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Targetable *ERBB2* mutation status is an independent marker of adverse prognosis in estrogen receptor positive, *ERBB2* non-amplified primary lobular breast carcinoma: a retrospective in silico analysis of public datasets

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Abstract

Background: Invasive lobular carcinoma (ILC) accounts for 10–15% of primary breast cancers and is typically estrogen receptor alpha positive (ER+) and *ERBB2* non-amplified. Somatic mutations in *ERBB2/3* are emerging as a tractable mechanism underlying enhanced human epidermal growth factor 2 (HER2) activity. We tested the hypothesis that therapeutically targetable *ERBB2/3* mutations in primary ILC of the breast associate with poor survival outcome in large public datasets.

Methods: We performed in silico comparison of *ERBB2* non-amplified cases of ER+ stage I–III primary ILC ($N = 279$) and invasive ductal carcinoma (IDC, $N = 1301$) using METABRIC, TCGA, and MSK-IMPACT information. Activating mutations amenable to HER2-directed therapy with neratinib were identified using existing functional data from in vitro cell line and xenograft experiments. Multivariate analysis of 10-year overall survival (OS) with tumor size, grade, and lymph node status was performed using a Cox regression model. Differential gene expression analyses by *ERBB2* mutation and amplification status was performed using weighted average differences and an in silico model of response to neratinib derived from breast cancer cell lines.

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Results: ILC tumors comprised 17.7% of all cases in the dataset but accounted for 47.1% of *ERBB2*-mutated cases. Mutations in *ERBB2* were enriched in ILC vs. IDC cases (5.7%, $N = 16$ vs. 1.4%, $N = 18$, $p < 0.0001$) and clustered in the tyrosine kinase domain of HER2. *ERBB3* mutations were not enriched in ILC (1.1%, $N = 3$ vs. 1.8%, $N = 23$; $p = 0.604$). Median OS for patients with *ERBB2*-mutant ILC tumors was 66 months vs. 211 months for *ERBB2* wild-type ($p = 0.0001$), and 159 vs. 166 months ($p = 0.733$) for IDC tumors. Targetable *ERBB2* mutational status was an independent prognostic marker of 10-year OS—but only in ILC (hazard ratio, HR = 3.7, 95% CI 1.2–11.0; $p = 0.021$). Findings were validated using a novel *ERBB2* mutation gene enrichment score (HR for 10-year OS in ILC = 2.3, 95% CI 1.04–5.05; $p = 0.040$).

Conclusions: Targetable *ERBB2* mutations are enriched in primary ILC and their detection represents an actionable strategy with the potential to improve patient outcomes. Biomarker-led clinical trials of adjuvant HER-targeted therapy are warranted for patients with *ERBB2*-mutated primary ILC.

Keywords: Lobular, Breast cancer, *ERBB2*, HER2, Mutation, Prognosis, Therapeutic biomarker, Adjuvant

Background

Invasive lobular carcinoma of the breast (ILC) accounts for 10–15% of all breast cancer with an estimated 250,000 cases per year worldwide [1–4]. Nearly all cases of ILC derive from luminal cells that express estrogen receptor alpha but lack E-Cadherin (*CDH1*) expression or *ERBB2* amplification [5].

Clinical evidence suggests that despite favorable prognostic indicators, e.g., ER+ and/or progesterone receptor positive, low Ki67 proliferation index and HER2– status, patients with ILC have similar or worse long-term outcomes compared to those with invasive ductal carcinoma (IDC, otherwise known as invasive carcinoma of no special type) [5, 6]. Adjuvant treatment of ER+ ILC with letrozole, an aromatase inhibitor, may be superior to tamoxifen, and ILC cells demonstrate resistance to tamoxifen in vitro [7, 8]. However, patients with ILC are treated according to identical protocols as those with IDC [9, 10].

HER2-targeted therapy is indicated for patients whose tumors are HER2+ by immunohistochemistry (IHC) or, if IHC is equivocal, where *ERBB2* is amplified as detected by in situ hybridization (ISH) [11, 12]. Recent evidence indicates that in *ERBB2* non-amplified breast cancer, somatic mutation of *ERBB2* (*ERBB2*mut) and/or *ERBB3* (*ERBB3*mut) may provide an alternative mechanism for upregulation of HER2 activity that is therapeutically tractable using second generation HER2 tyrosine kinase inhibitors such as neratinib [13, 14].

The epidermal growth factor (EGF) family of receptor tyrosine kinases (HER1–4) are activated by ligand-dependent homo-/heterodimerisation and regulate cellular proliferation and tumor progression [15, 16]. In *ERBB2*-amplified cells, the oncogenic effect of HER2 is mediated by heterodimerisation with HER3 in a ligand-independent manner [17]. Thus HER3 is necessary for HER2 oncogenic activity, and both HER2 and HER3 are therapeutic targets in *ERBB2*-amplified breast cancer

[18]. Mutations in *ERBB2* cluster in the tyrosine kinase and extra-cellular domains of HER2 and exert their oncogenic effects by activating tyrosine kinase activity or increasing HER2 dimerization, respectively [13]. In vitro studies of HER2 activity in cell line and xenograft models identified 13 mutations (listed in the “Patients and methods” section) that enhanced proliferation and/or demonstrated growth inhibition with the irreversible HER2/EGFR tyrosine kinase inhibitor, neratinib [13]. Mutations in HER3, a critical binding partner for HER2, have been shown to promote ligand (EGF)-independent transformation of breast epithelial cells only in the presence of kinase-active HER2 [19]. This indicates that known oncogenic mutations in *ERBB3*, e.g., G284R and E928G, may also be therapeutically targetable via HER2 inhibition [19].

