Molecular Diagnostics

a report by

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The human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor (EGFR) family of transmembrane protein receptor tyrosine kinases, which mediate cell growth, differentiation and survival in normal and abnormal breast cells. HER2 over-expression, which is observed in approximately 20–25% of human breast cancers, confers significant prognostic and predictive information.[1] Specifically, HER2 over-expression is associated with an increased risk of breast cancer recurrence and poor outcomes compared with non-over-expressing cohorts.[1,2] Furthermore, HER2 is an important predictive factor not only for clinical responses to the recombinant HER2 targeted monoclonal antibody trastuzumab (Herceptin®) but also to other systemic agents and hormonal therapy. Consequently, accurate determination of HER2 status is clinically relevant, not only for determination of eligibility for trastuzumab-based therapy but also for decisions regarding other systemic strategies. The issues and controversies surrounding HER2 status determination will be reviewed here.

HER2 Biology

HER2 (or ErbB2 or HER2neu) is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases which mediate cell growth, differentiation and survival through complex signal transduction pathways. Signalling cascades are initiated with the binding of an as yet unspecified ligand to the HER2 receptor. Ligand binding induces homodimerisation of the HER2 receptor with other HER2 receptors or heterodimerisation with other members of the EGFR family. Receptor dimerisation permits phosphorylation of intracellular tyrosine kinase residues and the consequent initiation of complex downstream signalling pathways, which have not yet been completely elucidated.[3]

Methods of HER2 Detection

HER2 status is most frequently determined by either immunohistochemistry (IHC) or fluorescence *in situ* hybridisation (FISH). Since the HER2 protein is present in all breast epithelial cells, the HER2 IHC assay is unique from many other IHC assays in that it is a semi-quantitative, rather than qualitative, method of protein level determination. Currently, two IHC assays are US Food and Drug Administration (FDA)-approved for HER2 status determination. The DAKO HercepTest® and Ventana PATHWAY® IHC assays utilise HER2-directed antibodies and chemical detection methods to identify HER2 proteins, typically on formalin-fixed, paraffin-embedded tissue samples.[4,5] IHC tests are scored on a scale of 0 to 3+ based on the pathologistís interpretation of staining intensity (see *table 1*). The second FDA-approved method of HER2 detection is FISH, whereby amplification of HER2 gene copies is evaluated using fluorescently labelled probes that are complimentary to the gene itself or the centromere of chromosome 17 on which the target gene is located. The PathVysion® method reports an average ratio of HER2 gene copy number to chromosome 17 copy number per cell with ratios of 1.8 to 2.2 considered 'borderline'.[6]

The INFORM® method uses a single HER2 gene probe and results are reported as the average number of copies per cell, with values of 4-6 considered 'borderline'.[7] The advantage of FISH compared with IHC testing is a more objective scoring system. However, FISH testing has the disadvantages of higher cost, longer times required for slide scoring, specific equipment requirements and the inability to preserve slides for storage or later review. Another method of HER2 status determination is chromogenic *in situ* hybridisation (CISH), which combines the advantages of IHC (lower cost and light microscopy) and FISH (DNA targeting, subjective scoring, internal control). The CISH method recently demonstrated 97% concordance with FISH testing for HER2 status determination but is not yet FDA-approved.[8]

Standardisation Issues in HER2 Testing

Accurate HER2 status determination is of critical clinical importance, given that a false negative result

denies patients the potential benefits of trastuzumab therapy while a false-positive result unnecessarily exposes patients to the potential toxicity of trastuzumab therapy, including cardiotoxicity. However, standardisation of HER2 status determination has been difficult. This issue was highlighted in a central review of early participants in the recently reported National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 study of adjuvant trastuzumab. Investigators demonstrated that 18% of community-based IHC determinations could not be confirmed by centralised IHC or FISH testing.[9] However, the NSABP investigators subsequently demonstrated concordance rates approaching 98% among laboratories performing high volumes of HER2 testing when tumours reported as 3+ by IHC were reanalysed by IHC and FISH at a central NSABP laboratory.[10] Consequently, only certified laboratories have been permitted to perform HER2 testing on patients accrued to NSABP adjuvant clinical trials of HER2-targeted therapy. Similarly, the poor concordance of 74% between local and central laboratories for patients enrolling in the Intergroup N9831 adjuvant trastuzumab clinical trial led to subsequent modifications in eligibility.[11]

