Clinical Cancer Research

Estrogen Receptor Expression in 21-Gene Recurrence Score Predicts Increased Late Recurrence for Estrogen-Positive/HER2-Negative Breast Cancer

Mitch Dowsett[1], Ivana Sestak[2], Richard Buus[1], Elena Lopez-Knowles[1], Elizabeth Mallon[3], Anthony Howell[4], John F. Forbes[5], Aman Buzdar[6], and Jack Cuzick[2]

Abstract

Purpose: To identify the individual genes or gene modules that lead to the OncoptypeDx 21-gene recurrence score's reduced performance after 5 years and thereby identify indices of residual risk that may guide selection of patients for extended adjuvant therapy.

Experimental Design: We conducted a retrospective assessment of the relationship between (i) the individual genes and gene modules of the Recurrence Score and (ii) early (0–5 years) and late (5–10 years) recurrence rates in 1,125 postmenopausal patients with primary estrogen receptor–positive breast cancer treated with anastrozole or tamoxifen in the Arimidex, Tamoxifen, Alone or Combined (ATAC) randomized clinical trial.

Results: In the HER2-negative population (n = 1,009), estimates of recurrence risk were similar between years 0–5 and 5–10 for proliferation and invasion modules but markedly different for the estrogen module and genes within it (all split at the median): for low estrogen module, annual recurrence rates were similar across the two time windows (2.06% vs. 2.46%, respectively); for high estrogen module, annual rates were 1.14% versus 2.72%, respectively ($P_{\text{interaction}}$ = 0.004). Estrogen receptor transcript levels showed inverse prediction across the time windows: HR, 0.88 (0.73–1.07) and 1.19 (0.99–1.43), respectively ($P_{\text{interaction}}$ = 0.03). Similar time-, module-, and estrogen-dependent relationships were seen for distant recurrence.

Conclusions: Patients with tumors with high estrogen receptor transcript levels benefit most from 5 years' endocrine therapy but show increased recurrence rates after 5 years and may benefit from extended therapy. Improved prognostic profiles may be created by considering period of treatment and follow-up time. *Clin Cancer Res; 21(12); 2763–70. ©2015 AACR.*

Introduction

Endocrine therapy for 5 years is standard treatment for patients with primary estrogen receptor–positive (ER^+) breast cancer (1). Therapy beyond this time is beneficial overall but no prognostic tools have been developed to identify which patients are likely to benefit (2).

A number of multiparameter molecular profiles have been developed that estimate the prognosis of patients with primary ER^+ breast cancer treated with 5 years' endocrine therapy (3–6). They have clinical use in helping select appropriate patients who may avoid chemotherapy based on estimates of risk of recurrence on endocrine therapy alone. The OncotypeDx 21-gene recurrence score (RS) is the most widely used. It was developed to estimate the risk of distant recurrence over 10 years in patients treated with tamoxifen and was subsequently validated for use in postmenopausal patients treated with aromatase inhibitors (7).

It has become clear that rate of recurrence is not linear with time and that different patterns occur with different molecular groups of tumors. This is revealed in comparisons between ER^- and ER^+ tumors (8) but also extends to subtypes of ER^+ disease. For example, in a study of more than 10,000 breast cancers, all-cause mortality was higher in years 0–5 for luminal $HER2^+$ than for luminal $HER2^-$ tumors, but thereafter the 2 luminal subgroups showed increasingly comparable mortality risk (9). We have recently reported that the prediction of recurrence risk by RS and IHC4 [a prognostic profile defined by the measurement of ER, progesterone receptor (PgR), Ki67, and HER2; ref. 10] is largely confined to recurrence in years 0–5 whereas that of the PAM50 risk of recurrence score (ROR) and breast cancer index (BCI) extends to post 5-year recurrence (11, 12). The EndoPredict score has also been reported to predict late recurrence (13).

The different time-related performance of these multiparameter molecular profiles suggests that molecular features of ER^+ breast cancer may be identifiable, which relate differently to recurrence risk in the 0–5 year on-treatment and 5–10 year

[1]Royal Marsden Hospital and Breakthrough Breast Cancer Centre, Institute of Cancer Research, London, United Kingdom. [2]Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, United Kingdom. [3]Department of Pathology, Western Infirmary, Glasgow, United Kingdom. [4]Christie Hospital, Manchester Breast Centre and Breakthrough Breast Cancer Research Unit, Manchester, United Kingdom. [5]Department of Surgical Oncology, University of Newcastle, Newcastle Mater Hospital, New South Wales, Australia. [6]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Mitch Dowsett, Academic Department of Biochemistry, Royal Marsden Hospital, and Breakthrough Breast Cancer Centre, Institute of Cancer Research, Fulham Road, London SW3 6JJ, United Kingdom. Phone: 44-0-20-7808-2885; Fax: 44-0-20-7376-3918; E-mail: mitch.dowsett@icr.ac.uk

doi: 10.1158/1078-0432.CCR-14-2842

Translational Relevance

This is the first study to examine the contribution of the individual genes and gene modules of the OncotypeDx to estimations of risk of recurrence. The study was conducted in a set of 1,125 tissue samples from postmenopausal patients with estrogen receptor–positive primary breast cancer treated with either tamoxifen or an aromatase inhibitor (anastrozole) in the Arimidex, Tamoxifen, Alone or Combined (ATAC) trial. Clinically relevant findings include: (i) tumors with higher ER levels have double the risk of recurrence after compared with before 5 years: this may relate to endocrine treatment cessation at that time and have importance for identifying patients for extended adjuvant endocrine therapy; (ii) the so-called "carryover" effect of endocrine therapy varies according to tumor phenotype; and (iii) estimating risk of recurrence may be improved by the derivation of prognostic instruments that separately consider early and late recurrence.

