Relapsed Classic E-Cadherin (CDH1)–Mutated Invasive Lobular Breast Cancer Shows a High Frequency of HER2 (ERBB2) Gene Mutations

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Abstract

Purpose: We queried whether comprehensive genomic profiling using a next-generation sequencing–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 conducted 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 formalin-fixed paraffin-embedded 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 ERBB2 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 of 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 of 286, 2%) was significant (P = 0.0006).

Conclusions: Comprehensive genomic profiling of relapsed CDH1-mutated ILC revealed actionable genomic alterations in 86% of cases, featured a high incidence of ERBB2 alterations, and can reveal actionable alterations that can inform treatment decisions for patients with ILC. Clin Cancer Res; 19(10); 2668–76. ©2013 AACR.

Introduction

Substantial progress has been made over the past 3 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 [immunohistochemistry (IHC)] currently play a more significant role in clinical trial design than routine histologic subtyping (1). 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 (2). In drug development, strategies have approached invasive ductal and invasive lobular breast carcinomas as essentially the same disease (3–6).

Most published studies have found that the overall prognosis for invasive lobular carcinoma (ILC) of the breast is similar to that of invasive ductal carcinoma (IDC; refs. 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 with ILC. In addition, although ER-positive ILC 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 United States each year, may be well characterized by a distinct genotype featuring a mutation in CDH1 that encodes the E-cadherin protein product, in contrast with the far more frequent ductal breast cancer, which typically has an unaltered CDH1 (11–15). In this study, restricted to 22 CDH1-mutated ILC from patients with relapsed disease, we queried whether a next-generation sequencing (NGS)-based assay could identify novel and unanticipated targets of therapy for these patients with relapsed, metastatic, and therapy-resistant disease.

Materials and 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 CDH1-mutated ILCs all of which had relapsed after primary surgical and one or more systemic treatment approaches. NGS was conducted on hybridization-captured, adaptor ligation–based libraries using DNA extracted from 4 formalin-fixed paraffin-embedded sections cut at 10 μm. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin (H&E)–stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor cells. DNA sequencing was conducted 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 libraries (Agilent SureSelect Custom Kit) and fully sequenced using 49-bp paired reads on the Illumina HiSeq 2000 to at an average depth of 877× and evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (CNA; 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% or more mutant allele frequency (MAF) and indels with a 10% or more MAF with 99% or more 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 with those expected from base substitutions present in individual cell line constituents as previously described (17–20).

CNAs had a validated accuracy of more than 95%. Actionable genomic alterations were defined as those identifying anticancer drugs in 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, 44–74). 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 1, 16 (73%) grade 2, and 5 (23%) grade 3 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 of 19 (88%) were ER-positive; 11 of 18 (61%) were PR-positive; and 1 of 19 (5%) was HER2-positive by either IHC or FISH. Sequencing was conducted on the primary ILC in n = 9 (41%) cases and biopsies of metastatic sites in n = 13 (59%) cases including lymph node metastases in n = 3 (14%) cases, liver metastases in n = 3 (14%) cases, pleural fluid cell blocks and bone metastases in n = 2 (9%) cases each, and brain metastases in n = 1 (5%) cases.

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;
Table 1. Clinical features, standard biomarker status, and genomic alterations in 22 cases of CDH1-mutated invasive lobular breast cancer

