Nottingham Clinico-Pathological Response Index (NPRI) after Neoadjuvant Chemotherapy (Neo-ACT) Accurately Predicts Clinical Outcome in Locally Advanced Breast Cancer

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Abstract

Purpose: There is a need to identify more sensitive clinicopathologic criteria to assess the response to neoadjuvant chemotherapy (Neo-ACT) and guide subsequent adjuvant therapy.

Experimental Design: We performed a clinicopathologic assessment of 426 patients who had completed Neo-ACT for locally advanced breast cancer (LABC) with a median follow-up of 70 months. Patients were divided into a training set treated with anthracycline combination chemotherapy (n = 172); an internal validation set treated with anthracycline and taxane (n = 129); and an external validation set treated with anthracycline with or without taxane (n = 125).

Results: A multivariate Cox regression model demonstrated a difference in overall survival and disease progression between patients who achieved pathologic complete response (pCR) and those who did not (P < 0.01, while reduction of tumor size was associated with DFS (P = 0.022). Nottingham Clinico-Pathological Response Indexes (NPRI) were calculated, and four prognostic groups (NPRI-PG) were identified. Patients in prognostic group 2 (NPRI-PG2) for BCSS (66 of 172; 38.4%) have the same prognosis as those who achieved pathologic complete response (pCR; NPRI-PG1; 15%). Receiver-operating characteristic (ROC) curves indicated that the NPRI outperformed the currently used prognostic factors and adding the NPRI improved their performance as a predictor for both BCSS (area under the curve [AUC], 0.88) and DFS (AUC, 0.87).

Conclusions: The NPRI predicts BCSS and DFS, with a higher sensitivity than pCR. The NPRI can also improve the specificity of clinicopathologic response as a study endpoint, for assessing response to Neo-ACT, and can serve as a valuable tool for the discovery of future predictive molecular markers. Clin Cancer Res; 1–11. ©2014 AACR.

Introduction

A meta-analysis combining data from more than 3,900 patients (1) with locally advanced breast cancer (LABC) demonstrated no difference in overall survival and disease progression between neoadjuvant and adjuvant chemotherapy. Several clinical trials have confirmed the safety and efficacy of neoadjuvant chemotherapy (Neo-ACT) and have established its utility in the management of LABC (2). Response to Neo-ACT is informative about a cancers biology (3), and provides some indication about the potential response of the tumor to further treatment (4). However, the assessment of response and its impact on survival is yet to be established and there are no universally accepted criteria (5, 6).

Studies have shown that achieving pathologic complete response (pCR) after Neo-ACT predicts overall survival, independent of treatment regimen (7–9). However, other trials comparing different Neo-ACT regimens have failed to demonstrate an association between pCR rate and improved outcome (10). Furthermore, pCR is an imperfect surrogate for clinical outcome, because: (i) only a small fraction of Neo-ACT patients achieve pCR (3%–28%; depends on the definition of pCR; ref. 11); (ii) patients who do not achieve pCR may still have a good prognosis, whereas patients who achieve pCR can still experience recurrent disease (12), and (iii) the presence of residual cancer cells observed after neoadjuvant therapy reflects a wide range of responses from near-pCR to complete resistance (3).

In this study, we identified clinicopathologic criteria that could grade response to Neo-ACT and correlate with clinical outcome. We used these criteria to develop a new clinicopathologic
Translational Relevance
We developed a new pathologic response index that can divide our patients into four distinct prognostic groups based on breast cancer–specific survival; the index was validated in internal and external independent cohorts. Patients with higher Nottingham Clinico-Pathological Response Index (NPRI) scores showed statistically significant associations with shorter survivals. The specificity and sensitivity of NPRI as a prognostic tool are superior to currently used prognostic indexes [e.g., pathologic complete response (pCR)]. For example, the good prognosis groups included 52% of all patients based on NPRI scores, compared with only 15% of patients by using the pCR criteria. This score has the potential to become the best prognostic tool after neoadjuvant chemotherapy, and to standardize important factors for the reporting of results in this setting. However, the NPRI has to be tested as a clinical tool to guide, and make a difference to, the choice and outcome of adjuvant therapy in a prospective clinical trial.

