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Original contribution

HER2 somatic mutation analysis in breast cancer: correlation with clinicopathological features☆,☆☆,★

Qingqing Ding MD, PHD[a,*], Hui Chen MD, PHD[a], Bora Lim MD[b], Senthil Damodaran MD, PHD[b], Weiwei Chen MD, PHD[a], Debu Tripathy MD[b], Sarina Piha-Paul MD[c], Rajyalakshmi Luthra PHD[d], Funda Meric-Bernstam MD[c], Aysegul A. Sahin MD[a,*]

[a]*Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030*
[b]*Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030*
[c]*Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030*
[d]*Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030*

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Summary *HER2* mutations have been reported in approximately 2% of breast cancers. Regardless of HER2 overexpression or amplification status, breast cancer with *HER2* mutations may respond to HER2-targeted therapy. As *HER2* mutation is rare, the clinical and pathological features of *HER2*-mutated breast cancers, such as hormonal status, histological grade, and metastasis, remain poorly defined. Therefore, the identification of *HER2*-mutated breast cancer has clinical significance. We retrospectively screened patients with metastatic breast cancer in whom molecular profiling had been performed using next-generation sequencing from 2012 to 2015; we identified 18 patients with *HER2* mutation. Mutations were found on next-generation sequencing-based panels, including Ion AmpliSeq Cancer Hotspot, Oncomine, FoundationOne, and Guardant360. *HER2* mutations were identified in both the tyrosine kinase (n = 14) and extracellular (n = 4) domains. Of the 14 cases with tyrosine kinase domain mutations, 13 were estrogen receptor positive; the 4 cases with extracellular domain mutations were exclusively estrogen receptor negative. In addition, 11 of 14 patients with tyrosine kinase domain mutations had bone metastasis, whereas no patients with *HER2* extracellular domain mutations had bone metastasis. Histologically, 13 patients had invasive ductal carcinoma, 1 had metaplastic carcinoma, and 4 had invasive lobular carcinoma (ILC). All 4 ILCs were high grade and pleomorphic, and not only had an *HER2* mutation in the kinase domain but also had an *HER2* mutation involving the L755 site. Specific mutation sites may be involved in the pathogenesis of nonclassic ILC.

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* Corresponding authors.
E-mail addresses: qqding@mdanderson.org (Q. Ding), asahin@mdanderson.org (A. A. Sahin).

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1. Introduction

Breast cancer is the most common malignancy and the second most common cause of cancer death in women in the United State [1,2]. Breast cancers with HER2 overexpression or gene amplification are classified as HER2-positive carcinomas. These cancers account for approximately 20% of all breast carcinomas and are usually associated with high histological grade, large tumor size, and lymph node metastasis; patients with HER2-positive breast cancer tend to be younger, have a more aggressive clinical course, and have an increased risk of recurrence compared with those with HER2-negative breast cancer [3-6].

As HER2 plays a key role in breast cancer progression, it is an ideal target for specific therapeutic approaches. Trastuzumab, an antibody that specifically targets HER2, has been used to treat HER2-positive cancers, along with neoadjuvant and adjuvant chemotherapy, since 1998. Trastuzumab has greatly improved the outcome of HER2-positive breast cancer patients. Serial studies have shown that, in these patients, the pathological complete response rate following neoadjuvant chemotherapy plus trastuzumab is significantly higher than it is following only neoadjuvant chemotherapy [7,8]. Moreover, recent large long-term studies have shown that adjuvant chemotherapy with trastuzumab to treat early-stage HER2-positive breast cancer significantly improves overall survival and disease-free survival compared to chemotherapy alone [9,10]. In addition to trastuzumab, multiple other agents have been approved for clinical use, including pertuzumab, lapatinib, and TDM-1 [11,12].

Patients with HER2-negative tumors are typically not eligible for HER2-positive targeted therapy. Recent gene sequencing studies have identified *HER2* mutations in approximately 2% of breast cancers, most of which are not associated with *HER2* amplification or overexpression. However, some *HER2* mutations have stronger tyrosine kinase activity than does wild-type HER2, indicating the presence of HER2 activation without amplification or overexpression [13-16]. Regardless of HER2 overexpression or amplification status, breast cancers with *HER2* mutations may respond to HER2-positive targeted therapy.

