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ORIGINAL ARTICLE

Identifying oncogenic drivers associated with increased risk of late distant recurrence in postmenopausal, estrogen receptor-positive, HER2-negative early breast cancer: results from the BIG 1-98 study

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Background: In postmenopausal, estrogen receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative early breast cancer, the risk for distant recurrence can extend beyond 5 years of adjuvant endocrine therapy. This study aims to identify genomic driver alterations associated with late distant recurrence.

Patients and methods: Next generation sequencing was used to characterize driver alterations in primary tumors from a subset of 764 postmenopausal estrogen receptor-positive/HER2-negative patients from the BIG 1-98 randomized trial. Late distant recurrence events were defined as $\geq$5 years from time of randomization). The association of driver alterations with distant recurrence-free interval in early and late time periods was assessed using Cox regression models. Multivariable analyses were carried out to adjust for clinicopathological factors. Weighted analysis methods were used in order to correct for over-sampling of distant recurrences.

Results: A total of 538 of 764 (70%) samples were successfully sequenced including 88 (63%) early and 52 (37%) late distant recurrence events after a median follow up of 8.1 years. In univariable analysis for late distant recurrence, *PIK3CA* mutations (58.8%) were significantly associated with reduced risk [hazard ratio (HR) 0.40, 95% confidence interval (CI) 0.20—0.82, $P = 0.012$], whereas amplifications on chromosome 8p11 (10.9%) (HR 4.79, 95% CI 2.30—9.97, $P < 0.001$) and *BRCA2* mutations (2.3%) (HR 5.39, 95% CI 1.51—19.29, $P = 0.010$) were significantly associated with an increased risk. In multivariable analysis, only amplifications on 8p11 ($P = 0.002$) and *BRCA2* mutations ($P = 0.013$) remained significant predictors.

Conclusions: In estrogen receptor-positive/HER2-negative postmenopausal early breast cancer, *PIK3CA* mutations were associated with reduced risk of late distant recurrence, whereas amplifications on 8p11 and *BRCA2* mutations were associated with increased risk of late distant recurrence. The characterization of oncogenic driver alterations may aid in refining treatment choices in the late disease setting, and help identify potential drug targets for testing in future trials.

Key words: breast, cancer, hormone, late, prognosis, recurrence

INTRODUCTION

In hormone receptor-positive breast cancer, the risk of disease recurrence and death can persist well beyond the standard 5 years of adjuvant endocrine therapy, even in patients with limited tumor burden at diagnosis.[1—3] Clinical trials investigating the use of extended adjuvant endocrine therapy have reported conflicting results, with some demonstrating small but statistically significant benefits,[4—8] while others have not.[9,10] This emphasizes the need for biomarkers to aid in individualizing adjuvant endocrine therapy decisions.

Routinely utilized clinicopathological parameters including tumor size, nodal status, and grade have

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demonstrated continued prognostic significance even after 5 years of endocrine therapy.[3,11] The Clinical Treatment Score after 5 years (CTS5), which was developed from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) dataset and validated in the BIG 1-98 dataset, is a tool with potential clinical utility for decision making regarding extended endocrine therapy use.

The added value of tumor molecular testing to further refine prognostic estimates for late distant recurrence has also been investigated with mixed results. Some gene expression-based tests have shown diminished prognostic significance in the setting of 5 years and beyond,[12−14] possibly due to the influence of genes that correlate strongly with increased proliferation and hence early disease recurrence. On the other hand, a recent comparison of six gene-expression signatures in the TransATAC study population demonstrated significant added value for three gene expression assays in the prognostication for risk of late disease recurrence in years 5 to 10 after randomization. This suggests that genomic assays could have clinical utility for improving prediction of late disease recurrence risk in this setting; however, as biological factors mediating the risk of early and late risk of recurrence may differ, novel assays are likely to be required.