ERBB2 mutations have previously been linked with worse prognosis in *CDH1*-altered ILC: a study of ILC cases in the TCGA dataset ($N = 169$) found that *ERBB2* mutations ($N = 6$) occurred exclusively in *CDH1*-altered tumors ($N = 100$) [20]. However, prognostic data on the 6 *ERBB2*mut cases were limited to 2 patient events for both disease-free survival and OS analyses. The overall rate of *ERBB2*mut in this study of primary ILC was 3.6% ($N = 6$ out of 169). A study of relapsed *CDH1*-mutated ILC found *ERBB2*mut in 18% of cases ($N = 4$ of 22), suggesting further acquisition of *ERBB2* mutations in *CDH1*-altered ILC due to the selective pressure of treatment [21].

To demonstrate the potential clinical benefit of targeting low frequency somatic mutations, prognostic analyses using large clinical datasets are required. In the MA12 trial comprising 328 premenopausal patients with ER+ primary breast cancer of all histological subtypes, non-silent *ERBB2* mutations occurred in 5.2% of patients ($N = 17$) and were adversely prognostic of OS ($p = 0.0114$) [22]. A study of 5605 cases of relapsed breast cancer found *ERBB2*mut in 2.4% of cases ($N = 138$), of

which 20% ($N = 27$ of 138) were in ILC tumors [23]. However, neither of these larger studies stratified clinical outcome by histological subtype.

An ILC-specific study of 630 cases of primary ILC found *ERBB2* and *ERBB3* mutations in 5.1 and 3.6% of tumors, respectively [24]. Comparison of cases of ER+, HER2- ILC from the same study ($N = 371$) with cases of ER+, HER2- IDC from TCGA ($N = 338$) indicated significant enrichment of both *ERBB2* and *ERBB3* mutations in ILC (4.3 and 3.5% in ILC vs. 1.5 and 0.6% in IDC) [24]. The study reported limited statistical evidence of a time-dependent effect of *ERBB2* mutational status associated with short-term breast cancer-specific survival. However, confirmation in datasets including patients with long-term follow-up and decoupling of activating from silent mutations is needed.

We hypothesized that low-frequency somatic mutations in *ERBB2* and *ERBB3* are enriched in ER+, *ERBB2* non-amplified primary ILC cases and may have a demonstrable prognostic effect. We tested these hypotheses by mining a combined dataset of the three largest primary breast cancer series with data on tumor *ERBB2* and *ERBB3* mutational status, gene expression, clinicopathological features, and patient survival outcomes. Our overall goal was to determine the association between targetable *ERBB2/3* mutations and survival in primary ER+ ILC, and thereby provide evidence for a clinically actionable strategy to improve patient outcomes.

Patients and methods

Patients and outcome measures

Genomic and clinical outcome data associated with tumor samples from patients with primary breast cancer in TCGA 2015 ($N = 817$), METABRIC 2012 and 2016 ($N = 2509$), and MSK-IMPACT 2018 ($N = 918$) were accessed online via CBioportal [25–27]. From these datasets, ER+ and HER2- cases of stage I–III ILC and IDC with both clinical outcome and mutational data called from next-generation sequencing (NGS) analyses were selected ($N = 1580$). Cases of mixed or non-ILC/IDC histology, ER-negative/undetermined, HER2+/undetermined, carcinoma in situ, and stage 4/undetermined were excluded. For TCGA and MSK datasets, HER2 status was determined by IHC (positive/negative) or, where IHC was indeterminate, by ISH assessment of *ERBB2* amplification, in line with standard clinical practice [11]. For METABRIC cases, HER2 status was determined using the Affymetrix SNP6 copy number inference pipeline.

The primary outcome measure, available in all datasets, was OS. Variables included *ERBB2* and *ERBB3* mutational status. *ERBB2*mut status was subcategorized as oncogenic or uncharacterized by cross-reference with

existing data that identified mutations targetable by HER2-inhibition: G309A/E, S310F, L755S, del755-759, S760A, D769H, D769Y, V777L, P780ins, V842I, and R896C [13]. Cases were denominated onc*ERBB2*mut if tumors harbored at least one oncogenic *ERBB2* mutation.

Clinical and NGS mutation data were integrated with clinicopathological features including histological subtype, lymph node (LN) status, and tumor size and grade. Normalized gene expression data were publicly available for METABRIC (Illumina HT12 microarray) and TCGA (RNA-seq) datasets.

Statistical analysis

For analysis of binary somatic mutation status, combined cohort analysis was performed. Enrichment for cases with mutations in candidate genes (*ERBB2/3*mut) by histological subtype (ILC vs. IDC) was determined by χ^2 test for association between categorical variables. For ILC and IDC separately, Kaplan-Meier (KM) survival curves stratified by mutation status were compared using logrank and generalized Wilcoxon tests. Multivariate analysis of OS was performed using a Cox regression model. Covariates included classic prognostic markers tumor grade, size (< 20 mm or ≥ 20 mm), and LN status (positive or negative). Adjustment was made for age at diagnosis (< 50 or ≥ 50 years). Tumor grade was classified as low (grade 1–2) or high (grade 3).