posttreatment time windows that are characteristic of conventional endocrine therapy. Identifying these would be expected to provide better understanding of the determinants of recurrence and improved strategies for disease management particularly beyond 5 years. To address this, we assessed whether the individual genes and gene modules (estrogen, HER2, invasion, and proliferation) of the RS estimated risk of recurrence differently between years 0–5 and 5–10 in 1,125 patients treated with either tamoxifen or anastrozole from the Arimidex, Tamoxifen, Alone, or in Combination (ATAC) clinical trial (14).

Materials and Methods

Tissue samples were from women presenting with primary breast cancer participating in the ATAC trial that compared the efficacy of 5 years of tamoxifen with that of anastrozole and of the combination of both treatments in postmenopausal women with localized ER^+ breast cancer, 51.6% of patients had completed their randomized treatment before the first reports of benefit from extended adjuvant therapy (15). No recommendation was made for patients to receive extended therapy. Although no formal recording of post 5-year therapy was conducted, the timing of the end of our trial and of the report on benefit from extended adjuvant therapy (15) together with the relatively low risk of the population studied, in which such extended therapy is given less frequently, we estimate that less than 5% of the tamoxifen arm and none of the aromatase inhibitor arm would have received extended adjuvant therapy. This would have imperceptible effect on the current analyses. Formalin-fixed, paraffin-embedded (FFPE) blocks were collected from 2006 patients, the large majority being from the UK on which the analyses reported here were based. Data on the rates of recurrence and distant recurrence according to the RS from 1,231 of the patients in the first 9 years of follow-up have previously been published (7). The cohort analyzed in this report consisted of the 1,125 cases previously reported for both the RS and IHC4 (10) to allow comparisons between features of the 2 scores. The cohort was selected to exclude patients who had received adjuvant chemotherapy given the primary role of these prognostic signatures for identifying patients who could safely avoid this treatment. No patient received targeted anti-HER2 as adjuvant therapy. A consort diagram shows the derivation of this cohort from the whole trial population (Supplementary Fig. S1). The current analyses were performed on the outcome data from the 10-year median follow-up analysis (14). Methods of immunohistochemical (IHC) and FISH (for HER2) analyses were as previously reported (16).

Expression of the 21 genes in the RS was measured by Genomic Health Inc. (GHI) as previously described (7). Throughout the current report, gene modules are referred to as in Paik and colleagues (3). As in the creation of the RS, the expression of each of the 16 prognosis-related genes was normalized relative to the expression of the 5 reference genes (ACTB, GAPDH, GUS, RPLPO, and TFRC). The scores for the proliferation, estrogen, invasion, and HER2 modules were calculated as in the RS algorithm and thresholds for the proliferation and HER2 modules were applied as in that algorithm. The latter 2 modules were also considered without the application of a threshold. In the whole cohort, 251 (22.3%) and 358 (31.8%) of patients, respectively, showed values above these thresholds and therefore given a numerical value other than the thresholds. In the $HER2^-$ population, the proportions with values above the threshold were 206 (20.4%) and 251 (24.9%), respectively.

The primary study endpoint was any recurrence defined as the earliest of local or distant recurrence, new primary breast cancer, or death. Secondary endpoint was distant recurrence, for which contralateral disease, local/regional recurrence, and other second primary cancers were not considered as events. Any death before a distant recurrence was also considered a censoring event.

All analyses were performed according to a prespecified statistical analysis plan.

Cox proportional hazard models with associated 95% confidence intervals (CI) were used to assess the association between recurrence and the 16 prognosis-related genes and gene modules. This was done separately for years 0–10, 0–5, and 5–10. Changes in likelihood ratio values (LR-X^2) were used to measure and compare the relative amount of information of one gene compared with another in each time period. For the direct comparison of RS–related genes with the IHC4 markers, the HRs were adjusted for a difference between the 25^{th} and 75^{th} percentiles of the continuous variable. *P* values were 3-sided, based on normal approximation and all CIs were at the 95% level. Analyses were performed using STATA version 12.1.

