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Original article

EarlyR signature predicts response to neoadjuvant chemotherapy in breast cancer

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ABSTRACT

Background: EarlyR gene signature uses ESPL1, SPAG5, MKI67, PLK1 and PGR to classify ER+ breast cancer (ER+ BC) into EarlyR-Low, EarlyR-Int, and EarlyR-High risk strata and is prognostic in patients treated with adjuvant chemotherapy. The ability of EarlyR to predict pathological complete response (pCR) and long-term survival following neoadjuvant chemotherapy (NACT) is evaluated herein.

Materials: The ability of EarlyR gene signature to predict pCR was assessed in publicly available Affymetrix microarray datasets (Cohort A; n = 659; 74 pCR events) derived from NACT-treated ER+ BC patients. Distant relapse-free survival (DRFS) results were analyzed in patients treated with NACT and adjuvant hormone therapy (AHT) (n = 281) and compared with patients treated with AHT alone (n = 455) (Cohort B; n = 736; 142 events).

Results: In cohort A, EarlyR was a significant predictor of pCR ($p = 5.8 \times 10^{-11}$) (EarlyR-Low, n = 400, pCR = 40, 5%; EarlyR-Int, n = 69, pCR = 7, 15% and EarlyR-High, n = 190, pCR = 47, 24%). In EarlyR-Low of Cohort B, the 5-year DRFS was not significantly (p = 0.55) different between NACT + AHT [0.81 (95%CI 0.73–0.90)] and AHT-only [0.85 (95%CI 0.81–0.90)]. In contrast, in EarlyR-High, the 5-year DRFS was higher (p = 0.019) in NACT + AHT [0.81 (95%CI 0.70–0.93)] as compared to AHT-only [0.60 (95%CI 0.51–0.71)].

Conclusions: High EarlyR is strongly associated with pCR in patients treated with neoadjuvant chemotherapy. EarlyR also predicts poor DRFS outcomes for patients in EarlyR-High not receiving NACT, and improved survival in NACT-treated EarlyR-High patients. EarlyR is not only a prognostic assay but also a predictive assay that identifies patients, who are also likely to respond to chemotherapy.

1. Introduction

The molecular heterogeneity of primary breast cancer significantly complicates the selection of the most effective treatment regimen. Although prognosis for early stage estrogen receptor positive (ER+) breast cancer patients is significantly improved by endocrine therapy alone, approximately 20% of patients will suffer a distant recurrence within ten years without additional treatment. Traditionally, clinico-pathological factors such as tumor size, grade, the number of positive lymph nodes, and measures of proliferation rate such as Ki67, have been used to assess the ability of systemic chemotherapy to reduce the long-term risk of recurrence. However, these factors alone fail to define all molecular traits that have a significant effect on response to chemotherapy.

Multi-gene signatures, e.g., Oncotype DX Recurrence Score [1], Mammaprint [2,3], Prosigna Risk of Recurrence [4], Endopredict [5] that are prognostic of distant recurrence have been proposed to help decide whether or not to administer chemotherapy. Hypothetically, patients identified as low risk by the signature will not benefit from adding chemotherapy to hormone therapy, while high-risk patients will benefit from chemotherapy. However, evidence of the predictive significance of these signatures, especially for chemotherapy including a taxane, is limited or equivocal. For example in the adjuvant settings, Oncotype DX was shown to predict benefit of CMF (cyclophosphamide, methotrexate and fluorouracil) chemotherapy

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for a subset of ER+, lymph-node negative (LN-) patients from NSABP B-20 clinical trial [6]. The recent analysis of the TAILORx clinical trial [7] did not show any benefit for the addition of chemotherapy in patients with intermediate Oncotype DX recurrence score. Similarly, there was limited chemotherapy benefit in the MINDACT trial for patients with discordant clinical and molecular risks [8]. Since all chemotherapy regimens for ER+ breast cancer recommended by the National Comprehensive Cancer Network (NCCN) contain a taxane, there is a significant clinical need for a molecular assay that can differentiate between the small percentage of ER+ breast cancer patients who may benefit from AT chemotherapy, and the majority who can safely avoid it. Of note, the use of taxanes is associated with significant increase in likelihood of bone marrow suppression (principally neutropenia), complete alopecia, and hypersensitivity reactions [9]. Taxane-induced neuropathy is a common, dose-limiting side effect of taxane therapy that affects the quality of life of patients [10].