To derive a novel gene expression signature of HER2 activity that accounted for the effect of potentially targetable *ERBB2*mut in *ERBB2* non-amplified tumors, we applied a weighted average difference (WAD) method to gene expression data in cases from the METABRIC 2012 ($N = 1980$) and TCGA 2015 ($N = 817$) cohorts [25, 28, 29]. Gene expression in *ERBB2*mut cases ($N = 38$, selected by *ERBB2* non-amplified status and patient age > 50) was compared with the same order of magnitude of *ERBB2* wild-type cases ($N = 79$, selected by *ERBB2* non-amplified status, grade > 1, stage > I, patient age > 50). This was repeated for onc*ERBB2*mut cases ($N = 23$) using the same comparator and selection criteria. To incorporate the effect of HER2 activity via *ERBB2* amplification, the overlap of differentially expressed genes DEGs shared by both comparisons (*ERBB2*mut and onc*ERBB2*mut vs. *ERBB2* wild-type) with DEGs from a further comparison of *ERBB2* amplified ($N = 247$) vs. non-amplified ($N = 1733$) cases in METABRIC was calculated. Finally, to incorporate the downstream phenotype (HER2 status), the overlap of this list with DEGs from a comparison of clinical HER2+ vs. HER2- cases in TCGA was calculated. In contrast to *ERBB2*mut cases, matching was not performed for *ERBB2* amplified or HER2+ cases because numbers were higher and within an order of magnitude across groups, such that

similar variation in gene expression could reasonably be assumed.

Multiple gene expression signatures of HER2 activity have been derived using cell line models and patient tumors [30–33]. We compared our novel gene signature with the HER2 activity signature established by Desmedt et al [31] with respect to its ability to detect potentially targetable *ERBB2*mut cases in our ILC/IDC dataset. This was achieved by multivariate regression modeling of response to neratinib for each gene signature using breast cancer cell line pharmacogenomic data from the BROAD Institute, accessed online via the CellMinerCDB portal [34, 35]. The Pearson coefficient for each significantly correlated signature gene was used to calculate normalized signature scores for each METABRIC case in the current dataset. To validate the prognostic effect of *ERBB2* in ILC, cases were then stratified by gene signature score (upper vs. lower quartiles) and the signatures compared by histological subtype using a Cox regression model of a 10-year OS.

Results

Clinicopathological landscape of the combined cohort

All cases of primary (stage I–III) ER+ and HER2– ILC and IDC from METABRIC, TCGA, and MSK-

IMPACT cohorts were selected (total $N = 1580$, see Table 1). Baseline clinicopathological characteristics by individual cohort are summarized in Table 1.

To detect prognostic effects of low-frequency mutations and add to the existing body of literature on ILC-specific mutational drivers, a combined cohort of three public datasets was collated. We first evaluated the implications of combining cases of ILC and IDC from potentially disparate datasets.

In the combined cohort, long-term follow-up of patients is dominated by the largest dataset (METABRIC, $N = 702$: mean follow-up 133 months) while early events are enriched by the two smaller datasets (TCGA and MSK, $N = 878$: mean follow-up 33 months) (see Supplemental Figure S1(A) in Additional file 1 for KM plot). Compared to the smaller datasets with shorter follow-up (TCGA and MSK), patients in METABRIC were more likely to be over 50 years of age and have T1 tumors (< 20 mm diameter), but no significant difference in grade or LN status was found (see Table 2). As the principle skew in the combined dataset is towards longer follow-up in METABRIC cases, OS analysis was limited to 10 years—thus providing a clinically meaningful endpoint for all patients, irrespective of age. Comparison of

Table 1 Baseline clinicopathological characteristics of the combined cohort

		METABRIC ($N = 702$)		TCGA ($N = 330$)		MSK-IMPACT ($N = 548$)		Total ($N = 1580$)	
		N	%	N	%	N	%	N	%
Histology*	ILC	76	10.8	100	30.3	103	18.8	279	17.7
	IDC	626	89.2	230	69.7	445	81.2	1301	82.3
Age	< 50 years	125	17.8	87	26.4	190	34.7	402	25.4
	≥ 50 years	577	82.2	243	73.6	358	65.3	1178	74.6
Menopause	Pre-	125	17.8	90	27.2	234	42.7	449	28.5
	Post-	577	82.2	219	66.4	309	56.4	1105	69.9
	unknown	0	0	21	6.4	5	0.9	26	1.6
Stage*	I	238	33.9	67	49.3	270	49.3	575	36.4
	II	418	59.5	188	34.3	188	34.3	794	50.3
	III	46	6.6	75	16.4	90	16.4	211	13.4
Tumor size	< 20 mm	215	30.6	96	29.1	330	60.2	641	40.6
	≥ 20 mm	487	69.4	234	70.9	218	39.8	939	59.4
Tumor grade	1	61	8.7	49	14.8	51	9.3	161	10.2
	2	344	49.0	190	57.6	191	34.9	725	45.9
	3	268	38.2	81	24.5	288	52.6	637	40.3
	Unknown	29	4.1	10	3.0	18	3.3	57	3.6
Follow-up	< 5 years	137	19.5	261	82.8	454	82.8	852	53.9
	5–10 years	195	27.8	61	11.7	64	11.7	320	20.3
	≥ 10 years	370	52.7	8	5.5	30	5.5	408	25.8
Status	Alive**	310	44.2	299	90.1	494	90.1	1103	69.8
	Deceased	392	55.8	31	9.9	54	9.9	477	20.3

*Cases of ILC/IDC histology, stage I–III, ER+ and HER2– status with clinical outcome and mutational data were selected via CBioportal

**At last follow-up

Table 2 Comparison of long vs. short follow-up cohorts: METABRIC (largest dataset, long follow-up) vs. TCGA and MSK (combined smaller datasets, short follow-up). Significant difference was found with respect to age and tumor size, but not tumor grade or LN status. Table excludes “unknown” cases for each variable

		METABRIC (N = 702)		TCGA AND MSK (N = 878)		χ^2 test
		N	%	N	%	
Age	< 50 years	125	17.8	277	31.5	$p < 0.00001$
	≥ 50 years	577	82.2	601	68.5	
Tumor size	< 20 mm	215	30.6	426	48.5	$p < 0.00001$
	≥ 20 mm	487	69.4	452	51.5	
Tumor grade	1–2	405	60.2	481	56.6	$p = 0.158$
	3	268	39.8	369	43.4	
LN status	Negative	370	53.9	489	55.8	$p = 0.457$
	Positive	316	46.1	387	44.2	

METABRIC with combined TCGA and MSK-IMPACT cohorts revealed no significant difference in a 10-year OS (logrank $p = 0.225$, see Supplemental Figure S1(B) in Additional file 1).