We have recently reported the prognostic relevance of oncogenic drivers in postmenopausal early-stage estrogen receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative patients enrolled in the BIG 1-98 study after 8.1 years median follow up.[15] In this *post hoc* exploratory study, our primary objective was to identify oncogenic drivers predictive of increased risk of late distant recurrence. These data may provide clinically useful information to guide the use of extended endocrine therapy, and aid in prioritizing future research into potentially targetable molecular drivers in the adjuvant setting.

METHODS

Patient cohort

This analysis includes patients enrolled in the BIG 1-98 clinical trial who were selected for next generation sequencing of tumor DNA. BIG 1-98 was an international, randomized, double-blind phase III trial (NCT00004205) that enrolled 8010 postmenopausal patients with hormone receptor-positive operable invasive breast cancer. Patients were randomly assigned to receive endocrine monotherapy with letrozole (2.5 mg orally daily) or tamoxifen (20 mg orally daily) for 5 years, or a sequential strategy of tamoxifen for 2 years, followed by letrozole for 3 years, or the reverse. The study details have been previously reported.[1,16] Written informed consent was provided by all patients. Ethics committees and relevant health authorities approved the protocol. Tumor samples were collected retrospectively in accordance with institutional guidelines and national laws.

Details of statistical power calculations and patient selection for tumor sequencing is shown in supplementary Figure S1 and described in supplementary Methods (available at https://doi.org/10.1016/j.annonc.2020.06.024), and has additionally been previously published.[15] A total of 938 patients were selected from 2706 patients with banked DNA extracted from formalin-fixed, paraffin-embedded archival tumor samples, using a sampling plan that included all patients who had a distant recurrence and a stratified random sampling of those without distant recurrence. After further exclusions due to DNA quality control, 764 samples were sent for tumor sequencing. In order to correct for over-sampling of distant recurrences, weighted analysis methods were used. Calculation of sampling weights has been previously described[15] (supplementary Methods, available at https://doi.org/10.1016/j.annonc.2020.06.024).

Tumor DNA sequencing and variant calling

Library preparation, hybridization capture, and sequencing were carried out using Foundation Medicine's T5 targeted panel of 287 cancer genes (supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2020.06.024). Samples with a median exon depth of coverage of 150× or greater were eligible for analysis. Variant pathogenicity was annotated as 'known', 'likely', 'ambiguous' or 'unknown', after filtering for known germline variants from publicly available databases. In order to reduce false positives, only alterations annotated as 'known' or 'likely' to be pathogenic were included for analysis. Driver alterations were defined categorically as present or absent (for short variants, as mutated versus wild-type; for amplifications, as amplified versus non-amplified). *PIK3CA* mutations were further annotated by affected protein domain (kinase domain, helical domain, other domain, multiple domains).

Objectives, end points, and statistical analysis

The primary objective of this study was to assess the associations of oncogenic drivers with the risk of late distant recurrence, with a secondary objective of assessing associations with the risk of early distant recurrence. The primary end point for this analysis was distant recurrence-free interval, which is defined as the time from randomization to recurrence at a distant site. Patients without recurrence at a distant site were censored at the date of last follow-up or death without recurrence. Distant recurrence events were defined arbitrarily as 'early' if it occurred less than 5 years from the time of randomization, and 'late' if it occurred 5 years or greater from the time of randomization.

The generalized Horvitz-Thompson weighted method (inverse probability weighting) was applied to all analyses.[17] All prognostic associations were determined using weighted Cox proportional hazards regression models, stratified for treatment assignment. Multivariable Cox proportional hazard models included the following variables which have previously been shown to be prognostic [age ($\geq$65 years versus $<$65 years), tumor size ($>$2 cm versus $\leq$2cm), nodal status (positive versus negative), and grade (3 versus 1/2)]. For risk of early distant recurrence (0−5 years after randomization), all patients who had not experienced an event and were still

in follow-up at 5 years were censored at 5 years. For risk of late distant recurrence (≥5 years after randomization), only patients who had had not experienced an event and were still in follow-up at 5 years were included, thus the predictors of late recurrence were conditional on remaining recurrence-free and alive at 5 years. Hazard ratios (HR) and 95% confidence intervals (95% CI) were generated using robust standard errors, with a Wald test for significance. Survival plots for visualization were generated using the 1—Kaplan—Meier (weighted) function and were unadjusted.