To address limitations of current assays, we developed the EarlyR assay computed from gene expression values of *ESPL1, SPAG5, MKI67, PLK1 and PGR* [11]. EarlyR, which can be applied as a continuous score (0–100) or as discrete risk strata (EarlyR-Low, EarlyR-int, EarlyR-High), has been shown to be prognostic of distant recurrence within 8 years of diagnosis in multiple cohorts treated with adjuvant hormone therapy [11] and adjuvant chemotherapy [12]. We have also shown that EarlyR score and risk stratification are prognostic of 8-year distant relapse-free interval (DRFI) in a cohort of BIG 1-98 adjuvant clinical trial [13,14] with gene expression measured from FFPE tissues.

Neoadjuvant (pre-surgical) chemotherapy (NACT) can significantly increase the likelihood of breast conserving surgery [15]. In addition, pathological complete response (pCR) following NACT has been significantly associated with prolonged relapse-free survival [16,17]. There is increasing evidence for the validity of pCR as a surrogate endpoint for long-term distant disease-free survival [18]. Importantly, the FDA has accepted pCR as an endpoint for accelerated approval of drugs (https://www.fda.gov/downloads/drugs/guidances/ucm305501.pdf).

There are very few studies that have assessed the role of gene signatures in predicting response to neoadjuvant chemotherapy (NACT). Most studies regarding the use of gene signatures in selecting NACT are limited by small sample (<100 patients) sizes [19] or are not restricted to ER+ breast cancers [20–22]. In this study, we analyzed the significance of EarlyR as a predictor of chemotherapy benefit in a cohort of ER+ patients (Cohort A; N = 659; 74 pCR events) treated with neoadjuvant AT or AC chemotherapy (NACT) in univariate analysis, and multivariate analysis with clinical factors and expression of biomarkers. The ability of EarlyR to predict a benefit of chemotherapy was also assessed with long-term (≥5 years) relapse-free survival in matched cohorts of ER+ patients treated with neoadjuvant chemotherapy plus adjuvant hormone therapy (NACT + AHT) or adjuvant hormone therapy alone (AHT) (Cohort B; N = 736).

2. Patients and methods

2.1. ER + breast cancer patients treated with neoadjuvant chemotherapy (Cohort A)

Clinical data and Affymetrix hgu133a CEL files were collected from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/gds/) for the ER+ samples with pCR data recorded from seven data series GSE20194 [23] (N = 164), GSE20271 [24] (N = 98), GSE22093 [25] (N = 42), GSE23988 [25] (N = 32), GSE25055 [26] (N = 172), GSE25065 [26] (N = 113), GSE42822 [27] (N = 38). Gene expression values for all cohorts were normalized and quantified using fRMA [28], which further avoids batch effects. The resulting gene expression data and merged clinical data comprise Cohort A (N = 659, Table 1). All patients have received anthracycline and cyclophosphamide (AC) based neoadjuvant chemotherapy. In this cohort, AC without a taxane was used for 91 patients, the rest received additional taxanes (AT; N = 568).

2.2. A cohort of matched chemotherapy-treated and untreated ER + breast cancer data (Cohort B)

A cohort in which the patients treated with neoadjuvant chemotherapy plus adjuvant hormone therapy (NACT + AHT) and those treated with adjuvant hormone therapy alone (AHT) have matching clinical features, including follow up for distant recurrence, was constructed as follows. A subset of Cohort A with available distant relapse, grade and lymph node status data, was selected from the series GSE25055 and GSE25065 (N = 281, NACT + AHT dataset) [26]. Four strata were created from the possible combinations of binary grade (grade 1/2 versus grade 3) and lymph node status (negative versus positive) to match proportions. The percentages of samples in each stratum were computed as the sampling constraints for creating the cohort treated only with adjuvant hormone therapy. The METABRIC cohort [29] was randomly sampled to identify patients with large (T-stage ≥ 2), ER+ tumors, treated with hormone therapy only, so that the proportions of samples in each of the four sampling strata match those generated within the NACT + AHT set (AHT-only N = 455). Cohort B, formed by combining NACT + AHT and AHT-only, has overall clinical features as described in Table 2. In addition to the published [29] outcome end point (breast cancer specific death), distant recurrence data for METABRIC was obtained by private communication. PAM50 data for 310 patients in Cohort A was obtained from prior publications [26,29]. This was used for comparative analysis with EarlyR.