ERBB2 mutations are enriched in primary ILC and cluster in the HER2 tyrosine kinase domain

We next assessed the prevalence of *ERBB2/3*mut in our dataset of ILC and IDC ($N = 1580$). Overall prevalence of *ERBB2*mut was 2.2% ($N = 34$). *ERBB2*mut was enriched in ILC, with prevalence of 5.7% ($N = 16$) vs. 1.4% in IDC ($N = 18$) ($p < 0.0001$). In contrast, prevalence of *ERBB3*mut was lower (1.6% overall, $N = 26$), and there was no enrichment of *ERBB3*mut in ILC vs. IDC (1.1%, $N = 3$ vs. 1.8%, $N = 23$; $p = 0.604$). Due to the small number of cases in ILC, further analysis of *ERBB3*mut was not performed.

In ILC, *ERBB2*mut clustered in the tyrosine kinase domain of HER2 (15 out of 16 cases; 93.8%). Of these, the majority have been characterized as oncogenic and potentially targetable using neratinib (onc*ERBB2*mut, 11 out of 15 kinase domain mutations in ILC; 73.3%). In IDC, all kinase domain mutations were known oncogenic ($N = 8$) and non-characterized mutations were distributed evenly across protein domains (see Fig. 1). The most frequently occurring *ERBB3*mut coded for E928G, which lies in the tyrosine kinase domain of HER3 ($N = 7$ out of 26; 27%).

ERBB2 mutation is an adverse prognostic indicator of survival for patients with primary ILC

Using our meta-cohort of 1580 cases, we next assessed *ERBB2*mut as a prognostic marker of OS in ILC ($N = 279$) and IDC ($N = 1301$). Median duration of patient follow-up was 50 months (range 0–351 months). In patients with ILC, median OS was significantly shorter if tumors were *ERBB2*mut positive (inclusive of oncogenic and uncharacterized mutations) vs. *ERBB2*mut negative (66 vs. 211 months, $p = 0.0001$). In contrast, there was

no significant difference in OS for *ERBB2*mut cases of IDC (159 vs. 166 months, $p = 0.733$) (see Fig. 2).

*ERBB3*mut status was not a significant prognostic indicator of OS in cases of IDC (HR = 1.46, 95% CI 0.65–3.28; $p = 0.359$). There were no events (patient deaths) at 10 years of follow-up in the three cases of *ERBB3*mut ILC.

Targetable ERBB2 mutation status is an independent adverse prognostic marker of 10-year overall survival in ER+, ERBB2 non-amplified ILC

To test the effect of therapeutically actionable *ERBB2* mutations on a clinically relevant endpoint, we stratified 10-year OS by onc*ERBB2*mut status. In ER+, *ERBB2* non-amplified ILC, onc*ERBB2*mut status was prognostic of 10-year OS independently of LN status, tumor grade, and size (HR 3.65, 95% CI 1.21–11.00; $p = 0.021$, see Fig. 3). The independent prognostic value of onc*ERBB2*mut status on 10-year OS was retained after further adjusting for age at diagnosis (HR 3.19, 95% CI 1.06–9.62; $p = 0.039$).

Unselected *ERBB2*mut status (including oncogenic and uncharacterized mutations) was also adversely prognostic of 10-year OS in univariate analysis (HR 3.66, 95% CI 1.54–8.73; $p = 0.003$), but in contrast to characterized onc*ERBB2*mut, this prognostic effect was not independent of LN stage, tumor grade, and/or size. The independent prognostic value of onc*ERBB2*mut was further verified at 5 years of follow-up (HR 3.668, 95% CI 1.096–12.275; $p = 0.035$).

A novel gene signature of targetable HER2 activity using ERBB2mut status

Since existing gene signatures of HER activity derive from HER2 status (by IHC and/or ISH), we generated a novel gene signature incorporating DEGs in *ERBB2*mut and onc*ERBB2*mut cases, as described in the “Patients and methods” section and outlined in Fig. 4. Our aim was to establish a novel signature that reflects HER2