Materials and Methods

Patients
Four hundred and twenty-six patients with clinical stage IIA–IIIC disease (T1-4, N0-3, and M0) who completed Neo-ACT for LABC were included in this study and divided into three cohorts according to treatment center and regimen:

1. The training cohort (n = 172) were diagnosed at the Nottingham University Hospitals (NUH) between 1996 and 2011 and treated with standard anthracycline regimens in the form of 6 cycles of FEC (5-fluorouracil, 500 mg/m²; epirubicin 75–100 mg/m²; and cyclophosphamide, 500 mg/m², on day 1 of a 21-day cycle). This cohort was used to characterize the NPRIs and to determine cutoff points for prognostic groups (NPRI-PG) based on breast cancer–specific survival (BCSS) and disease-free survival (DFS).

2. The internal validation cohort (n = 129) included patients who were treated at the same institution (NUH) between 2002 and 2011 and received a taxane in addition to the anthracycline regimens (AC-T) as part of clinical trial protocols.

3. The external validation cohort (n = 125) were treated at the Breast Clinical Trials Unit at Mount Hospital (Perth, WA, Australia) between 1999 and 2011 and received anthracycline regimens with or without a taxane.

Detailed patient demographics and clinicopathologic characteristics were prospectively assessed and regularly updated (summarized in Supplementary Table S1). All patients underwent Neo-ACT, mastectomy, or breast-conserving surgery and axillary dissection, followed by adjuvant radiotherapy. All patients involved in our study received endocrine therapy for 5 years if >1% of tumor was positive for ER. A number of patients received adjuvant chemotherapy if (i) the tumor is known to be triple negative from base line assessments, (ii) The surgical specimen demonstrates three or more involved lymph nodes, (iii) the patients showed no response to, or progression on, Neo-ACT, and (iv) significant residual tumor is present in the surgical specimen. The median follow-up time was 70 months for the entire population and all patients gave their informed consent before initiation of therapy. The Nottingham Research Ethics Committee approved this work. The Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) criteria were followed throughout this study (13).

Pathologic review
Six authors (I.O. Ellis, S. Pinder, A.H.S. Lee, B. Latham, D. Palmer, and T.M. Abdel-Fatah) contributed to a comprehensive review of the pathology reports and hematoxylin and eosin–stained slides from pair-matched diagnostic core biopsies and surgical resection specimens (breast and regional lymph nodes). All slides were centrally reviewed by T.M. Abdel-Fatah. Pathologic features were assessed and their evaluation criteria are summarized in Supplementary Table S2. In view of the subjectivity and subclassification of fibrosis (none, mild, moderate, and severe), we had cause to simplify classification of fibrosis into present or absent. Thus, the absence of fibrosis with or without granulation tissue/necrosis in both the tumor bed and dissected regional lymph nodes was considered as evidence for the absence of any pathologic response reaction to chemotherapy. Intra- (k; range, 0.75–0.88, Cohen k test) and inter- (k; range, 0.70–0.82; using multirater k tests) observer agreements were moderate for the evaluation of fibrosis. In cases where discordant results were obtained, the slides were reevaluated by I.O. Ellis and T.M. Abdel-Fatah together and a consensus reached. The pCR was defined as the absence of residual invasive carcinoma in both the breast and regional lymph nodes. The number of histologically positive lymph nodes was determined by examination of serial macroscopic sections of each lymph node. On average, 16 breast blocks and all submitted lymph nodes were examined for each case before a diagnosis of pCR was reached.

Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) measurements were available for all patients and re-assessed according to the most recent American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (14, 15). The tumor histologic grade was assessed according to the Nottingham histological system (16). The primary tumor size and lymph node stage at the time of diagnosis were determined by physical examination and imaging, including mammography and sonography. The sonography measurements for primary tumor size at time of diagnosis (baseline size) and the pathologic measurements of invasive carcinoma after surgery (final primary tumor size) were used to calculate the reduction in the primary tumor size. Clinical staging of the breast cancer at the time of diagnosis (clinical-TNM stage) and the pathologic staging after Neo-ACT (yp-TNM stage) were determined using the revised American Joint Committee on Cancer (y-AJCC) staging system for breast cancer (17). Pretreatment Ultrasound assessment of the axilla is routine and if any morphologic abnormality is detected, an Ultrasound guided biopsy is performed of one of the nodes, even if it is not palpable. No patients in our study had their sentinel lymph node biopsied as part of their baseline diagnosis.

The Miller–Payne system for classification of Neo-ACT
The histologic grading system for response of Neo-ACT based on the percentage of reduction in tumor cellularity has been
assessed according to the Miller–Payne system as previously described (18).