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Cover-age</th>
<th>Depth</th>
<th>Specimen tested by NGS</th>
<th>Tumor grade</th>
<th>Tumor stage</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
<th>Actionable genomic alterations</th>
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<td>842</td>
<td>Pleural fluid CB</td>
<td>IHC</td>
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<tr>
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<td>897</td>
<td>Primary tumor</td>
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<td>Neg 2 1</td>
<td>T823fs/C3 23 H1047R</td>
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<td>4</td>
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<td>885</td>
<td>Liver metastasis</td>
<td>III IV Pos Pos Neg (IHC)</td>
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<td>I594fs/C3 19 Amplified</td>
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<td>Neg 3 2</td>
<td>Amplified E167/C3 H1047R</td>
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<td>6</td>
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<td>Neg Neg Neg (IHC)</td>
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<td>L355fs/C3 1 N1044K</td>
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<td>7</td>
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<td>II IV</td>
<td>Unk Unk Neg (IHC)</td>
<td>NA 3 2</td>
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<td>Liver metastasis</td>
<td>II III</td>
<td>Pos Pos Neg (IHC)</td>
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<td>NA 3 2</td>
<td>A719fs/C3 29 A775_G776ins</td>
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<td>10</td>
<td>76</td>
<td>454</td>
<td>Primary tumor</td>
<td>II III</td>
<td>Pos Pos Neg (IHC)</td>
<td>NA 2 1</td>
<td>E763/C3 H1047L</td>
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<td>Amplified I650fs/C3</td>
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<td>Pleural fluid CB</td>
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<td>E17K D337_L343del</td>
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<td>13</td>
<td>54</td>
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<td>Brain metastasis</td>
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<td>Pos Neg IHC2+/FISH equivocal (ratio 1.9)</td>
<td>NA 4 2</td>
<td>Amplified P309fs/C3</td>
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<tr>
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<td>58</td>
<td>987</td>
<td>Liver metastasis</td>
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<td>Pos Pos Neg (IHC)</td>
<td>NA 4 3</td>
<td>Amplified Q23/C3</td>
<td></td>
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<tr>
<td>15</td>
<td>54</td>
<td>454</td>
<td>Primary tumor</td>
<td>III IV</td>
<td>Pos Pos Neg (IHC)</td>
<td>NA 3 2</td>
<td>Amplified Q611fs/C3</td>
<td></td>
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<tr>
<td>16</td>
<td>62</td>
<td>987</td>
<td>Primary tumor</td>
<td>II IV</td>
<td>Unk Unk Unk (IHC)</td>
<td>NA 4 2</td>
<td>Amplified E308/C3</td>
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<tr>
<td>17</td>
<td>65</td>
<td>454</td>
<td>Primary tumor</td>
<td>III IV</td>
<td>Pos Pos Neg (IHC)</td>
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<td>Amplified Q23/C3</td>
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<td>68</td>
<td>211</td>
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<td>Pos Pos Neg (IHC)</td>
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<tr>
<td>19</td>
<td>62</td>
<td>1,098</td>
<td>Liver metastasis</td>
<td>II IV</td>
<td>Pos Pos Neg (IHC)</td>
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<td>Amplified</td>
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<tr>
<td>20</td>
<td>68</td>
<td>454</td>
<td>Cecal metastasis</td>
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<td>Pos Pos Neg (IHC)</td>
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<td>Amplified</td>
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<td>Primary tumor</td>
<td>III IV</td>
<td>Unk Unk Unk (IHC)</td>
<td>NA 5 3</td>
<td>Amplified</td>
<td></td>
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<tr>
<td>22</td>
<td>68</td>
<td>454</td>
<td>Primary tumor</td>
<td>II IV</td>
<td>Pos Pos Neg (IHC)</td>
<td>NA 5 2</td>
<td>Amplified</td>
<td></td>
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</table>

NOTE: , actionable alteration.
Abbreviations: CB, cell block; NA, not available.

Ross et al. (2013)
ERBB2 tumors evaluated, 4 (44%) had an ERBB2 mutation or amplification. Of the 9 primary ERBB2 alterations with 3 (60%) of 5 grade 3 tumors having either an ERBB2 mutation and 1 case with amplification (3 cases with mutations and 1 case with amplification) compared with 2 (15%) of 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 3 tumor with a L755S ERBB2 mutation in a liver metastasis sample from an ERBB2–mutated stage IV high-grade ERBB2–mutated breast cancer with papillary features, an ERBB2 gene copy number gain was detected by NGS, which correlated with the positive HER2 overexpression on immunohistochemical staining and gene amplification detected by FISH testing of the same specimen. This tumor also had an amplification of the CCND1 gene and a H1047R 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 CDH1–mutated stage IV high-grade ERBB2–mutated case also had amplification of the ERBB2 gene and amplification of MCL1 and RICTOR, respectively (Fig. 2C). In case number 22, a woman with relapsed metastatic ILC, her primary tumor was ERBB2+/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 (Fig. 2D). In case study number 18, an ERBB2–mutated stage IV high-grade ERBB2–mutated bone metastasis from a 62-year-old patient, a novel ERBB2–GRB7 fusion was identified (Figs. 2E and 3). This tumor also had an amplification of the CCND1 gene and a H1047R 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 CDH1–mutated stage IV high-grade ERBB2–mutated breast cancer with papillary features, an ERBB2 gene copy number gain was detected by NGS, which correlated with the positive HER2 overexpression on immunohistochemical staining and gene amplification detected by FISH testing of the same specimen. This tumor also had amplifications in the CCND1 and MCL1 genes and a TP53 mutation. Only this 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 ERBB2 were uniformly HER2-negative by IHC and/or FISH testing.

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 (Fig. 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 2 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.