The Breast Cancer International Research Group (BCIRG) investigators also demonstrated a 79% concordance rate between outside/local HER2 determination by IHC and centralised determinations by FISH but a 92% concordance rate between outside/local and centralised FISH determinations.[12] Consequently, methods for HER2 determination have varied between the pivotal adjuvant trastuzumab studies. The NSABP B31, Intergroup N9831 and HERceptin Adjuvant (HERA) eligibility criteria defined HER2 over-expression as 3+ by IHC or FISH-positive while the Breast Cancer International Research Group (BCIRG) 006 investigators used FISH methodology alone to define eligibility.[13,14,15] The clinical relevance of these differences in HER2 determination have not yet been determined.

Clinical Relevance of Accurate HER2 Status Determination

HER2 Status as a Prognostic Factor

Historically, HER2 over-expression has been associated with adverse outcomes among trastuzumab-naïve breast cancer patients.[1,16] However, the independent prognostic impact of HER2 status has not been consistent across studies, with several studies reporting prognostic impact on univariate analysis, but not multivariate analysis, and several studies demonstrating no correlation between HER2 status and outcomes.[17-23] These discrepancies may reflect variations in HER2 status determination across studies.[3,24-26] Furthermore, the prognostic impact of HER2 status has diminished with the advent of HER2 targeted therapy.[13,14,15]

Table 1. IHC (HercepTestTM) Scoring of HER2 Over-Expression

Staining pattern	Score	Interpretation
No staining	0	Negative
Faint incomplete staining of cell membrane in >10% of tumor cells	1+	Trace Negative
Weak to moderate complete staining of cell membrane in >10% of tumor cells	2+	Weak Positive
Strong complete staining of cell membrane in >10% of tumor cells	3+	Positive

HER2 Status as a Predictive Factor

HER2 status is also an important predictive factor, not only for clinical responses to trastuzumab but also for responses to other systemic agents. For example, enhanced sensitivity has been observed in models where trastuzumab is administered in combination with taxanes or vinorelbine.[27,28] In a recent study among pre-menopausal women, HER2 over-expression was also associated with increased clinical responsiveness to an adjuvant anthracycline-containing regimen compared with a non-anthracycline regimen.[29] This enhanced sensitivity is postulated to reflect co-amplification HER2 and topoisomerase II-alpha, the DNA replication and recombination enzyme targeted by anthra-cyclines.[30,31] However, co-amplification studies have not demonstrated consistent results.

HER2 status may also confer important predictive information about response to hormone therapy. For example, HER2-positive breast cancers appear to be relatively resistant to tamoxifen therapy, but not aromatase inhibitor therapy, although again, these studies have not demonstrated consistent results.[32-36] However, in one recently reported randomised clinical trial, significant progression-free survival (PFS) benefits were observed with the addition of trastuzumab to anastrazole among post-menopausal women with oestrogen receptor-positive HER2-positive metastatic breast cancer.[37] Thus, accurate HER2 determination may have important clinical implications beyond decisions regarding trastuzumab therapy.

Conclusion

HER2 status determination is an integral component of clinical decision-making, not only for decisions about trastuzumab therapy, but also potentially for other systemic and hormonal therapeutic strategies.

However, clinical decisions based on HER2 determination have been complicated by inconsistencies in reporting between laboratories and the variations in HER2 determination methodology between clinical trials. Furthermore, there is no clinical information available on the potential benefit of HER2-targeted therapy among patients with HER2 positive breast cancer by FISH but IHC scores of 0 or 1+. Similarly, there is little information regarding the potential benefit of trastuzumab in patients with FISH-negative but IHC 3+ breast cancers. Furthermore, whether a relationship exists between the extent of HER2 gene amplification or protein over-expression and degree of clinical benefit has not yet been determined. Recently, FISH assays have gained popularity as a result of the growing body of evidence suggesting that HER2 status by FISH may more accurately predict response to trastuzumab-based therapy when compared with IHC determinations.

