IN THE SPOTLIGHT

Activating Mutations in *HER2*: Neu Opportunities and Neu Challenges

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Summary: Twenty-five years after the publication of seminal studies showing that *HER2* gene amplification and protein overexpression are oncogenic in breast cancer, Bose and colleagues report on the identification of activating *HER2* somatic mutations that, albeit rare, may determine the response of breast cancer cells to anti-HER2 agents. These results herald a new era for the potential use of existing HER2-targeted agents for the treatment of patients with *HER2*-mutant breast cancer. *Cancer Discov; 3(2); 145–7. ©2012 AACR.*

See related article by Bose et al., p. 224 (7).

The *HER2* (*ERBB2*) proto-oncogene is amplified and its product overexpressed in approximately 15% to 20% of breast cancers (1). The characterization of the impact of *HER2* gene amplification and overexpression on the behavior of breast cancers resulted in the development of targeted therapies for the treatment of patients with HER2-positive breast cancer (2). Furthermore, fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and immunohistochemical assays for HER2 have been introduced as predictive biomarkers to determine which patients may benefit from therapy with anti-HER2 agents (i.e., the monoclonal antibodies trastuzumab and pertuzumab and the tyrosine kinase inhibitor lapatinib; refs. 1–4).

Over the past two decades, preclinical and clinical studies have revealed that breast cancer cells harboring *HER2* amplification and overexpression are "addicted" to (i.e., physiologically dependent on) the continued activation of HER2 and its downstream signaling pathways, and have identified multiple mechanisms of resistance to HER2-targeted agents (reviewed in ref. 2). In breast cancer, in contrast with non–small cell lung cancers, in which *HER2* activation was shown to be preferentially the result of somatic mutations (5, 6), the potential therapeutic opportunities offered by rare *HER2* somatic mutations (6) was largely neglected, given the high prevalence of *HER2* gene amplification.

In this issue of *Cancer Discovery*, Bose and colleagues (7) report on the identification of 27 *HER2* somatic mutations, of which 17 unique mutations, in 1.67% (25 of 1,499) of the breast cancers analyzed. Most cases harboring *HER2* mutations (20 of 25; 80%) were considered to be HER2-negative by means of immunohistochemistry and FISH, whereas 3 were HER2-positive and 2 were equivocal according to current guidelines for HER2 assessment (1). Different mutations were shown to result in different phenotypes and sensitivity to specific anti-HER2 agents (Fig. 1). Taken together, these tantalizing findings provide a rationale for the development of HER2 sequencing-directed clinical trials assessing the treatment of patients with *HER2*-mutant breast cancer using HER2-targeted agents.

Massively parallel sequencing studies have revealed that the repertoire of somatic mutations in breast cancers is complex, with a limited number of genes that are highly recurrently mutated, even fewer highly recurrent hotspot mutations, and a plethora of genes affected by mutations in a small minority (i.e., 1%–3%) of breast cancers (8). In this context, with so many mutations being found in a minority of breast cancers, concepts that are germane for the translation of massively parallel sequencing results into benefits for patients with cancer include which mutations constitute drivers of the disease and which mutations confer resistance to specific therapeutic agents. Will functional analyses of individual mutations be required to establish their biologic relevance or can one extrapolate the impact of mutations on the functions of a gene based on their recurrence rates, or on the analyses of related genes, or the same gene in other cancers?

Bose and colleagues (7) decided to face the challenge of investigating the functional impact of 13 *HER2* somatic mutations, and the results of this Herculean task could not be more fascinating. The *HER2* somatic mutations identified in breast cancers clustered around 2 domains of the gene: 20% mapping to exon 8, affecting amino acid residues 309–310 of the HER2 extracellular domain, and 68% mapping to exons 19–20, affecting residues 755–781 of the tyrosine kinase domain (7). The likelihood of these *HER2* mutations to affect tyrosine kinase activity was assessed by comparison with known activating *HER2*, *EGF receptor (EGFR)*, and *anaplastic lymphoma kinase (ALK)* mutations in other tumor types and protein structure visualization. On the basis of this analysis, some of the *HER2* mutations identified in breast cancer were suggested to be activating mutations (e.g., the *HER2* in-frame deletion of amino acids 755–759 was similar to the *EGFR* exon 19 deletions known to be drivers in non–small cell lung cancer), whereas others were suggested to mediate resistance to some modalities of HER2 inhibition (e.g., L755S), and some were of unknown significance.