Clinical-Pathologic Scoring System and CPS-ER histological grade score

Two prognostic scoring systems, Clinical-Pathologic Scoring System (CPS) and CPS-ER histological grade (CPS-EG) systems, based on clinical tumor–node–metastasis (TNM) stage, yp-TNM stage, ER status, and histologic grade were calculated as previously described (19).

Residual cancer burden

Residual cancer burden (RCB) was estimated from routine pathologic sections of the primary breast tumor site and the regional lymph nodes after completion of Neo-ACT according to the MD Anderson Cancer Center (Houston, TX) criteria (3). A calculated RCB index for each patient was generated using the calculation formula at the MD Anderson Cancer Center's website (http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3; last accessed 2014 June 19).

Detailed statistical methods

Statistical analyses were performed using STATISTICA (Stat Soft Ltd.) and SPSS (version 17). Where appropriate, Pearson χ², Student t test, and ANOVA tests were used. All tests were two-sided with a 95% confidence interval (CI) and a P < 0.05 was considered to be indicative of statistical significance. Survival data, including survival time, DFS, and development of local-regional and distant metastases (DM), were maintained on a prospective basis. BCSS was defined as the number of months from diagnosis to the occurrence breast cancer–related death. DFS was defined as the number of months from time of surgery to the occurrence of recurrence or DM relapse. Survival was censored if the patient was still alive, lost to follow-up, or died from other causes. Cumulative survival probabilities and 5-year BCSS and DFS were estimated using the univariate Cox models and the Kaplan–Meier plot method where appropriate, and differences between survival rates were tested for significance using the log-rank test.

Development and calculation of NPRI scores

After the definition of factors associated with BCSS and DFS, multivariate Cox proportional hazards models (with backward stepwise exclusion of factors, using a criterion of P < 0.05 for retention of factors in the model) were used to identify which factors were independently associated with clinical outcomes. The statistical significance of the model was assessed on the basis of the likelihood ratio test. The proportional hazards assumption was tested using both standard log–log plots and by generating the Kaplan–Meier survival estimate curves, and observing that the curves did not intersect with each other. Hazard ratios (HR) for death risks and relapse and 95% CIs were calculated from the Cox proportional hazards analysis. Subsequently, NPRI scores for both BCSS and DFS were calculated using the summations of P_coefficient values of the factors/measurements retained in the final model after controlling for both hormonal and chemotherapies.

Determination of the NPRI cutoff

Thresholds were determined to define four NPRI prognostic groups (NPRI-PG) with distinct prognoses: NPRI-PG1 included those with no traces of residual disease (i.e., those who achieved pCR), NPRI-PG2 included those with good response (near-pCR), and NPRI-PG3 and NPRI-PG4 those with moderate and the minimal responses, respectively. To determine the first cutoff point (between NPRI-PG4 and other NPRI score groups), a multivariate Cox regression model was used on the basis of cutoff points selected between the 5% and the 95% quartiles of the NPRI score distribution. The optimal cutoff point was selected as the quartile that maximized the profile log-likelihood of this model. A second cutoff point (between NPRI-PG2 and NPRI-PG3) was determined similarly by maximizing the profile log-likelihood of a Cox model that included all clinical covariates and the first dichotomous PRI score factor (i.e., NPRI-PG4 vs. NPRI-PG2/3).

Clinical impact of the NPRI and model discrimination

To assess the potential clinical impact of the NPRI, multivariable analyses using the Cox proportional hazards model were performed with the NPRI score as a continuous variable, while controlling for both the neoadjuvant and adjuvant chemotherapy regimens.

Receiver operating characteristic (ROC) curves were generated to compare NPRI performance as a prognostic tool and the other currently used prognostic models. Logistic fit of low versus high survival category by cumulative hazard (the product of the HRs of each incorporated variable) was performed. Area under the curve (AUC) values were calculated from ROC curves. An AUC of 0.8 or above was considered a good classifier.

Model discrimination was evaluated on the basis of the Harrell concordance index (c index), which is a generalized area under the receiver operating curve (AUC) for censored observations and is equal to the probability of concordance between the predicted probability of relapse and the relapse outcome (20). The c index was adjusted for bias using bootstrap resampling with 300 replications. The CI for the c index was obtained on the basis of approximate normality using the variance estimate of the unadjusted index.

