Relapsed classic E-cadherin (CDH1) mutated invasive lobular breast cancer demonstrates a high frequency of HER2 (ERBB2) gene mutations

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Conflict of Interest Statement:

Authors JS Ross, K Wang, G Otto, SR Downing, J Sun, J He, JA Curran, S Ali, G Palmer, VA Miller and PJ Stephens are employees and own stock in Foundation Medicine, Inc.

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Statement of Clinical Relevance

This study describes the application of a novel comprehensive next generation sequencing-based diagnostic test on active clinical cases of relapsed lobular breast cancer and how the results of the analysis can drive the selection of treatment for these patients by discovering unanticipated therapeutic targets. Use of this approach could facilitate accrual to clinical trials of small molecules of antibodies in breast cancer patients with \textit{ERBB2} mutations.
Abstract

**Purpose:** We queried whether comprehensive genomic profiling using a next generation sequencing (NGS)-based assay could identify novel and unanticipated targets of therapy for patients with relapsed invasive lobular carcinoma (ILC).

**Experimental Design:** DNA sequencing (Illumina HiSeq 2000) was performed for 3,320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer on indexed, adaptor ligated, hybridization-captured libraries using DNA isolated from FFPE sections from 22 histologically verified ILC.

**Results:** A total of 75 genomic alterations were identified with an average of 3.4 alterations per tumor (range 1-6), of which 35 were actionable for an average of 1.59 actionable alterations per patient (range 0-3). Nineteen of 22 (86%) of the ILC samples harbored at least one actionable alteration. Six (27%) cases featured alterations in **ERRB2** including 4 (18%) with **ERBB2** mutation, 1 (5%) with an **ERBB2** gene fusion and 1 (5%) with an **ERBB2** copy number gain (amplification). The enrichment of **ERBB2** mutations/fusion in **CDH1** mutated ILC (5/22, 23%) compared with the 5 **ERBB2** mutations in a series of 286 non-**CDH1** mutated breast cancers from which the ILC cases were obtained (5/286, 2%) was significant (p=0.0006).

**Conclusions:** Comprehensive genomic profiling of relapsed **CDH1** mutated ILC revealed actionable genomic alterations in 82% of cases, featured a high incidence of **ERBB2** alterations and can reveal actionable alterations that can inform treatment decisions for ILC patients.
**Key Words**: lobular breast cancer; CDH1; E-cadherin; HER2; mutation; ER; PR; next generation sequencing; personalized medicine; targeted therapy
Introduction

Substantial progress has been made over the past three decades in our understanding of the epidemiology, clinical course and basic biology of breast cancer and the integration of routine and molecular biomarkers into patient management. Modern techniques designed to detect the disease at an earlier stage combined with new methods of determining risk assessment and more optimized combined modality treatment that have enhanced our ability to manage, and in some cases, achieve a cure for the disease. The morphologic classification of invasive carcinomas of the breast has had a significant role in managing individual patients, but has not been a major driver of therapy development and clinical trials. In addition, the application of standard slide-based biomarker status for the estrogen receptor (ER), progesterone receptor (PR) and HER2 gene copy number (FISH) and/or protein expression (IHC) currently play a more significant role in clinical trial design than routine histologic subtyping (5). This is also true when the mRNA expression profile-based molecular portraits classification of invasive breast cancer including the luminal A, luminal B, Normal, HER2 positive and basal-like (typically “triple negative”) nomenclature has been used to classify the tumors (6). In drug development strategies have approached invasive ductal and invasive lobular breast carcinomas as essentially the same disease (1-4).