Results

There were 215 recurrences and 164 distant recurrences in the first 10 years of follow-up. This is 20 recurrences and 19 distant recurrences more than reported in our earlier report on the same cohort of patients who had RS and IHC4 because of longer follow-up in this study (10). The number of recurrences and distant recurrences and the annual recurrence and distant recurrence rates both overall and according to HER2 status are shown in Table 1. The annual overall recurrence rate was 1.82% in years 0–5 and 2.60% in years 5–10. This is similar to the 2.26% and 2.48%, respectively, in the 1,993 patients in the United Kingdom cohort of ATAC patients untreated with chemotherapy. A total of 116 cases (10.3%) were $HER2^+$ and the annual recurrence rates for these were 4.10% (years, 0–5) and 2.68% (years, 5–10), respectively. For the 1,009 $HER2^-$ cases, a lower rate than for $HER2^+$ cases was seen in years 0–5 (1.56%, $P < 0.001$) but a similar rate was seen in years 5–10 (2.60%, $P = 0.9$).

Table 1. Number and annual rates of recurrence and distant recurrence for all patients and separately for patients with $HER2^+$ or $HER2^-$ disease according to time period of follow-up

All recurrences		**All**	**$HER2^+$**	**$HER2^-$**	
Numbers of patients		**1,125**	**116**	**1,009**	***P* ($HER2^+$ vs. $HER2^-$)**
Years					
0–10	No. of recurrences	215	34	181	
	Recurrence/y (%)	2.10%	3.12%	1.98%	0.002
0–5	No. of recurrences	100	23	77	
	Recurrence/y (%)	1.82%	4.10%	1.56%	<0.001
5–10	No. of recurrences	115	11	104	
	Recurrence/y (%)	2.60%	2.68%	2.60%	0.9
Distant recurrences (DR)		**All**	**$HER2^+$**	**$HER2^-$**	
Numbers of patients		**1,125**	**116**	**1,009**	***P* ($HER2^+$ vs. $HER2^-$)**
Years					
0–10	No. of DR	164	33	131	
	DR/y (%)	1.60%	3.06%	1.43%	<0.001
0–5	No. of DR	80	21	59	
	DR/y (%)	1.46%	3.74%	1.20%	<0.001
5–10	No. of DR	84	12	72	
	DR/y (%)	1.88%	2.92%	1.78%	0.2

The (univariate) HRs, LR-X^2, and *P* values for heterogeneity tests for each of the 16 genes and each of the 4 gene modules (both with and without application of thresholds where relevant) are shown separately for all cases, $HER2^-$ cases and $HER2^-$/node-negative cases in Supplementary Table S2 for all recurrences and in Supplementary Table S3 for distant recurrences. The analyses presented below focus on all recurrences, but similar patterns for distant recurrences were observed (data not shown).

The results in this report deal only with $HER2^-$ cases, as the results from the $HER2^+$ cases are largely of academic interest nowadays given the lack of application of HER2-directed therapies in the ATAC trial population. The data for all recurrences in $HER2^-$ cases are shown in Table 2. As we have previously reported (12), the prognostic significance of the RS is stronger in years 0–5 than in years 5–10 (LR-X^2, 23.5 and 6.7, respectively; $P_{\text{heterogeneity}} = 0.09$). In this series, each of the modules for proliferation (with and without threshold), estrogen, and invasion and the individual genes BAG1 and GSTM1 showed significant association with risk of recurrence for years 0–10. Of the 4 genes in the estrogen module only, BCL2 was significantly prognostic over this time period. The HER2 module was significantly associated with

Table 2. HRs (95% CI) and χ^2 values for all individual genes and gene modules according to time periods in $HER2^-$ patients