At present, the American Society of Clinical Oncology (ASCO) and National Comprehensive Care Network (NCCN) recommend HER2 testing for all patients with invasive breast cancer.[38,39] The NCCN has also developed algorithm-based recommendations for HER2 testing that include FISH confirmation for IHC 2+ determinations at laboratories which meet quality assurance standards for HER2 testing methodology. Certainly, as clinical decision-making is becoming increasingly tailored to the individual and the specific biology of their cancer, the clinical relevance and methodology of accurate HER2 determination will continue to be an active area of investigation. ■

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References

1. *Slamon DJ, Clark GM, Wong SG, et al., "Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene",* Science *(1987);235: pp. 177–182.*
2. *Hayes DF, Thor AD, "C-erbB-2 in breast cancer: development of a clinically useful marker",* Semin Oncol *(2002);29: pp. 231–245.*
3. *Ross JS, Fletcher JA, Linette GP, et al., "The HER2/neu gene and protein in breast cancer 2003: biomarker and target of therapy",* The Oncologist *(2003);8: pp. 307–325.*
4. *DAKO HercepTest® [Package insert]. Carpinteria,Calif: DAKO Corp., (2004).*
5. *Pathway® HER2 [Package insert]. Tucson, Ariz: Ventana Medical Systems, Inc. (2004).*
6. *PathVysion® HER2/neu method [Package insert]. Downers Grove, Ill. Vysis, Inc. (2001).*
7. *INFORM® [Package insert]. Tucson, Ariz: Ventana Medical Systems, Inc. (2001).*
8. *Hanna W, "Testing for HER2 status",* Oncology *(2001);61: pp. 22–30.*
9. *Paik S, Bryant J, Tan-Chiu E, et al., "Real-world performance of Her2 testing - National Surgical Adjuvant Breast and Bowel Project Experience",* J Natl Cancer Inst *(2002);94: pp. 852–854.*
10. *Paik S, Tan-Chiu E, Bryant J, et al., "Successful quality assurance program for Her2 testing in the NSABP trial for Herceptin",* Breast Cancer Res and Treat *(2002);76(Suppl 1): pp. S31.*
11. *Roche PC, Suman VJ, Jenkins RB, et al., "Concordance between local and central laboratory HER2 testing in the Breast Intergroup Trial N9831",* J Natl Cancer Inst *(2002);94: pp. 855–857.*
12. *Press MF, Sauter G, Bernstein L, et al., "Diagnostic evaluation of HER2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials",* Clin Cancer Res *(2005);11: pp. 6598–6607.*
13. *Romond EH, Perez EA, Bryant J, et al., "Trastuzumab plus Adjuvant Chemotherapy for Operable HER2-Positive Breast Cancer",* N Engl J Med *(2005);353(16): pp. 1673–1684.*
14. *Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al., "Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer",* N Engl J Med *(2005);353(16): pp. 1659–1672.*
15. *Slamon D, Eiermann W, Pienkowski RN, et al., "Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC-T) with doxorubicin and cyclophosphamide followed by docetaxel and*

trastuzumab (AC-TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study", 28th San Antonio Breast Cancer Symposium *(2005); Abstract 1.*