Most published studies have found that the overall prognosis for invasive lobular carcinoma (ILC) of the breast is similar to that of invasive duct carcinoma (IDC) (7-9). However, most of these outcome studies have excluded the less common, but more aggressive pleomorphic variant of ILC in the clinical outcome data analysis (10) which could overestimate the prognosis of patients of ILC. In addition, although ER positive invasive lobular carcinoma of the breast generally has a favorable prognosis, relapsed lobular breast cancer originally treated only with hormonal therapy may, on occasion, follow an aggressive clinical course (7-9). All of these observations favor the study of ILC as a separate entity from the more frequent invasive ductal breast carcinoma.
Recent studies suggest that lobular breast cancer, which makes up 10% of all invasive breast cancers and approximately 20,000 new cases in the US each year, may be well characterized by a distinct genotype featuring a mutation in \textit{CDH1} that encodes the E-cadherin protein product, in contrast with the far more frequent ductal breast cancer which typically has an unaltered \textit{CDH1} (11-15). In this study restricted to 22 \textit{CDH1} mutated ILC from patients with relapsed disease, we queried whether a next generation sequencing-based assay could identify novel and unanticipated targets of therapy for these patients with relapsed, metastatic and therapy resistant disease.

\textbf{Methods}

We reviewed the records of a series of 308 invasive breast cancers submitted for NGS-based diagnostic testing at Foundation Medicine, Inc. and identified 22 \textit{CDH1} mutated ILCs all of which had relapsed after primary surgical and one or more systemic treatment approaches. NGS was performed on hybridization-captured, adaptor ligation based libraries using DNA extracted from 4 FFPE sections cut at 10 microns. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor cells. DNA sequencing was performed for 3,320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer (1.14 million total base pairs) on indexed, adaptor ligated, hybridization-captured (Agilent SureSelect custom kit) and fully sequenced using 49 bp paired reads on the Illumina HiSeq 2000 to at an average depth of 877X and evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements as previously described (16). To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was optimized and validated.
to detect base substitutions at a ≥5% mutant allele frequency (MAF) and indels with a ≥10% MAF with ≥99% accuracy. To validate base substitution detection, 2 pools of 10 normal cell-lines were used each containing a total of 2,057 known base substitutions representing a broad range of allele frequencies. We compared the alterations detected to those expected from base substitutions present in individual cell-line constituents as previously described (17-20). To validate indel detection, 28 tumor cell lines were used containing a total of 47 known somatic indel alterations in 22 genes to generate 41 pools of 2-10 cell-lines each creating a test set of 227 indels spanning a wide range of allele frequencies and indel lengths (17-20). To validate copy number alteration (CNA) detection, 7 tumor cell-lines bearing 19 focal gene amplifications (6-15 copies, 15 genes) and 9 homozygous gene deletions (6 genes) were pooled with their matched normal cell lines (thereby maintaining consistent genotypes) in 5 ratios ranging from low to high tumor content (20-75%), creating a test set of 210 copy number alterations (17-20).

Copy number alterations had a validated accuracy of >95%. Actionable genomic alterations were defined as those identifying anti-cancer drugs on the market or available in registered clinical trials the mechanism of action of which was predicted to be relevant based on the genomic alteration identified. Local site permissions to use clinical samples were used for this study.

Results

The mean age of the female patients in this study was 56 years (range 44 to 74). Tissue samples obtained from 22 cases of ILC originate from 22 unique patients. Either the primary tumor or a recurrent/metastatic tumor sample was available for each patient and histologically graded as follows: 1 (5%) grade I, 16 (73%) grade II and 5 (23%) grade III tumors. Six ILC (27%) were Stage III and 16 (73%)
were Stage IV at the time of sequencing (Table 1). Of the cases for which hormone receptor and HER2 slide based test results were available, 15/19 (88%) were ER positive; 11/18 (61%) were PR positive; and 1/19 (5%) was HER2 positive by either immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). Sequencing was performed on the primary ILC in n= 9, (41%) cases, biopsies of metastatic sites n=13, (59%) including lymph node metastases n=3 (14%), liver metastases n=3, (14%) cases, pleural fluid cell blocks and bone metastases in n=2 (9%) each, and brain metastases n=1 (5%).