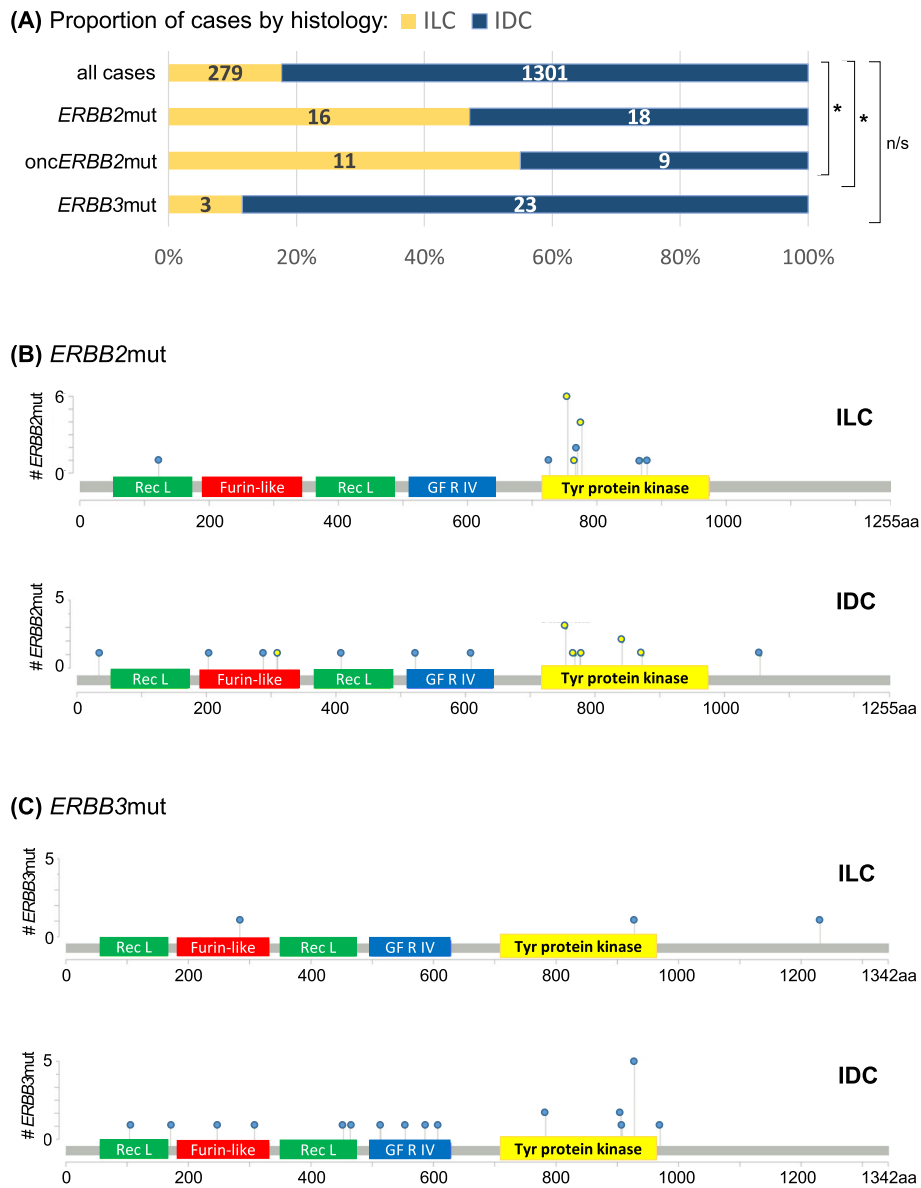
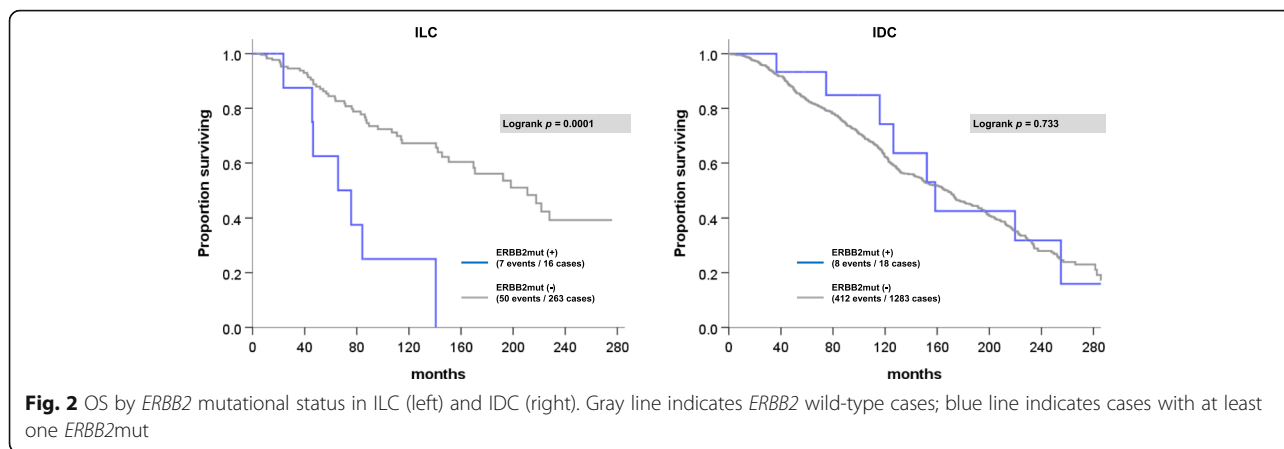


Fig. 1 Rate of *ERBB2* and *ERBB3* mutations and their spatial distribution on HER2/3 in ILC and IDC. *ERBB2*mut were found to be **a** enriched in ILC (yellow bar) vs. IDC (blue bar) and **b** clustered in the tyrosine kinase domain of HER2; **c** *ERBB3*mut occurred at lower frequency, with the high-frequency outlier in IDC coding for known oncogenic HER3 kinase domain alteration E928G ($N=6$). Y-axes show the number of cases harboring at least one *ERBB2/3* mutation at a specific amino acid (aa) of HER2/3, shown along the x-axes. Yellow-filled circles indicate onc*ERBB2*mut. Extracellular domains of HER2/3: Receptor L, Furin-like and Growth Factor Receptor IV; intracellular: tyrosine protein kinase. * $p < 0.001$; n/s = not significant

pathway activity more completely than existing signatures, whether induced by *ERBB2* mutations or amplifications, and to be able to apply it in a wider range of patients. A list of up and downregulated *ERBB2* “mutant” DEGs ($N=20$) was generated by combining the overlap between DEGs for METABRIC *ERBB2* amplified vs. non-amplified, *ERBB2* mutated vs. wild-type (*ERBB2*-mut and onc*ERBB2*mut separately), and TCGA HER2+ vs. HER2- (Fig. 4a, and see Additional file 2 for Supplemental Tables S1–3 with all gene lists).