Functional characterization of 13 *HER2* somatic mutations by means of *in vitro* kinase assays and HER2/EGFR signaling pathway analyses, as well as studies on the effect of these

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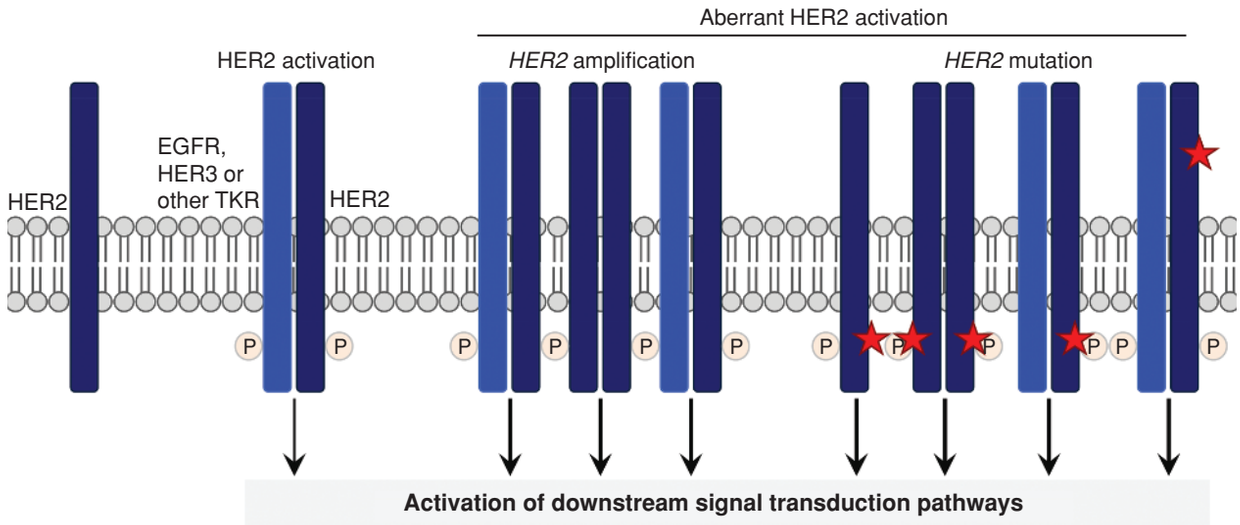

B

Mutation	HER2 domain	Number of cases	HER2 status	Functional characterization	Activating mutation	Lapatinib IC_{50} (nmol/L)
G309A	Extracellular	1	–	P	Yes	470 ± 50
G309E	Extracellular	1	+	NP	Yes¶	NP
S310F*	Extracellular	3	–	NP	Yes¶	NP
R678Q**	Juxtamembrane	1	–	P	No	NP
L755S*	Kinase	6	5–, 1 Eq	P	No	>10,000
L755W**	Kinase	1	–	NP	No	NP
del755-759	Kinase	2	–	P	No	660 ± 90
del.755-759+S760A	Kinase	1	–	P	No	NP
I767M	Kinase	1	–	P	No	NP
D769H	Kinase	1	+	P	Yes	980 ± 950
D769Y	Kinase	1	–	P	Yes	NP
V777L	Kinase	3	2–, 1 Eq	P	Yes	1,040 ± 570
P780-Y781insGSP	Kinase	1	–	P	Yes	NP
Y835F	Kinase	1	–	P	No	NP
V842I	Kinase	1	–	P	Yes	650 ± 210
R896C	Kinase	1	–	P	Yes	NP
G1201V	Kinase	1	+	NP	No	NP

Figure 1. *HER2* somatic mutations in breast cancer. **A,** diagrammatic representation of the different mechanisms of HER2 activation in breast cancer, including *HER2* gene amplification and protein overexpression, and activating mutations (red star) affecting either the HER2 tyrosine kinase domain or the extracellular domain. **B,** summary of characteristics of the *HER2* somatic mutations reported by Bose and colleagues (7). * and ** denote mutations found in the same breast cancer; –, negative; +, positive; ¶, described as activating mutation by Greulich and colleagues (5); Eq, equivocal according to current guidelines (1); NP, not performed; P, performed. HER2 status according to FISH and/or immunohistochemistry. Note that one case harboring the V777L mutation and another harboring the L755S mutation could be interpreted as equivocal following the current American Society of Clinical Oncology and College of American Pathologists guidelines for HER2 status assessment (1); however these cases were shown not to harbor *HER2* gene amplification by orthogonal methods (7).

mutations on cell growth using *in vitro* and *in vivo* assays, revealed that 7 of these mutations are activating and probably constitute *bona fide* driver events (i.e., G309A, D769H, D769Y, V777L, P780-Y781insGSP, V842I, and R896C; Fig. 1; ref. 7). Elegant *in vitro* models were used to test the impact of these mutations on the response to HER2-targeted agents, including trastuzumab, lapatinib, and neratinib. These experiments confirmed that the *HER2* L755S mutation, found in 6 of 25 patients, is likely to result in resistance to the reversible tyrosine kinase inhibitor lapatinib, but cells harboring this mutation remain sensitive to the irreversible inhibitor neratinib (7). In addition, 2 mutations affecting the *HER2* extracellular domain (G309E and S310F) have recently been shown to have activating properties *in vitro* (5).