Among the 22 ILCs we identified 75 genomic alterations with an average of 3.4 alterations per tumor (range 1-6), of which 35 were considered actionable for an average of 1.59 actionable alterations per patient (range 0-3) (Figure 1, Table 1). Nineteen of 22 (86%) of the ILC harbored at least one actionable alteration potentially associated with clinical benefit of targeted therapies.

As a required study entry criterion, all 22 (100%) of the ILC featured a mutation in CDH1. There was a wide variety of CDH1 base substitutions and splice site mutations identified with no repeated pattern of sequence alteration in any of the 22 CDH1 mutated ILC cases in this study (Table 1).

Frequency of Genomic Alterations in ERBB2

Six (27%) of the CDH1 mutated ILC cases featured alterations in ERBB2 including 4 (18%) with ERBB2 mutations, 1 (5%) with an ERBB2-GRB7 gene fusion and 1 (5%) with an ERBB2 copy number gain (amplification). There was a trend for high grade tumors to harbor ERBB2 alterations with 3 (60%) of the 5 grade III tumors having either ERBB2 mutations or amplification. Of the 9 primary tumors evaluated, 4 (44%) had an ERBB2 alteration (3 cases with mutations and 1 case with amplification) compared to 2 (15%) of the 13 metastatic cases (1 case with an ERBB2 mutation and 1 case with an ERBB2 fusion). The 4 cases with ERBB2 mutations included study case number 7, a Grade III tumor with a L755S ERBB2 mutation in a liver metastasis sample from an ER-/PR-/HER2 FISH- 63 year old patient (Figure 2a). This
tumor also featured an amplification of \textit{ESR1} as well as amplification in \textit{MYC} and mutations in \textit{RUNX1} and \textit{TP53}. Case study number 9, a primary tumor sequence from an ER-/PR-/HER2 IHC- ILC harbored 2 \textit{ERBB2} mutations: a A775\_G776insYVMA mutation and a R1153* truncation and no additional genomic alterations (Figure 2b). Case study number 17 demonstrated a P780\_Y781insGSP \textit{ERBB2} mutation in the primary tumor from a 65 year old patient. In addition to the \textit{ERBB2} mutation, this tumor also featured a splice site mutation in the \textit{MAP2K4} gene and amplification of \textit{MCL1} and \textit{RICTOR}, respectively (Figure 2c). In case number 22, a woman with relapsed metastatic ILC, her primary tumor was ER+/PR-/HER2- (FISH). When this primary tumor was sequenced, two discrete \textit{ERBB2} mutations were found (S310F and V842I) as well as mutations in the \textit{MEN1} and \textit{SMAD4} genes (Figure 2d). In case study number 18, a ER+/PR+/HER2- bone metastasis from a 62 year old patient, a novel \textit{ERBB2-GRB7} fusion was identified (Figure 2e and Figure 3). This tumor also had an amplification of the \textit{CCND1} gene and a H1047R \textit{PIK3CA} mutation. There was insufficient archived extracted DNA or tumor tissue remaining on this sample for further DNA and RNA-based characterization of this gene fusion. Finally, in case study number 21, a \textit{CDH1} mutated stage IV high grade ER-/PR- pleomorphic breast cancer with papillary features, an \textit{ERBB2} gene copy number gain was detected by NGS which correlated with the positive HER2 overexpression on IHC staining and gene amplification detected by FISH testing of the same specimen. This tumor also had amplifications in the \textit{CCND1} and \textit{MCL1} genes and a \textit{TP53} mutation. Only this \textit{ERBB2} amplification case also had a positive HER2 slide based test result (both IHC and FISH) and the 5 ILC cases with either a mutation or fusion of \textit{ERBB2} were uniformly HER2 negative by IHC and/or FISH testing.