As shown in Fig. 4b, *ERBB2*mut and onc*ERBB2*mut clustered in the upper quartile of the novel gene signature score in cases of ER+, HER2- ILC/IDC from METABRIC. In contrast, *ERBB2*mut and onc*ERBB2*mut cases did not cluster when scored by the established gene signature of HER2 pathway activation but were evenly distributed across the cohort. Both the novel and established HER2 pathway gene signatures were predictive of response to neratinib in multivariate analysis using a pharmacogenomic breast cancer cell line model



(Pearson $R = 0.9$, $p < 1e^{-14}$ for both; see Supplemental Figure S2 in Additional file 3 for the original plots from the CellMinerCDB online portal). The regression model used incorporates drug response and gene expression data from $N = 36$ breast cancer cell lines [35, 36]. Our findings using this model verify that the novel signature derived from DEGs in *ERBB2mut* vs. wild-type cases incorporates oncogenic HER2 activity due to *ERBB2mut*. This suggests that a gene signature may have value in predicting targetable *ERBB2* alterations including *ERBB2mut* in HER2- breast cancer. Finally, Fig. 4c demonstrates that the novel gene signature score (stratified into upper vs. lower quartiles) was adversely associated with 10-year OS (HR = 2.3, 95% CI 1.04–5.05; $p = 0.040$), thus providing mRNA level validation of *ERBB2mut* as an adverse prognostic marker.

Using the same dataset (CellMinerCDB), it was not possible to associate *ERBB2mut* status with neratinib

response as only one breast cancer cell line (MDA-MB-468) was *ERBB2mut* positive. Response to neratinib for MDA-MB-468 was in the upper tertile of breast cancer cell lines (data in the public domain via CellMinerCDB online portal).

Discussion

In this study, we mined clinical and NGS data from the largest clinical cohorts with data in the public domain to test whether there was an ILC-specific association of *ERBB2* mutations with OS. In our meta-cohort of ER+, *ERBB2* non-amplified cases of ILC and IDC, we found that *ERBB2* mutations are enriched ILC, cluster in the functional kinase domain of HER2, and robustly associate with adverse clinical outcomes—independently of known prognostic clinicopathological features including LN status and tumor grade. In contrast, there was no ILC-specific enrichment of *ERBB3mut*.

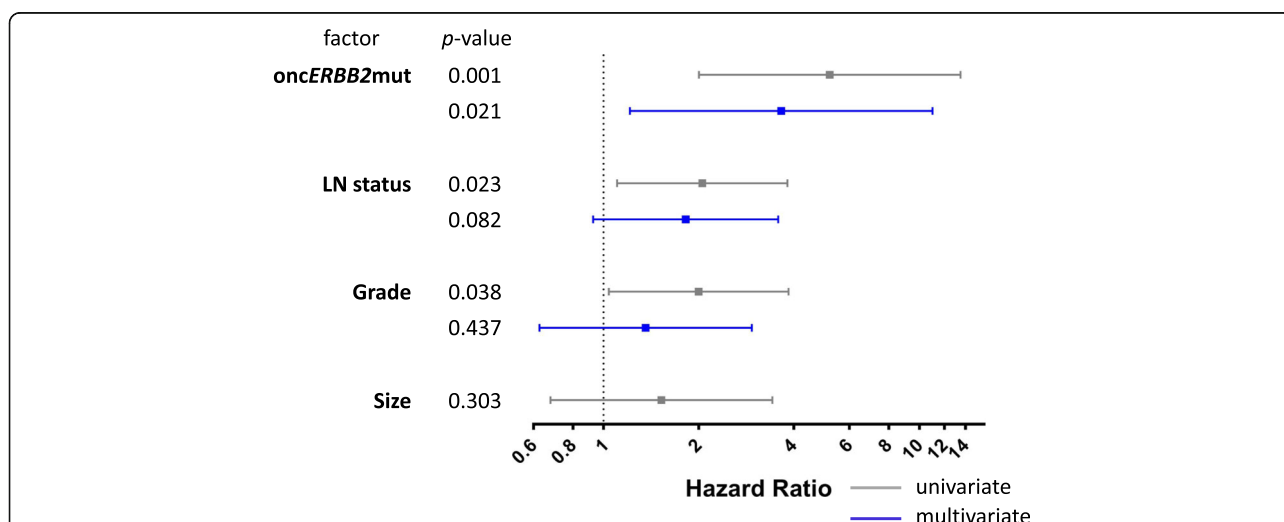
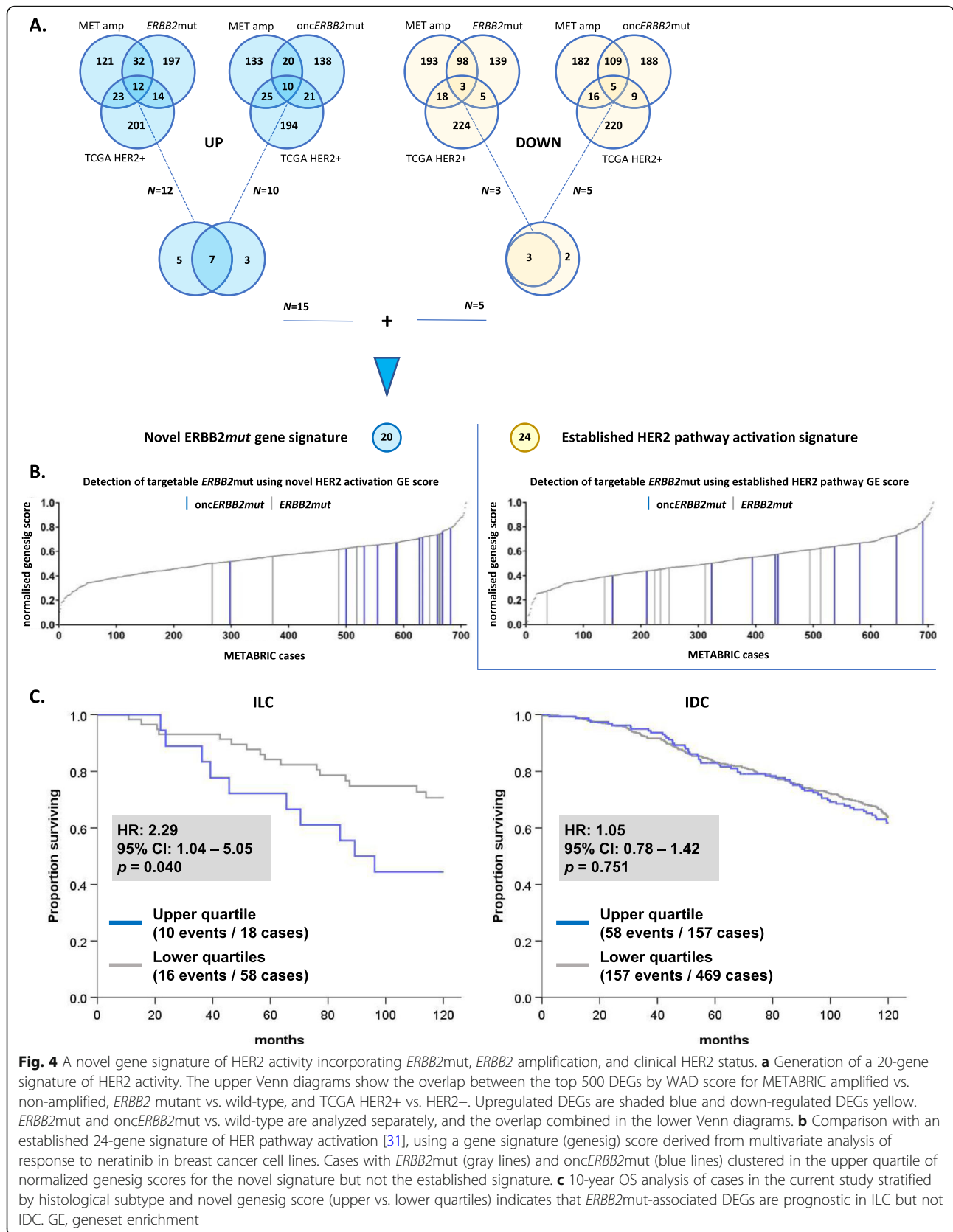