As *HER2* mutation is rare, the clinical and pathological features of *HER2* mutation breast cancers, such as hormonal status, histological grade, and metastasis, remain poorly defined.

2. Materials and methods

2.1. Patient population

We retrospectively screened patients with metastatic breast cancer for whom molecular profiling had been performed by next-generation sequencing (NGS) from January 1, 2012, to December 31, 2015. Patients' clinicopathological features, including age, clinical and pathological stage, tumor histological type, tumor nuclear grade, and hormone and HER2 status, were evaluated to determine their correlation with NGS results. HER2 status was evaluated on the basis of the American Society of Clinical Oncology/College of American Pathologists guidelines; a 3+ on immunohistochemical staining or *HER2* amplification by fluorescence in situ hybridization (FISH) was considered positive for HER2. Estrogen receptor (ER) and progesterone receptor (PR) were considered positive if 1% or more of tumor cells were stained on immunohistochemical analysis [17].

2.2. Immunohistochemical analysis and FISH

We obtained patients' ER, PR, and HER2 expression and FISH-based *HER2* amplification status from their medical records. The monoclonal antibody ER clone 6F11 (Leica Biosystems, Buffalo Grove, IL), PR clone PgR 1294 (Dako, Carpinteria, CA), and HER2 clone e2-4001 (Thermo Fisher Scientific, Waltham, MA) were used to detect the α forms of ER, PR, and HER2, respectively. A FISH analysis was performed with a dual-color PathVysion HER2 DNA Probe kit (Abbott Molecular, Des Plaines, IL) using standard laboratory procedures according to the manufacturer's recommendations [18].

2.3. Next-generation sequencing

Hematoxylin and eosin–stained sections of formalin-fixed, paraffin-embedded tissue were reviewed for each sample to circle areas that were enriched for invasive tumors and ensure at least 20% tumor content. Microdissection was performed on corresponding consecutive unstained sections within the circled area for genomic DNA extraction and purification. The NGS panels AmpliSeq Cancer Hotspot v1 and v2 and Oncomine were run on an Ion Torrent Personal Genome Machine and Ion Proton, respectively (Thermo Fisher Scientific, Waltham, MA) [19-21]. Sequence alignment and analysis were performed using Torrent Suite software (Thermo Fisher Scientific, Waltham, MA). A minimum coverage depth per amplicon of 250 was required; a variant frequency of 10% and higher was considered positive. A variant frequency of less than 10% required additional confirmation by either orthogonal testing or repeat NGS. Normal matched control DNA was analyzed in an Oncomine panel but not in an AmpliSeq Cancer Hotspot panel. Synonymous substitution and variants that were known to be of germline origin, on the basis of laboratory-developed OncoSeek and dbSNP databases, were not reported in this study. Sample testing using FoundationOne and Guardant360 panels was performed at outside facilities.

2.4. Statistical analysis

The association between genetic alterations and clinical and biological characteristics was analyzed by χ^2 and Fisher exact tests. The level of significance was set at .05.

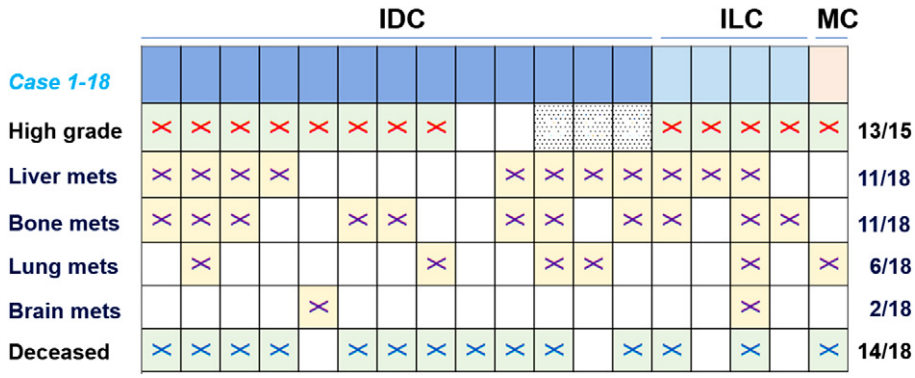

Fig. 1 Clinicopathological features of 18 cases of breast cancer with *HER2* mutation.