We used R software version 3.4.3 for statistical analyses and deemed an unadjusted two-sided *P* value less than 0.05 to be significant.

RESULTS

Patient characteristics

In total, 538 samples (70%) successfully underwent DNA sequencing and met sequencing quality metrics, with a median exon depth ranging from 151×—1397× for target genes. Patient characteristics of the selected sequenced cohort and all eligible patients from the BIG 1-98 trial are shown in supplementary Table S2 (available at https://doi.org/10.1016/j.annonc.2020.06.024).[15] This includes 140 samples from patients who had a distant recurrence, of which 88 occurred early (<5 years from randomization) and 52 late (≥5 years from randomization) (Figure 1). The median follow-up for distant recurrence events was 8.1 years. An additional 13 patients were excluded from the 'late' analysis due to follow-up of <5 years. The weighted frequencies of driver alterations are shown in Figure 2 and supplementary Figure S2 (available at https://doi.org/10.1016/j.annonc.2020.06.024).

Associations of driver alterations for late distant recurrence

The univariable and multivariable prognostic associations of driver alterations for early and late distant recurrence are shown in Table 1 and Figure 2. Driver alterations present in 5% or greater frequency were included for this analysis, as well as selected alterations of interest [*AKT1* (4.1%) and *BRCA2* (2.3%) mutations].

For the risk of distant recurrence in patients ≥5 years from randomization, *PIK3CA* mutations were significantly associated with reduced risk of late distant recurrence in univariable analysis (HR 0.40, 95% CI: 0.20—0.82, $P = 0.012$) but not in multivariable analysis ($P = 0.087$). Conversely, amplifications on 8p11 (HR 4.79, 95% CI 2.30—9.97, $P < 0.001$) and *BRCA2* mutations (HR 5.39, 95% CI 1.51—19.29, $P = 0.010$) were significantly associated with higher risk of late distant recurrence in univariable analysis and also remained significant predictors in multivariable analysis ($P = 0.002$, $P = 0.013$, respectively). There is an increased risk of late distant recurrence associated with *CDH1* mutations (HR 1.80, 95% CI 0.70—4.64, $P = 0.223$), *GATA3* mutations (HR 1.82, 95% CI 0.78—4.22, $P = 0.164$), and amplifications on 20q13 (HR 2.86, 95% CI 0.96—8.52, $P = 0.060$); however, these were not statistically significant. Survival plots by driver alteration status are shown in Figure 3 and supplementary Figure S3 (available at https://doi.org/10.1016/j.annonc.2020.06.024).

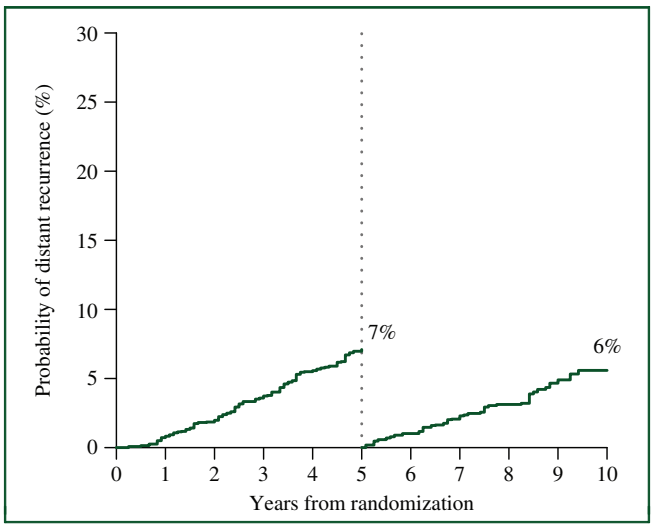

Figure 1. Early and late distant recurrences in BIG 1-98.
Weighted survival plot for distant recurrence in patients who underwent DNA sequencing.