Fitted polynomial function curves were calculated that summarize the broad relativity between the NPRI value and both 5- and 10-year BCSS and DFS. These were constructed from the raw data by applying median BCSS and DFS of each NPRI-PG against the 5- and 10-year BCSS and DFS for each division. Predictions from the curves were compared with the actual values.

Predictive accuracy of the NPRI compared with other prognostic clinicopathologic factors

To evaluate whether the NPRI-PGS add new independent prognostic information to current prognostic systems, we performed separate Kaplan–Meier analyses by NPRI-PG within each ER status, AJCC stage stratum, CPS, CPS-EG, RCB classes, and non-pCR subgroup. The significance of the additional stratification provided by the NPRI was evaluated on the basis of the log-rank test.

Results

Clinicopathologic factors associated with higher risk of death and relapse after FEC chemotherapy in univariate Cox analysis included the absence of fibrosis in primary tumor site and regional lymph nodes, the presence of lymphovascular invasion (LVI), increasing number of lymph node metastases, and hormonal therapy. These factors maintained significance as independent
predictors for both BCSS and DFS after controlling for adjuvant chemotherapy and other covariates by using the multivariate Cox proportional hazards models with backward stepwise exclusion (Table 1 and Supplementary Table S3). The percentage of reduction in primary tumor size showed statistical significant association with DFS only (Supplementary Table S3 and Supplementary Fig. S1). None of the covariates exhibited significant deviations from the proportionality assumption or had time-dependent effects (code system used is summarized in Supplementary Table S4). Subsequently, the summations of $F$ coefficient values produced by the Cox analysis were used to calculate the NPRIs for each patient as follows:

Developing the NPRI for BCSS

The following formula has been used to calculate the NPRI for BCSS:

$$
\text{NPRI (BCSS)} = \text{fibrosis status (0, 1)} \times 1.618028 + \text{LVI status (0, 1)} \times 1.048666 + \text{number of positive lymph nodes (0–43)} \times 0.063750 + \text{planned hormonal therapy status (0, 1)} \times -1.093202.
$$

The prognostic value of the NPRI compared with prognostic pathologic and clinical factors

We examined the predictive accuracy of the NPRI score compared with other prognostic clinicopathologic factors by performing the Cox proportional hazards univariate and multivariable analyses controlling for neoadjuvant and adjuvant chemotherapy. In the training cohort, patients had almost a 3-fold increase in death (HR, 2.83; 95% CI, 2.17–3.68; $P = 1.1 \times 10^{-14}$) for each unit of increase in the NPRI (Supplementary Table S5). When the NPRI was included in a multivariate Cox regression model (Fig. 1A), the overall predictive power of the model was significantly improved ($P = 3.6 \times 10^{-15}$), and the NPRI was significantly associated with a 2-fold increase of the risk of death (HR, 2.14; 95% CI, 1.37–3.36; $P = 0.001$). Using univariate analysis, a similar statistically significant association between the NPRI and BCSS was found in both the internal and external validation cohorts (Supplementary Table S5). When the Cox proportional hazard multivariable analysis was repeated in the two validation sets controlling for neoadjuvant and adjuvant chemotherapy, the NPRI score outperformed other pathologic and clinical covariates and was independently associated with BCSS (Figs. 1B and 2A). All other pathologic and clinical covariates failed to show a consistent association with prognosis (Figs. 1 and 2). Similar results were confirmed when the three cohorts were combined together ($n = 426$; Fig. 2B).

To assess the contribution of the NPRI toward the prediction of clinical outcome of Neo-ACT, the Cox proportional hazards statistical models containing relevant pathologic and other clinical predictors controlling for neoadjuvant and adjuvant

### Table 1. Univariate and multivariate backward stepwise analysis for factors associated with BCSS in the training cohort