3. Results

3.1. Clinical characteristics and clinical courses of patients with *HER2* mutation

We identified 18 breast cancer patients with *HER2* mutations by NGS-based panels, including Ion AmpliSeq Cancer Hotspot (v1 and v2; n = 13), Oncomine (n = 1), FoundationOne (n = 3), and Guardant360 (n = 1). The NGS samples included 6 primary breast cancers and 14 metastatic tumors (6 from the liver; 3 from bones; and 1 each from the brain, lungs, contralateral breast, lymph nodes, and blood). The 18 breast cancers included 13 invasive ductal carcinomas (IDCs), 4 invasive lobular carcinomas (ILCs), and 1 metaplastic carcinoma (MC). Most patients (13 of 15, including 8 IDCs, 4 ILCs, and 1 MC) had high-grade tumors. Fourteen of 18 patients had multiple organ metastases and died of disease. The liver and bones were the most common metastasis sites, with 11 cases each. In addition, there were 6 cases of lung metastasis and 2 cases of brain metastasis (Fig. 1) (Supplemental Data 1).

3.2. Mutated *HER2* domains were associated with ER status and distant metastasis

HER2 mutations were identified in both the tyrosine kinase domain (n = 14) and extracellular domain (ECD) (n = 4). Tyrosine kinase domain mutations were found at the following locations: L755S (4), L755_E757delinsS (1), V777L (4), D769H (2), D769Y (1), I767M (1), and L841V (1) (n = 14). Of the 4 cases with mutations in the ECD, 2 were located at R47H, 1 at S22N, and 1 at V424I (Fig. 2). Most cases (13 of 14) with mutations in the tyrosine kinase domain were positive for ER, whereas the 4 cases with ECD mutations were exclusively ER negative, indicating a correlation between ER positivity and *HER2* mutation in the tyrosine kinase domain ($P = .0003$, Table 1). However, positive *HER2* amplification and overexpression were not related to the *HER2* mutation domain. *HER2* amplification was detected in 6 of 14 cases with mutations in the tyrosine kinase domain and 1 of 4 cases with mutations in the ECD ($P > .05$, Table 1). In addition, there were 3 triple-negative breast cancer cases that had *HER2* mutations in the ECD (2 R47H and 1 S22N).

Bone metastasis was associated with an *HER2* mutation in the kinase domain. All 11 cases with bone metastasis had an *HER2* mutation in the kinase domain; the 4 cases with an *HER2* mutation in the ECD were all negative for bone metastasis (Table 2, Fig. 3). However, liver metastasis was not related to the *HER2* mutation domain. There were 11 cases with liver metastasis; 9 had an *HER2* mutation in the kinase domain, and 2 had a mutation in the ECD (Table 2). In addition, histological grade and histological type were not associated with the *HER2* mutation domain. Of the 13 high-grade

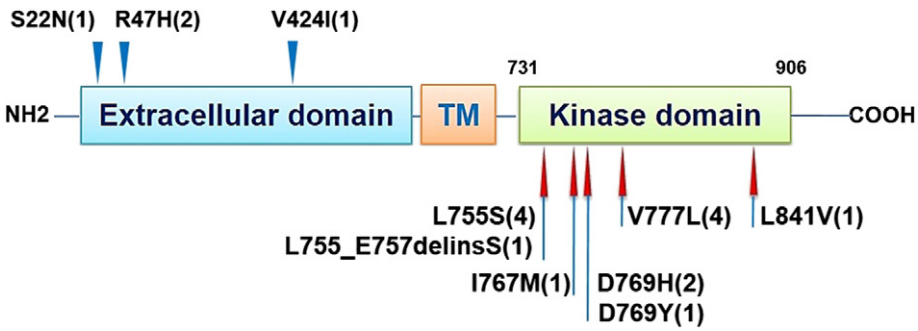

Fig. 2 *HER2* mutations were identified in both the tyrosine kinase domain and ECD. Eighteen breast cancer cases with *HER2* mutation including 14 mutations in the tyrosine kinase domain and 4 mutations in the ECD were identified by NGS-based panels.