Associations of driver alterations for early distant recurrence

For the risk of distant recurrence within the first 5 years, *TP53* mutations (HR 1.94, 95% CI 1.11—3.38, $P = 0.020$) and amplifications on 11q13 (HR 2.49, 95% CI 1.46—4.25, $P = 0.001$) and 8p11 (HR 2.57, 95% CI 1.40—4.69, $P = 0.002$) were associated with greater risk of early distant recurrence in univariable analysis; however, only amplifications on 11q13 remained significant in multivariable analysis ($P = 0.005$). *PIK3CA* mutations demonstrated a numerically reduced risk of early distant recurrence but were not statistically significant (HR 0.66, 95% CI 0.39—1.11, $P = 0.115$). Survival plots by driver alteration status are shown in Figure 3 and supplementary Figure S3 (available at https://doi.org/10.1016/j.annonc.2020.06.024).

Association of PIK3CA mutations by affected functional domain for late distant recurrence

Supplementary Table S3 (available at https://doi.org/10.1016/j.annonc.2020.06.024) shows the 'early' and 'late' prognostic associations by affected functional domain. Reduced risk of distant recurrence was observed for both early and late time periods in all subtypes of *PIK3CA* mutation as compared with wild type. Interestingly, kinase domain *PIK3CA* mutations as compared with wild type seemed to have a very low risk of late distant recurrence in univariable (HR 0.18, 95% CI 0.06—0.55, $P = 0.003$) and multivariable analysis ($P = 0.009$).

DISCUSSION

In this study, we found that the risk of late distant recurrence was significantly lower in patients with *PIK3CA* mutation, although it should be noted that this did not remain