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>Age</td>
<td>Continuous</td>
<td>1.04 (1.01-1.07)</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>Yes</td>
<td>0.50 (0.25–0.98)</td>
</tr>
<tr>
<td>Presenting tumor size mm</td>
<td>Continuous</td>
<td>1.01 (1.00–1.02)</td>
</tr>
<tr>
<td>Presenting grade</td>
<td>Low/Intermediate</td>
<td>1</td>
</tr>
<tr>
<td>Tumor type</td>
<td>Other</td>
<td>1.20 (0.54–2.84)</td>
</tr>
<tr>
<td>ER expression</td>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>HER2 status</td>
<td>Normal</td>
<td>1.74 (0.91–3.34)</td>
</tr>
<tr>
<td>PR expression</td>
<td>Positive</td>
<td>0.59 (0.27–1.30)</td>
</tr>
<tr>
<td>Triple-negative phenotype</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Clinical TNM (c-TNM)</td>
<td>Stage II</td>
<td>1</td>
</tr>
<tr>
<td>Pathologic TNM (yp TNM)</td>
<td>Stage II/III</td>
<td>6.39 (1.96–20.83)</td>
</tr>
<tr>
<td>Number of positive lymph nodes</td>
<td>Continuous</td>
<td>1.06 (1.03–1.10)</td>
</tr>
<tr>
<td>Residual inv CA size</td>
<td>Continuous</td>
<td>1.01 (1.01–1.02)</td>
</tr>
<tr>
<td>Percentage of tumor size reduction</td>
<td>Continuous</td>
<td>0.99 (0.98–1.00)</td>
</tr>
<tr>
<td>Percentage of cellularity of residual inv CA</td>
<td>Continuous</td>
<td>1.03 (1.01–1.04)</td>
</tr>
<tr>
<td>Percentage of reduction in cellularity of inv CA (Miller-Payne system)</td>
<td>$&lt;90%$</td>
<td>1</td>
</tr>
<tr>
<td>Extension and distribution of inv</td>
<td>No cell/scattered</td>
<td>1.81 (1.31–2.50)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>LVI</td>
<td>Yes</td>
<td>4.28 (2.22–8.23)</td>
</tr>
<tr>
<td>Ductal carcinoma</td>
<td>Yes</td>
<td>1</td>
</tr>
</tbody>
</table>

*Statistically significant $P < 0.05$.

Abbreviations: IDC-NS, invasive ductal carcinoma-no special type; inv-CA, invasive carcinoma.

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Chemotherapy were constructed. ROC analyses were performed as follows:

1. NPRI score alone.
2. RCB score alone.
3. pCR vs. none pCR
4. c-TNM
5. yp-TNM
6. The Miller–Payne system alone
7. CPS alone
8. CPS-EG alone
9. A prognostic model including RCB score, yp-TNM, c-TNM, histologic grade, HER2, ER, pCR status, age, and neoadjuvant and adjuvant chemotherapy without the NPRI score.
10. The same prognostic model as number 9 with NPRI scores.

AUC values for BCSS confirmed that the NPRI (AUC, 0.85) was superior to other prognostic models, and that it could be a good prognostic tool (Fig. 3A and Supplementary Fig. S2A), which remained consistent when the analysis was repeated for both internal (Fig. 3B and Supplementary Fig. S2B) and external (Fig. 2C and Supplementary Fig. S2C) validation cohorts, and after combining the three cohorts together (Fig. 3D and Supplementary Fig. S2D).

The NPRI identifies distinct prognostic groups of non-pCR patients

We identified two cutoff points to assign patients with residual disease (non-pCR; or non-NPRI-PG1) after FEC treatment into one of three classes: NPRI-PG2 (good prognosis group), NPRI-PG3 (moderate prognostic group), and NPRI-PG4 (poor prognostic group). The first cutoff point (NPRI-PG4 vs. NPRI-PG2/3) was selected as the 92nd percentile (NPRI, 1.87383) for BCSS. The second cutoff point (NPRI-PG2 vs. NPRI-PG3) corresponds to the 53.5th percentile for BCSS. The cutoff points defined subgroups of NPRI-PG1 to NPRI-PG4 with increasingly poor prognosis (Fig. 4).

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The cumulative incidence estimate of the overall probability of...
death within 5 years was 4% for the pCR group (NPRI PG1) and 5% for NPRI-PG2, whereas it was 33% and 71% for NPRI-PG3 and NPRI-PG4, respectively. Applying the NPRI in both the internal and the external independent cohorts defined groups with increasingly poor 5-year prognoses (Fig. 4B and C). The separation of death rates was somewhat smaller in the training set than for the internal validation cohort. To explore this further, we combined the poor prognosis groups together (NPRI-PG4 and NPRI-PG3) and compared the 5-year BCSS with those in the NPRI-PG1 (pCR patients) and found the separation of both the 5-year death rates were still wider in AC-T cohort versus FEC-only cohort (12% for BCSS).