\textit{Frequency of Genomic Alterations in Primary Tumor Samples versus Metastatic Tumor Biopsies.}

Although all ILCs in this study ultimately relapsed, 9 of the ILCs sequenced in this study were archived specimens of the primary disease. The incidence of genomic alterations in the primary tumors and metastatic site biopsies were virtually identical. When the original primary tumor was used for
sequence analysis in this cohort of advanced and metastatic ILC, the 9 tumors yielded 31 total genomic alterations and 3.44 alterations per tumor (Figure 1). In comparison, the 13 ILC cases where a metastatic site provided the sample for the NGS assay yielded 43 total alterations and 3.31 alterations per tumor. Interestingly, the incidence of actionable alterations was higher in the primary tumor sample group (2.0 actionable alterations per tumor) than in the metastatic site biopsy group (1.23 actionable alterations per tumor). However, given the relatively small numbers of cases in these two groups this observation is preliminary and requires validation on a larger series of cases.

Additional genomic alterations identified in the cohort included mutations in PIK3CA (8 cases; 36%); TP53 (6 cases; 27%); RB1 (2 cases; 9%); KRAS (2 cases; 9%); AKT1 (2 cases 9%) and ERBB4 (1 case; 5%). The PIK3CA mutation frequency was higher in the 9 cases where the primary tumors were sequenced (56%) than in the metastatic site biopsy samples (23%) whereas the TP53 mutation frequency was higher in the metastatic lesions (31%) than for the cases where the primary tumor samples (11%) was used for genomic analysis. Mutations in PIK3CA and TP53 have been reported in 41% and 50% of ILC, respectively (COSMIC, Oct 2012). Whether the observed differences between the two groups are significant requires validation on a larger series of cases. DNA copy number gains were seen in CCND1 (6 cases; 27%); FGFR1 (3 cases; 14%); in MYC (2 cases; 9%), and ESR1 (1 case; 5%).

Finally, in order to assess clonality, the mutant allele frequencies of the 43 reported mutations in the 4 most commonly mutated genes in the study: CDH1, TP53, PIK3CA and ERBB2 were assessed. Considering tumor suppressors (CDH1, TP53) and oncogenes (PIK3CA, ERBB2) separately, we observed mean mutant allele frequencies of 39.7% and 18.5%, respectively (Supplemental Table 2). Taken in the context of an average estimated tumoral purity of approximately 40% [range 10%-95%] in the study, these mutant allele frequencies are consistent, on average, with a clonal homozygous model in the case of tumor suppressor mutations and the clonal heterozygous model in the case of oncogene mutations.
Discussion