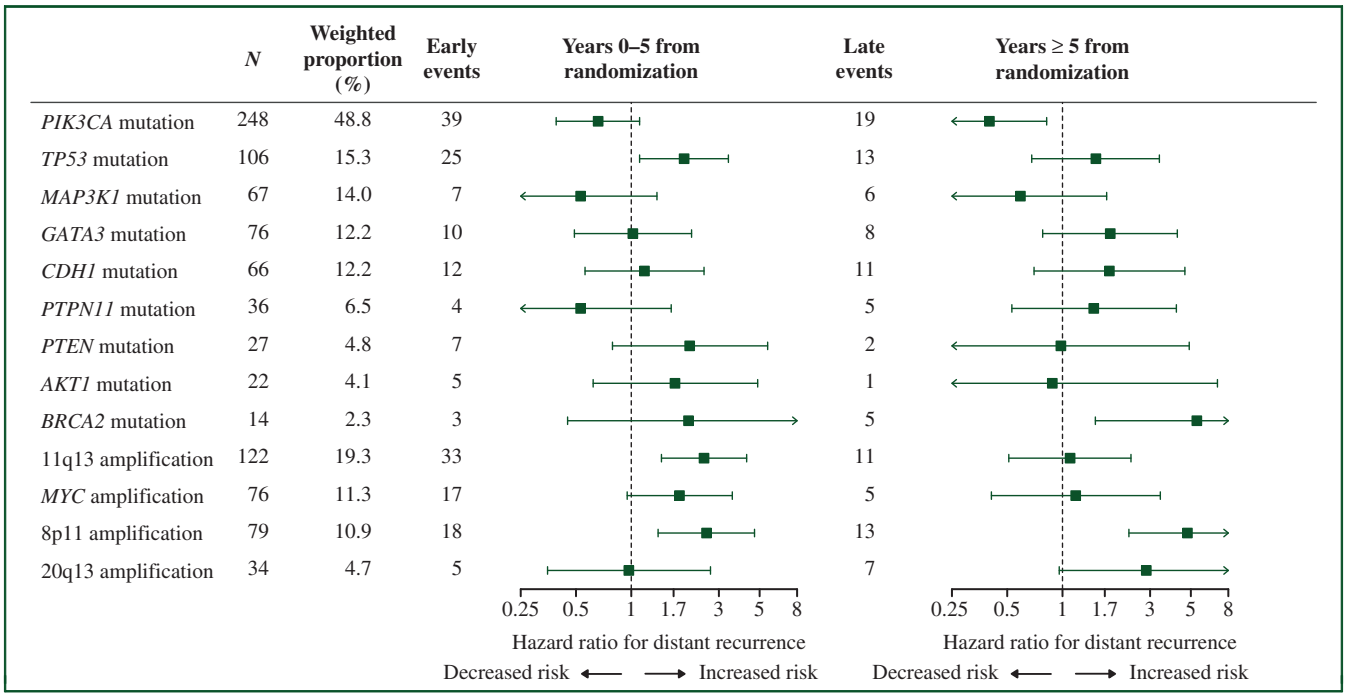

Figure 2. Prognostic associations of driver alterations.
Forest plots demonstrating the prognostic associations of driver alterations in early (0–5 years from randomization) and late (≥5 years from randomization) time periods using univariable Cox regression models. Boxes demonstrate the hazard ratio point estimate with bars demonstrating 95% confidence intervals.

significant in multivariable analysis. This effect was seemingly more pronounced than in the first 5 years. On the other hand, amplifications on chromosome 8p11 were significantly associated with increased risk of both early and late distant recurrence. *BRCA2* mutations were also significantly associated with increased risk of late distant

Table 1. Prognostic associations of driver alterations in early and late time periods using Cox regression models

	N	Weighted proportion (%)	Early events	Late events	Univariable analysis				Multivariable analysis			
					Early distant recurrence		Late distant recurrence		Early distant recurrence		Late distant recurrence	
					HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
PIK3CA mutation	248	48.8	39	19	0.66 (0.39–1.11)	0.115	0.40 (0.20–0.82)	0.012	0.79 (0.48–1.31)	0.360	0.54 (0.27–1.09)	0.087
TP53 mutation	106	15.3	25	13	1.94 (1.11–3.38)	0.020	1.52 (0.68–3.37)	0.309	1.36 (0.81–2.29)	0.247	0.94 (0.45–1.93)	0.857
MAP3K1 mutation	67	14.0	7	6	0.53 (0.21–1.38)	0.195	0.59 (0.20–1.74)	0.341	0.56 (0.22–1.41)	0.215	0.69 (0.24–1.98)	0.489
GATA3 mutation	76	12.2	10	8	1.02 (0.49–2.13)	0.964	1.82 (0.78–4.22)	0.164	0.97 (0.45–2.06)	0.930	1.54 (0.66–3.62)	0.322
CDH1 mutation	66	12.2	12	11	1.18 (0.56–2.49)	0.664	1.80 (0.70–4.64)	0.223	1.43 (0.65–3.15)	0.369	2.31 (0.99–5.42)	0.054
PTPN11 mutation	36	6.5	4	5	0.53 (0.17–1.65)	0.271	1.48 (0.53–4.17)	0.455	0.59 (0.20–1.75)	0.339	1.33 (0.50–3.56)	0.570
PTEN mutation	27	4.8	7	2	2.08 (0.79–5.53)	0.140	0.98 (0.20–4.90)	0.984	2.15 (0.85–5.46)	0.106	0.94 (0.20–4.38)	0.940
AKT1 mutation	22	4.1	5	1	1.73 (0.62–4.88)	0.298	0.88 (0.11–6.98)	0.901	1.28 (0.43–3.79)	0.655	0.92 (0.11–7.88)	0.941
BRCA2 mutation	14	2.3	3	5	2.05 (0.45–9.35)	0.353	5.39 (1.51–19.29)	0.010	1.68 (0.43–6.59)	0.455	4.2 (1.36–12.97)	0.013
11q13 amplification	122	19.3	33	11	2.49 (1.46–4.25)	0.001	1.10 (0.51–2.36)	0.802	2.07 (1.24–3.44)	0.005	0.85 (0.40–1.83)	0.684
MYC amplification	76	11.3	17	5	1.83 (0.95–3.55)	0.072	1.18 (0.41–3.41)	0.759	1.08 (0.53–2.20)	0.830	0.74 (0.28–1.99)	0.555
8p11 amplification	79	10.9	18	13	2.57 (1.40–4.69)	0.002	4.79 (2.30–9.97)	<0.001	1.67 (0.91–3.09)	0.100	3.03 (1.52–6.06)	0.002
20q13 amplification	34	4.7	5	7	0.97 (0.35–2.7)	0.949	2.86 (0.96–8.52)	0.060	0.65 (0.24–1.81)	0.414	1.46 (0.46–4.65)	0.519