2.3. Computation of EarlyR signature and strata in Cohorts A and B

The EarlyR genomic score (0–100) for ER+ breast cancer was computed from expression data for the genes *SPAG5, ESPL1, MKI67, PLK1* and *PGR* as previously published [11] for Affymetrix hgu133a array data and METABRIC, and summarized in Supplementary Methods. The EarlyR risk strata EarlyR-Low (EarlyR ≤ 25), EarlyR-Int (25 < EarlyR ≤ 75), EarlyR-High (75 < EarlyR) were defined using the declared pre-specified thresholds. Notably, clinical data from these cohorts were not used for computing EarlyR.

2.4. Computation of surrogate for IHC4

IHC4 equation [30] is derived from protein expression (by IHC)

Table 1
Clinical features of ER+ samples treated with neoadjuvant chemotherapy (Cohort A).

feature	Cohort A (N = 659)
pCR events	74 (11%)
LN (-/+/NA)	230/410/19
Grade (1/2/3/NA)	55/325/238/41
PR (+/-/NA)	436/149/74
Her2 (+/-/NA)	45/536/78
T-stage (1/2/3/4/NA)	36/334/172/108/9
N-stage (0/1/2/3/NA)	230/286/76/48/19
treatment (AC only[a]/AT[b]/Herceptin[c])	91/568/13
EarlyR-Low/EarlyR-Int/EarlyR-High	400 (61%)/69 (10%)/190 (29%)

[a] Anthracycline and cyclophosphamide.
[b] Anthracycline, cyclophosphamide plus taxane.
[c] These patients also received neoadjuvant AT.

Table 2
Clinical features of matched hormone therapy only (AHT-only) and hormone therapy plus chemotherapy (NACT + AHT[a]) cohorts for comparison of survival in EarlyR strata.

feature	AHT-only (N = 455) (number, %)	NACT + AHT (N = 281) (number, %)
LN-, Grade 1 or 2	116 (26%)	73 (26%)
LN-, Grade 3	47 (10%)	29 (10%)
LN+, Grade 1 or 2	176 (39%)	108 (38%)
LN+, Grade 3	116 (26%)	71 (25%)
T-stage (1/2/3/4)	0/422 (93%)/33 (7%)/NA	20 (7%)/146 (52%)/74 (26%)/41 (16%)
EarlyR-Low/EarlyR-Int/EarlyR-High	291 (64%)/63 (14%)/101 (22%)	176 (63%)/29 (10%)/76 (27%)
age (median)	69.6	49
PAM50 (Basal/Her2/LumA/LumB/Normal)	2%/7%/46%/38%/7%	9%/7%/51%/24%/9%
5-year distant relapse event (yes/no or censored)	99 (22%)/356 (78%)	41 (15%)/240 (85%)
time to 5-year relapse or censoring (median)	5	3.1

[a] All patients received neoadjuvant chemotherapy including taxanes.

of ESR1, PGR, MKI67, and HER2 status. As a surrogate for IHC4 we replace protein level measures by gene expression levels assessed by Affymetrix array. So that the ranges of values of ESR1 and PGR match those in the original computation, the expression values were linearly scaled to a range of 0–10. Specifically, if v denotes the tuple of expression values of, say ESR1, in a cohort, the scaled value for v_i is $10 \times (v_i - \min(v))/(\max(v) - \min(v))$. Expression values of MKI67 were similarly scaled to the range 0–100. Her2 status was reported in the clinical data provided for the samples used here. In the IHC4 equation, Her2+, Her2- were represented by 1, 0, respectively. With these changes, IHC4 = 94.7 × { −0.100 × ESR1 - 0.079 × PGR + 0.586 × Her2 + 0.240 × log (1 + 10 × MKI67)}.

2.5. Statistical analysis

All statistical analyses and visualizations were performed using R (https://www.r-project.org), including the packages fRMA, survival and ggplot2. The significance of interaction between categorical variables was assessed with a Chi-squared test. Survival model significance was measured with the log-rank test in a Cox proportional hazards model.

3. Results

3.1. EarlyR was predictive of pCR due to neoadjuvant chemotherapy in ER+ breast cancer

The significance of EarlyR as a predictor of pCR was evaluated in Cohort A as a continuous score (Fig. 1a) and as discrete risk strata (Fig. 1b). EarlyR score was a significant predictor of pCR in Cohort A ($p = 1.83 \times 10^{-10}$). The likelihood of pCR was less than 5% for 50% of patients and 25% for over 15% of samples (Fig. 2). Analysis of the population by EarlyR-Low, EarlyR-Int and EarlyR-High strata showed increasing likelihood of pCR due to NACT ($p = 5.8 \times 10^{-11}$ by Chi-squared test). The rates of pCR in EarlyR-Low, EarlyR-Int and EarlyR-High were 5% (n = 20/400), 15% (n = 7/69) and 24% (n = 47/190), respectively. Notably, although EarlyR-High consisted of just 29% of Cohort A, it contained 61% of pCR cases.