Table 2 Statistical analysis of bone and liver metastases with *HER2* mutation domains

HER2 mutation	Kinase domain	ECD	*P*
Bone metastases +	11	0	.0045
Bone metastases –	3	4	
Liver metastases +	9	2	NS
Liver metastases –	5	2	

NOTE. Bone metastasis was associated with *HER2* mutation in the kinase domain.

Table 1 Statistical analysis of ER and HER2 status with *HER2* mutation domains

HER2 mutation	Kinase domain	ECD	*P*
ER+	13	0	.0003
ER–	1	4	
HER2+	6	1	NS
HER2–	8	3	

NOTE. Positive ER was associated with *HER2* mutations in the kinase domain.

breast cancers, 10 had an *HER2* mutation in the kinase domain and 3 in the ECD. Of the 13 IDCs, 10 had an *HER2* mutation in the kinase domain and 3 in the ECD. All 4 ILCs had an *HER2* mutation in the kinase domain.

3.3. *HER2* L755 mutation was associated with pleomorphic lobular carcinoma

All 4 ILCs were high grade and pleomorphic, and not only had an *HER2* mutation in the kinase domain but also had a mutation involving the L755 site (Table 3, Fig. 4); these comprised 80% of cases (4 of 5) with *HER2* mutations at the L755 site. Of the 12 cases with mutations at other sites, all were IDCs. These cases indicate that breast cancers with *HER2* L755 mutation are more likely to have lobular features.

Table 3 Statistical analysis of *HER2* mutation sites (L755 and non-L755) with breast cancer types

HER2 mutation	Nonclassic lobular carcinoma	Ductal carcinoma	*P*
L755	4	1	.002
Non-L755	0	12	

NOTE. Breast cancers with *HER2* L755 mutation were more likely to have lobular features.

3.4. Concurrent gene alterations

Most of the reviewed breast cancer patients with *HER2* mutations (14 of 18) had several different gene alterations; of these, 5 had more than 1 gene alteration. The most common gene alterations were *TP53* (n = 8) and *PIK3CA* (n = 7). Other miscellaneous genes included *CDH1*, *IDH2*, *SMAD4*, and *JAK3* (n = 1 each) (Fig. 5).

4. Discussion

Multiple novel *HER2* mutations in the ECD were identified in breast cancer patients. Specific mutation sites may be involved in the pathogenesis of nonclassic ILC, and the identification of these mutations may allow us to predict the development of site-specific metastases. Of the 14 cases with *HER2* mutations in the kinase domain, 13 developed ER-positive breast cancer and 11 had bone metastasis. Mutations in the ECD were seen exclusively in ER-negative breast cancer and were not associated with bone metastasis. To our knowledge, this was the first study that documented the association between the tyrosine kinase domain mutation of *HER2* and ER-positive breast cancer and bone metastasis. It is known that bone metastasis is common in ER-positive breast cancer cases, so the correlation of *HER2* mutation in tyrosine kinase domain with bone metastasis may be indirect, and mediated through ER. In addition, we found that specific mutations are involved in the pathogenesis of nonclassic

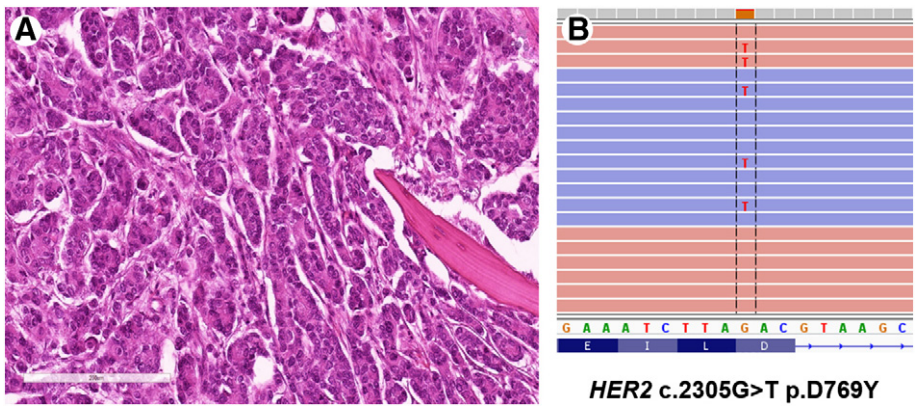

Fig. 3 Bone and liver were the most common metastasis sites in breast cancer patients with *HER2* mutations. A representative breast cancer bone metastasis case with *HER2* mutation in the kinase domain: A, The histological image (original magnification ×20) of a breast carcinoma bone metastasis with *HER2* mutation. B, The NGS result of a breast cancer case with *HER2* D769Y mutation in the kinase domain.