Fig. 3 Univariate and multivariate analyses of 10-year OS in $N = 279$ cases of ER+, *ERBB2* non-amplified ILC. Gray square dot indicates hazard ratio (HR) in univariate analysis and the gray bar indicates the 95% confidence interval (CI). Significant prognostic variables in univariate analyses (where 95% CI does not span HR = 1) are included in multivariate analysis, shown in blue. For each variable, cases with unknown values are excluded from the analysis



Compared to the largest previous study with data on *ERBB2* mutational status in primary ILC ($N=371$) [24], the current study found higher frequency of *ERBB2*mut (5.7 vs. 4.3%) but lower frequency of *ERBB3*mut (1.1 vs. 3.5%). Hazard proportionality in the study by Desmedt et al 2016 suggested that *ERBB2* mutational status had a time-dependent effect associated with short-term risk of breast cancer relapse [24]. The current study adds data on the prognostic effect of specific *ERBB2* mutations that were previously shown in cell line, in vitro and xenograft studies to respond to existing clinical compounds such as trastuzumab or neratinib [13]. We demonstrated that the status of these therapeutically targetable *ERBB2* mutations is an independent adverse prognostic marker of overall survival in a large cohort of patients with ILC.

Findings from the current study imply that targeted sequencing of ILC tumors for *ERBB2* mutations may be an actionable and viable strategy to improve outcomes for patients with primary ILC by providing a biomarker for HER2-targeted therapy in the adjuvant setting. Based on worldwide ILC incidence of 250,000 [1–4], 10% rate of ER- and/or HER2- status in ILC, and 5% rate of potentially targetable *ERBB2*mut in ER+, *ERBB2* non-amplified ILC, we conservatively estimate that 11,250 additional ILC patients per year who have *ERBB2*-mutated tumors may benefit from HER2-targeting therapy.

Existing clinical studies of HER2-targeting therapy are underway in advanced breast cancer. For example, neratinib (alone or in combination with fulvestrant) was tested in phase II study for patients with advanced, *ERBB2* non-amplified, *ERBB2*mut breast cancer (clinical trial registration number NCT01670877). Of the 16 patients who had evaluable tumor responses, $N=2$ had partial response by RECIST criteria (13%). Out of 14 patients with tumors harboring *oncERBB2*mut, $N=5$ (36%) derived clinical benefit, defined as stable disease or partial/complete response by RECIST criteria [37]. Given that the patients in this trial had received multiple prior lines of treatment, it is conceivable that patients with *ERBB2* non-amplified primary tumors harboring *oncERBB2*mut will also derive benefit from (neo) adjuvant HER2-targeted therapy such as neratinib.