In the patients from Cohort A, who were treated with a taxane containing NACT regimen (N = 568), EarlyR score was significantly predictive of pCR ($p = 1.23 \times 10^{-9}$). EarlyR score was also predictive (p = 0.04) in the subset of NACT patients (N = 91), who did not receive a taxane.

3.2. EarlyR was significantly predictive of pCR in multivariate logistic regression including expression of genes comprising IHC4

IHC4 equation [30], derived from protein expression (by IHC) of ESR1, PGR, MKI67, and HER2 status, has been shown to be predictive of outcomes in ER+ breast cancer [31]. As we did not have the protein expression values, we assessed the relative predictive significance of EarlyR compared to expression levels of the component genes in Cohort A and a surrogate of IHC4 (Methods) using univariate logistic regression (Table 3). (Expression values and EarlyR score were linearly scaled to a range of 0–10 so that coefficients and standard errors would be comparable.) Results showed that increasing expression of MKI67 was highly predictive of pCR, and decreasing levels of ESR1 and PGR were also statistically significant.

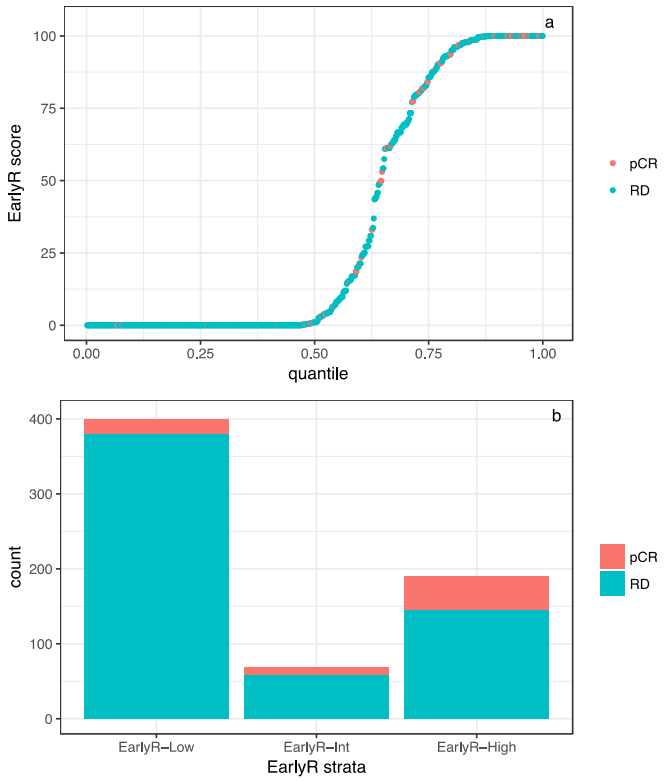

Fig. 1. The relationships between EarlyR and pathological complete response in Cohort A are displayed for (a) the EarlyR continuous score, and (b) the EarlyR risk strata. (a) EarlyR score values are plotted versus quantiles for the score, with points colored by pCR (red) and residual disease (blue). There are very few pCR cases when EarlyR score is near zero, with increasing frequency of cases as the score increase. EarlyR score is a significant predictor of pCR by logistic regression ($p = 1.83 \times 10^{-10}$). (b) The percentages of Cohort A in EarlyR-Low, EarlyR-Int and EarlyR-High were 61%, 10%, 29%, respectively. The rates of pCR in EarlyR-Low, EarlyR-Int and EarlyR-High were 5%, 15% and 24%, respectively. EarlyR stratification is a significant predictor of pCR ($p = 5.8 \times 10^{-11}$ by Chi-squared test).

