VOLUME 34 · NUMBER 16 · JUNE 1, 2016

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Genomic Characterization of Primary Invasive Lobular Breast Cancer

Christine Desmedt, Gabriele Zoppoli, Gunes Gundem, Giancarlo Pruneri, Denis Larsimont, Marco Fornili, Debora Fumagalli, David Brown, Françoise Rothé, Delphine Vincent, Naima Kheddoumi, Ghizlane Rouas, Samira Majjaj, Sylvain Brohée, Peter Van Loo, Patrick Maisonneuve, Roberto Salgado, Thomas Van Brussel, Diether Lambrechts, Ron Bose, Otto Metzger, Christine Galant, François Bertucci, Martine Piccart-Gebhart, Giuseppe Viale, Elia Biganzoli, Peter J. Campbell, and Christos Sotiriou

See accompanying editorial on page 1838

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on February 29, 2016.

Supported by Susan G. Komen, Fondation MEDIC, Les Amis de Bordet, Fonds National de Recherche Scientifique, the Breast Cancer Research Foundation, the Wellcome Trust Clinical Research Training Fellowship Programs, the Italian Association for Leukemia and Lymphoma, and the Italian Association for Cancer Research.

C.D., G.Z., G.G., P.J.C., and C.S. contributed equally to this work.

Presented at the 37th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 9-13, 2014.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Christine Desmedt, PhD, Breast Cancer Translational Research Laboratory, Institut Jules Bordet, 125 Boulevard de Waterloo, 1000 Brussels, Belgium; e-mail: christine.desmedt@bordet.be.

0732-183X/16/3416w-1872w/$20.00

DOI: 10.1200/JCO.2015.64.0334

ABSTRACT

Purpose
Invasive lobular breast cancer (ILBC) is the second most common histologic subtype after invasive ductal breast cancer (IDBC). Despite clinical and pathologic differences, ILBC is still treated as IDBC. We aimed to identify genomic alterations in ILBC with potential clinical implications.

Methods
From an initial 630 ILBC primary tumors, we interrogated oncogenic substitutions and insertions and deletions of 360 cancer genes and genome-wide copy number aberrations in 413 and 170 ILBC samples, respectively, and correlated those findings with clinicopathologic and outcome features.

Results
Besides the high mutation frequency of *CDH1* in 65% of tumors, alterations in one of the three key genes of the phosphatidylinositol 3-kinase pathway, *PIK3CA*, *PTEN*, and *AKT1*, were present in more than one-half of the cases. *HER2* and *HER3* were mutated in 5.1% and 3.6% of the tumors, with most of these mutations having a proven role in activating the human epidermal growth factor receptor/ERBB pathway. Mutations in *FOXA1* and *ESR1* copy number gains were detected in 9% and 25% of the samples. All these alterations were more frequent in ILBC than in IDBC. The histologic diversity of ILBC was associated with specific alterations, such as enrichment for *HER2* mutations in the mixed, nonclassic, and *ESR1* gains in the solid subtype. Survival analyses revealed that chromosome 1q and 11p gains showed independent prognostic value in ILBC and that *HER2* and *AKT1* mutations were associated with increased risk of early relapse.

Conclusion
This study demonstrates that we can now begin to individualize the treatment of ILBC, with *HER2, HER3,* and *AKT1* mutations representing high-prevalence therapeutic targets and *FOXA1* mutations and *ESR1* gains deserving urgent dedicated clinical investigation, especially in the context of endocrine treatment.

J Clin Oncol 34:1872-1881. © 2016 by American Society of Clinical Oncology

INTRODUCTION

Invasive lobular breast cancer (ILBC) is the most frequent special histologic type of breast cancer (5% to 15%) after invasive ductal breast cancer (IDBC).[1] This neoplasia is typically characterized by small, discohesive epithelial cells, which mostly express the estrogen receptor (ER, encoded by the *ESR1* gene), lack *HER2* (*ERBB2*) amplification, and lost the cell adhesion molecule E-cadherin (CDH1). Besides the most prevalent classic subtype, several special ILBC subtypes have been described on the basis of their architecture (alveolar, solid, and trabecular) or cytonuclear characteristics (apocrine, histiocytoid, pleomorphic, and signet ring, further grouped into the mixed-nonclassic subtype).[2] The solid and mixed nonclassic subtypes have been associated with worse survival.[2]

From a clinical perspective, ILBCs generally show an indolent clinical behavior and tend to exhibit a peculiar metastatic pattern.[3] Although patients with ILBC tend to have lower response rates to conventional chemotherapeutic agents than patients with IDBC, some results have suggested that they might derive increased benefit

from treatment with aromatase inhibitors.[4,5] Despite these histologic and clinical differences, patients with ILBC are treated as those with IDBC because of the relative paucity of research dedicated to this disease.

Several studies have devoted efforts to characterize the mutational landscape of breast cancer (reviewed in Goncalves et al[6]), with a clear underrepresentation of ILBC, which limits the understanding of the genomic alterations that define these cancers. In the current study, we assessed genomic alterations that recur in cancer-related genes in, to our knowledge, the largest cohort of primary ILBC tumors collected for this purpose. We aimed to correlate those recurrent alterations with clinical and pathologic features and to identify those associated with breast cancer–free interval (BCFI).

METHODS

Patients and Samples

Formalin-fixed paraffin-embedded primary ILBC samples from 630 patients (Data Supplement) were selected from four institutional biobanks (Institut Jules Bordet and Cliniques Universitaires Saint Luc, Brussels, Belgium; European Institute of Oncology, Milan, Italy; and Institut Paoli-Calmettes, Marseille, France) according to the following criteria: availability of a formalin-fixed paraffin-embedded sample from the surgical specimen of the primary tumor; no distinct invasive neoplastic components other than pure lobular histologic subtype by central revision; minimal tumor cellularity of 10% to have an unbiased representation of ILBC histotypes (Data Supplement); and availability of at least 600 ng of double-stranded DNA. Patients who received neoadjuvant treatment and/or had previous malignancies at the time of ILBC diagnosis were excluded. All patients received a diagnosis between January 1994 and December 2008 and were treated with surgery followed by medical treatment per local guidelines at the time of presentation. For 235 of 630 patients, we also collected material from an uninvolved lymph node as a source of germline DNA. ILBC histologic subtypes were defined as in Iorfida et al.[2] Other patient characteristics are provided in Table 1. The study protocol was approved by the internal ethics committee of each participating center.

Table 1. Patient and Sample Characteristics (n = 413)

Characteristic	Patients No.	Patients %
Age, years		
< 50	130	31.5
50-64	165	39.9
≥ 65	118	28.6
pT(m)		
Unifocal	314	76.0
Multifocal	99	24.0
Tumor size, cm		
< 2	169	40.9
≥ 2	244	59.1
Nodal status		
Negative	197	47.7
Positive	204	49.4
Unknown	12	2.9
Tumor grade		
1	47	11.4
2	301	72.9
3	63	15.3
Unknown	2	0.5
Ki-67 status		
< 20%	283	68.5
≥ 20%	124	30.0
Unknown	6	1.5
Receptor status*		
ER-negative	25	6.1
ER-positive/PgR-negative	85	20.6
ER-positive/PgR-positive	302	73.1
Unknown	1	0.2
HER2 status*		
Negative	391	94.7
Positive	20	4.8
Unknown	2	0.5
Histologic subtype		
Classic	197	47.7
Alveolar	66	16.0
Solid	65	15.7
Mixed, nonclassic	57	13.8
Trabecular	28	6.8

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; Ki-67, protein encoded by the *MKI67* gene; PgR, progesterone receptor.
*The ER and HER2 status denote the pathologic assessment by immunohistochemistry and combined immunohistochemistry/fluorescent in situ hybridization status of these markers.