Multivariate analysis included adjustment for treatment assignment, age, tumor size, nodal status, and grade as described in the methods section.
95% CI, 95% confidence interval; HR, hazard ratio.

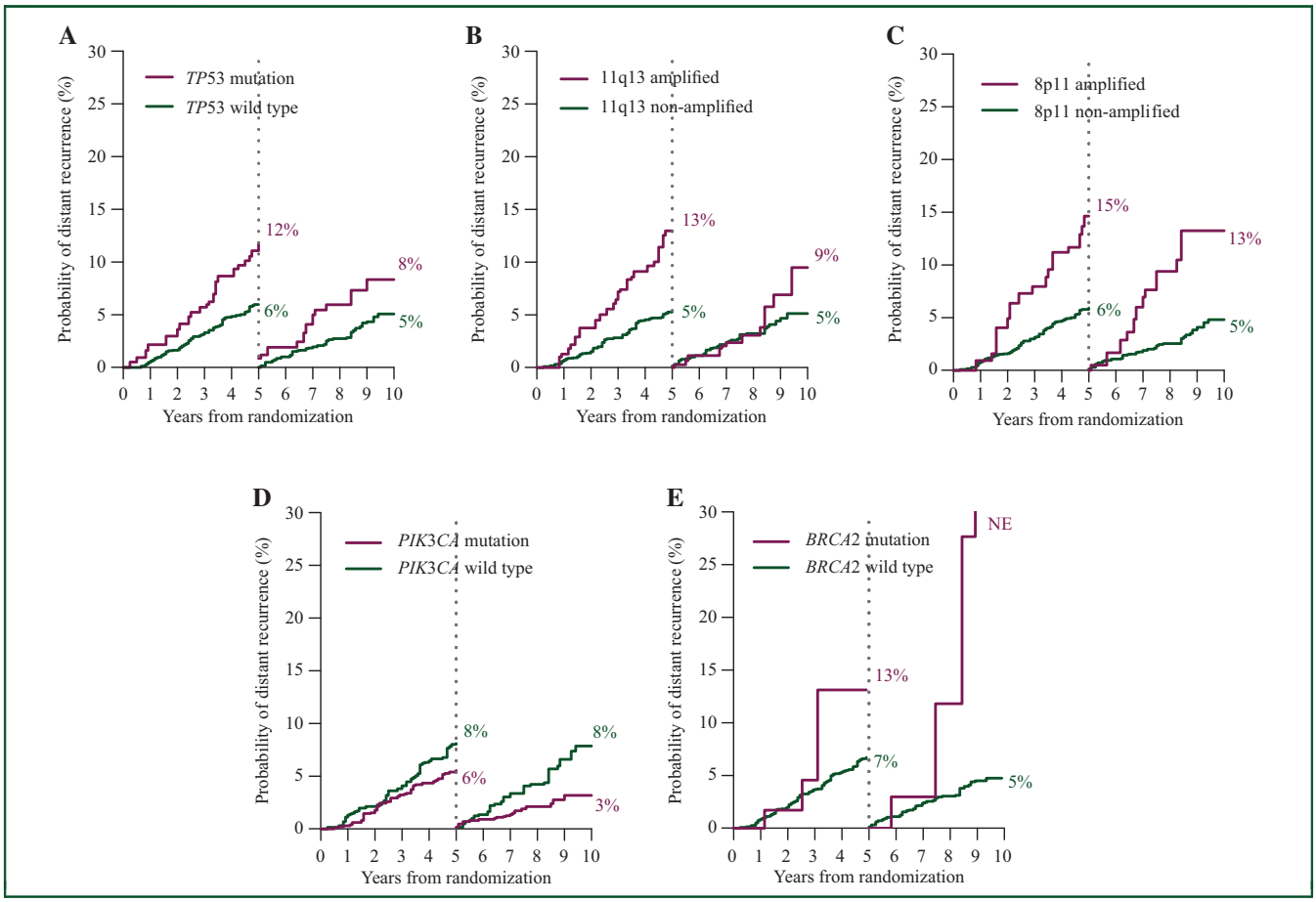

Figure 3. Cumulative incidence for distant recurrence by driver alteration status.
Weighted cumulative incidence curves for distant recurrence shown by driver alteration status for *TP53* mutations (A), 11q13 amplifications (B), 8p11 amplifications (C), *PIK3CA* mutations (D), and *BRCA2* mutations (E).
NE, not evaluable.

recurrence. However, this should be interpreted with caution given the low number of patients and events.

PIK3CA mutations are the most common somatic genetic alteration in estrogen receptor-positive/HER2-negative breast cancer.[15] Similar to a recently published report, a favorable disease outcome was observed in both early and late time settings for patients with tumors harboring *PIK3CA* mutations.[18] This may be in part due to a relative mutual exclusion with somatic alterations associated with higher proliferation and increased risk of recurrence such as *TP53* mutations, and amplifications on chromosomes 11q13, and *MYC*.[15] However, given the relatively small number of patients with these alterations, this would need confirmation in larger studies. In our dataset, kinase domain *PIK3CA* mutations are associated with the most favorable and statistically significant reduction in the risk of late distant recurrence as compared with other types of *PIK3CA* mutations. It should be noted, however, that a recent large pooled analysis found no significant difference between helical and kinase domain mutations for recurrence.