The **NPRI prognostic groups stratify clinical outcome of breast cancer molecular subgroups**

Subgroup analysis of the combined patient cohort confirmed that the NPRI is a valid prognostic tool regardless of the molecular classification of breast cancer (Figs. 4 and 5). In previous studies, pCR and RCB have been demonstrated to be a much more reliable prognostic factor in endocrine receptors (ER) negative disease. In our study, the NPRI has separated both ER-positive and ER-negative cohorts into distinct prognostic groups (Fig. 4E and F). Applying the NPRI to the ER-positive subgroup demonstrated that 46% of patients have poor clinical outcome despite receiving adjuvant hormone therapy after completing Neo-ACT, whereas 49% of ER-negative cancers had an excellent prognosis. Moreover, only 55% of HER2 overexpression breast cancers had a favorable outcome despite receiving adjuvant trastuzumab following neoadjuvant therapy (Fig. 5A). Although patients with triple-negative breast cancer did not receive targeted adjuvant...
therapy after neoadjuvant therapy, our results demonstrated that 49% of those patients had excellent prognosis (Fig. 5B). The NPRI prognostic groups stratify prognoses of c-TNM, yp-TNM, CPS, and CPS-EG stages. Subgroup analyses of the TNM stage at diagnosis also confirmed the prognostic power of the NPRI to separate clinical TNM stage III cancers into distinct BCSS prognostic groups \((P = 4.5 \times 10^{-11}; \text{data not shown})\). Results demonstrated that 41% of those patients achieved excellent prognosis. In addition, we evaluated the contribution of the NPRI-PCs to the prognostic power of each posttherapy yp-TNM stage group (Fig. 5C). Regarding BCSS, the NPRI classified yp-TNM stage II/III patients into three distinct prognostic subgroups \((P < 0.001; \text{Fig. 5C})\). In addition, applying the NPRI to yp-AJCC stage 0/I indicated that 10% of those patients have a less favorable outcome \((P = 0.00001; \text{data not shown})\). Therefore, the NPRI classification appears to add significant prognostic power compared with posttreatment pathologic yp-AJCC stage. Moreover, applying the NPRI to the recently developed CPS and CPS-EG prognostic systems (that included information on c-TNM, yp-TNM, ER, and histologic grade) has added prognostic power to each system (Fig. 5D and E). For instance, the NPRI has reclassified patients with either CPS scored 2/3 \((n = 245)\) or CPS-EG scored 3/4 \((247)\) into three distinct prognostic subgroups \((P = 9.4 \times 10^{-8} \text{ and } 6.7 \times 10^{-8}; \text{respectively; Fig. 5D and E})\).

The NPRI adds significant prognostic power compared with RCB classes

Applying the NPRI to RCB classes in all three cohorts of patients improved the prognostic stratification of patients who were designated as RCB classes II \((n = 195; \text{predicted to have a } 5\text{-year } \text{BCSS } 78\%) \text{ and III } (n = 110; \text{predicted to have a } 5\text{-year } \text{BCSS of } 63\%)\). Each class has been stratified into three distinctive prognostic groups (Fig. 5F and G; \(P < 0.00001\)). Applying the NPRI to

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**Figure 3.** ROC analysis of the NPRI score and other clinicopathologic covariates were performed for predicting BCSS in the training (A), internal validation (B), external validation (C), and combined three cohorts (D). The AUC was calculated for ROC curves, and sensitivity and specificity were calculated to assess the performance of RCB alone (1), NPRI alone (2), and a statistical prognostic model that was constructed on the basis of the multivariable Cox proportional hazards incorporating known clinicopathologic prognostic variables, including pCR, RCB score, presenting clinical TNM stage, revised pathologic TNM stage (yp-TNM) stage, histologic grade based on Nottingham grading system, ER expression status, HER2 status, and age at diagnosis (3). **A**, **B**, and **C**, ROC analysis was also performed for the aforementioned prognostic model after incorporating the NPRI score (4). Dashed gray lines indicate the 45° angle tangent line marked at a point that provides best discrimination between true positives and false positives, assuming that false positives and false negatives have similar costs. T, taxane.
RCB class II split the patients in this class to two groups: those who have a 12% worse, and those who have a 45% better prognosis than that predicted (Fig. 5F). Similarly, the NPRI has identified that 25% of RCB class III had a worse, and 27% a better, prognosis than that predicted for that class of patients (Fig. 5G).

The Pearson correlation coefficient and fitted polynomial function curves showed that there is an excellent inverse linear correlation between the NPRI and BCSS. In Fig. 5I, the fitted polynomial function summarizes a broad relationship between the NPRI value and median 5- and 10-year survival.