The experiments carried out by Bose and colleagues (7) and others (5) show that predicting the biologic impact of a

mutation in a rather well known oncogene is by no means a trivial task. Although the mutations analyzed clustered around 2 specific HER2 domains, 6 were shown not to cause any detectable phenotype, whereas others were activating, resulted in drug resistance, or were neomorphic (i.e., del755–759 led to a phenotype characterized by increased phosphorylation of EGFR and HER3, two of HER2's dimerization partners). Hence, inferring their behavior based on recurrence, on whether they affected highly conserved regions, or by analogy with mutations in other tyrosine kinases may not be sufficient (7). Although it is possible that in the future, computational biology algorithms may reliably differentiate between passenger and driver mutations, this study (7) shows that, currently, good old molecular biology assays are still required.

The findings reported by Bose and colleagues (7) have a profound impact on our understanding of breast cancer. First, tumors considered to be HER2-negative according to current guidelines (1) may still be "addicted" to HER2 signaling due to *HER2* activating mutations; hence, the population of breast cancer patients who may benefit from anti-HER2 agents, in particular tyrosine kinase inhibitors, is likely to expand. Second, these *HER2* activating mutations may in part explain the subset of patients with breast cancer who benefit from anti-HER2 agents despite having HER2-negative disease (9). Third, if prospective clinical trials confirm that patients whose breast cancers harbor *HER2* activating mutations benefit from anti-HER2 therapies, then the companion diagnostics for these therapeutic agents will also fundamentally change. In fact, the findings reported by Bose and colleagues (7) herald a new era for breast cancer patient management and suggest that the days of defining HER2 status solely by FISH/CISH and immunohistochemistry are likely to be numbered. It is plausible that HER2 clinical testing will evolve into a system in a way akin to that used for the diagnostic and predictive workup of lung cancers, with *HER2* gene sequencing (and sequencing of other genes known to result in resistance to anti-HER2 agents) inevitably being included in the standard diagnostic armamentarium of breast cancer pathologists. The results described by Bose and colleagues (7), however, should not be perceived necessarily as practice changing but rather as the cornerstone for the development of clinical trials to test whether patients with *HER2*-mutant breast cancers are responsive to HER2-targeted drugs, and if so, which mutations are predictive of sensitivity to which agent. It is difficult to conceive a better example of breast cancer precision medicine in 2013.

A few points, however, still need further exploration. The role of coexisting *HER2*-activating mutations and gene amplification in the same tumor has yet to be elucidated, given that 3 patients with *HER2*-mutant breast cancer, including one harboring the D769H mutation, were HER2-positive by FISH/immunohistochemistry. Another interesting observation was that one of the *HER2* activating mutations was restricted to 16% of the alleles sequenced (7), potentially suggesting that this mutation was restricted to a subclone of the neoplastic cells. Given the burgeoning data on the prevalence of intratumor genetic heterogeneity and its impact on drug resistance (10), it is plausible that if post–anti-HER2 therapy breast cancer samples were sequenced, an even higher prevalence of *HER2* mutations would be identified. Given that some *HER2* somatic mutations predict sensitivity and others predict resistance to anti-HER2 agents, the development of molecular biomarkers to define the patient population to be enrolled in clinical trials will have to be carefully considered.

More than 6,500 articles on HER2 in breast cancer have been published; however, only now have we come to terms with the fact that an old oncogene in a rather well characterized cancer type may be activated through a known mechanism that had not been previously carefully considered. The advent of massively parallel sequencing has given us new ways of approaching well-known questions, and the results from Bose and colleagues (7) do remind us of the old Proustian maxim that the voyage of discovery lies not in finding new landscapes but in having new eyes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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REFERENCES

1. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–45.
2. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol 2012;9:16–32.
3. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012;366:109–19.
4. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.
5. Greulich H, Kaplan B, Mertins P, Chen TH, Tanaka KE, Yun CH, et al. Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. Proc Natl Acad Sci U S A 2012;109:14476–81.
6. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, et al. Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. Clin Cancer Res 2006;12:57–61.
7. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, et al. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013;3:224–37.
8. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
9. Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. N Engl J Med 2008;358:1409–11.
10. Turner NC, Reis-Filho JS. Genetic heterogeneity and cancer drug resistance. Lancet Oncol 2012;13:e178–85.