Targeted Sequencing

The 630 primary ILBC samples underwent targeted sequencing analysis of 360 cancer-related genes (Data Supplement). We discarded samples that did not have the required coverage to detect mutations for any cellularity with a prespecified type I error probability $\alpha = .05$ and power of $1 - \beta = .80$, on the basis of Carter et al.[7] At a median sequencing coverage over exonic regions of 103X, 413 tumors were retained for final analysis of substitutions and insertions and deletions (Data Supplement). Germline DNA was interrogated for 196 (47.5%) patients. Single base substitutions were called independently in each sample by using CaVEMan (Cancer Variants through Expectation Maximization),[8] whereas small somatic insertions and deletions were identified by using a modified version of Pindel.[9] To avoid the inclusion of potential germline variants in samples without germline DNA and to focus on mutations with a likely role in cancer, we further considered only oncogenic mutations as defined in Desmedt et al,[10] henceforth referred to as mutations for the sake of simplicity. The Cancer Genome Atlas (TCGA) database was used for comparison of frequencies after applying the same definition for oncogenicity.

Copy Number and Gene Expression Analysis

Log_2 ratios were calculated from the sequencing coverage, normalized with single nucleotide polymorphism data derived from sequencing data of nontumor DNA samples, and smoothed by guanine-cytosine correction. One hundred seventy samples passed the quality criteria for copy number aberration (CNA) analysis. GeneChip U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA) gene expression microarray data were available for 117 patients with ILBC with mutation data.[11]

Statistical Analysis

For association analysis of genomic alterations with clinical and pathologic parameters, we limited the investigations to oncogenic mutations observed in ≥ 2% of the 413 samples retained for mutational analysis and to CNAs that occurred in ≥ 5% of the 170 samples with adequate quality criteria. These cutoffs were warranted by exploratory power considerations. In survival analyses, we considered BCFI as the primary end point on the basis of STEEP System criteria outlined by Hudis et al.[12] Follow-up was curtailed at 12 years in consideration of the declining number of patients after that time point. Details are provided in the Data Supplement.

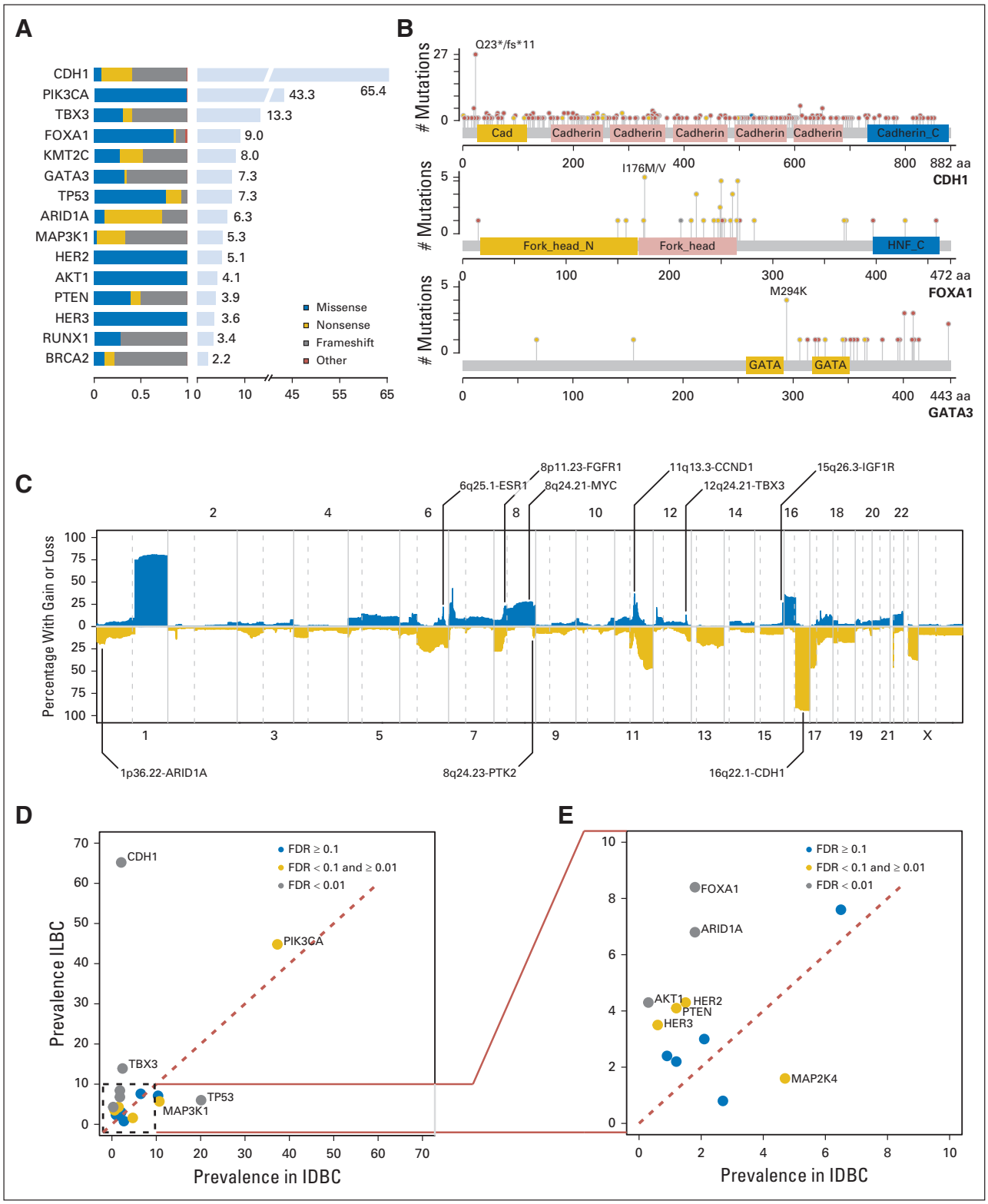

Fig 1. Genomic landscape of ILBC. (A) Histogram of the frequency and type of mutations of the genes recurrently mutated in more than 2% of the primary ILBC tumors. (B) Lollipop plots of protein coding sequences and major domains of *CDH1*, *TBX3*, and *FOXA1*. Gold and red circles represent missense and truncating mutations (nonsense, nonstop, frameshift insertion and deletion, and splice site substitution), respectively. The length of the vertical lines corresponds to the frequency of the respective mutation. (C) Genome-wide view of copy number alterations present in ILBC. Gains are indicated in blue and losses in gold. (D and E) Scatter plots of the prevalence of the mutated genes in estrogen receptor (ER)-positive/HER2-negative IDBC (n = 338) from The Cancer Genome Atlas on the *x*-axis and the prevalence of mutated genes in ER-positive/HER2-negative ILBC (n = 371) from the present cohort on the *y*-axis. The color of the dots represents the significance of the comparison, with gold and orange representing FDRs < 10%. Cad, Caherin prodomain like; Cadherin_C, Cadherin cytoplasmic; FDR, false discovery rate; HER2, human epidermal growth factor receptor 2; Fork_head_N, forkhead N-terminal region; HNF_C, HNF3 C-terminal domain; IDBC, invasive ductal breast cancer; ILBC, invasive lobular breast cancer.