Developing of NPRI for DFS

The following formula has been used to calculate the NPRI for DFS:

\[
NPRI_{DFS} = \text{Fibrosis status (0, 1)} \times 1.2830 + \text{LVI status (0, 1)} \times 0.8431 + \text{number of positive lymph nodes (0–43)} \times 0.0537 + \text{percentage of reduction of primary tumor size (+100% to -100%) } \times -0.008162 + \text{planned hormonal therapy status (0, 1)} \times -0.7521.
\]

In the training internal and external validation cohorts, patients had almost a 3-fold increase in relapse \((P < 0.0001)\) for each unit of increase in the NPRI\(_{DFS}\) (Supplementary Table S5). When the NPRI\(_{DFS}\) was included in a multivariate Cox regression model controlling for other prognostic models and neoadjuvant and adjuvant chemotherapy (Supplementary Figs. S3A and S3B and S4), the overall predictive power of the model was significantly improved \((P < 0.0001)\) and the NPRI\(_{DFS}\) outperformed other clinicopathologic covariates that failed to show a consistent association with prognosis.

NPRI\(_{DFS}\) identifies distinct DFS prognostic groups of non-pCR patients

The first cutoff point \((NPRI_{DFS}-PG4 vs. NPRI_{DFS}-PG2/3)\) was selected as the 83rd percentile \((NPRI_{DFS}, 0.91021)\) for DFS. The second cutoff point \((NPRI_{DFS}-PG2 vs. NPRI_{DFS}-PG3)\) corresponds to the 64.5th percentile \((NPRI_{DFS}, 0.522257)\) DFS. The cutoff points defined subgroups of NPRI\(_{DFS}-PG1\) to NPRI\(_{DFS}-PG4\) with increasingly poor prognosis (Supplementary Fig. S5). Applicability of the NPRI\(_{DFS}\) was evaluated in both the internal and the external independent cohorts and NPRI\(_{DFS}\) defined groups with increasingly poor 5-year prognoses (Supplementary Fig. S5). The \(c\) index of the prognostic model on the internal and external validation cohorts was 0.776 (95% CI, 0.67–0.87) and 0.841 (95% CI, 0.76–0.92), respectively (Supplementary Fig. S6).
Discussion

Up to 20% of patients with breast cancer present with locally advanced disease that is associated with a poor prognosis (21). In those tumors that lack a specific therapeutic target (i.e., ER or HER2), conventional chemotherapy remains the mainstay of systemic therapy. Knowledge of the primary tumor's sensitivity or resistance to Neo-ACT can predict the efficacy of these agents on micrometastatic disease. A trial of the effectiveness of adjuvant chemotherapy, following poor response to Neo-ACT, is desperately needed. However, at present, there are no effective prognostic tools to guide adjuvant therapy based on response to Neo-ACT. Although the pathologic evaluation of tumor response is still the gold standard, the lack of uniform reporting of pathologic response remains a problem (22).

In this study, a comprehensive clinicopathologic evaluation of a cohort of patients who received neoadjuvant anthracycline/FEC chemotherapy at a single center was conducted and the NPRI was developed, which was then validated in two independent cohorts.

Patients with higher NPRI scores showed statistically significant associations with shorter DFS and BCSS. The NPRI identifies an important subset of patients with combined insensitivity to chemo- and hormone therapies that may benefit from novel therapy in a trial setting. For example, the NPRI identifies a second good prognostic group (NPRI-PG2) that have responded as well as the cohort with pCR (PG1) and can be spared further multiple cycles of unnecessary (and
potentially toxic) treatments. Likewise, ER-positive patients in the NPRI-PG3 and PG4 groups had a moderate to poor prognosis despite receiving adjuvant hormone therapy after completing Neo-ACT.

The separation of the 5-year relapse and death rates were somewhat better in patients who received AC-T chemotherapy compared with those who received anthracycline only, indicating some benefit from the addition of a taxane. But this is uncertain due to the lower number of patients in the NPRI-PG4 subgroup. However, after combining NPRI-PG3 and NPRI-PG4, we found the same separation, confirming that those patients might benefit from adding a taxane to anthracycline chemotherapy, in agreement with a recent large meta-analysis study (23). Moreover, the $c$ index of the prognostic model on the internal and external validation cohorts showed similar sensitivity and specificity.