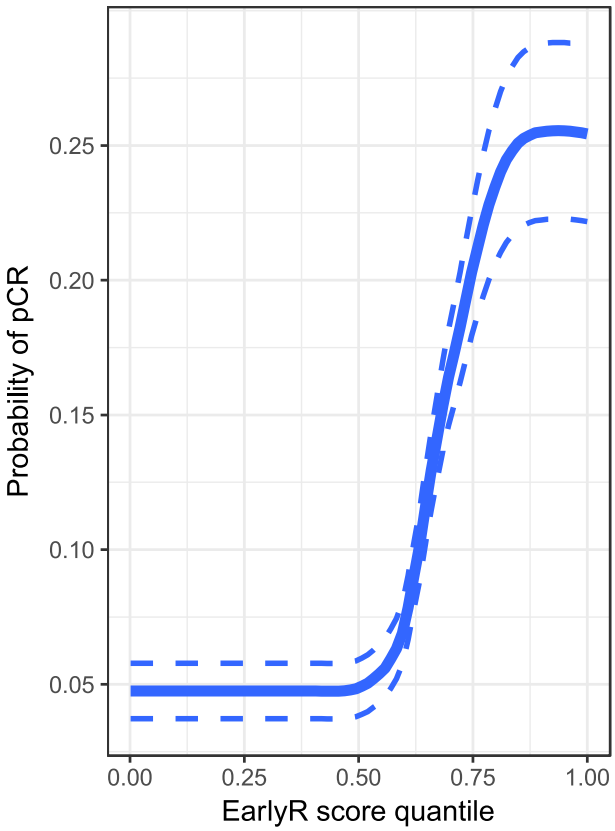

Fig. 2. The probability of pCR was plotted for Cohort A by quantiles of EarlyR score. Probabilities were predicted from a logistic regression fit with pCR/RD as the response variable and EarlyR score as the sole covariate. Dotted lines indicate the 95% confidence intervals. A smooth curve was plotted using a loess fit to the data points.

Expression of ERBB2 as a continuous variable was not significantly predictive of pCR.

To compare the relative predictive significance of EarlyR and the IHC4 equation genes, we evaluated a multivariate logistic regression model with variables EarlyR score, ESR1, PGR, MKI67 and ERBB2 (Table 4A). In this model, only EarlyR score was statistically significant ($p = 0.0018$). In a multivariate logistic regression model with variable EarlyR score and IHC4 score, only EarlyR score was significant ($p = 2.02 \times 10^{-4}$, Table 4B). This showed that EarlyR had superior predictive value as compared to any combination of IHC4 genes expression values.

3.3. EarlyR was significantly predictive of pCR in subgroups defined by grade, lymph-node status, T-stage and HER2 status

Guidelines, e.g., NCCN, use clinical features such as lymph node status and tumor size to help decide which patients to recommend for chemotherapy treatment. To gauge the ability of EarlyR to augment such information, the predictive significance of EarlyR score was evaluated using logistic regression in Cohort A restricted to grade 2/3, LN+/-, T-stage 2/3/4, and HER2- (Table 5). In this analysis, EarlyR was highly predictive of pCR ($p < 0.01$) in all subgroups except T-stage 4. There aren't enough HER2+, grade 1, or T-stage 1 samples in Cohort A for a meaningful analysis.

Thus, EarlyR refines the predictive significance of traditional clinico-pathological parameters across the spectrum of subtypes.

Table 3
Significance of EarlyR score, IHC4 score, and specific genes as separate predictors of pCR in univariate logistic regression.

variable[a]	coefficient	standard error	statistic	p value
EarlyR score	0.18	0.03	5.27	1.4×10^{-7}
MKI67	0.87	0.17	5.25	1.5×10^{-7}
ESR1	−0.15	0.06	−2.38	0.02
PGR	−0.26	0.11	−2.38	0.02
ERBB2	−0.04	0.08	−0.52	0.60
IHC4	0.41	0.10	4.20	2.72×10^{-5}

[a] Each continuous variable x was linearly scaled to a range 0–10 by the formula 10 *(x − min)/(max − min).

Table 4
Significance of (A) EarlyR score and specific genes as predictors of pCR in multivariate logistic regression using all variables, and (B) EarlyR score and IHC4 score in multivariate logistic regression.

variable[a]	coefficient	standard error	statistic	p value
A				
EarlyR score	0.14	0.05	3.12	0.0018
MKI67	0.14	0.11	1.24	0.21
ESR1	−0.07	0.06	−1.16	0.25
PGR	−0.03	0.11	−0.28	0.78
ERBB2	−0.04	0.08	−0.52	0.60
B				
EarlyR score	0.15	0.04	3.72	2.02×10^{-4}
IHC4 score	0.17	0.12	1.47	0.14

[a] Each continuous variable x was linearly scaled to a range 0–10 by the formula 10 *(x − min)/(max − min).