[18] Taken together, these data suggest that tumor *PIK3CA* status could potentially serve as a useful adjunctive biomarker to clinicopathological variables for determining if a patient has sufficiently low risk to be unlikely to derive a significant benefit from extended endocrine therapy. Furthermore, we have previously reported *PIK3CA* mutations to be associated with increased dependency on estrogen receptor signaling, and predictive of benefit with letrozole as compared with tamoxifen.[15] In the setting of occult metastatic disease, prolonged endocrine treatment in these patients could hypothetically increase selective pressure for the acquisition of genetic resistance mechanisms such as *ESR1* mutations.[19]

Previous reports regarding the prognostic implications of *BRCA2* mutations have largely focused on germline mutations, with a number of studies having shown no significant differences in survival outcomes between germline *BRCA2* mutation carriers and non-carriers.[20] Conversely, the prognostic implications of somatic *BRCA2* mutations on the risk of late distant recurrence remain unknown. This alteration was associated with a higher risk of late recurrences in our dataset. However, our results are limited by small numbers of patients and events, and must be interpreted with caution. The utilized sequencing pipeline in this study did not include germline sample analysis, and so cannot conclusively determine whether mutations are somatic or germline in origin. Reasons as to why *BRCA2* mutations might confer higher risk of recurrence in the late setting are

unclear, but may result from unique biology as a consequence of homologous recombination deficiency,[21] aberrant mutational processes,[22] or even antitumor immunity as has been described for *BRCA1* mutations.[23] Adjuvant strategies that induce synthetic lethality, such as with poly (ADP-ribose) polymerase (PARP) inhibition, may have potential benefits for these patients. Results of the OlympiA study, a randomized phase III trial of olaparib as adjuvant therapy in patients with high-risk HER2-negative breast cancer and a germline BRCA1/2 mutation (NCT02032823) are eagerly awaited.