In order to define a more genetically homogenous population of ILC cases, patient entry into this study required the presence of a CDH1 mutation (21-22). Using this approach we found that a striking 19 of 22 (86%) ILC cases had one or more actionable alterations including changes in PIK3CA, CCND1, ERBB2, FGFR1, MCL1, KRAS, NF1, AKT1, and BRCA2. The incidence of CDH1 mutations in ILC varies significantly in public databases and literature from a low of 31% (COSMIC, July 2012) to 62% (23). Most investigators consider a mutation in CDH1 to represent a genomic alteration characteristic of the lobular subtype of breast cancer (21-22). Other mechanisms that lead to loss of E-cadherin function include methylation of CDH1 (24). The CDH1 mutations in the 22 ILC cases included in this study were all unique and involved point mutations in 20 (91%) cases and splice site mutations/deletions in 2 (9%) cases. In triple negative invasive ductal carcinoma of the breast, loss of E-cadherin expression has been linked to adverse clinical outcome (25). Finally, neither CDH1 mutation, nor loss of E-cadherin function by other mechanisms, are considered targets for anti-cancer therapy at this time (26). Six (27%) of the CDH1 mutated ILC cases featured alterations in ERBB2 including 4 cases with mutations (L755S in one case; A775_G776 and insYVMA in one case, R1153* in one case; P780_Y781insGSP in one case; and S310F and V842I in one case), 1 case with an ERBB2-GRB7 fusion and 1 case with ERBB2 amplification. For ILC, HER2 gene amplification and protein overexpression are generally considered to be rare events typically below a 5% incidence in most published studies (27-29). ERBB2 mutations are rare in breast cancer and were not listed in a recently published major DNA sequence analysis study of more than 100 cases that also included whole genome analysis in 22 cases (30). In the COSMIC database, 14 (1.52%) of 919 listed breast cancers have an ERBB2 mutation that alters the Her2 protein (COSMIC Jan 2013). Similarly, in a currently unpublished prior assessment of a database of 308 clinical breast cancer samples
of all types sequenced at Foundation Medicine, Inc. from which the 5 ILC with ERBB2 mutations that are also included in the current study were identified, only 10 (3.25%) total invasive breast cancers (5 ductal and 5 CDH1 mutated lobular) featured ERBB2 mutations. When the 5/22 (23%) CDH1 mutated ILC with ERBB2 mutation/fusion are compared to the 5/286 (2%) of the CDH1 WT (non-mutated) invasive breast cancers with ERBB2 mutations, this difference is statistically significant (p=0.0006). When the ERBB2-GRB7 fusion case is excluded from analysis and only ERBB2 mutated cases are included, the enrichment of ERBB2 alterations in CDH1 mutated ILC remains significant (p=0.003). Thus, the filtering of breast cancers by combining lobular histologic phenotype with CDH1 mutation significantly enriches for the presence of ERBB2 mutations. Given that the 5 ERBB2 mutated CDH1 WT tumors did not display a lobular histologic phenotype, this data indicates that it is unlikely that subsets of ILC in which E-cadherin is inactivated by other mechanisms such as methylation are also enriched for ERBB2 mutations.

Interestingly, ERBB2 mutations have been reported more frequently in lung cancer than in breast cancer reaching as high as 10% in the adenocarcinoma subtype (31-32). The mutational spectrum of ERBB2 in breast cancer identified in this study is distinct from the mutations described for lung cancer which has also recently been reported by others (33). The L755S mutation identified in case 7 of this series of ILC is located in the kinase domain of Erbb2, and has been shown to be an activating mutation, capable of inducing oncogenic transformation in cell culture (34-35). Mutations in exon 20 of ERBB2, such as A775_G776insYVMA found in case 9 of this study, have been associated with Erbb2 activation and sensitivity to Erbb2 inhibitors and dual inhibitors of Erbb2 and Egfr (32, 36-39). Preclinical studies have shown that the L755S ERBB2 mutation is resistant to the reversible dual Egfr/Erbb2 inhibitor lapatinib, but may be sensitive to irreversible dual Egfr/Erbb2 inhibitors, such as afatinib, which are under investigation in clinical trials (40). The P780_Y781insGSP mutation is located at exon 20 of ERBB2 and has also been linked to Erbb2 activation and sensitivity to Erbb2 inhibitors and dual inhibitors of
Erbb2 and Egfr (36-39). The monoclonal antibodies trastuzumab and pertuzumab have been approved for use in ERBB2-amplified breast cancer, but their efficacy in the context of the L755S mutation is unknown.

The ERBB2-GRB7 putative fusion seen in study case 18 has not been previously reported. The fusion retains the kinase domain of ERBB2 (uniprot.org) which suggests that it could result in ERBB2 activation. The 17q12-21 amplicon which includes both ERBB2 and GRB7 is frequently amplified in breast cancer and preclinical studies suggest that it may be a recombination hotspot (41). An expression screening study has reported that GRB7 can function as an ERBB2-dependent oncogene (42). Interestingly, two previous studies focused on breast cancer rearrangements did not report this fusion although the number of CDH1 mutated lobular cancers in these studies may have been limited (43-44). GRB7 encodes an adaptor protein that interacts with ERBB2 and has been shown in a preclinical study to enhance its transformative capacity and increase ERBB2 phosphorylation in fibroblasts (44). In that the sample that yielded the ERBB2-GRB7 fusion was a small bone metastasis biopsy, there was insufficient remaining material available to further characterize the alteration at the RNA level. However, this fusion protein has not been functionally characterized, and its effect is therefore unknown and the relevance to the efficacy of clinical use of anti-HER2 targeted therapies is unclear.