	0–10 years		**0–5 years**		**5–10 years**		
	HR (95% CI)	**LR-χ2**	**HR (95% CI)**	**LR-χ2**	**HR (95% CI)**	**LR-χ2**	**$P_{\text{heterogeneity}}$**
HER2 genes							
GRB7	0.79 (0.64–0.97)	4.91	0.71 (0.52–0.97)	4.56	0.79 (0.59–1.06)	2.49	0.6
HER2	0.90 (0.73–1.11)	0.94	0.70 (0.51–0.96)	4.89	1.01 (0.75–1.36)	0.01	0.09
HER module	0.79 (0.63–0.98)	4.64	0.69 (0.50–0.95)	4.87	0.80 (0.59–1.08)	2.12	0.5
HER module (with threshold)	0.89 (0.50–1.60)	0.15	0.44 (0.14–1.42)	2.31	1.17 (0.58–2.38)	0.19	
Proliferation genes							
Cyclin B1	1.85 (1.51–2.26)	35.54	1.81 (1.32–2.47)	13.85	1.81 (1.37–2.39)	17.96	0.9
Ki-67	1.64 (1.40–1.91)	42.01	1.52 (1.19–1.93)	12.31	1.66 (1.35–2.04)	24.63	0.6
MYLB2	1.51 (1.34–1.70)	44.92	1.59 (1.32–1.91)	23.1	1.46 (1.24–1.71)	20.45	0.5
STK15	1.95 (1.61–2.36)	40.94	2.02 (1.49–2.73)	18.42	1.83 (1.41–2.39)	18	0.6
Survivin	1.45 (1.29–1.64)	37.71	1.50 (1.24–1.81)	18.05	1.42 (1.21–1.66)	18.19	0.6
Proliferation module	1.90 (1.60–2.27)	53	1.94 (1.47–2.56)	22.71	1.84 (1.45–2.33)	26.11	0.8
Proliferation module (with threshold)	2.86 (1.84–4.43)	17.13	3.18 (1.68–6.05)	9.58	2.72 (1.46–5.06)	7.85	
Estrogen genes							
BCL2	0.61 (0.52–0.71)	35.83	0.57 (0.45–0.73)	19.86	0.64 (0.52–0.81)	14.52	0.5
SCUBE2	0.96 (0.88–1.04)	0.94	0.84 (0.74–0.96)	6.67	1.07 (0.94–1.20)	1.1	0.009
ER	1.05 (0.92–1.20)	0.51	0.88 (0.73–1.07)	1.61	1.19 (0.99–1.43)	3.54	0.03
PgR	0.94 (0.87–1.01)	2.62	0.86 (0.76–0.96)	6.87	1.00 (0.90–1.11)	0.001	0.05
Estrogen module	0.83 (0.72–0.96)	6.13	0.66 (0.54–0.81)	14.41	1.01 (0.82–1.23)	0.001	0.004
Invasion genes							
Cathepsin L2	1.26 (1.07–1.48)	7.6	1.19 (0.93–1.54)	1.84	1.27 (1.02–1.59)	4.62	0.7
Stromelysin 3	1.25 (1.12–1.39)	16.35	1.30 (1.09–1.55)	9.24	1.23 (1.06–1.42)	7.71	0.6
Invasion module	1.56 (1.30–1.87)	23.39	1.60 (1.20–2.14)	10.67	1.53 (1.20–1.96)	11.93	0.8
Other genes							
BAG1	0.73 (0.58–0.90)	8.92	0.52 (0.36–0.73)	14.79	0.85 (0.64–1.13)	1.28	0.03
CD68	1.03 (0.80–1.32)	0.04	1.08 (0.73–1.60)	0.15	1.03 (0.73–1.45)	0.03	0.9
GSTM1	0.77 (0.68–0.87)	17.12	0.73 (0.61–0.87)	11.25	0.80 (0.68–0.95)	6.29	0.4
RS	1.033 (1.022–1.045)	27.02	1.045 (1.028–1.062)	23.45	1.024 (1.007–1.042)	6.74	0.09

recurrence without thresholding, but this significance was lost after application of the threshold. It is notable that for each of GRB7, HER2 and the HER2 module higher values were associated with lower recurrence risk in contrast to the incorporation of each of these in the RS as predictors of higher risk. CD68 showed no association with recurrence in years 0–10 (Table 2).

Each of the proliferation genes was strongly and similarly prognostic for years 0–5 and 5–10 as was the entire proliferation module ($P < 0.0001$). A weaker, but still statistically significant, prognostic effect was observed by the module after application of the threshold for both time periods.

The HRs for the individual genes as continuous variables were all in the same direction for the 2 time periods (i.e., either consistently greater or consistently lower than 1.0) except for ER and SCUBE2 (Fig. 1). Both of the latter are in the estrogen module and both were associated with lower risk in years 0–5 [ER, 0.88 (95% CI, 0.73–1.07); SCUBE2, 0.84 (95% CI, 0.74–0.96)] but higher risk in years 5–10 [ER, 1.19 (95% CI, 0.99–1.43); SCUBE2, 1.07 (95% CI, 0.94–1.20)]. The test for heterogeneity between the time periods was significant for these 2 genes ($P = 0.03$ and 0.009, respectively). Overall, the HR for the estrogen module was 0.66 (95% CI, 0.54–0.81) for years 0–5 and 1.01 (95% CI, 0.82–1.23) for years 5–10. Again the test for heterogeneity was significant ($P = 0.004$).

The annual recurrence rate according to high or low ER expression is shown in Fig. 2. The annual recurrence rate was relatively stable for cases with low ER (i.e., below the median) between years 0–5 and 5–10 and was higher than that for those with high ER in years 0–5. However, during years 5–10, the rate increased in cases with high ER to a level similar to or higher than cases with low ER. In the $HER2^-$ population, the cutoff between high and low Oncotype ESR1 expression (i.e., the median) was 11.01 and the median level in the low and high populations was 10.27 and 11.76, respectively.

Table 3 shows the numbers and rates of recurrence according to time and according to both the proliferation module and estrogen module. In each time interval and ER category, there was a higher recurrence rate in the high than in the low proliferation tumors (e.g. high Estrogen module: years 0–5, 8.6% vs. 3.3%, $P = 0.02$; years 5–10, 17.5% vs. 10.7%, $P = 0.05$). In contrast, in years 0–5, the recurrence rate in both the low and high proliferation tumors was higher for the low estrogen module, but in years 5–10, the rate was higher for the high estrogen module in both cases.