Amplifications on 8p11 are present in around 10% of hormone receptor-positive, HER2-negative breast cancers, and were also found to be associated with increased risk of distant metastasis in the late setting. We have previously described this genetic alteration to be associated with increased proliferation and worse prognosis overall.[15] Furthermore, amplifications of fibroblast growth factor receptor 1 (*FGFR1*) and *ZNF703* have been reported to be associated with endocrine resistance *in vitro*.[24,25] The mechanisms underlying the significantly increased risk of distant metastasis in the late setting remain uncertain; however, the risk profile for distant recurrence is persistently elevated in both early and late settings for these patients, suggesting *de novo* endocrine resistance as a plausible contributing cause. Thus far, FGFR inhibitors have been disappointing as targeted treatments for *FGFR1* amplification in breast cancer,[26] and it has been suggested that other oncogenes in both the 8p11 amplicon, as well as in co-occurring amplicons in other regions may be important co-existing molecular targets.[27] One example of such a candidate is the histone lysine methyltransferase *NDS3*, which may be therapeutically tractable.[28] This serves to emphasize the need to improve our understanding of the biology of this genomic subset of patients in order to develop novel and effective targeted therapeutic strategies.

There are limitations to this exploratory study. While this analysis utilizes only a selected subset of patients from the total trial cohort, the sampling design and weighted analysis is adequate for an exploratory biomarker study.[17] It is strengthened by the use of a phase III clinical trial dataset with robust end point data. However, the retrospective nature and small number of patients and events in each alteration category mean that our findings must be interpreted with caution and require validation in other datasets. Finally, while this study has an extensive 8 years median follow-up, it is well established that patients can develop disease recurrence beyond these time frames,[3] emphasizing the ongoing need for extended patient follow up in both clinical trials and patient registries.

In conclusion, we have shown that DNA-based classification of tumor oncogenic drivers can provide unique insights into the risk of late recurrence. It is likely that prognostic estimates for patients can be refined by incorporating clinical scores such as CTS5 in conjunction with molecular information. Further studies will be required to best integrate clinicopathological, gene expression-based, and DNA-based molecular variables for optimal risk stratification.[29] This may assist clinicians in balancing the potential benefits and risks for the use of extended endocrine therapy, as well as provide potential new drug targets.

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DISCLOSURES

CL declares honoraria (outside of this submitted study) from AstraZeneca, Novartis, Pfizer, Roche, Boehringer Ingelheim; consulting or advisory role with AstraZeneca, Novartis, Pfizer, Boehringer Ingelheim; research funding to his institution from AstraZeneca; travel funding from AstraZeneca, and Boehringer Ingelheim. PS has acted as an uncompensated consultant for Roche-Genentech. BT declares stocks for Novartis and Roche; has acted as a consultant for Roche. GV has received honoraria (outside of this submitted study) from MSD Oncology, Roche, Pfizer, Novartis, Bayer, Daiichi Sankyo, and Dako Agilent. MR declares institutional research funding and/or provision of drug supply for clinical trials from Novartis, Pfizer, Ipsen, AstraZeneca, Pierre Fabre, Roche, TerSera; institutional research funding from Novartis, Bayer, and Bristol-Myers Squibb (BMS); institutional advisory role from Ipsen; advisory role, travel support, and honoraria from BMS. SL has no conflicts related to the current manuscript; receives research funding to her institution from Novartis, BMS, Merck, Roche-Genentech, Puma Biotechnology, and Pfizer; consultant (not

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