It has been recommended that the predictive ability of a new score should be evaluated on the basis of whether the score improves an already optimized multivariate model of available risk factors (24). On the basis of this, a statistical prognostic model, including the NPRI score and known prognostic factors, has shown to be superior to all the prognostic models without the NPRI.

Several studies have attempted to provide criteria for response after Neo-ACT (22) by using clinical, magnetic resonance imaging (MRI), or sonography findings (19, 25) or by bi-dimensional measurement of the primary tumor bed in resection specimens (3, 22). In fact, these systems have incorporated macro-anatomic features of breast cancer (viz. residual tumor size and lymph node status). The NPRI, with inclusion of LVI, host response, and changes in tumor size, also highlights the importance of the tumor microenvironment as a predictor for response to chemotherapy.

In agreement with other studies (3, 10, 26), we found that lymph node status after Neo-ACT is still the single-most important prognostic factor. However, the increasing use of sentinel lymph node biopsy either before or after Neo-ACT leads to difficulties in evaluating the prognostic importance of lymph node status. With regard to the interpretation of sentinel lymph node status after Neo-ACT, the current data are inconsistent and requires further evaluation (27). However, it is possible that with adequate standardization of the techniques and data from prospective clinical trials, sentinel lymph node status after Neo-ACT could be added to our index for patient selection to reduce surgical morbidity in the good prognostic groups.

In this study, the presence of LVI after Neo-ACT was an independent predictor of clinical outcomes, in agreement with previous studies (28, 29). It has been shown that tumor emboli in vascular spaces are relatively resistant to treatment when compared with carcinoma invading the stroma (30). It should be noted that the identification of LVI may sometimes be difficult as the residual tumor nests or ductal carcinoma in situ (DCIS) may show marked retraction artifact in the fibrous stroma mimicking invasion (5). Thus, proper tissue fixation and immunohistochemical staining for lymphatic channel markers may be useful to distinguish tissue retraction from lymphatic invasion.

Although residual tumor size has been proposed as a prognostic factor for breast cancer (11, 29), we found that the reduction in primary tumor size was more predictable of DFS than actual residual tumor size after Neo-ACT. In fact, using residual tumor size does not discriminate between large contiguous carcinomas (which have shown minimal treatment effect) and microscopic foci scattered in a tumor bed with equally great dimensions (demonstrating significant treatment effect). Many examinations have been proposed to monitor the extent of the residual disease extent during Neo-ACT, such as physical examination, mammography, and sonography, but most studies demonstrate modest accuracy when compared with final pathologic assessment (31). There is increasing evidence that MRI is an excellent imaging tool with high specificity for both early-response monitoring and the assessment of residual disease (32). However, relatively few studies reported direct comparisons between MRI and other cost-effective tests (32). Partridge and colleagues (33) found that MRI tumor volume was more predictive of DFS than tumor diameter, suggesting that volumetric changes measured using MRI may provide a more sensitive assessment of treatment efficacy. Furthermore, changes of metabolic volume measured by functional MRI could reflect early outcomes of Neo-ACT (34). In fact, future incorporation of MRI results into the NPRI score could add another dimension to the NPRI for early assessment of response to Neo-ACT.

Limitations of the study and future directions

Although we validated our findings in two independent cohorts and we have shown that the NPRI can supplement existing methods to define pathologic response, the utility of the NPRI requires further validation in larger patient populations. It also requires prospective evaluation to demonstrate its role as a prognostic tool and its potential to select patients for novel systemic therapies following Neo-ACT. In addition, further studies are needed to address interobserver variability, standardization of the NPRI measurements, and develop a more objective methodology to quantify such factors as fibrosis, LVI, and changes in tumor size. In particular, the effect of new treatment regimens on the components of the index will need to be assessed in more recent cohorts. Further refinement of the scoring system through the addition of new molecular or biologic markers is also needed.

Conclusion

We suggest that the incorporation of the NPRI in assessing clinical outcome following Neo-ACT helps individualization of systemic treatment in patients with locally advanced breast cancer. In particular, it may identify patients who fail to benefit from standard chemotherapy regimens and in whom treatment with novel therapies is warranted. We believe that this scoring system may provide a standardized approach to reporting the tumor response to Neo-ACT.

Disclosure of Potential Conflicts of Interest

G. Ball is an employee of CompanDX Limited. A.H.S. Lee reports receiving a commercial research grant and speakers’ bureau honoraria from Roche. No potential conflicts of interest were disclosed by the other authors.

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