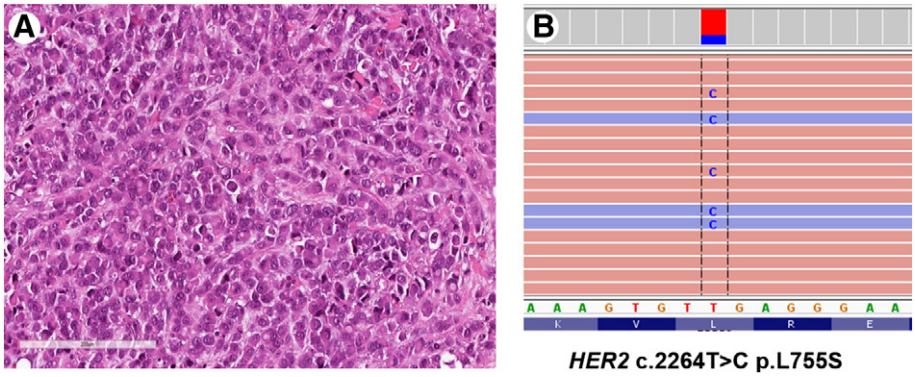

Fig. 4 Invasive lobular carcinomas with *HER2* mutation were high grade and pleomorphic. A representative ILC with *HER2* mutation at the L755 site: A, The histological image (×20) of a pleomorphic ILC with *HER2* mutation. B, The NGS result of an ILC with *HER2* L755S mutation in the kinase domain.

ILC, as the 4 cases with ILC all had an *HER2* mutation involving the L755 site within tyrosine kinase domain.

Receptor tyrosine kinase HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Overexpression of HER2 or the formation of a heterodimer between HER2 and other HER family members could result in the activation of HER2 kinase activity, including the initiation of a variety of oncogenic signaling pathways, such as the PI3K/AKT and MAPK pathways [22,23].

HER2 amplification has been identified in multiple human cancers besides breast cancer, including lung, gastric, and gynecological cancers, and is associated with an increase in recurrent disease with a poor prognosis [24-28]. In addition, *HER2* mutations have been observed in a small subset of lung and breast cancers. *HER2* mutations have been found in 1%-5% of lung adenocarcinomas and 2% of breast cancers, which exhibit activated HER2 signaling [13,16,29,30].

Recent reports have demonstrated *HER2* mutations in nonamplified *HER2* (negative HER2) breast cancers [13,16]. In our 18 cases with *HER2* mutations, only 7 (39%) exhibited *HER2* amplification; the other 11 cases did not. Most *HER2* mutations were in the tyrosine kinase domain (14 of 18 [78%]). In the kinase domain, several mutation sites that we observed, such as V777L and D769H, were found to be activating mutations with upregulated tyrosine kinase activity, resulting in activation of downstream oncogenic signalings such as PI3K/AKT and MAPK pathways [14,16]. Thus, even without *HER2* amplification, these mutations can drive tumorigenesis and tumor development through activated HER2 signaling in breast cancer. There were 4 cases with an *HER*2 mutation in the ECD; 3 were HER2 negative (not amplified). Recent studies have shown that alteration of the *HER2* ECD leads to increased dimerization of the HER2 and HER3 protein, which is also associated with activated HER2 signaling in the absence of *HER2* amplification [16,31]. We will further investigate the impact and molecular mechanisms of newly identified *HER2* mutations, including the 3 found in the ECD (R47H, S22N, and V424I) and the 1 found in the kinase domain (L841V).