3.4. EarlyR significantly refined the ability of intrinsic subtypes to predict pCR following AT treatment

The predictive significance of PAM50 subtypes (Basal, Luminal A, Luminal B, Her2, Normal), previously published [26], was assessed for the NACT + AHT subset of cohort B (Table 2). PAM50 was predictive of pCR ($p = 8.0 \times 10^{-4}$) with Basal and Luminal B subtypes enriched for pCR cases (Table 6). In this same cohort, EarlyR stratification was also predictive of pCR ($p = 1.8 \times 10^{-5}$). Furthermore, in analysis restricted to Basal and Luminal B tumors, EarlyR stratification was significantly predictive of pCR ($p = 0.01$). Note that EarlyR-High contained 100% and 83% of the pCR cases in the Basal and Luminal B subgroups, respectively.

3.5. EarlyR predicted improved 5-year distant survival due to NACT chemotherapy plus adjuvant hormone therapy compared to hormone therapy alone

While pathological complete response alone may increase surgical options for some patients, the principal goal of chemotherapy is to lower a patient's long-term risk of recurrence. To test the differences in relapse risk between patients treated only with hormone therapy (AHT-only) and those treated with additional chemotherapy (NACT + AHT) relative to EarlyR strata, we formed Cohort B (N = 736) of matched AHT-only (N = 455) and NACT + AHT (N = 281) samples (Table 2). The difference between estimated 5-year distant relapse-free survival (DRFS) proportions in AHT-only and NACT + AHT (Fig. 3) was not statistically significant although the AHT-only cohort contained 22% relapse events and NACT + AHT contained 15% relapse events. This lack of statistical significance was likely due to the modest sample size and relatively short median follow-up time in NACT + AHT (3.1 years). The median ages in AHT-only and NACT + AHT were significantly different (patients in NACT + AHT being younger; average 49 vs 69.6), however age did not significantly influence relapse risk in this cohort. The proportions of samples in each EarlyR strata were nearly the same in AHT-only and NACT + AHT (Table 2, $p = 0.17$ for

Table 5
Significance of EarlyR score[a] as a predictor of pCR within subtypes defined by clinical features.

subtype	coefficient	standard error	statistic	p value
grade 2	0.16	0.06	2.64	0.0084
grade 3	0.19	0.05	3.83	0.00013
LN-	0.22	0.05	4.12	3.84×10^{-5}
LN+	0.18	0.04	4.58	4.64×10^{-6}
T-stage 2	0.18	0.05	4.01	6.00×10^{-5}
T-stage 3	0.25	0.05	4.75	2.09×10^{-6}
T-stage 4	0.07	0.08	0.87	0.39
HER2-	0.19	0.04	5.4	7.62×10^{-8}

[a] EarlyR score was linearly scaled to a range 0–10 by the formula 10 *(x − min)/(max − min).

Table 6
Distribution of pCR cases for EarlyR strata in PAM50 intrinsic subtypes in NACT + AHT subgroup of Cohort B.

EarlyR stratum	PAM50 intrinsic subtype				
	Basal	Her2	Luminal A	Luminal B	Normal
EarlyR-Low (pCR/RD)	0/4	1/3	3/125	1/21	2/16
EarlyR-Int (pCR/RD)	0/1	1/3	2/10	1/11	1/0
EarlyR-High (pCR/RD)	7/14	1/11	0/5	10/26	0/5

Chi-squared test), further evidence of the matching characteristics of the two treatment groups.

We analyzed the differences in incidence of relapse due to treatment relative to EarlyR strata in EarlyR-Low (Fig. 4a) and EarlyR-High (Fig. 4b). In EarlyR-Low, the 5-year estimated DRFS proportion was not significantly (p = 0.55) different between NACT + AHT [0.81 (95%CI 0.73–0.90)] and AHT-only [0.85 (95%CI 0.81–0.90)]. In contrast, in EarlyR-High, the 5-year estimated DRFS proportion was significantly (p = 0.019) higher in NACT + AHT [0.81 (95%CI 0.70–0.93)] than in AHT-only [0.60 (95%CI 0.51–0.71)].