Figure 2. ERBB2 mutations in invasive lobular breast cancer detected by NGS. Actionable alterations detected on 5 ERBB2 mutation patients were listed next to H&E images. A, core needle biopsy of a grade 3 invasive ILC metastatic to the liver in a 65-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. B, this grade 2 primary ILC was ER+/PR+/HER2 IHC- and featured only a CDH1 mutation in addition to 2 distinct ERBB2 mutations: A775_G776insYVMA and R1153*. C, this grade 3 ILC showed a P780_G776insGSP 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. D, this grade 2 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. E, this ER+/PR+/HER2- bone metastasis from a 62-year-old patient showed a novel ERBB2-GRB7 fusion. This tumor also had an amplification of the CCND1 gene and a H1047R PIK3CA mutation.
ERBB2 Mutations in Lobular Breast Cancer

Figure 3. Schematic illustration of the ERBB2–GRB7 gene fusion. The schematic is created from a ER/PR/HER2+ ILC bone metastasis showing a novel ERBB2–GRB7 fusion. This tumor also had an amplification of the CCND1 gene and a H1047R PIK3CA mutation. The figure shows an 18-kb fusion spanning genomic breakpoint. Forty-nine high-quality paired-end reads span the chimeric structure of ERBB2–GRB7 fusion; a subset is shown as dashed black lines. Fourteen reads span the exact genomic breakpoint, mapping it unambiguously to the single-nucleotide resolution. E1–25, exons 1 to 25; E12–15, exons 12 to 15; exons 26–27 of ERBB2 and exons 1–11 of GRB7 in gray 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 (44, 45).

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. Considering tumor suppressors (CDH1, TP53) and oncogenes (PIK3CA, ERBB2) separately, we observed mean mutant allele frequencies of 39.7% and 18.5%, respectively (Supplementary Table S2). Taken in the context of an average estimated tumor 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

To define a more genetically homogenous population of ILC cases, patient entry into this study required the presence of a CDH1 mutation (7, 21). 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% (22). Most investigators consider a mutation in CDH1 to represent a genomic alteration characteristic of the lobular subtype of breast cancer (7, 21). Other mechanisms that lead to loss of E-cadherin function include methylation of the CDH1 gene and a H1047R PIK3CA mutation. The incidence of CDH1 mutations in ILC varies significantly in public databases and literature from a low of 31% (COSMIC; July 2012) to 62% (22). Most investigators consider a mutation in CDH1 to represent a genomic alteration characteristic of the lobular subtype of breast cancer (7, 21).
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, 10 (3.25%) total invasive breast cancers (5 ductal and 5 CDH1 mutated lobular) featured ERBB2 mutations. When the 5 of 22 (23%) CDH1-mutated ILC with ERBB2 mutation/fusion are compared with the 5 of 286 (2%) of the CDH1 WT (nonmutated) 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, these data indicate 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 (28, 29). 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 (30). 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 (31, 32). 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 (29, 33–36). 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 (37). 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 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 ERBB2 (HER2) somatic mutation was capable of activating the gene in breast cancer and concluded that ERBB2 somatic mutations are potential drug targets for breast cancer treatment (30).

Several questions are raised by this study. Are all lobular breast cancers, or just CDH1-mutated ILCs enriched for ERBB2 mutations? If this relationship holds true, other CDH1-mutated cancers such as diffuse gastric carcinoma may also have a disproportionate enrichment of ERBB2 mutation. Moreover, the biologic mechanism for this apparent enrichment of ERBB2 alterations in CDH1-mutated ILCs is not known at this time. Interestingly, however, both the loss of cell adhesion associated with CDH1 mutation and the well-documented enhancement of cell motility attributed to ERBB2 activation (42, 43) could conceivably synergize to drive invasion and metastasis in these patients with difficult to treat, relapsed ILC. Finally, is 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 ERBB2 alterations in 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 explain whether 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 ERBB2 mutation between lung and breast cancer.

Clinical trials focused on ERBB2 mutations and fusions in breast cancer using both on the market antibody therapeutic and tyrosine kinase inhibitors as well as new agents in late stages of development seem 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 patients with ILC 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.

Disclosure of Potential Conflicts of Interest

I.S. Ross, K. Wang, C. Otto, S.R. Downing, J. Sun, J. He, J.A. Curran, S. Ali, R. Yelensky, D. Lipson, G. Palmer, V.A. Miller, and P.J. Stephens are employees and stock owners of Foundation Medicine, Inc. V.A. Miller is a consultant/advisory board member of Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: J.S. Ross, R. Yelensky
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.S. Ross, C.E. Sheehan, A.B. Boguniewicz, S.R. Downing, J.A. Curran
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