The concept of targeting of ERBB2 mutations for anti-HER2 therapies has recently been expanded to breast cancer (33). Investigators identified 25 patient samples with ERBB2 mutations from a series of 8 independent breast cancer sequencing projects. Using in vivo kinase assays, analysis of protein structure and animal models, the investigators were able to identify 7 activating ERBB2 mutations, G309A, D769H, D769Y, V777L, P780ins, V842I, and R896C that were sensitive to the kinase inhibitor neratinib (33). The P780_Y781insGSP mutation of ERBB2 in study case 17 is included in this list of activating ERBB2 alterations. Neither the A775_G776insYVMA, R1153*mutation identified in case 9
nor the novel \textit{ERBB2-GRB7} fusion from study case 18 were reported by these investigators. The L755S mutation found in case 7 of the current report was considered not to be an activating mutation by these investigators but conferred resistance to the drug lapatinib in their experimental systems. The authors concluded that \textit{ERBB2 (HER2)} somatic mutation was capable of activating the gene in breast cancer and concluded that \textit{ERBB2} somatic mutations are potential drug targets for breast cancer treatment (33).

Several questions are raised by this study. Are all lobular breast cancers, or just \textit{CDH1} mutated ILCs enriched for \textit{ERBB2} mutations? If this relationship holds true, other \textit{CDH1} mutated cancers such as diffuse gastric carcinoma may also have a disproportionate enrichment of \textit{ERBB2} mutation. Moreover, the biologic mechanism for this apparent enrichment of \textit{ERBB2} alterations in \textit{CDH1} mutated ILCs is not known at this time. Interestingly, however both the loss of cell adhesion associated with \textit{CDH1} mutation and the well-documented enhancement of cell motility attributed to \textit{ERBB2} activation (45-46) could conceivably synergize to drive invasion and metastasis in these patients with difficult to treat, relapsed ILC. Finally, is \textit{ERBB2} mutation simply associated with relapsed breast cancer, rather than specifically being enriched in relapsed ILC? At this time, is not known whether a similar enrichment of \textit{ERBB2} alterations in \textit{CDH1} mutated ILC would be seen in cases that were successfully treated in the primary setting and, unlike the ILC in the current study, did not relapse or metastasize. This may speak to whether \textit{ERBB2} mutation is typically present at the early stages of cancer for the patient, or is an additionally acquired mutation as the cancer evolves, perhaps under selection pressure. Understanding the chronologic nature of this mutation could also explain distinct spectrums of \textit{ERBB2} mutation between lung and breast cancer.

Clinical trials focused on \textit{ERBB2} mutations and fusions in breast cancer using both on the market antibody therapeutics and tyrosine kinase inhibitors as well as new agents in late stages of development appear highly warranted. In conclusion, deep sequencing of genomic DNA using an
optimized clinical grade diagnostic assay can provide a broad cancer-related gene survey at a depth of coverage that provides sensitive detection for all classes of cancer-related genomic alterations, and when applied to ILC patients can reveal actionable alterations that have the potential to inform treatment decisions in the majority of patients. Wider use of this testing strategy could accelerate accrual to therapeutic trials of agents targeting these (and other mutations) and allow more efficient identification of more active single agents or combinations.

References


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Figure Legend

**Figure 1. Relapsed/Metastatic ILC: Primary Tumor Genomic Profiles.** The figure is divided into two groups: 1) relapsed/metastatic ILC where the patient’s primary tumor was sequenced and 2) relapsed/metastatic ILC where a metastatic lesion biopsy was sequenced.