RESULTS

Recurrent Mutations in Protein-Coding Genes

We observed mutations in 103 of the 360 genes interrogated, of which 15 were recurrently mutated in at least 2% of the tumors (Figs 1A and 1B; Data Supplement). Some genes were mainly affected by truncating mutations (eg, *CDH1*, *GATA3*), whereas for others, missense substitutions prevailed (eg, *PIK3CA*, *FOXA1*). Besides the expected high mutation frequency of *CDH1*,[3] present in 65% of cases, we observed that 50% of the tumors were mutated in at least one of the three key genes of the phosphatidylinositol 3-kinase (PI3K) pathway, namely *PIK3CA*, the negative regulator *PTEN*, and the downstream effector kinase *AKT1*. In agreement with the literature,[13] these mutations were mutually exclusive in all but five of 207 tumors that harbored a mutation in one of these genes. Of note, the activating *AKT1* substitution E17K was found in all but two patients who carried *AKT1* mutant tumors.

Several transcriptional regulators were recurrently altered with high prevalence, such as *TBX3*, *FOXA1*, *KMT2C*, *GATA3*, and *ARID1A*. Mutations in *TBX3*, a member of the T-box transcription factors family, were observed in 13% of cases, principally in the protein T-box domain. FOXA1 and GATA3, mutated in 9% and 7% of the tumors, respectively, are key players in the recruitment of the ER transcription complex.[14] Mutations in the chromatin regulatory factors *KMT2C* and *ARID1A* were present in 8% and 6%, respectively. With the exception of *KMT2C* mutations, which have been demonstrated to facilitate cell cycle progression,[15] the transcriptional consequences of the described alterations are currently unknown.

Finally, two members of the human epidermal growth factor receptor (HER)/ERBB family, *HER2* and *HER3*, were mutated in 5.1% and 3.6% of the tumors, respectively (Fig 2; Data Supplement), with three of 35 mutated cases being simultaneously *HER2* amplified, as revealed by routine fluorescent in situ hybridization analysis. We observed 16 different substitutions in these genes. The majority of those were concentrated in the tyrosine kinase domain of the respective genes, each associated with a highly recurrent mutation (L755S for *HER2* and E928G for *HER3*; Fig 2A). Each of these mutations, together with the S310F, D769Y, and V777L *HER2* substitutions, and the S846I and Q809R *HER3* mutations are known to activate the HER/ERBB pathway[16-19] (Figs 2C-2F). Although the recurrent *HER2* L869R substitution has never been characterized in breast cancer, it is homologous to substitutions observed in the activation loops of the *EGFR*, *FLT3*, and *BRAF* kinases[20,21] (Data Supplement). Hence, the functional relevance of at least eight of the 16 substitutions, present in 27 of 35 of the *HER2* and *HER3* mutated tumors (77%), is supported by structural and/or in vitro evidence. In the subset of tumors with available gene expression data, we further observed that the scores of an established microarray signature of HER2 pathway activation[22] were above the median for the majority of these *HER2* and *HER3* mutated tumors (Fig 2B).

Recurrent CNAs

We identified 25 arm-level changes and 20 focal gains/deletions, recurrent in at least 5% of the 170 tumors with CNA data (Fig 1B; Data Supplement). Of note, the *HER2* amplification status identified by routine assessment was confirmed by the present analysis in all tumors but one.

CDH1 deletion was present in 94% of the patients (160 of 170, 152 as arm-level loss and eight as a focal event). In the subset of tumors stained for CDH1 by immunohistochemistry (156 of 170), absence of expression was observed in 133 (85%) cases (Fig 2G), with protein loss being statistically more frequent in tumors that harbored both a deletion and a mutation compared with those that retained at least one intact copy of the gene ($P < .001$).

We also observed recurrent, focal copy number gains at 6q25.1, which encompasses *ESR1*, in 25.3% (43 of 170) of the cases. This focal gain was associated with a higher ESR1 mRNA signature score[22] ($P = .03$; Fig 2H) as well as with increased mRNA expression of TFF1,[23] a canonical ESR1 transcriptional target ($P = .04$), which altogether point to a functional role for this aberration in ILBC. Among recurrent focal alterations, noteworthy gains were found in 11q13.3 (*CCND1*, 38.2%), 8q24.21 (*MYC*, 31.2%), 15q26.3 (*IGF1R*, 31.2%), 8p11.23 (*FGFR1*, 25.3%), and 12q24.21 (*TBX3*, 18.8%), and losses were found in 1p36.22 (*ARID1A*, 22.9%) and 8q24.23 (*PTK2*, 18.2%), often associated with significant *in-cis* transcriptional modulation (Data Supplement).

Comparison With Publicly Available Data

We used the publicly available data from TCGA to compare the mutation frequencies in the present ILBC cohort with the ILBC cases analyzed by TCGA (n = 159) and to investigate the differences between ER-positive/HER2-negative ILBCs from the present series (n = 371; Data Supplement) and ER-positive/HER2-negative IDBCs from TCGA (n = 338). Sequencing results generated independently on these two ILBC cohorts were comparable, with significant differences observed only for *CDH1* and *TBX3*, which had a higher prevalence in the present cohort compared with TCGA ILBC cases (Data Supplement). A possible higher prevalence was also observed for *AKT1*, whereas there was a lower prevalence for *NF1* and *RUNX1*. These differences in mutation prevalence might be explained by clinicopathologic differences between the two ILBC cohorts (TCGA series characterized by larger, more frequently HER2-positive and progesterone receptor–positive tumors as well as by older patients; Data Supplement). The smaller number of ILBC cases assessed by TCGA with the associated lesser precision in frequency estimation and the selection of high cellularity samples by TCGA could also account for part of the observed differences (especially for the classic ILBC subtype, which is characterized by low cellularity).[24]

With regard to the comparison of the two main breast cancer histologies, we observed significant prevalence differences for several genes. *AKT1*, *ARID1A*, *CDH1*, *HER2*, *HER3*, *FOXA1*, *PIK3CA*, *PTEN*, and *TBX3* were more frequently altered in ER-positive/HER2-negative ILBC than in ER-positive/HER2-negative IDBC, whereas the opposite was true for *MAP3K1*, *MAP2K4*, and *TP53* (Fig 1D; Data Supplement). Finally, despite methodological differences that hampered a precise comparison, we could identify CNAs, which in addition to the expected *CDH1* losses, were significantly more prevalent in ILBC than IDBC, such as *ESR1* and *ETV1* gains (Fig 1C; Data Supplement).