Subdivision of the tumors according to treatment as well as estrogen module and time interval of follow-up creates small subgroups with consequent limited confidence in comparisons (Table 4). The median RS for the anastrozole- and tamoxifen-treated patients was 15.16 [interquartile range (IQR), 10.28–21.20] and 15.64 (IQR, 10.39–22.73), respectively ($P = 0.16$). For all patients, the 5-year recurrence rate was higher for both anastrozole and tamoxifen in years 5–10 than in years 0–5 (anastrozole, 1.50% vs. 2.80%; tamoxifen, 2.02% vs. 2.40%), with a greater difference for anastrozole. Considering the $HER2^-$ patients, the difference between the recurrence rates in the first 5 and second 5 years was greatest for those with high estrogen module scores with the difference being again greater for anastrozole (1.04% vs. 3.34%) than tamoxifen (1.24% vs. 2.06%).

Both of ER and PgR when assessed by IHC and scored as H-score and % cells positive, respectively (as integrated in the IHC4) showed greater prognostic significance for years 0–5 than years 5–10 (Supplementary Table S4). However, the HR for ER by IHC remained below 1.00 in contrast to the change in the direction of prognostic information for mRNA levels of ER.

Discussion

The key message from this study is that while, as expected, patients with tumors with high ER mRNA expression showed a relatively low recurrence rate in the years 0–5 this was followed by an approximately doubling of risk in years 5–10. Conversely, those patients with tumors with low ER mRNA showed relatively constant risk of recurrence across 10 years after diagnosis. Genes other than ER in the estrogen module of the RS, particularly SCUBE2 (also known as CEGP1) also showed a higher HR in years 5–10 than 0–5, suggesting that this phenomenon may be associated with the relative estrogen sensitivity of the tumors. A rational hypothesis is that this may have been due to effective suppression of recurrence in tumors with high estrogen sensitivity during adjuvant treatment but loss of control at, and because of, the withdrawal of treatment at 5 years. This is contrary to the concept of the "carryover" effect on risk reduction for tamoxifen that was developed from observations in overview analyses (17) and more recently for aromatase inhibitors, although there are more limited data on the extent of this for the latter because of the later initiation of the relevant trials (18). It should be noted that the data underpinning this carryover concept include patients with $HER2^+$ disease untreated with HER2-targeted therapy. As shown here and by others (9), such patients have a higher recurrence risk over the first 5 years. In addition, those overview data have not been subdivided according to measures of estrogen sensitivity as here. Our data suggest that carryover effects of therapy that are apparent when considering all patients may differ substantially if analyzed according to molecular phenotype.

The population studied here is only a subgroup of the overall ATAC population. Although it constitutes a highly annotated population of patients that were subject to registration standard follow-up throughout the 10 years, it cannot be seen to be sufficiently representative of the trial as a whole or sufficiently powered for studies of treatment interactions. Thus, the apparently stronger different time-related recurrence rates between the low and high estrogen module for the anastrozole-treated than tamoxifen-treated patients may be spurious but it merits further investigation in other trials of aromatase inhibitors versus tamoxifen.

Support for the current findings is provided by the recent report of the time-related patterns of relapse derived from analysis of publically deposited data on 965 patients treated with 5 years' adjuvant tamoxifen according to a mitotic kinase score (MKS, proliferation-related) and an ER-related score (ERS) based on the four genes in OncotypeDX (19). A high recurrence rate was found in the first 5 years for those with high MKS and low ERS that decreased in the second 5 years, whereas the other 3 groups showed increased recurrence rates after 5 years. The data have substantial similarities to those reported here but are difficult to compare directly, in part, because of the incomplete follow-up in their study through the second 5 years.

The only other publication of the impact of individual genes and gene modules in the OncotypeDx considered the predictive value of the individual genes and the gene modules for benefit