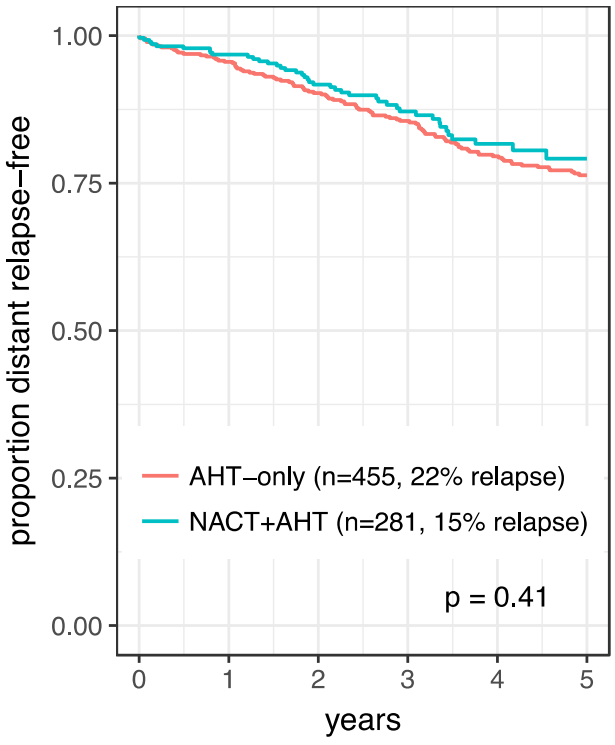

Fig. 3. Kaplan-Meier survival estimates are plotted for Cohort B stratified by treatments hormone therapy plus neoadjuvant chemotherapy (NACT + AHT) and hormone therapy alone (AHT-only). In a Cox proportional hazards model, the hazard ratio of AHT-only versus NACT + AHT, 0.86 (95%CI 0.59–1.2) is not statistically significant, although the percentage of relapse cases is lower in NACT + AHT (15%) than in AHT-only (22%).

These analyses provide evidence that ER+ breast cancer patients in EarlyR-Low do not obtain significant benefit in terms of reduced risk of 5-year distant relapse with NACT + AHT compared to AHT-only; while, in contrast, patients in EarlyR-High who are treated with NACT + AHT have a significant lower risk of relapse than patients treated with AHT-only.

4. Discussion

A critical decision in the treatment of ER+ breast cancer is whether or not to add chemotherapy to hormone therapy. Because all preferred chemotherapy regimens for ER+ breast cancer in the NCCN guidelines (https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf; Version I.2018, March 20, 2018) include a taxane, and taxane therapy is associated with considerable toxicities, there is a critical need for a diagnostic test to identify the patients who will benefit from chemotherapy.

In this study, we showed that EarlyR genomic signature was significantly predictive of pCR resulting from NACT in a large cohort (N = 581) of ER+ breast cancer patients. Moreover, using a cohort of matched samples treated with hormone therapy alone (AHT-only) or hormone therapy plus chemotherapy (NACT + AHT), we showed that patients in EarlyR-High had significantly reduced risk of relapse after 5 years, while no such benefit was observed in EarlyR-Low.

To deepen understanding of the predictive significance of EarlyR, we performed a robust multivariate analysis of the comparative predictive significance of EarlyR, clinical factors, biomarker expression and intrinsic subtypes. EarlyR was shown to be universally predictive of pCR in subgroups defined by LN status, T-stage, N-stage and grade, with the sole exceptions of T4 tumors or subgroups too small for analysis. Thus, EarlyR provides clinically useful information in all ER+ breast cancer patients. EarlyR was also shown to be superior in predictive significance to expression of *MKI67*, a clinical biomarker previously reported to be predictive of chemotherapy benefit [32].

The predictive significance of intrinsic subtypes is an important factor because of their roles in the Prosigna signature and the Mammaprint/Blueprint panel (Agendia). Intrinsic subtypes were found to be predictive of chemotherapy benefit in the NACT + AHT arm of Cohort B, confirming results previously reported [33,34]. EarlyR was also predictive of pCR in the same cohorts. In addition, EarlyR was also predictive (p = 0.01) of pCR in the Basal and Luminal B subtypes (the two PAM50 subtypes significantly enriched with pCR cases). In fact, of the 19 pCR cases found in these two subtypes, 17 were in EarlyR-High. In contrast, studies of the ability of Oncotype DX Recurrence Score to predict a benefit of neoadjuvant taxane-based chemotherapy have been negative [22] or included ER- samples [35,36].