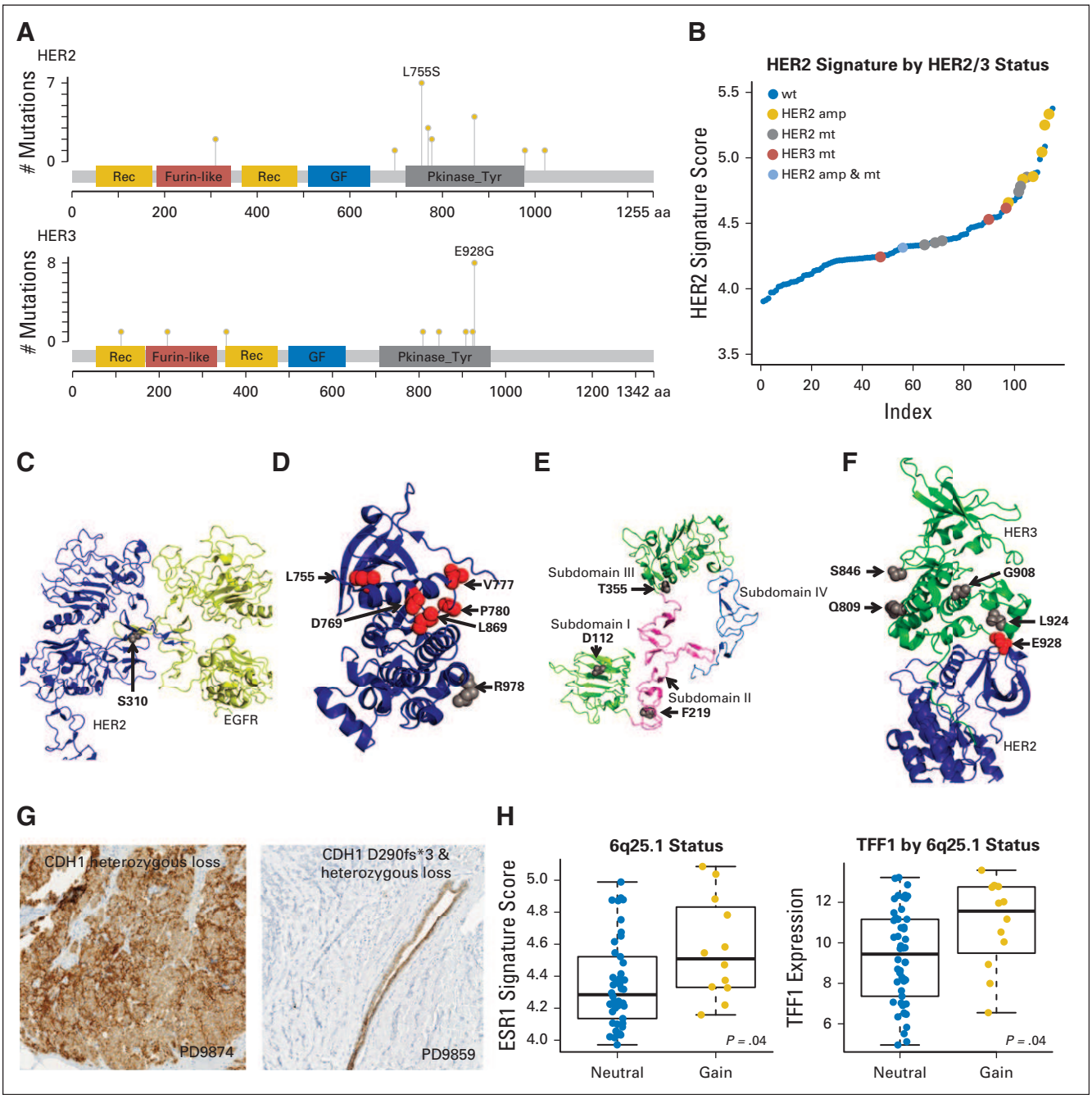

Fig 2. Characterization of recurrent genomic alterations. (A) Lollipop plots of protein coding sequences and major domains of *HER2* and *HER3*. (B) Waterfall plot of HER2 mRNA signature score for primary tumors with available gene expression data. Color and symbol codes represent *HER2* and/or *HER3* alterations in the set. (C) Homology model of HER2-EGFR extracellular domain heterodimer constructed by using Protein Data Bank (PDB): 1N8Y (HER2) and PDB: 1IVO (EGFR). (D) HER2 kinase domain PDB: 3PP0. (E) HER3 extracellular domain PDB: 1M6B. (F) Homology model of HER3-HER2 kinase domain heterodimer constructed by using PDB: 3PP0 (HER2) and PDB: 3KEX (HER3). For panels C to F, residues mutated in two or more tumors from the present set are highlighted in red, and those found only in one tumor are highlighted in gray. Protein structural alignments and visualizations of the *HER2* and *HER3* mutations were performed using PyMol (Delano Scientific, Palo Alto, CA). (G) Immunohistochemical stains of CDH1 expresser and nonexpresser cases. (H) Box plots of the ESR1 gene expression signature score and TFF1 mRNA expression levels as functions of the copy number status of the 6q25.1 segment (wild type *v* gain). aa, amino acid; amp, amplification; EGFR, epidermal growth factor receptor; GF, growth factor receptor domain IV; HER, human epidermal growth factor receptor; mt, mutation; Pkinase_Tyr, protein tyrosine kinase; rec, receptor L domain; wt, wild type.

Association of Genomic Alterations With Clinical and Pathologic Features

We first investigated the association of recurrent mutations and CNAs with clinicopathologic features (Table 2; Data Supplement). *TP53* mutations were more frequent in older patients and in high-grade, highly proliferative tumors, as assessed by Ki-67 (protein encoded by the *MKI67* gene). On the contrary, *PIK3CA* mutations were found to be associated with low proliferative tumors. *HER2* mutations were further associated with high grade and progesterone receptor negativity and *MAP3K1* mutations with node-negative

tumors. At the copy number level, among others, 6q25.1 (*ESR1*) and 8p11.23 (*FGFR1*) gains were more frequent in older and younger patients, respectively, whereas 1p36.22 (*MTOR/ARID1A*) deletions were associated with high proliferative and *HER2*-amplified tumors (Table 2). Of note, *PTEN* and *TBX3* mutations as well as 11q14.1 gains (*PAK1*) were associated with the presence of multifocal tumors, which corroborates the clinical perspective that those genes are involved in cell motility and tissue invasion.[25-27]

We then sought to identify genomic alterations associated with the various histologic subtypes of ILBC. In addition to the already-described clinical associations with such subtypes,[2] we found that the mixed nonclassic and solid subtypes were enriched in *ERBB2* and *ARID1A* mutations, respectively, whereas both were associated with *TP53* mutations (Data Supplement). By copy number analysis, the solid subtype was more often characterized by 11p and 6q25.1 (*ESR1*) gains and by 1p36.22 (*ARID1A*) deletions, the mixed nonclassic subtype frequently presented with 1p36.22 (*ARID1A*) deletions, and the alveolar subtype was enriched in 11q13.3 (*CCND1*) and 11q14 (*PAK1*) gains. Altogether, these results show that distinctive genomic alterations likely underlie the phenotypic appearance of ILBC histologic subtypes (Fig 3).