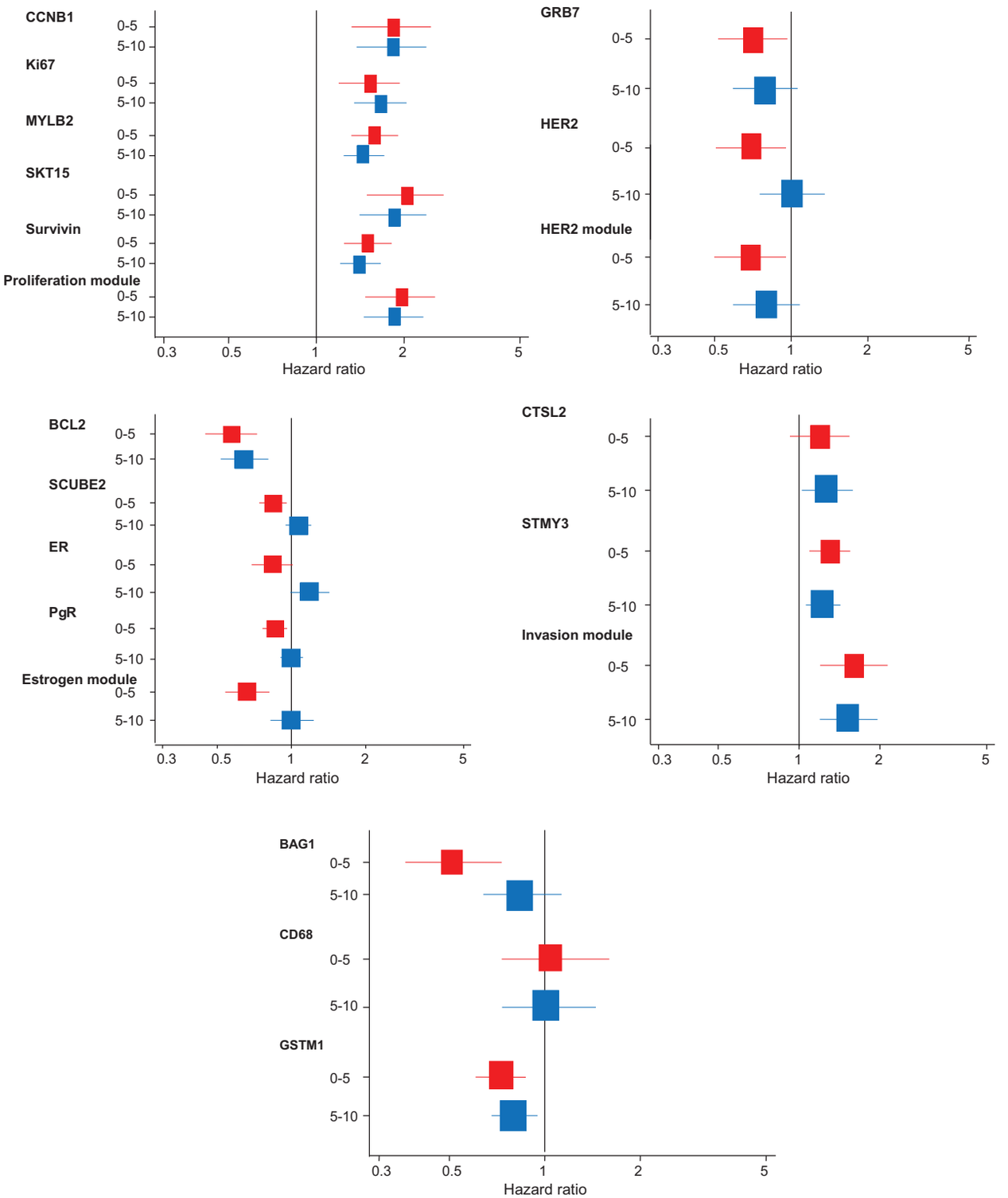

Figure 1.
Forest plots for all genes and modules in HER2$^-$ patients shown separately for years 0–5 (red) and years 5–10 (blue).

from adjuvant tamoxifen in the NSABP B14 trial (20). In that report, the RS overall had borderline significance ($P = 0.06$) for a higher score to indicate relatively poor benefit but higher scores in the estrogen module were indicative of significantly reduced recurrence with tamoxifen ($P_{interaction} = 0.008$). It is particularly notable that the 2 genes that showed statistically significant

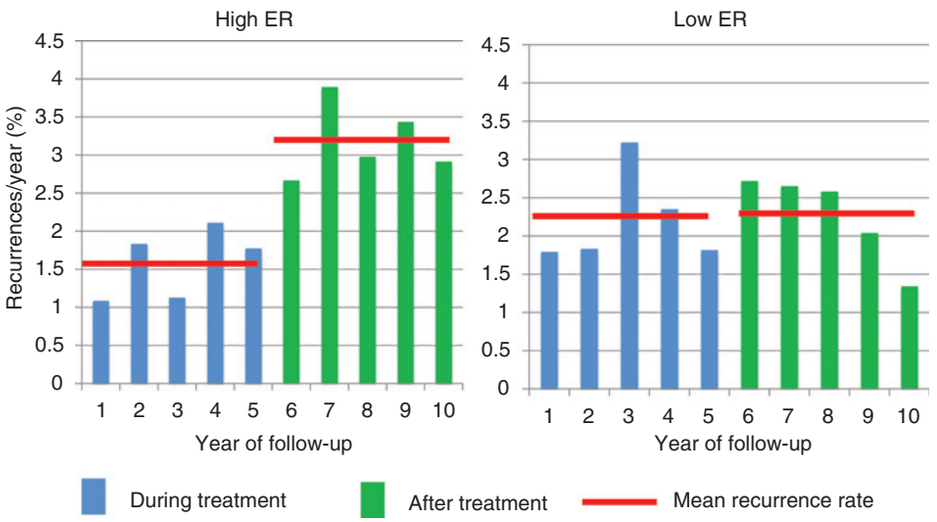

Figure 2.
Annual recurrence rates according to the expression of ER (RNA) in HER2$^-$ patients.

interaction with time in the current analysis (ER and SCUBE2) were the only genes that individually showed a significant interaction with benefit from tamoxifen in this NSABP B14 study. This supports the hypothesis that the increase in recurrence seen after 5 years in association with the expression of these genes is a treatment-dependent as opposed to strictly time-dependent effect.

In the current analysis, the HRs for ER expression as assessed by IHC did not alter as greatly over the 2 time periods as those for ER mRNA. This may be because IHC measures of ER do not show linearity with mRNA levels (20). Modern ER IHCs show compressed ranges for ER expression in ER$^+$ tumors and therefore less distinct separation between different levels of ER expression than is achieved by mRNA estimates (21).