This study documented the ability of EarlyR to predict a reduced risk of recurrence due to chemotherapy treatment using Cohort B. Cohort B did not consist of samples from a clinical trial with randomized arms of treated and untreated patients, a recognized limitation of the analysis. To compensate, we selected treated and untreated samples with proportional strata defined by LN status and grade. In this way, we attempted to create sets of chemotherapy treated and untreated sets of samples with comparable pre-treatment risks of recurrence (Table 2). In contrast, the evaluation of the predictive significance of Oncotype DX Recurrence Score in NSABP B-20 [6] was performed in a subset of samples from a clinical trial, however clinical features of each treatment arm in

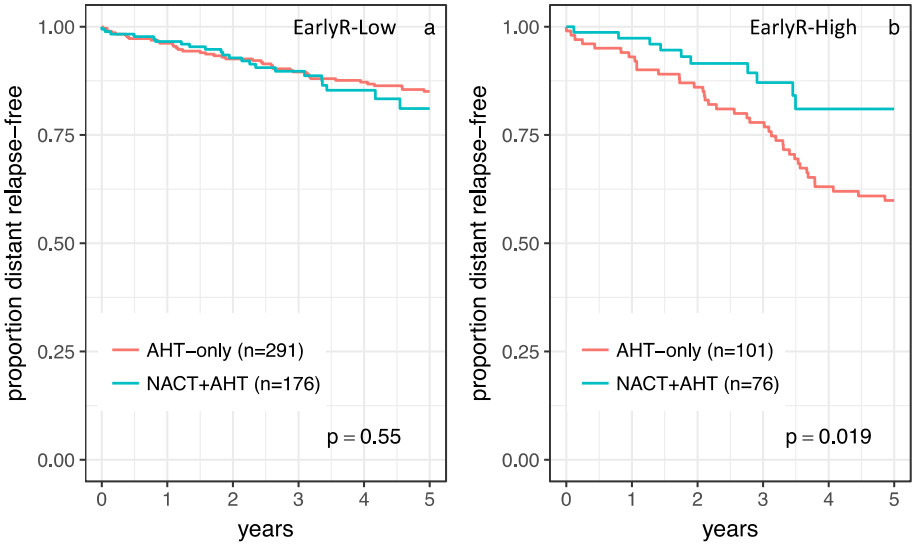

Fig. 4. Kaplan-Meier survival estimates were plotted for AHT-only versus NACT + AHT in Cohort B for (a) EarlyR-Low and (b) EarlyR-High.

this subgroup were not reported. Further analyses of EarlyR in subsets of patients from clinical trials are being currently undertaken.

There are number of gene signatures that are commercially available for ER+ breast cancer. Mammaprint and Oncotype Dx have been analyzed in prospective randomized clinical trials while others such as EndoPredict and Prosigna have been analyzed using a retrospective-prospective design. Each of the assays has significant limitation. The MINDACT trial documented limited benefit for patients with a mismatch between clinical risk and (Mammaprint-determined) genomic risk. The study of the significance of Oncotype Dx to predict chemotherapy benefit in NSABP B-20 was limited due to differences in the clinical traits of the treatment arms analyzed [6] and published results for Oncotype DX in NACT-treated samples is lacking. EndoPredict and Prosigna are both significantly influenced by clinico-pathological variables in their assessment of risk strata. We conclude that there is still an unfulfilled need for an assay that is significantly superior to "subjective" clinical risk assessment in prediction of chemotherapy benefit. We posit that EarlyR signature merits consideration in satisfying this need. With this study, we have now documented the utility of EarlyR in patients treated with neoadjuvant chemotherapy therapy in addition to adjuvant therapy.

5. Conclusion

We have previously documented the utility of EarlyR gene signature in patients treated with adjuvant chemotherapy in multiple cohorts [12] and in BIG 1–98 clinical trial [13,14]. It has consistently identified a large subset of patients having low scores that can possibly be spared from chemotherapy. For example, in the BIG 1–98 cohort, EarlyR-Low, EarlyR-int, EarlyR-High consisted of 67%, 19% and 14% of samples, respectively. Patients with low EarlyR had an excellent survival (95% at 8 year for LN-). Herein, we further document that EarlyR gene signature is predictive of response to neoadjuvant (AT and/or AC) chemotherapy in ER+ breast cancer patients (N = 659). Patients with EarlyR-High (~29% of samples) had the greatest likelihood of developing pCR. Importantly, EarlyR-Low patients (61%) were largely non-responsive. The long-term outcomes of patients in EarlyR-High were significantly improved by the use of NACT, documenting clinical utility of chemotherapy in these patients. Taken together, we document that EarlyR is a predictive assay wherein high scores are associated with response to neoadjuvant chemotherapy as manifest by pCR and improved survival.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2018.11.006.

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