Breast Cancer–Free Survival Analyses

We took advantage of the long-term follow-up data available for this cohort (median follow-up, 9.5 years) to explore whether specific genomic aberrations could be associated with prognosis (BCFI; Data Supplement). Univariable assessment of mutated genes and CNAs identified *TP53* mutations and several CNAs as associated with the investigated end point (Figs 4A and 4C). Only three CNAs were, however, retained by the stability selection with the lasso-penalized Cox proportional hazard regression model, which also included classic clinicopathologic variables. Specifically, 1q gains were associated with a better outcome, whereas 17q12 and 11p gains were associated with a worse outcome (Figs 4D-4F). Hazard proportionality assessment suggested that *AKT1* and *HER2* mutations may have time-dependent effects associated with short-term risk, even after adjusting for standard clinicopathologic variables (Fig 4B; Data Supplement).

DISCUSSION

ILBC represents a significant proportion of the breast cancer population and is associated with distinct clinical and pathologic features compared with IDBC. However, no specific treatment recommendation exists for this disease. We therefore aimed to identify and characterize the recurrent genomic alterations associated with ILBC in the largest cohort investigated, to our knowledge, at the genomic level to identify biologically and clinically meaningful determinants for such disease.

We show that ILBC presents a different mutational landscape from that of IDBC. In addition to the expected increased frequency of genomic alterations that involve *CDH1*, ILBC is characterized by a peculiarly high prevalence of mutations that affect PI3K and HER/ERBB family members, ESR1-specific and other transcriptional regulators as well as CNAs that involve, among others, *ESR1* gains in one-fourth of the cases. Of note, no novel ILBC-specific mutated genes, not included in our analysis, were discovered

Table 2. Association of the Recurrent Genomic Alterations Retained by the Stability Selection With Clinical and Pathologic Characteristics: Genes Recurrently Mutated in > 2% of the Patient Sample

Variable	Mutated Genes	Mutated Tumors Per Category (%)	Odds Ratio (95% CI)	*P*
ER (positive *v* negative)	*HER2*	4.6 *v* 12.0	0.37 (0.11 to 1.61)	.167
	FOXA1	8.2 *v* 20.0	0.44 (0.16 to 1.43)	.161
	RUNX1	2.8 *v* 12.0	0.19 (0.06 to 0.85)	.032*
	TP53	6.7 *v* 16	0.34 (0.12 to 1.19)	.087†
PgR (positive *v* negative)	*BRCA2*	1.6 *v* 3.9	0.37 (0.10 to 1.41)	.140
	HER2	3.9 *v* 8.8	0.40 (0.17 to 0.99)	.047*
HER2 (positive *v* negative)	*HER3*	10 *v* 3.3	4.12 (0.74 to 15.91)	.095†
	KMT2C	25 *v* 7.2	5.85 (1.83 to 16.89)	.003‡
	PIK3CA	20 *v* 44.8	0.31 (0.09 to 0.84)	.021*
Grade (3 *v* 1 and 2)	*HER2*	11.1 *v* 4	2.89 (1.02 to 7.55)	.046*
	PIK3CA	33.3 *v* 45.1	0.62 (0.34 to 1.10)	.104
	TP53	23.8 *v* 4.3	6.48 (2.97 to 14.18)	< .001§
Ki-67 (≥ 20 *v* < 20)	*PIK3CA*	35.5 *v* 46.6	0.64 (0.41 to 0.99)	.048*
	TP53	15.3 *v* 3.9	4.30 (2.03 to 9.51)	< .001§
Age (50-64 *v* < 50 years)	*FOXA1*	4.2 *v* 9.2	0.45 (0.17 to 1.14)	.092†
	TP53	6.1 *v* 3.8	1.5 (0.54 to 4.68)	.442
Age (≥ 65 *v* < 50 years)	*FOXA1*	15.3 *v* 9.2	1.82 (0.85 to 4.02)	.125
	TP53	12.7 *v* 3.8	3.51 (1.35 to 10.57)	.009‡
Nodal status (positive *v* negative)	*MAP3K1*	2.5 *v* 8.6	0.28 (0.10 to 0.71)	.006‡
Multifocal (yes *v* no)	*CDH1*	72.7 *v* 63.1	1.53 (0.94 to 2.56)	.087†
	PTEN	8.1 *v* 2.5	3.28 (1.21 to 8.94)	.021*
	TBX3	19.2 *v* 11.5	1.90 (1.02 to 3.45)	.045*

Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; Ki-67, protein encoded by the *MKI67* gene; PgR, progesterone receptor.
*$P < .05$.
†$P < .1$.
‡$P < .01$.
§$P < .001$.