**Figure 2. ERBB2 mutations in invasive lobular breast cancer detected by next generation sequencing.** Actionable alterations detected on 5 ERBB2 mutation patients were listed next to H&E images. **Figure 2a.** Core needle biopsy of a grade III invasive ILC metastatic to the liver in a 63 year old woman with a triple negative (ER-/PR-/HER2 FISH -) tumor. The tumor featured a L755S ERBB2 mutation. In addition to the CDH1 mutation, this ER IHC negative tumor also had amplifications of the ESR1 and MYC genes, and TP53 and RUNX1 mutations.

**Figure 2b.** This grade II primary ILC was ER+/PR+/ HER2 IHC – and featured only a CDH1 mutation in addition to 2 distinct ERBB2 mutations: A775_G776insYVMA and R1153*.

**Figure 2c.** This grade III ILC demonstrated a P780_Y781insGSP ERBB2 mutation in the primary tumor from a 65 year old patient. In addition to the ERBB2 mutation, this tumor also featured a splice site mutation in the MAP2K4 gene and amplification of MCL1 and RICTOR, respectively.
**Figure 2d.** This grade II relapsed metastatic ILC featured a primary tumor that was ER+/PR-/HER2- (FISH). When this primary tumor was sequenced, 2 discrete ERBB2 mutations were found (S310F and V842I) as well as mutations in the MEN1 and SMAD4 genes.

**Figure 2e.** This ER+/PR+/HER2- bone metastasis from a 62 year old patient demonstrated a novel ERBB2-GRB7 fusion. This tumor also had an amplification of the CCND1 gene and a H1047R PIK3CA mutation.

**Figure 3. Schematic illustration of the ERBB2-GRB7 gene fusion.**

The schematic is created from a ER+/PR+/HER2- ILC bone metastasis demonstrating a novel ERBB2-GRB7 fusion. This tumor also had an amplification of the CCND1 gene and a H1047R PIK3CA mutation. The figure demonstrates an 18Kb segment on chromosome 17 is deleted fusing ERBB2 and GRB7. ERBB2 is represented in blue as 5’ partner, and GRB7 is represented in orange as 3’ partner. Forty-nine high-quality paired-end reads span the chimeric structure of ERBB2-GRB7 fusion, a subset are shown as dashed black lines. Fourteen reads span the exact genomic breakpoint, mapping it unambiguously to the single nucleotide resolution. E1-25, exons 1-25; E12-15, exons 12-15; Exons 26-27 of ERBB2 and Exons 1-11 of GRB7 in grey box are within the deleted segment. Receptor L, Receptor L domain; Furin-like, Furin-like cysteine rich region; TM, transmembrane; Tyrosine Kinase, Tyrosine kinase domain; and SH2, Src Homology 2 domain (47-48).
Figure 1 (Ross)

Relapsed/Metastatic ILC: Primary Tumor Used for NGS

Relapsed/Metastatic ILC: Metastatic Tumor Used for NGS

- Substitution
- Gene amplification
- Gene copy number loss
- Translocation
- Gene fusion
Figure 3 (Ross)

