the main study showed a marginal, but nonsignificant, improvement in DFS for tamoxifen over placebo (HR, 0.77; 95% CI, 0.59–1.01). Somewhat surprising, the trend in benefit was irrespective of hormone receptor status, with the clinically ER-positive patients having a HR of 0.82. One possible explanation for this observation may be the limited accuracy of hormone receptor testing in community hospitals. When we conducted central immunohistochemistry-based testing to classify the luminal subtypes, the HR improved (HR 0.75), but this was still not statistically significant—in part perhaps due to the smaller sample size and subsequent limited power. Only when we used the RT-PCR approach with the PAM50 classifier was there a statistically significant benefit of adjuvant tamoxifen among patients with luminal subtypes (HR, 0.52; 95% CI, 0.32–0.86; \( P = 0.009 \)). Furthermore, the benefit of adjuvant hormonal therapy in this premenopausal population is additive to the gains from adjuvant chemotherapy (all patients received adjuvant chemotherapy in MA.12), which is in keeping with the recent update from the EBCTCG.
overview of adjuvant tamoxifen (22). Our study illustrates the importance of accurate testing of predictive biomarkers, and the potential improvements that can be made with more advanced assay methodologies that also more comprehensively reflect pathway biology. The concordance between IHC assessment of limited biomarkers (ER and PR) versus the measurement globally of multiple activated genes in the luminal pathways, as with PAM50, was modest in our study at approximately 80%, suggesting the later approach is more reflective of the responsiveness of biologic pathways.

The luminal subtypes comprise the majority of breast cancer cases. Adjuvant hormonal therapies potentially have the greatest clinical impact (with relative risk reductions approximating 50%) in improving breast cancer outcomes, but the benefit is only in hormone receptor–positive breast cancers (10). Patients with hormone receptor–negative breast cancer would be at risk of treatment toxicities without potential benefit. Both the previous methodology of quantitative ligand-binding biochemical assay and current methodology using immunohistochemistry are subject to preanalytical and analytical variability, leading to estimates that, up to 20% of current hormone receptor testing worldwide, may be inaccurate (23). A recent guideline developed by a joint American Society of Clinical Oncology and College of American Pathologists taskforce recommended testing algorithms for more accurate and reproducible assays in hormone receptor testing (24).

qRT-PCR measures gene expression using RNA extracted from FFPE tumor tissue. Although this newer methodology has advantages in its ability to precisely quantitate multiple gene transcripts simultaneously, it could also potentially suffer from variabilities in RNA extraction, primer selection, PCR protocols, and measurement platforms. Recent studies, however, comparing PCR-based assays and IHC techniques have showed high correlation between these 2 methodologies (25–28).

The intrinsic subtypes luminal A, luminal B, HER-2–enriched, basal-like, and normal-like have been extensively studied by microarray-based gene expression profiling...
These subtypes have been shown to not only segregate based on biology, but also are independently prognostic (2). The PAM50 assay was developed with the aim to identify these intrinsic subtypes, and has also been validated across multiple cohorts and platforms (19). An advantage of intrinsic subtype determination using the PAM50 assay over other gene expression signatures is its ability to provide outcome prediction not only in ER-positive but also in ER-negative tumors. By virtue of the design of MA.12, which permitted enrolment of patients with ER-negative cancers, we were able to show that PAM50 was superior to IHC classification for prognosis across the full spectrum of breast cancer subtypes. Furthermore, in a recently published study of 786 primary tumors linked to long-term outcome, the PAM50 assay was able to identify a very low-risk prognostic group (>95% DFS at 10 years) in a cohort of women treated with tamoxifen but no chemotherapy (18). These results were similar in discriminatory magnitude to those obtained from the 21-gene assay when it was applied to clinical trial material (4, 5).

In conclusion, intrinsic subtype classification by RT-PCR with the PAM50 assay is feasible on routinely collected FFPE tumor material. Within the context of the randomized MA.12 study, this methodology and assay was superior to conventional local and central IHC ER assessment for both prognosis and more importantly prediction of benefit to adjuvant tamoxifen compared with placebo in a population of younger women showing the PAM50 model is effective regardless of the age of the patient at presentation.

Disclosure of Potential Conflicts of Interest

J. Parker is an author of the patent (in part) for the PAM50 assay. P.S. Bernard, C.M. Perou, M.J. Ellis, and T.O. Nielsen have ownership interest (including patents) in BioClassifier LLC. No potential conflict of interests were disclosed by other authors.

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References


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