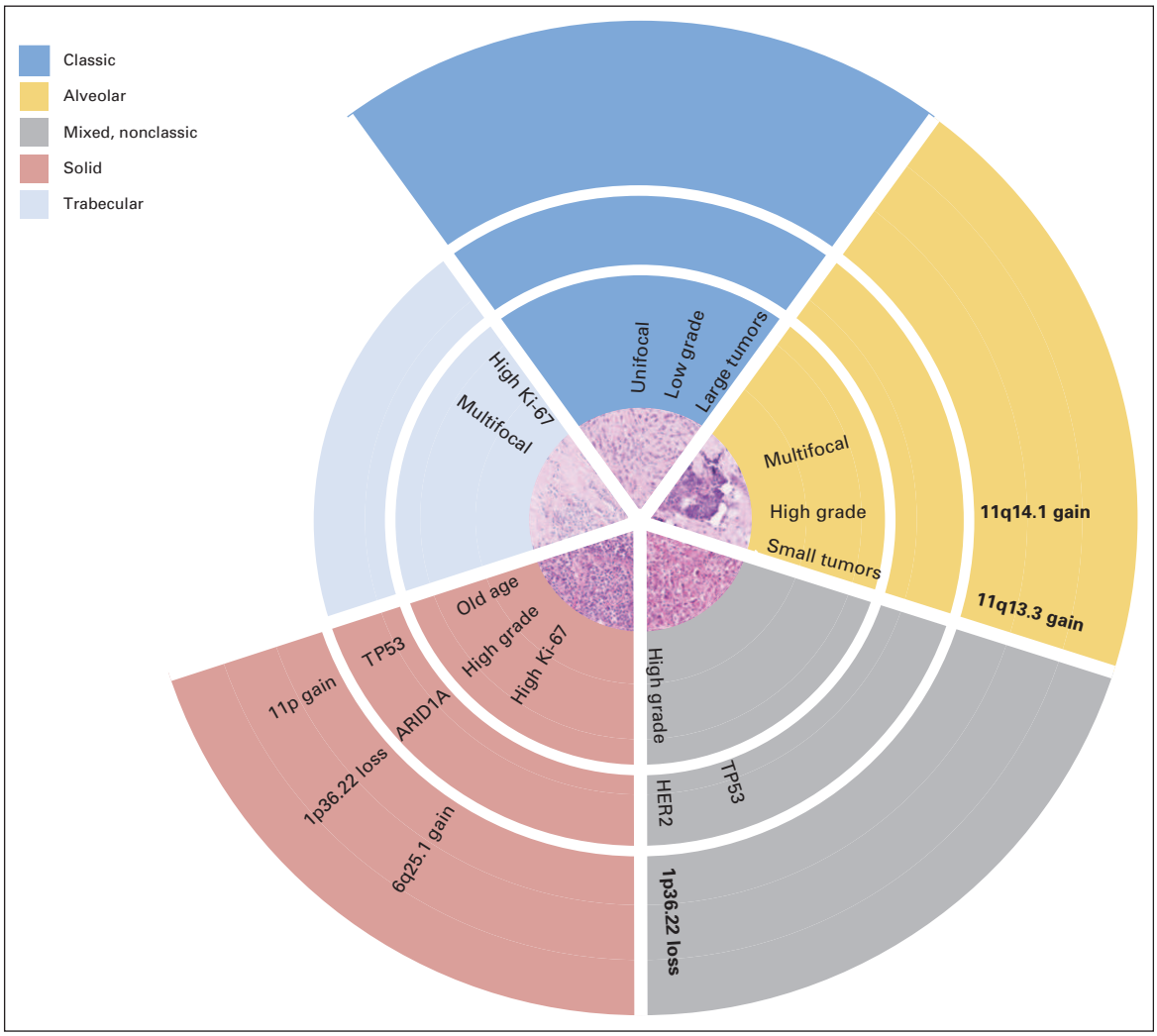

Fig 3. Clinicopathologic variables and genomic alterations associated with the histologic subtypes of invasive lobular breast cancer (ILBC). For the association analyses of genomic alterations with the histologic subtypes, we limited the investigations to mutations observed in ≥ 2% of 413 samples and to copy number aberrations (CNAs) that occurred in ≥ 5% of 170 samples. The alterations reported in boldface are those that are significant after adjusting for standard clinicopathologic variables. For the statistical correlations of the ILBC subtypes with CNAs, we excluded patients with trabecular ILBC because of the insufficient sample size of that subtype. HER2, human epidermal growth factor receptor 2; Ki-67, protein encoded by the *MKI67* gene.

through the exome sequencing approach adopted by TCGA,[28] which supports the maturity of biology-driven next-generation sequencing–targeted panels, such as the one we deployed.

The clinically relevant findings of the present work can be unfolded into three main categories. First, we identified alterations in one of three key genes of the PI3K pathway, *PIK3CA*, *PTEN*, and *AKT1*, in 50% of ILBC cases, each more frequently mutated in ER-positive/HER2-negative ILBC than in ER-positive/HER2-negative IDBC tumors. Whereas *PIK3CA* mutant cancers were associated with low proliferation rates, as defined by Ki-67, patients affected by *AKT1* mutated tumors (4.1%) were associated with a short-term risk of relapse.

Second, in a sufficiently large cohort of well-annotated primary ILBC, this study points out that the prevalence of *HER2* and *HER3* mutations is significantly higher than in primary IDBC, with 8.5% (35 of 413) of the investigated ILBC cases carrying an *HER2* and/or *HER3* mutation. The functional evidence of oncogenicity for the majority of mutations (71%)[16-21] together with the demonstration of transcriptional activation of the pathway in most *HER2* and *HER3* mutated tumors emphasize the actionable nature of these alterations. Overall, 60 (14.5%) tumors either were *HER2* amplified (as determined by fluorescent in situ hybridization) or carried an *HER2*/*HER3* mutation. This proportion of HER/ERBB pathway alterations was even higher (23.1%) in the mixed, non-classic subtype, which suggests that the treatment of nearly one-fourth of these aggressive tumors could be individualized. With the large number of ILBC cases in this study, we confirmed that *HER2* mutations occur at a higher frequency in ILBC than in invasive breast cancer overall.[29-32] Moreover, we point out a potential association between *HER2* mutations and short-term risk of relapse in breast cancer. The imminent results of the ongoing phase II trials that target patients with *HER2*-mutated, nonamplified breast cancer (NCT01670877 and NCT01953926) will shed more light on the clinical relevance of such genetic alterations.

Finally, we identified mutations in genes involved in the genomic action of ER, such as *GATA3* (7.3%) and *FOXA1* (9.0%),