Prolonged exposure to targeted therapies can cause secondary genomic alterations, such as *BCR-ABL* T315I mutation with imatinib in Chronic myelogenous leukemia (CML) and *EGFR* T790M mutation with gefitinib in lung cancer, leading to resistance to these targeted therapies [32-34]. All 18 of the reviewed cases had advanced breast cancer with multiple metastases, and 14 metastatic tumor samples from the liver, bones, brain, and lymph nodes were submitted for NGS; thus, we cannot rule out the possibility that the

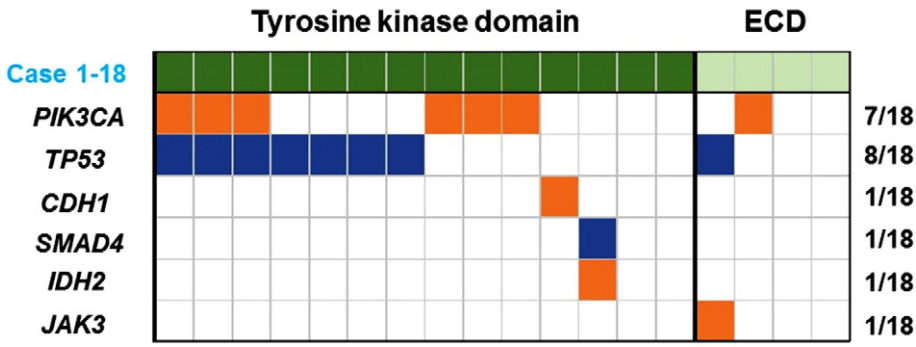

Fig. 5 Coexisting gene mutations with *HER2* mutations in breast cancer cases. Based on the NGS results, 14 of 18 breast cancer patients with *HER2* mutation had several different gene alterations. The most common gene alterations were *TP53* (n = 8) and *PIK3CA* (n = 7); other miscellaneous genes included *CDH1*, *IDH2, SMAD4*, and *JAK3* (n = 1 each).

detected *HER2* mutation is a late event (ie, the tumor's response to previous chemotherapy, hormonal therapy, or HER2-targeted therapy). However, taken together the variant frequency of *HER2* and coexisting mutations from sequencing data and the tumor fraction of tested samples, these HER2 mutations were likely oncogenic mutations driving the pathogenesis of breast cancer and not a passenger alteration arising from a subclonal population. The same mutations were detected in both primary and metastatic tumors in 2 patients in our study (Supplemental Data 1), further supporting the likelihood of these *HER2* mutations as a driver in the pathogenesis of breast cancer other than chemotherapy-induced mutation, and these *HER2* mutations are involved in tumor development and metastasis.

Several case reports and animal studies have shown that lung and breast cancers that harbor an activating *HER2* mutation are sensitive to HER2-targeting monoclonal antibodies (trastuzumab and pertuzumab) and tyrosine kinase inhibitors (afatinib and neratinib). Currently, there are ongoing clinical trials to evaluate the efficacy of targeted therapies using trastuzumab and neratinib in patients with *HER2* mutation [35-38]. On the basis of the outcome of these trials, the metastatic breast cancers with negative HER2 but with *HER2* mutation might benefit from alternative therapies including the tyrosine kinase inhibitors targeting to HER2. For mutations in the ECD, pertuzumab can be added to disrupt the dimerization of HER2 and HER3 and block downstream signaling. In addition, as the *PIK3CA* gene mutation is one of the most common alterations found with *HER2* mutations, it is possible that it is related to the resistance to HER2-targeted therapy in breast cancer [39,40]. As our knowledge and treatment regimen evolve in the future, NGS might become one of the tests to help us identify potential targets including *HER2* mutations for the patients without appropriate treatment strategy. A recent study showed that ER+ breast cancer with *HER2* mutation is not responsive with hormone therapy only, and dual blockade of the HER2 and ER pathways will help the treatment of ER+ *HER2* mutant breast cancers [41], so the identification of *HER2* mutation may help oncologists to redesign specific therapy.

In the current study, we identified 18 breast cancer patients with *HER2* mutation by NGS-based panels, most patients had high grade tumors, and liver and bone were the most common metastasis sites. *HER2* mutations were identified in both the tyrosine kinase domain (n = 14) and ECD (n = 4). Most cases with mutations in the tyrosine kinase domain were positive for ER (13 of 14) and associated with bone metastasis (11 of 14). In addition, all 4 ILCs with *HER2* mutation were high grade and pleomorphic, and breast cancers with *HER2* L755 mutation within tyrosine kinase domain were more likely to have lobular features.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2019.07.006.

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