In the current cohort of primary ER+, HER2- ILC and IDC, *ERBB2* mutations in ILC tumors clustered almost exclusively in the active HER2 tyrosine kinase domain ($N=15$ of 16). Our retrospective clinical outcome data indicates an ILC-specific adverse survival association for patients with *ERBB2* non-amplified tumors harboring potentially targetable *ERBB2* mutations. This primary finding was validated by existing

mRNA data via a novel gene signature of HER2 activity, which linked *ERBB2*mut with response to neratinib. Subject to independent external validation, a gene signature of HER2 activity could have clinical application as a biomarker of response to HER2-targeted small molecules such as neratinib and may be more cost-effective than targeted NGS in the detection of *ERBB2*mut in primary ILC.

Taken together, these new findings suggest that focusing early phase studies of HER2-inhibition (e.g., with neratinib) in patients with *ERBB2* non-amplified, *ERBB2*mut primary ILC may be an effective strategy to demonstrate feasibility and clinical benefit in the (neo) adjuvant setting.

Limitations of the current study include cohort size, quality of clinical outcome data, and bias in long-term follow-up towards cases from the largest dataset (METABRIC). Statistical power is limited by the small number of patient deaths in cases of ILC and IDC with *ERBB2*mut ($N=7$ and 8, respectively). However, the clear deviation of the KM plot of OS in ILC stratified by *ERBB2*mut (Fig. 2) is reflected in a low type I error of one in 10,000 ($p=0.0001$). In contrast, there was no ILC-specific enrichment of *ERBB3*mut in our database. However, in IDC there was enrichment of the known oncogenic HER3 kinase domain mutation, E928G. To demonstrate the prognostic effect of mutations affecting HER3, larger datasets will be required.

The effect of incomplete clinical outcome data on the primary endpoint (OS) is difficult to quantify but is accounted for statistically in Cox regression modeling by censoring patients at last follow-up. Patients in METABRIC presented at older age, which is associated with more indolent, less biologically aggressive breast cancer [38]. This implies an underestimation of the adverse impact of *ERBB2* mutations on 10-year survival outcomes in the current study. Since the METABRIC dataset did not have complete HER2 IHC or reverse-phase protein array data, it was not possible to associate activating *ERBB2*mut directly with downstream HER2 protein expression. Instead, we generated a gene signature incorporating clinical HER2 status to validate our findings.

Conclusions

Targetable kinase domain *ERBB2* mutations are enriched in primary ILC and their detection by targeted sequencing or validated gene signature surrogate may provide an actionable strategy to improve patient outcomes. Biomarker-led clinical trials of adjuvant HER2-targeted therapy to treat breast tumors with activating *ERBB2* mutations are warranted in HER2- primary ILC.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13058-020-01324-4>.

Additional file 1: Supplemental Figure S1. (A) 10-year OS KM plot stratified by individual study cohort: blue – METABRIC, solid gray – TCGA, dotted gray – MSK-IMPACT; (B) 10-year OS KM plot stratified by study category: blue – long follow-up (METABRIC), gray – short follow-up (TCGA and MSK-IMPACT combined); Logrank $p = 0.225$.

Additional file 2: Supplemental Tables. (S1) list of genes in the novel ($N = 20$) and established (Desmedt et al [31], $N = 24$) gene signatures of HER2 activity; **(S2 and S3)** top 500 DEGs by WAD score stratified into genes that are up-regulated (S2) and down-regulated (S3) for METABRIC *ERBB2* amplified vs. non-amplified (*ERBB2amp*), METABRIC *ERBB2*mut vs. wild-type (*ERBB2mut*) and onc*ERBB2*mut vs. wild-type (onc*ERBB2*mut), and TCGA HER2+ vs. HER2- (TCGA HER2+).

Additional file 3: Supplemental Figure S2. Correlation between the observed response of breast cancer cell lines ($N = 36$) to neratinib and the response predicted by expression of (A) novel ($N = 20$) and (B) established (Desmedt et al [31], $N = 24$) gene panels. Plots were generated using CellMinerCDB online portal and cell line data from the BROAD Institute [34, 35].

Abbreviations

CI: Confidence interval; DEG: Differentially expressed gene; EGF: Epidermal growth factor; ER: Estrogen receptor alpha; *ERBB2/3mut*: *ERBB2/3* mutant; HER2/3: Human epidermal growth factor receptor 2/3; HR: Hazard ratio; IDC: Invasive ductal carcinoma of the breast; IHC: Immunohistochemistry; ILC: Invasive lobular carcinoma of the breast; ISH: In situ hybridization; KM: Kaplan-Meier; LN: Lymph node; NGS: Next-generation sequencing; onc*ERBB2*mut: Oncogenic *ERBB2* mutant; OS: Overall survival; RECI ST: Response evaluation criteria in solid tumors; WAD: Weighted average difference

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Authors' contributions

SK, DMH, WZ, SO, and SJJ conceived, designed, and supervised the study. SK, MA, CM, KB, NPM, and SJJ analyzed and interpreted the data. SK, WZ, SO, and SJJ prepared the manuscript. ARG, IOE, and EAR had supervisory and administrative roles. SJ, TF, and KS had consultative roles in study design. All authors were involved in manuscript preparation and reviewing for submission, gave their final approval, and agreed to be accountable for all aspects of the work.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available via the CBIOPortal online portal (<http://www.cbioportal.org>) and in the Genome-Phenome Archives EGAS00000000098 and phs000178 (<https://ega-archive.org/studies/EGAS00000000098> and <https://ega-archive.org/studies/phs000178>).

Ethics approval and consent to participate

Ethics approvals and consent for each cohort (METABRIC, TCGA, and MSK-IMPACT) was obtained in the original studies.

Consent for publication

Not applicable

Competing interests

Since the first submission of the article, the corresponding author (SJJ) has moved employment to AstraZeneca, with no competing interests to declare. All other authors declare that they have no competing interests.

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