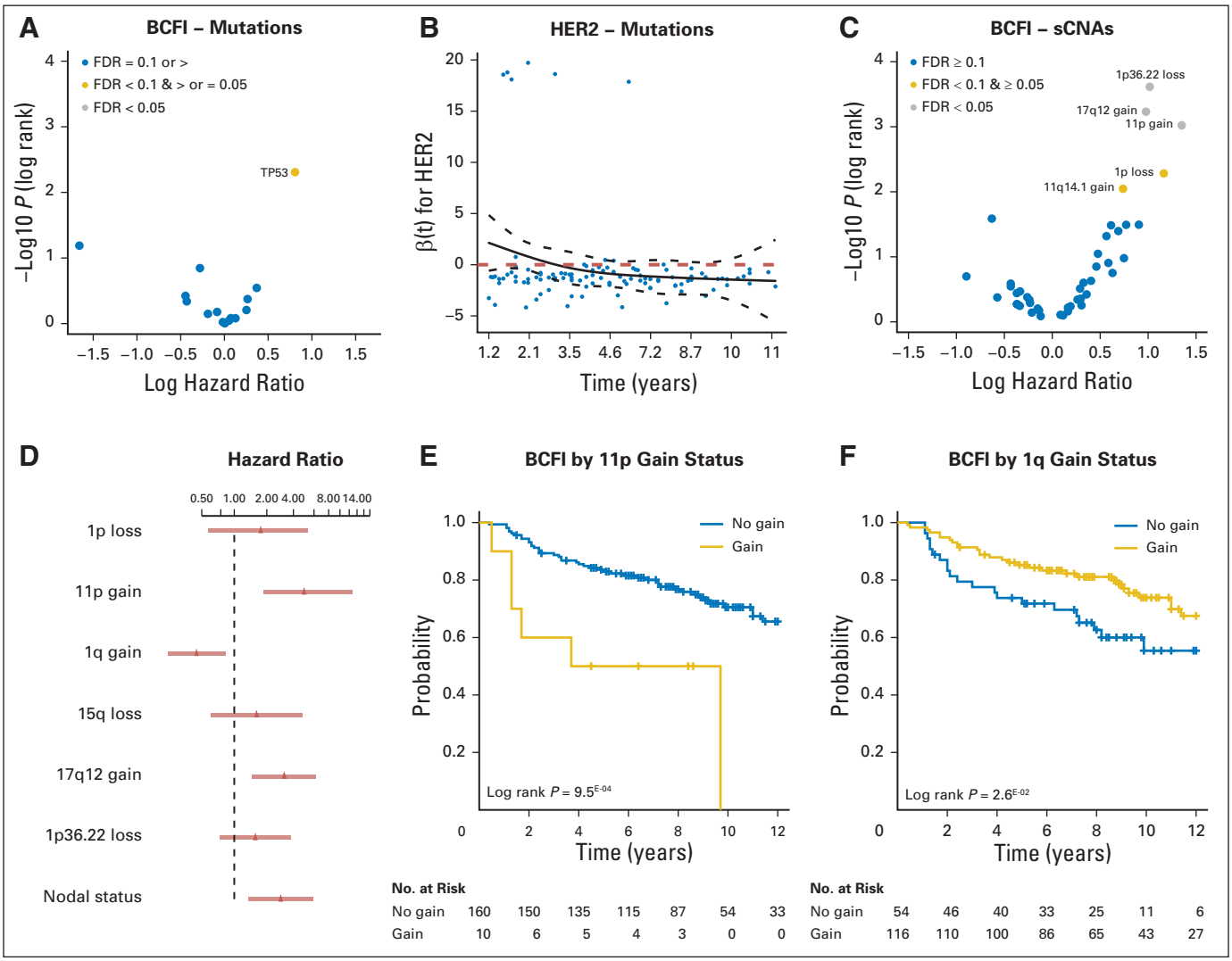

Fig 4. Breast cancer–free interval (BCFI) analyses. (A and B) Volcano plots of the logarithm of *P* values (−log10) *v* the hazard ratio (log) estimated by Cox proportional hazards regression on mutations (A) and copy number abberations (CNAs; C), with prevalence ≥ 2% and 5% in the set, respectively. Color codes are by FDR. (B) Plot of adjusted Schoenfeld residuals (*y*-axis) *v* time for *HER2* mutations. The solid line is a natural cubic spline fit of the data points, with dashed lines representing 95% confidence intervals. (D) Hazard ratio estimates for the variables retained by the stability selection with the lasso-penalized Cox proportional hazard regression model. Horizontal lines show the 95% confidence intervals for adjusted hazard ratios, while superimposed solid triangles represent point estimates. (E and F) Kaplan-Meier curves of CNAs that retained an independent prognostic role for BCFI on multivariable Cox proportional hazards regression. Blue lines represent the unaffected patients; gold lines represent patients with the assessed alterations. Small vertical lines indicate censored observations. sCNA, somatic copy number aberration; FDR, false discovery rate; HER2, human epidermal growth factor receptor 2.

which were present at significantly lower and higher frequencies than ER-positive/HER2-negative IDBC, respectively. Both FOXA1 and GATA3 are important for the expression of ESR1-target genes because of their key role in the transcription factor complex, which assembles at the estrogen response element of genes transcriptionally regulated by ESR1.[14] Therefore, we can expect these alterations to play a role in modulating response to hormone treatment. However, this will need to be investigated in the context of a clinical trial; the institutional and retrospective nature of the present cohort precluded such analysis. In addition to these mutations, copy number gains of *FOXA1* and 6q25.1 (*ESR1*) were further observed in 5.9% and 25.3% of the cases, respectively, which could also affect the ER-related transcriptional program. We have shown that *ESR1* copy number gains are associated with increased ESR1 mRNA together with an increased expression of the ESR1-target gene TFF1. Of note, this alteration had a higher prevalence in the aggressive solid histotype, with 44% of the cases presenting 6q25.1 gains compared with 17% of classic ILBC cases. Such a finding is especially relevant as a result of experimental data that suggested that tumors with increased *ESR1* copy number levels could show increased sensitivity to estradiol therapy.[33]

In conclusion, we provide evidence that the clinical and pathologic features that distinguish ILBC from IDBC are mirrored by a peculiar genomic landscape. Given the higher prevalence of *HER2*, *HER3*, and *AKT1* mutations in ILBC than in IDBC and the existence of drugs that target these alterations, we recommend the characterization of ILBC tumors for these genes. In addition, *FOXA1* mutations and *ESR1* gains urgently deserve dedicated clinical investigation, especially in the context of endocrine treatment. As our understanding of the molecular features that

characterize ILBC increases, we can begin to individualize the treatment of this disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Christine Desmedt, Giancarlo Pruneri, Giuseppe Viale, Christos Sotiriou

Financial support: Christine Desmedt, Christos Sotiriou
Administrative support: Christine Desmedt
Provision of study materials or patients: Denis Larsimont, Otto Metzger, Françoise Bertucci, Martine Piccart-Gebhart, Giuseppe Viale
Collection and assembly of data: Christine Desmedt, Gabriele Zoppoli, Gunes Gundem, Giancarlo Pruneri, Denis Larsimont, Debora Fumagalli, Françoise Rothé, Delphine Vincent, Naima Kheddoumi, Ghizlane Rouas, Samira Majjaj, Patrick Maisonneuve, Roberto Salgado, Thomas Van Brussel, Diether Lambrechts, Otto Metzger, Christine Galant, Françoise Bertucci, Giuseppe Viale, Peter J. Campbell
Data analysis and interpretation: Christine Desmedt, Gabriele Zoppoli, Gunes Gundem, Giancarlo Pruneri, Marco Fornili, David Brown, Sylvain Brohée, Peter Van Loo, Ron Bose, Martine Piccart-Gebhart, Giuseppe Viale, Elia Biganzoli, Peter J. Campbell, Christos Sotiriou
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Lakhani SR, Schnitt EI, Hoon Tan SJ, et al: WHO Classification of Tumors of the Breast (ed 4). Lyon, France IARC, 2012
2. Iorfida M, Maiorano E, Orvieto E, et al: Invasive lobular breast cancer: Subtypes and outcome. Breast Cancer Res Treat 133:713-723, 2012
3. Guiu S, Wolfer A, Jacot W, et al: Invasive lobular breast cancer and its variants: How special are they for systemic therapy decisions? Crit Rev Oncol Hematol 92:235-257, 2014
4. Metzger O, Giobbie-Hurder A, Mallon E, et al: Relative effectiveness of letrozole compared with tamoxifen for patients with lobular carcinoma in the BIG 1-98 trial. Cancer Res 72:S1-1, 2012 (abstr)
5. Knauer M, Gruber C, Dietze O, et al: Survival advantage of anastrozol compared to tamoxifen for lobular breast cancer in the ABCSG-8 study. Cancer Res 75:S2-06, 2015 (abstr)
6. Goncalves R, Warner WA, Luo J, et al: New concepts in breast cancer genomics and genetics. Breast Cancer Res 16:460, 2014
7. Carter SL, Cibulskis K, Helman E, et al: Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 30:413-421, 2012
8. Varela I, Tarpey P, Raine K, et al: Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 469:539-542, 2011
9. Ye K, Schulz MH, Long Q, et al: Pindel: A pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics 25: 2865-2871, 2009
10. Desmedt C, Fumagalli D, Pietri E, et al: Uncovering the genomic heterogeneity of multifocal breast cancer. J Pathol 236:457-466, 2015
11. Metzger-Filho O, Michiels S, Bertucci F, et al: Genomic grade adds prognostic value in invasive lobular carcinoma. Ann Oncol 24:377-384, 2013
12. Hudis CA, Barlow WE, Costantino JP, et al: Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: The STEEP system. J Clin Oncol 25:2127-2132, 2007
13. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al: An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 68:6084-6091, 2008
14. Liu Z, Merkurjev D, Yang F, et al: Enhancer activation requires trans-recruitment of a mega transcription factor complex. Cell 159:358-373, 2014
15. Gonzalez-Perez A, Jene-Sanz A, Lopez-Bigas N: The mutational landscape of chromatin regulatory factors across 4,623 tumor samples. Genome Biol 14:r106, 2013
16. Bose R, Kavuri SM, Searleman AC, et al: Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 3:224-237, 2013
17. Collier TS, Diraviyam K, Monsey J, et al: Carboxyl group footprinting mass spectrometry and molecular dynamics identify key interactions in the HER2-HER3 receptor tyrosine kinase interface. J Biol Chem 288:25254-25264, 2013
18. Jaiswal BS, Kljavin NM, Stawiski EW, et al: Oncogenic ERBB3 mutations in human cancers. Cancer Cell 23:603-617, 2013 [Erratum: Cancer Cell 25:543-544, 2014]
19. Littlefield P, Liu L, Mysore V, et al: Structural analysis of the EGFR/HER3 heterodimer reveals the molecular basis for activating HER3 mutations. Sci Signal 7:ra114, 2014
20. U M, Talevich E, Katiyar S, et al: Prediction and prioritization of rare oncogenic mutations in the cancer kinome using novel features and multiple classifiers. PLOS Comput Biol 10:e1003545, 2014
21. Wu JY, Yu CJ, Chang YC, et al: Effectiveness of tyrosine kinase inhibitors on "uncommon" epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. Clin Cancer Res 17:3812-3821, 2011
22. Desmedt C, Haibe-Kains B, Wirapati P, et al: Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res 14:5158-5165, 2008
23. Robinson JL, Holmes KA, Carroll JS: FOXA1 mutations in hormone-dependent cancers. Front Oncol 3:20, 2013
24. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490:61-70, 2012
25. Coniglio SJ, Zavarella S, Symons MH: Pak1 and Pak2 mediate tumor cell invasion through distinct signaling mechanisms. Mol Cell Biol 28:4162-4172, 2008
26. Peres J, Prince S: The T-box transcription factor, TBX3, is sufficient to promote melanoma formation and invasion. Mol Cancer 12:117, 2013
27. Tamura M, Gu J, Matsumoto K, et al: Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science 280:1614-1617, 1998
28. Ciriello G, Gatza ML, Beck AH, et al: Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163:506-519, 2015
29. Chmielecki J, Ross JS, Wang K, et al: Oncogenic alterations in ERBB2/HER2 represent potential therapeutic targets across tumors from diverse anatomic sites of origin. Oncologist 20:7-12, 2015
30. Fang Y, Jiang Y, Wang X, et al: Somatic mutations of the HER2 in metastatic breast cancer. Tumour Biol 35:11851-11854, 2014
31. Ross JS, Wang K, Sheehan CE, et al: Relapsed classic E-cadherin (CDH1)-mutated invasive lobular breast cancer shows a high frequency of HER2 (ERBB2) gene mutations. Clin Cancer Res 19:2668-2676, 2013
32. Lien HC, Chen YL, Juang YL, et al: Frequent alterations of HER2 through mutation, amplification, or overexpression in pleomorphic lobular carcinoma of the breast. Breast Cancer Res Treat 150:447-455, 2015
33. Li S, Shen D, Shao J, et al: Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Reports 4:1116-1130, 2013

Affiliations

Christine Desmedt, Gabriele Zoppoli, Denis Larsimont, Debora Fumagalli, David Brown, Françoise Rothé, Delphine Vincent, Naima Kheddoumi, Ghizlane Rouas, Samira Majjaj, Sylvain Brohée, Roberto Salgado, Martine Piccart-Gebhart, and Christos Sotiriou, Institut Jules Bordet; Christine Galant, Cliniques Universitaires Saint Luc, Brussels; Peter Van Loo, University of Leuven; Thomas Van Brussel and Diether Lambrechts, VIB Vesalius Research Center, Leuven, Belgium; Gabriele Zoppoli, University of Genoa and Istituto di Ricerca a Carattere Clinico-Scientifico San Martino-National Cancer Institute, Genoa; Giancarlo Pruneri, Patrick Maisonneuve, and Giuseppe Viale,

European Institute of Oncology; Marco Fornili and Elia Biganzoli, University of Milan, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Istituto Nazionale Tumori, Milan, Italy; Gunes Gundem and Peter J. Campbell, Wellcome Trust Sanger Institute, Cambridgeshire; Peter Van Loo, The Francis Crick Institute, London, United Kingdom; Ron Bose, Washington University School of Medicine, St Louis, MO; Otto Metzger, Dana-Farber Cancer Institute, Boston, MA; and François Bertucci, Institut Paoli-Calmettes, Marseille, France.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Genomic Characterization of Primary Invasive Lobular Breast Cancer

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or jco.ascopubs.org/site/ifc.

Christine Desmedt
No relationship to disclose

Gabriele Zoppoli
Travel, Accommodations, Expenses: Life Technologies

Gunes Gundem
No relationship to disclose

Giancarlo Pruneri
No relationship to disclose

Denis Larsimont
Travel, Accommodations, Expenses: Roche

Marco Fornili
No relationship to disclose

Debora Fumagalli
No relationship to disclose

David Brown
No relationship to disclose

Françoise Rothé
No relationship to disclose

Delphine Vincent
No relationship to disclose

Naima Kheddoumi
No relationship to disclose

Ghizlane Rouas
No relationship to disclose

Samira Majjaj
No relationship to disclose

Sylvain Brohée
No relationship to disclose

Peter Van Loo
No relationship to disclose

Patrick Maisonneuve
No relationship to disclose

Roberto Salgado
Travel, Accommodations, Expenses: Roche

Thomas Van Brussel
No relationship to disclose

Diether Lambrechts
Consulting or Advisory Role: Genentech, Sanofi, Bayer AG, Boehringer Ingelheim, Multiplicom
Speakers' Bureau: Eli Lilly, Roche
Research Funding: Genentech (Inst), Sanofi (Inst), Bayer AG (Inst)
Patents, Royalties, Other Intellectual Property: Named as an inventor on several patents related to response to antiangiogenesis

Ron Bose
Honoraria: Genentech
Consulting or Advisory Role: Genentech

Otto Metzger
Honoraria: Novartis, Eisai, AbbVie, Roche
Research Funding: Pfizer, Genentech, Eisai

Christine Galant
No relationship to disclose

François Bertucci
No relationship to disclose

Martine Piccart-Gebhart
Consulting or Advisory Role: AstraZeneca, Eli Lilly, Invivis Pharmaceutical, MSD, Novartis, Pfizer, Genentech, Synthon, Radius
Research Funding: Amgen (Inst), Astellas Pharma (Inst), AstraZeneca (Inst), Bayer (Inst), Eli Lilly (Inst), Invivis Pharmaceutical (Inst), MSD (Inst), Novartis (Inst), Pfizer (Inst), Genentech (Inst), Sanofi (Inst), Symphogen (Inst), Synthon (Inst), Verastem (Inst)

Giuseppe Viale
Consulting or Advisory Role: DakoCytomation, Genentech, AstraZeneca
Speakers' Bureau: Novartis
Travel, Accommodations, Expenses: Roche, Celgene

Elia Biganzoli
No relationship to disclose

Peter J. Campbell
Stock or Other Ownership: 14M Genomics
Consulting or Advisory Role: 14M Genomics

Christos Sotiriou
Consulting or Advisory Role: NanoString Technologies, Astellas Pharma, Vertex
Travel, Accommodations, Expenses: NanoString Technologies, Astellas Pharma, Vertex, Roche

Acknowledgment

We thank the patients who participated to this study as well as A. De Rose, M. Maetens, S. Martin, S. McLaren, and S. O'Meara for logistical, technical, and administrative assistance.