Chromosome 17

14 single split reads spanning genomic breakpoint

ERBB2

GRB7

49 paired reads

Fusion gene

ERBB2

GRB7

Receptor_L

Furin-like

Receptor_L

Furin-like

Tyrosine Kinase

SH2

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| Case | Age | Tumor Stage | Tumor Grade | Primary Tumor E-cadHER2 | Coverage Depth (bp) | Specimen tested by NGS | Actionable Genomic Alterations | ERBB2 | ERBB4 | ESR1 | FGFR1 | KRAS | MAP2K4 | MET | MYC | NCOA1 | NCOA3 | NFKBIA | NFKBIL1 | NPa | PDCD1 | PIK3CA | PIK3R1 | PR | PRO1C1 | PTEN | RB1 | RICTOR | RNF162 | SMAD4 | TERT | TP53 | VHL | MX1 | NLRP3 | ZC3H12D | ZNF542 |
|------|-----|-------------|-------------|-------------------------|-------------------|---------------------|------------------------|-------|-------|-------|-------|-------|--------|-----|-----|-------|-------|--------|--------|-----|-----|-------|-------|-----|--------|-------|-----|--------|--------|-------|-----|------|-------|-------|
| 1    | 65  | 842         | Pleural Fluid | CB II IV pos pos pos (IHC) | 500,000 | 842 | V577N (1) | E3S85K |
| 2    | 66  | 897         | Primary Tumor | II IV pos pos pos (FISH) | 500,000 | 897 | T828N (33) | H0470B |
| 3    | 65  | 1230        | Skin Recurrence | II pap. Inflammatory | 500,000 | 1230 | E545K | E3S85K |
| 4    | 66  | 835         | Liver Metastasis | II IV pos pos pos (IHC) | 500,000 | 835 | E545K (19) | Amplified | Amplified | E3S85K |
| 5    | 74  | 1085        | Primary Tumor | II pos pos pos (IHC) | 500,000 | 1085 | E167* (7) | H0470B |
| 6    | 64  | 1290        | Lymph Node Metastasis | II IV pos pos pos (IHC) | 500,000 | 1290 | L355K (1) | K0446K |
| 7    | 63  | 1125        | Liver Metastasis | II IV pos pos pos (IHC) | 500,000 | 1125 | A546N (8) | Amplified | Amplified | E3S85K |
| 8    | 66  | 301         | Lymph Node Metastasis | II IV pos pos pos (IHC) | 500,000 | 301 | E545K (15) | D134K | E3S85K |
| 9    | 61  | 454         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 454 | E793* (15) | H0446K |
| 10   | 68  | 764         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 764 | A408V (13) | Amplified | Amplified | E3S85K |
| 11   | 63  | 1426        | Lymph Node Metastasis | II IV pos pos pos (IHC) | 500,000 | 1426 | E3S85K (13) | Amplified | Amplified | E3S85K |
| 12   | 55  | 1464        | Pleural Fluid | CB II IV pos pos pos (IHC) | 500,000 | 1464 | E3S85K | E3S85K |
| 13   | 54  | 1098        | Brain Metastasis | II IV pos pos pos (IHC) | 500,000 | 1098 | E3S85K (15) | R51S | E3S85K |
| 14   | 54  | 647         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 647 | E3S85K (15) | G265S | E3S85K |
| 15   | 58  | 1162        | Liver Metastasis | II IV pos pos pos (IHC) | 500,000 | 1162 | E3S85K (19) | Amplified | Amplified | E3S85K |
| 16   | 54  | 547         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 547 | E3S85K (2) | Amplified | Amplified | E3S85K |
| 17   | 65  | 929         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 929 | E3S85K (novel) | Amplified | Amplified | E3S85K |
| 18   | 62  | 211         | Bone Metastasis | II IV pos pos pos (IHC) | 500,000 | 211 | E3S85K (novel) | Amplified | Amplified | E3S85K |
| 19   | 61  | 138         | Bone Metastasis | II IV pos pos pos (IHC) | 500,000 | 138 | E3S85K | Amplified | Amplified | E3S85K |
| 20   | 60  | 405         | Oral Metastasis | II IV pos pos pos (IHC) | 500,000 | 405 | L71V (19) | Amplified | Amplified | E3S85K |
| 21   | 44  | 1475        | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 1475 | A636V (4) | Amplified | Amplified | E3S85K |
| 22   | 59  | 821         | Primary Tumor | II IV pos pos pos (IHC) | 500,000 | 821 | K214del (8) | D134K | E3S85K |

= actionable alteration
Relapsed classic E-cadherin (CDH1) mutated invasive lobular breast cancer demonstrates a high frequency of HER2 (ERBB2) gene mutations

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