It has become clear that treatment with an aromatase inhibitor or tamoxifen after 5 years of tamoxifen, so-called extended adjuvant therapy, can markedly reduce recurrence rates compared with no treatment (2, 17). The potential benefit from such extended therapy to an individual patient needs to be balanced with its side effects. Estimating this balance would benefit from a better identification of those patients most likely to benefit. The current data suggest that those with the most estrogen-sensitive tumors are likely to be a group deriving major benefit from extended therapy. This possibility should be assessed in correlative science studies associated with the relevant trials. Of note, the BCI has been found to predict benefit from letrozole in the MA.17 trial that randomized patients to receive the aromatase inhibitor for 5 years or no further endocrine treatment in women who had remained recurrence-free after 5 years' adjuvant tamoxifen treatment (22). It would be helpful to know the features of the 7 genes in BCI that contributed to the prediction. The current work suggests that determining the degree to which molecular features that relate to endocrine dependence of tumors might also predict benefit from extended adjuvant therapy and help in creating optimized signatures for this purpose. Several studies of extended adjuvant treatment with tamoxifen or an aromatase inhibitor, including those that follow 5 years' aromatase inhibitor treatment have recently or will shortly mature and provide further valuable material for optimizing signatures that predict benefit from longer term treatment.

A further implication of this work is that gene signatures for recurrence might be significantly improved if they considered the effects of time/treatment, for example, by the derivation of time/treatment-specific signatures. Estimates from the RS score itself might also be improved by time-dependent consideration of the estrogen module and/or genes within it. It seems likely, however, that the impact of this would be modest in the algorithm as currently used, given the substantial prognostic contribution provided by other modules such as proliferation. This work would

Table 3. Number and 10-year recurrence rates according to proliferation, estrogen module, and time period in HER2$^-$ patients

Years 0–10	**Estrogen module low**	**Estrogen module high**	***P* (Estrogen module low vs. high)**
Proliferation module low	34/257 (13.2%)	25/211 (11.8%)	0.7
Proliferation module high	58/274 (21.2%)	64/267 (24.0%)	0.4
P (Proliferation module low vs. high)	0.02	0.0007	
Years 0–5	**Estrogen module low**	**Estrogen module high**	***P* (Estrogen module low vs. high)**
Proliferation module low	16/257 (6.2%)	7/211 (3.3%)	0.1
Proliferation module high	31/274 (11.3%)	23/267 (8.6%)	0.3
P (Proliferation module low vs. high)	0.04	0.02	
Years 5–10	**Estrogen module low**	**Estrogen module high**	***P* (Estrogen module low vs. high)**
Proliferation module low	18/225 (8.0%)	18/169 (10.7%)	0.4
Proliferation module high	27/254 (10.6%)	41/234 (17.5%)	0.03
P (Proliferation module low vs. high)	0.3	0.05	

Table 4. Overall number and annual rate of recurrences according to treatment and level of estrogen module (split at the median) in all patients and in the HER2$^-$ subgroup

Years			All	HER2$^-$	HER2$^-$ Low Estrogen-module	HER2$^-$ High Estrogen-module
0–5	All	No. of recurrences	100	77	47	30
		Recurrence/y	1.82%	1.56%	2.06%	1.14%
	Anastrozole	No. of recurrences	41	31	17	14
		Recurrence/y	1.50%	1.26%	1.52%	1.04%
	Tamoxifen	No. of recurrences	59	46	30	16
		Recurrence/y	2.02%	1.88%	2.58%	1.24%
5–10	All	No. of recurrences	115	104	45	59
		Recurrence/y	2.60%	2.60%	2.46%	2.72%
	Anastrozole	No. of recurrences	62	59	22	37
		Recurrence/y	2.80%	2.94%	2.48%	3.34%
	Tamoxifen	No. of recurrences	53	45	23	22
		Recurrence/y	2.40%	2.26%	2.50%	2.06%

NOTE: Overall number and annual rate of recurrences according to treatment and HER2 status. Data are compared with that with patients in the main ATAC trial not receiving chemotherapy and those from the UK not receiving chemotherapy.

need confirmation before such modifications should be undertaken.

The sample set from which the RS was originally developed included HER2$^+$ cases (3), yet the RS is clinically applied almost exclusively in HER2$^-$ cases. In the current analysis of patients with HER2$^-$ disease, higher levels of HER2 expression were associated with lower risk of recurrence (HER2 transcript expression is readily measurable in most cases conventionally considered as HER2$^-$). This is consistent with our earlier report including a different cohort of tamoxifen-treated patients with ER$^+$/HER2$^-$ disease (23). However, it conflicts with HER2 expression being given a positive score for risk in the RS algorithm partly irrespective of HER2$^+$/HER2$^-$ status. In that calculation, a threshold is applied to the HER2 module score such that cases with a score <8 are allocated a value of 8 and those ≥8 retain their numerical value. This latter group includes nearly all of the HER2$^+$ cases in the cohort studied but also about 30% of the HER2$^-$ cases. These relationships may explain the lower risk seen in the current study for cases with higher HER2 levels in years 0–5. The estimates of risk from HER2 and the closely associated GRB7 may be deleterious to the accuracy of RS in patients with HER2$^-$ disease.

In summary, several genes and gene modules of the RS associate differently with risk of early and late recurrence; most notably, ER mRNA levels predicted increased recurrence risk after 5 years at withdrawal of therapy. It is likely that recurrence in such patients may be restricted by extended adjuvant therapy and this possibility should be examined. Improved predictors of long-term outcome are likely to be derived by considering time of follow-up and focus on HER2$^-$ cases.

Disclosure of Potential Conflicts of Interest

M. Dowsett reports receiving other commercial research support from AstraZeneca, speakers bureau honoraria from Ventana, and is a consultant/advisory board member for Genoptix. E. Mallon reports receiving speakers bureau honoraria from Genomic Health. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Dowsett, I. Sestak, R. Buus, A. Howell, J.F. Forbes, A. Buzdar

Development of methodology: I. Sestak, A. Buzdar

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Dowsett, E. Lopez-Knowles, E. Mallon, A. Howell, J.F. Forbes

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Dowsett, I. Sestak, R. Buus, E. Mallon, A. Howell, A. Buzdar, J. Cuzick

Writing, review, and/or revision of the manuscript: M. Dowsett, I. Sestak, R. Buus, E. Lopez-Knowles, A. Howell, J.F. Forbes, A. Buzdar, J. Cuzick

Study supervision: M. Dowsett, J.F. Forbes, J. Cuzick

Other (*pathology review*): E. Mallon

Grant Support

This work was funded by grants from the Mary-Jean Mitchell Green Foundation, Breakthrough Breast Cancer, AstraZeneca, the Royal Marsden NIHR Biomedical Research Centre, and Cancer Research UK programme grant C569-10404.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 3, 2014; revised January 29, 2015; accepted February 2, 2015; published online June 15, 2015.

References

1. Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011;378:771–84.
2. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 2003;349:1793–802.
3. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–26.
4. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7.
5. Ma XJ, Salunga R, Dahiya S, Wang W, Carney E, Durbecq V, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. Clin Cancer Res 2008;14:2601–8.
6. Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clin Cancer Res 2011;17:6012–20.

7. Dowsett M, Cuzick J, Wale C, Forbes J, Mallon EA, Salter J, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. J Clin Oncol 2010;28:1829–34.
8. Saphner T, Tormey DC, Gray R. Annual hazard rates of recurrence for breast cancer after primary therapy. J Clin Oncol 1996;14:2738–46.
9. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med 2010;7:e1000279.
10. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol 2011;29:4273–8.
11. Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 2013;14:1067–76.
12. Sestak I, Dowsett M, Zabaglo L, Lopez-Knowles E, Ferree S, Cowens JW, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. J Natl Cancer Inst 2013;105:1504–11.
13. Dubsky P, Brase JC, Jakesz R, Rudas M, Singer CF, Greil R, et al. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2− breast cancer patients. Br J Cancer 2013;109:2959–64.
14. Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 2010;11:1135–41.
15. Goss PE, Ingle JN, Pater JL, Martino S, Robert NJ, Muss HB, et al. Late extended adjuvant treatment with letrozole improves outcome in women with early-stage breast cancer who complete 5 years of tamoxifen. J Clin Oncol 2008;26:1948–55.
16. Dowsett M, Cuzick J, Wale C, Howell T, Houghton J, Baum M. Retrospective analysis of time to recurrence in the ATAC trial according to hormone receptor status: an hypothesis-generating study. J Clin Oncol 2005;23:7512–7.
17. Davies C, Pan H, Godwin J, Gray R, Arriagada R, Raina V, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2013;381:805–16.
18. Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol 2010;28:509–18.
19. Bianchini G, Pusztai L, Karn T, Iwamoto T, Rody A, Kelly CM, et al. Proliferation and estrogen signaling can distinguish patients at risk for early versus late relapse among estrogen receptor positive breast cancers. Breast Cancer Res 2013;15:R86.
20. Kim C, Tang G, Pogue-Geile KL, Costantino JP, Baehner FL, Baker J, et al. Estrogen receptor (ESR1) mRNA expression and benefit from tamoxifen in the treatment and prevention of estrogen receptor-positive breast cancer. J Clin Oncol 2011;29:4160–7.
21. Collins LC, Botero ML, Schnitt SJ. Bimodal frequency distribution of estrogen receptor immunohistochemical staining results in breast cancer: an analysis of 825 cases. Am J Clin Pathol 2005;123:16–20.
22. Sgroi DC, Carney E, Zarrella E, Steffel L, Binns SN, Finkelstein DM, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. J Natl Cancer Inst 2013;105:1036–42.
23. Pinhel I, Hills M, Drury S, Salter J, Sumo G, A'Hern R, et al. ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer. Breast Cancer Res 2012;14:R46.

Clinical Cancer Research

Estrogen Receptor Expression in 21-Gene Recurrence Score Predicts Increased Late Recurrence for Estrogen-Positive/HER2-Negative Breast Cancer

Mitch Dowsett, Ivana Sestak, Richard Buus, et al.

Clin Cancer Res 2015;21:2763-2770.