16. *Paik S, Hazan ER, Sass RE, et al., "Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in primary breast cancer",* J Clin Oncol *(1990);8: pp. 103–112.*
17. *Tsuda H, Hirohashi S, Shimosato Y, et al., "Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast carcinoma. A retrospective analysis of 176 cases",* Cancer *(1990);65: pp. 1794–1800.*
18. *Borg A, tendon AK, Sigurdsson H, et al., "HER-2/neu amplification predicts poor survival in node-positive breast cancer",* Cancer Res *(1990);50: pp. 4332–4337.*
19. *Rilke F, Colnaghi MI, Cascinelli N, et al., "Prognostic significance of HER-2/neu expression in breast cancer and its relationship to other prognostic factors",* Int J Cancer *(1991);49: pp. 44–49.*
20. *Noguchi M, Koyasaki M, Ohta N, et al., "c-erbB-2 oncoprotein expression versus internal mammary lymph node metastases as additional prognostic factors in patients with axillary lymph node-positive breast cancer",* Cancer *(1992);69: pp. 2953–2960.*
21. *van de Vijver MJ, Peterrse JL, Mooi WJ, et al., "Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer",* N Engl J Med *(1988);319: pp. 1239–1245.*
22. *Heintz NH, Leslie KO, Rogers LA, et al., "Amplification of the c-erbB-2 oncogene and prognosis of breast adenocarcinoma",* Arch Pathol Lab Med *(1990);114: pp. 160–163.*
23. *Clark GM, McGuire WL, "Follow-up study of HER-2/neu amplification in primary breast cancer",* Cancer Res *(1991);51: pp. 944–948.*
24. *Pauletti G, Dandekar S, Rong H, et al., "Assessments of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry",* J Clin Oncol *(2000);18: pp. 3651–3664.*
25. *Volpi A, Nanni O, DePaola F, et al., "HER-2 expression and cell proliferation: prognostic markers in patients with node-negative breast cancer",* J Clin Oncol *(2003);21: pp. 2708–2712.*
26. *Schmidt M, Lewark B, Kohlschmidt N, et al., "Long-term prognostic significnce of HER-2/neu in untreated node-negative breast cancer depends on the method of testing",* Breast Cancer Res *(2005);7: pp. R256–R266.*
27. *Marty M, Cognetti F, Maraninchi D, et al., "Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group",* J Clin Oncol *(2005);23(19): pp. 4265–4274.*
28. *Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al., "Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer",* N Engl J Med *(2006);354(8): pp. 809–820.*
29. *Pritchard KI, Shepherd LE, O'Malley FP, et al., "HER2 and responsiveness of breast cancer to adjuvant chemotherapy",* N Engl J Med *(2006);354(20): pp. 2103–2111.*
30. *Tanner M, Isola J, Wiklund T, et al., "Topoisomerase IIalpha gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian Breast Group Trial 9401",* J Clin Oncol *(2006);24(16): pp. 2428–2436.*
31. *O'Malley FP, Chia S, Tu D, et al., "Prognostic and predictive value of topoisomerase II alpha in a randomized trial comparing CMF to CEF in premenopausal women with node positive breast cancer (NCIC CTG MA.5)",* J Clin Oncol *2006 ASCO Annual Meeting Proceedings (2006);24: Abstract 533.*
32. *Schiff R, Chamness GC, Brown PH, "Advances in breast cancer treatment and prevention: preclinical studies on aromatase inhibitors and new selective estrogen receptor modulators (SERMs)",* Breast Cancer Res *(2003);5(5): pp. 228–231.*
33. *Ellis MJ, Coop A, Singh B, et al., "Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial",* J Clin Oncol *(2001);19(18): pp. 3808–3816.*
34. *Elledge RM, Green S, Ciocca D, et al., "HER2 expression and response to tamoxifen in estrogen receptor-positive breast cancer: a Southwest Oncology Group Study",* Clinical Cancer Res *(1998);4: pp. 7–12.*
35. *Paik S, Shak S, Tang G, et al., "A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer",* N Engl J Med *(2004);351(27): pp. 2817–2826.*
36. *Berry DA, Muss HB, Thor AD, et al., "HER2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer",* J Clin Oncol *(2000);18: pp. 3471–3479.*
37. *Kaufman B, et al., "Trastuzumab plus anastrozole prolongs progression-free survival in postmenopausal women with HER2 positive, hormone-dependent metastatic breast cancer (MBC)",* Ann Oncol *(2006);17: Abstract LBA2.*
38. *Bast RC Jr, Ravdin P, Hayes DF, et al., "2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines for the American Society of Clinical Oncology",* J Clin Oncol *(2001);19: pp. 1865–1878.*
39. *NCCN® Practice Guidelines in Oncology – v.2.2005: Breast Cancer. National Comprehensive Cancer Network. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf*