

HER2 Testing in Breast Cancer: Opportunities and Challenges

Annette Lebeau

Pathologisches Institut, LMU München, Germany

Key Words

HER2 · Breast cancer · Background, molecular

Summary

Human epidermal growth factor receptor 2 (HER2) is overexpressed in 15–25% of breast cancers, usually as a result of HER2 gene amplification. Positive HER2 status is considered to be an adverse prognostic factor. Recognition of the role of HER2 in breast cancer growth has led to the development of anti-HER2 directed therapy, with the humanized monoclonal antibody trastuzumab (Herceptin®) having been approved for the therapy of HER2-positive metastatic breast cancer. Clinical studies have further suggested that HER2 status can provide important information regarding success or failure of certain hormonal therapies or chemotherapies. As a result of these developments, there has been increasing demand to perform HER2 testing on current and archived breast cancer specimens. This article reviews the molecular background of HER2 function, activation and inhibition as well as current opinions concerning its role in chemosensitivity and interaction with estrogen receptor biology. The different tissue-based assays used to detect HER2 amplification and overexpression are discussed with respect to their advantages and disadvantages, when to test (at initial diagnosis or pre-treatment), where to test (locally or centralized) and the need for quality assurance to ensure accurate and valid testing results.

Schlüsselwörter

HER2 · Mammakarzinom · Hintergrund, molekularer

Zusammenfassung

Der humane epidermale Wachstumsfaktor-Rezeptor-2 (HER2) wird in 15–25% der Mammakarzinome überexprimiert, üblicherweise auf dem Boden einer Genamplifikation. Ein positiver HER2-Status gilt als ungünstiger Prognosefaktor. Die Aufdeckung der Rolle von HER2 im Tumorgrowth des Mammakarzinoms hat zu der Entwicklung einer anti-HER2-gerichteten Therapie geführt. Der humanisierte monoklonale Antikörper Trastuzumab (Herceptin®) ist mittlerweile zur Behandlung des HER2-positiven metastasierten Mammakarzinoms zugelassen. Außerdem legen klinische Studien nahe, dass HER2 wichtige prädiktive Informationen bezüglich des Erfolgs und Versagens von bestimmten Hormon- und Chemotherapien liefern kann. Als Folge dieser Entwicklungen ist der Bedarf an HER2-Bestimmungen in aktuellem und archiviertem Tumormaterial deutlich angestiegen. Dieser Artikel gibt einen Überblick über den molekularen Hintergrund der Funktion, Aktivierung und Inhibition von HER2 sowie aktuelle Sichtweisen zu dessen Rolle im Hinblick auf die Chemosensitivität und Interaktion mit der Biologie des Östrogenrezeptors. Die verschiedenen gewebebasierten Verfahren zum Nachweis einer HER2-Amplifikation und -Überexpression werden im Hinblick auf ihre Vor- und Nachteile diskutiert. Außerdem wird auf die Fragen eingegangen, wann (bei Erstdiagnose oder unmittelbar vor einer Behandlung) und wo (lokal oder zentralisiert) die Bestimmung erfolgen sollte. Zusätzlich werden Qualitätssicherungsmaßnahmen erläutert, die notwendig sind, um genaue und valide Ergebnisse sicherzustellen.

Introduction

The human epidermal growth factor receptor-2 (HER2) proto-oncogene, also known as HER-2/neu or c-erbB2, is the human analogue of the oncogenic mutant *neu* gene identified in rat neuroblastomas in the early 1980s. Although this and other HER2 mutations are rarely, if at all, observed in human cancers, wild-type HER2 has been found to be amplified at the genomic level and/or overexpressed at the protein level in a number of human cancers [1–4]. This subject has been most extensively addressed in the domain of breast cancer in which HER2 is overexpressed or amplified with a 15–25% incidence. HER2 overexpression and amplification is associated with a significantly shorter overall survival rate and time to relapse, i.e. a worse prognosis, in breast cancer patients [5, 6]. An increasing body of evidence also supports the role of HER2 as an important predictive factor of response to chemotherapy and hormonal therapy in breast cancer [7–11]. Consequently, therapeutic agents that directly target HER2 protein have been developed. Nowadays trastuzumab (rhuMAb 4D5, Herceptin®, Genentech, South San Francisco, CA, USA) is the agent with the most extensive available data from randomized trials. Herceptin is a humanized monoclonal antibody (MAb) that directly targets the extracellular domain of the HER2 receptor. It was approved for HER2-positive metastatic breast cancer by the US Food and Drug Administration in 1998 and in the member states of the European Union in 2000.

Because of the recently published promising interim efficacy analyses of trials that added trastuzumab to adjuvant chemotherapy in HER2-positive breast cancer, Herceptin is going to be included in the adjuvant therapy of human breast cancer [12, 13]. Therefore, the history of HER2 and Herceptin can be seen as a successful example of translational research. None of the recent developments in the understanding of the molecular mechanisms underlying breast tumorigenesis has had a greater impact on both clinicians and pathologists. Furthermore, anti-HER2-directed therapy represents a model for the future of targeted therapies not only in breast cancer. This review will address the molecular basics of HER2 function, activation and inhibition in breast cancer as well as the currently available and approved tissue-based methods for the assessment of HER2 status, including the relevance of quality assurance.

Molecular Background

Structure, Function and Activation of HER2

Located on chromosome 17q21, the HER2 proto-oncogene encodes a 185-kDa glycoprotein, the HER2 protein or HER2 receptor. HER2 plays a key role in one of the best studied growth factor receptor systems in breast cancer, the HER (or erbB, or Type 1) tyrosine kinase receptor family. This family

comprises 4 homologous epidermal growth factor receptors, HER1 (EGFR/erbB1), HER2 (erbB2), HER3 (erbB3) and HER4 (erbB4), in which EGFR was the first to be molecularly cloned [14]. Each of these receptors comprises an extracellular ligand-binding domain, a transmembrane lipophilic segment and an intracellular protein kinase domain with a carboxyl terminal segment containing sites of phosphorylation or tyrosine residues [1, 4, 11]. The HER family plays an important role in regulating cell growth, survival and differentiation in a complex manner. Various ligands have been identified that activate individual HER receptors, although no ligand has been found that directly binds to the HER2 receptor. In common with many other growth factor receptors, members of the HER family dimerize upon ligand stimulation and transduce their signals by subsequent autophosphorylation catalyzed by the receptor cytoplasmatic tyrosine kinase activity. HER receptor monomers form homodimers with the same receptor or heterodimers with other members of the HER family in response to ligand binding. HER2 is the preferred heterodimerization partner within the family and can be stabilized and transactivated in heterodimers by ligands for the partner HER monomer, such as HER1 or HER3. This heterodimerization between HER2 and the other receptors of the family allows HER2 to participate in signal transduction, even in the absence of a cognate ligand. The type and amplitude of activated downstream signaling cascades are influenced by the type and number of receptors expressed by a particular cell and the amount and type of ligand that stimulates the cell.

In vitro and animal studies have indicated that HER2 gene amplification and protein overexpression play a pivotal role in oncogenic transformation, tumorigenesis and tumor progression. The normal epithelial cell possesses 2 copies of the HER2 gene and expresses low levels of HER2 protein on the cell surface, equivalent to some tens of thousands of receptors per cell. With oncogenic transformation, HER2 gene amplification generating more than the normal 2 gene copies is the most common mechanism leading to 10–100-fold increases in HER2 receptor monomers on the cell surface, i.e. HER2 overexpression equivalent to millions of receptors. In some cases, the extracellular domain of the HER2 receptor (ECD^{HER2}) may be shed from the cell surface and detected in the circulation. Overexpression of HER2 appears to have a number of effects that potentially result in carcinogenesis. The preferential formation of HER2-containing heterodimers leads to prolonged and enhanced downstream signaling due to increased stability compared with other heterodimers, reduced ligand dissociation and a decreased rate of endocytosis. When EGFR heterodimerizes with HER2, it is increasingly recycled to the cell surface, also resulting in intensified signaling. The formation of constitutively active homodimers may result in the initiation of downstream signaling pathways. On the basis of these effects, HER2 amplification disrupts normal control mechanisms by activation and suppression of numerous signal

transduction pathways with potential roles in tumor development and growth [1–3, 11].

HER2 and Response to Chemotherapy or Hormonal Therapy

HER2 status seems to influence the response to chemotherapy and hormonal therapy. But the evaluation of clinical trials has not been consistent, and the interpretation of these retrospective data is complicated and open to discussion. Nevertheless, most of the data suggest that a positive HER2 status predicts the likelihood of resistance to some conventional therapies, such as CMF (cyclophosphamide, methotrexate, 5-fluorouracil), on the one hand, but an increased chemosensitivity to anthracyclines on the other hand [11, 15]. However, the molecular background of these potential interactions is unclear. It has been suggested but not yet established that not the HER2 amplification but the co-amplification of the topoisomerase II-alpha (Topo II α) gene, which is located in the vicinity of the HER2 gene on chromosome 17q21, might be the predictive marker for responsiveness to anthracyclines. Topo II α is the known target for anthracyclines, and approximately 40% of breast cancers with HER2 amplification also show amplification of the Topo II α gene [16].

A range of studies have reported that patients with HER2-overexpressing tumors respond poorly to treatment with the selective estrogen receptor (ER) modulator (SERM) tamoxifen compared to patients with HER2-negative tumors [17]. In vitro evidence suggests that this effect is based on indirect activation of the EGFR by ERs located in the plasma membrane. A small pool of ERs seems to be located in the plasma membrane and the cytoplasm. These ERs can mediate rapid signals originating from the membrane, known as membrane-initiated estrogen signaling (MISS). One potential mechanism for the MISS activity involves indirect activation of the EGFR. Dimerization of the EGFR with another receptor like HER2 activates signaling pathways which in turn enhance nuclear ER signaling. In HER2-overexpressing tumors, a vicious cycle may be established in which estrogen activates HER signaling, and the HER signaling pathway further activates ERs. SERMs, such as tamoxifen, activate membrane ER in a manner similar to estrogen so that this molecular crosstalk might, in part, explain the resistance to this agent and seems to have important implications for the treatment of breast cancer. It is thought that estrogen deprivation therapy with aromatase inhibitors is more effective than SERMs in HER2-positive tumors as it shuts off not only the nuclear-initiated steroid signaling but also the MISS activities of ER [18]. However, this question can only be answered with additional large-scale, long-term prospective studies taking into account the inverse relationship between ER and progesterone receptor (PgR) status and HER2 overexpression. Only approximately 11–35% of ER- and/or PgR-positive cases show HER2 overexpression [6, 11, 19].

Targeting HER2 by Monoclonal Anti-HER2 Antibodies

Preclinical studies have demonstrated that the growth of human tumors in mice and human breast cancer cell lines overexpressing the HER2 receptor is inhibited by different anti-HER2 MAbs. The rate of tumor regression depends on the type of antibody. Whereas some antibodies almost completely abolish tumor growth, others are only partial antagonists. Some of the anti-HER2 MAbs were even found to enhance tumor volume [3]. The most efficient murine anti-HER2 antibody to be developed was muMAb 4D5 (Genentech) which was selected for further clinical development and humanized to circumvent the anti-globulin response. The therapeutic antibody Herceptin is the recombinant human anti-HER2 MAb derived from muMAb 4D5. It binds to the juxtamembrane region of the HER2 ectodomain [20], a region believed to be important for dimerization and transmembrane signaling. Data clearly indicate that Herceptin markedly inhibits tumor growth in breast cancer xenografts overexpressing HER2 unlike those not overexpressing HER2 [21–23]. The underlying mechanisms that mediate the anti-tumor effects are currently under investigation and have not yet been fully elucidated. Available data suggest that the major mechanisms include: i) acceleration of internalization and degradation of HER2 receptors from the cell membrane [3]; ii) recruitment of immune cells to attack and kill target tumor cells via antibody-dependent cell-mediated cytotoxicity [24–26]; iii) inhibition of cleavage of the extracellular domain of the receptor by metalloproteinase, preventing homodimerization of HER2 remnants and therefore antagonizing the constitutive growth signaling [27, 28]; iv) inhibition of its downstream phosphatidylinositol-3'-kinase (PI3K)-Akt pathway [29, 30]; v) the induction of G₁ arrest and the cyclin-dependent kinase inhibitor p27^{kip1} [27].

Clinical studies have demonstrated that Herceptin is well tolerated. It significantly improves survival of patients with HER2-positive advanced metastatic breast cancer, particularly in combination with chemotherapy [31, 32]. Despite careful patient selection on the basis of HER2 assessment, the overall Herceptin response rate is limited, especially if given as a single agent [33, 34]. These observations suggest that HER2 gene amplification and protein overexpression are necessary but not sufficient for Herceptin responsiveness. Insights are limited, not least because the determinants of Herceptin response are poorly understood. A recently published study suggests that the efficacy of Herceptin is dependent on the ability to inhibit PIK3 signaling which is involved in the regulation of cellular processes such as cell proliferation, survival and protein synthesis [35]. Herceptin specifically downregulates PIK3 signaling through activation of the tumor suppressor PTEN. Resistance to Herceptin occurs when PTEN lipid phosphatase function is lost, suggesting that PTEN activation is a critical component of the therapeutic effect. Additionally, PI3K inhibitors rescue PTEN loss-induced Herceptin resistance, sug-

gesting that PI3K-targeting therapies could overcome this resistance. Although the investigators provided in vitro and in vivo data that support their hypothesis, the results need further validation including the analysis of PTEN expression in correlation to the efficacy of Herceptin monotherapy.

Tissue-Based Assessment

Currently, there is great clinical demand for determining the HER2 status of breast cancer cases, because of its impact on treatment decisions for the patient. HER2 status obtained by tissue-based testing is at present the only accepted predictive marker for Herceptin therapy. Besides, HER2-positivity has been added for assessment of endocrine responsiveness and risk in the algorithm for selection of adjuvant systemic therapy for early breast cancer patients at the 'International Expert Consensus on the Primary Therapy of Early Breast Cancer 2005' in St. Gallen, Switzerland [36]. This underscores the need for reliable and robust methodologies to determine the HER2 status in breast cancer.

How to Test?

At least since the approval of Herceptin in the USA there has been considerable confusion among clinicians and pathologists with regard to which method and which reagents are most appropriate for HER2 testing in routine clinical practice. Herceptin is indicated for patients whose tumors have HER2 overexpression and/or amplification as determined by an accurate and validated assay. Figure 1 illustrates the recommended HER2 testing algorithm in accordance with the required preconditions for the use of Herceptin in Europe. Currently, the most frequently used HER2 testing methods are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).

IHC employs antibodies specifically directed against an epitope of the HER2 protein, thereby detecting HER2 on the cell surface. It is optimal for usage on formalin-fixed, paraffin-embedded tumor tissue which is the commonly used material in clinical practice. It is performed in the majority of pathology laboratories and represents a highly sensitive and specific technique if adequate antibodies and staining protocols are applied. HER2 expression is recognized by a typical staining pattern of the tumor cell membrane that is scored semiquantitatively by the observer. The scoring system used in the Herceptin pivotal trials is recommended in the guidelines for the interpretation of the HercepTest™ kit (DakoCytomation, Glostrup, Denmark). It is based on the percentage of positive cells and the intensity of staining, giving a score range of 0–3+. An IHC score of 3+ indicates an unequivocally positive result, whereas 0/1+ are considered as negative.

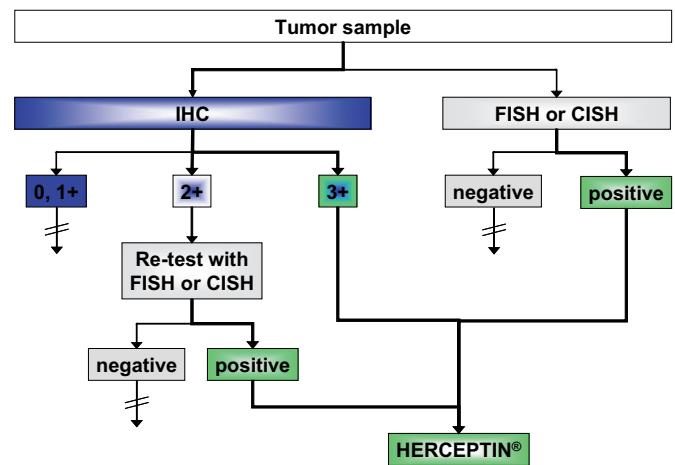


Fig. 1. HER2 testing algorithm demonstrating identification of patients eligible for Herceptin therapy (IHC = immunohistochemistry; FISH = fluorescence in situ hybridization; CISH = chromogenic in situ hybridization).

FISH allows the detection of HER2 gene amplification, the initial genetic event that results in HER2 overexpression. FISH can directly assess the HER2 gene copy number by using labeled complementary DNA probes to detect HER2-specific DNA sequences by hybridization. As with IHC, FISH is performed on formalin-fixed, paraffin-embedded tissue samples and also assesses the HER2 level on a cell-by-cell basis. Because DNA is more stable than protein, FISH is less affected by pre-analytical factors and handling of the tissue than IHC. The results are evaluated by quantification of the HER2 gene signals per tumor cell nucleus, complemented by counting the chromosome 17 signals if a dual color probe is used. According to the manufacturer's guidelines, HER2 gene amplification is either scored as an absolute value (> 4 gene copies per nucleus; Inform®, HER-2/neu test, Ventana Medical Systems, Tucson, AZ, USA) or as a ratio of average HER2 copy number to that of chromosome 17 (ratio ≥ 2 ; PathVysion®, HER-2 test, Abbott, Abbott Park, IL, USA).

Both methods are reliable, robust and highly specific when adequately performed and have both been widely accepted as the established standard HER2 testing methodologies. Nevertheless, both methods are attended with advantages and disadvantages. While IHC seems to be more susceptible to variations in the handling of the tissue and testing protocols as well as subjectivity in the scoring of immunoreactivity, FISH represents a relatively costly technique in which signals decay over time and occasional difficulties can occur when identifying areas of invasive carcinoma by fluorescence microscopy. The reliability of both techniques can be assured by use of validated assays and staining protocols under standardized test conditions as well as adequate staff training. Thus, it is preferable to use standardized tests, with the currently available tests being HercepTest and Pathway™ HER-2 (Ventana Medical Systems, Tucson, AZ, USA) for IHC and

PathVysion and Inform for FISH. Tumors showing protein overexpression by IHC scored 3+ and/or gene amplification detected by FISH are termed HER2-positive. Patients with HER2-positive tumors are eligible for Herceptin therapy. Herceptin has been shown to provide most benefit in women whose tumors overexpress the HER2 protein at the 3+ level or demonstrate amplification of the HER2 gene. The concordance rate between strong HER2 overexpression (score 3+) and gene amplification detected by FISH is around 90%. Furthermore, clinical outcomes are similar in IHC 3+ and FISH-positive populations [31, 33, 34, 37]. A proportion of cases, currently defined as equivocal by IHC (i.e. IHC score 2+) also demonstrates gene amplification, but only in up to 25% of tumors [38, 39]. Since some of these patients also responded to Herceptin in the pivotal trials [39], most of the national testing guidelines recommend to retest all IHC 2+ tumors by FISH [40].

Recently, chromogenic *in situ* hybridization (CISH) was introduced for the detection of gene amplification. With CISH, the HER2 gene is detected using a peroxidase enzyme-labeled probe with a chromogenic detection instead of fluorescent dye. As a consequence, technical equipment and costs are less extensive compared with FISH. CISH-stained signals can be viewed by standard light microscopy, histopathology of the specimen can be assessed simultaneously, and the staining remains stable over a long period of time [15, 41]. Currently, one standardized test is commercially available for CISH (Zymed SPoT-Light® HER2 CISH™ Kit, Invitrogen, South San Francisco, CA, USA). An international validation ring study has demonstrated that CISH results are reproducible between laboratories [42]. In the European Union, CISH is one of the approved testing methods in the assessment of patients for whom Herceptin treatment is being considered (Herceptin Prescribing Information, 2004). However, there is a relative lack of experience concerning the predictive value of CISH except for one congress contribution that suggests a sensitivity of CISH similar to FISH for predicting response to Herceptin [43]. Even though comparative studies have reported a high concordance (90–100%) between CISH and FISH and/or IHC [15, 41, 44], CISH seems to be less sensitive in the case of low-level amplification. Up to 43% of samples with low-level amplification detected by FISH are negative with CISH [42]. However, it should be noted that these cases occur at a low frequency in the general population (1–3%) but at a higher frequency in the critical group of IHC 2+ carcinomas (4–25%) [38, 42, 45]. Nevertheless, whichever method or testing algorithm is used to detect HER2 gene amplification or protein overexpression, one has to bear in mind that the capability to predict the response to Herceptin therapy is limited. Therefore, the rigorous pursuit of the status of critical downstream signaling molecules or alternative pathways in the tumors targeted for Herceptin, alternative MAbs or HER2 small molecule inhibitors should be a key aspect of the clinical approach in HER2-positive breast cancer in the future [46].

When to Test?

Guidelines from the American Society of Clinical Oncology (ASCO) recommend that HER overexpression should be evaluated in every primary breast cancer patient, either at diagnosis or at the time of recurrence [47]. To date, it is becoming more important that HER2 status is determined prospectively so that it can be used as a prognostic and predictive factor to guide therapeutic possibilities [36, 48]. If the determination of HER2 status was to guide the administration of adjuvant therapy, the analysis would clearly be required at the time of diagnosis. However, advocates of delayed testing (i.e. in metastatic disease, the setting in which Herceptin is currently licensed) point out the cost implications of testing all patients at time of diagnosis. This is particularly true if FISH is used for primary testing or re-testing of equivocal cases (i.e. tumors scored 2+ by IHC). Thus, answering the question of how to cover expenditure is becoming increasingly important. However, there is also a practical argument for determining and recording the HER2 status of samples at diagnosis, even if it is not used before the disease recurs. There is huge variation in national regulations on storing paraffin blocks: for example, while there is no legal requirement in Germany, pathologists in France and Canada are legally required to store blocks for 10 and 15 years, respectively. It is well known, that breast cancer can recur up to 20 years after the initial diagnosis [40]. Studies showing that HER2 status in the primary lesion reflects HER2 status of corresponding metastatic sites from the same patient also support testing at first diagnosis. The level of concordance between primary and metastatic sites ranges from approximately 80 to 100% and generally exceeds 90% [16]. Thus, early testing is the appropriate strategy to predict the status of metastatic lesions so that taking biopsies from metastatic patients is unnecessary if archival tissue of the primary tumor can be assessed [11].

Since core needle biopsies are increasingly performed within the scope of primary diagnosis of breast cancer, they are also increasingly used for the assessment of prognostic and predictive markers, especially if primary (neoadjuvant) systemic therapy is planned. Studies comparing the HER2 status in needle biopsies and surgical specimen have reported an overall concordance of 91–100% using IHC alone [49–53]. However, one investigation has presented data which suggest that the validity of IHC score 3+ in core biopsies is limited [53]. Additional FISH analysis of the needle biopsy for those patients who were HER2-negative (HercepTest score 0, 1+, 2+) in the surgical specimen and HER-positive (HercepTest score 3+) in the core biopsy confirmed the negative HER2 status of the surgically removed tissue in 11 out of 13 cases (84.6%). Overall, among 57 patients showing HER2 overexpression by IHC (HercepTest score 3+) in the core biopsies, 11 (19.3%) were identified as false-positive on the basis of IHC analysis of the surgical specimen and FISH. These results suggest that HER2 testing in surgical specimen is preferable if surgery is per-

formed as primary therapy. If a pre-treatment core biopsy represents the only available tissue, additional FISH analysis may be used in patients tested as HER2-positive (IHC score 3+) to validate the HER2 status and to avoid anti-HER2-directed therapy from which they are unlikely to benefit. However, it is important to note, that the above mentioned results represent data of a mono-institutional study so that large multi-institutional studies will be necessary to clarify if additional FISH analysis is actually required in case of carcinomas scored 3+ by IHC in core biopsies. Furthermore, methodological aspects which might explain the observed discrepancies should be investigated. Modifications in pre-analytical handling (i.e. fixation or embedding) of the core biopsies may be responsible for the increased sensitivity of IHC.

Where to Test?

Where to test is a contentious issue. According to the license of Herceptin in Europe, HER2 testing has to be performed in specialized laboratories that ensure validation of the testing methods so that reliable and reproducible results are obtained. Advocates for centralized testing claim that laboratories that are only processing a small number of samples annually cannot assure adequate quality of HER2 testing. Adhering to strict quality control, quality assurance and validation requires adequate equipment, standardized testing protocols and continuous training. Centralized facilities assay greater numbers of samples per year, leading to greater experience and accuracy. Rigid quality control and validation add to the level of accuracy. The higher accuracy of large-volume laboratories (≥ 100 assays per month) versus small-volume laboratories can be exemplified by a report from the adjuvant Herceptin trial led by the National Surgical Adjuvant Breast and Bowel Project [54]. The accuracy of testing laboratories was proven in 104 breast tumor samples by IHC and FISH in a central reference laboratory. The frequency of false-positive reporting was higher in small-volume laboratories, especially in those that used 'home-brew' immunohistochemical tests instead of standardized test kits. It was concluded that HER2 testing at a local laboratory by any assay other than FISH would need to be validated at a large-volume center.

However, it is important to note that local testing centers offer certain advantages. Results from local testing laboratories are usually available quicker, with close contact between the pathologist and the medical oncologist. In addition, tissue samples are prepared in a homogenous manner at a local level, for which interpretation of IHC testing can be more reliably adapted for consistent results. Variability of tissue sam-

pling and fixation can be a source of interpretation error for the heterogeneous samples received at central testing facilities. Moreover, the results of national quality assurance programs offered by several national pathologists associations indicate that the consistency and accuracy of local HER2 testing can potentially reach a level similar to that of central testing [16]. Requirements are the use of regular quality control/assurance programs and standardization of procedures. In addition, increased investment in education, training and experience exchange will help to achieve the goal, not only with regard to HER2 testing but also the detection of other therapy targets in the future.

Quality Control and Quality Assurance

Whichever method is used, accurate HER2 testing is essential for optimal patient management with Herceptin. False-negative results may deny patients the chance of life-extending therapy, while false-positive results waste resources, give rise to false hopes and expose patients to unnecessary adverse effects. There are several factors that can contribute to inaccuracy of testing results in the clinical setting, including method of tissue fixation, the assay method used, non-standardization of procedures, intra- und interlaboratory variability and interobserver variability. Thus, there should be standard operating protocols for the used assays, and the initial validation of assays against a gold standard is mandatory for the maximization of day-to-day and long-term accuracy. Moreover, internal quality controls, including positive and negative controls, should be included. Controls can be tissue arrays, tissue specimens of known HER2 status or cell lines. A regular audit of HER2-positive results in an unselected breast cancer population is recommended to check that these are within the reported limit (15–25%). If possible, the concordance between IHC and FISH/CISH should be analyzed (IHC 3+ > 90% FISH/CISH-positive; IHC 2+ $\leq 25\%$ FISH/CISH-positive). If there is an equivocal result, testing should be repeated, or retesting by another method, such as FISH, should be performed, if necessary in an external laboratory. Education and experience in practical interpretation of assay results is essential to avoid misinterpretation of artifacts and to minimize uncertainty at cut-off points, i.e. 2+/3+ cut-off. Staff training can also improve the performance of HER2 testing accuracy. External quality assessment procedures should be regularly implemented. Quality assurance can include regular circulation of test samples to other laboratories, i.e. reference testing laboratories, for confirmation of the testing results by the same or another method or participation in ring studies [16, 40].

References

- 1 Harari D, Yarden Y: Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. *Oncogene* 2000;19:6102-6114.
- 2 Prenzel N, Fischer OM, Streit S, Hart S, Ullrich A: The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. *Endocr Relat Cancer* 2001;8:11-31.
- 3 Yarden Y: Biology of HER2 and its importance in breast cancer. *Oncology* 2001;61(suppl 2):1-13.
- 4 Gschwind A, Fischer OM, Ullrich A: The discovery of tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer* 2004;4:361-368.
- 5 Ross JS, Fletcher JA: HER-2/neu (c-erb-B2) gene and protein in breast cancer. *Am J Clin Pathol* 1999;112:S53-S67.
- 6 Revillion F, Bonnetterre J, Peyrat JP: ERBB2 oncogene in human breast cancer and its clinical significance. *Eur J Cancer* 1998;34:791-808.
- 7 Hanna W, Gelmon KA: Review of the literature on HER2/neu testing and the role of HER2/neu as a prognostic and predictive factor in breast cancer – update December 2000 to July 2001. *Current Oncol* 2002;9:S2-S17.
- 8 Lohrisch C, Piccart MJ: HER2/neu as a predictive factor in breast cancer. *Clin Breast Cancer* 2001;2:129-135.
- 9 Piccart MJ, Di Leo A, Hamilton A: HER2: a 'predictive factor' ready to use in the daily management of breast cancer patients? *Eur J Cancer* 2000;36:1755-1761.
- 10 Piccart MJ, Lohrisch C, Di Leo A, Larsimont D: The predictive value of HER2 in breast cancer. *Oncology* 2001;61:73-82.
- 11 Dowsett M, Cooke T, Ellis I, Gullick WJ, Gusterson B, Mallon E, Walker R: Assessment of HER2 status in breast cancer: why, when and how? *Eur J Cancer* 2000;36:170-176.
- 12 Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CM, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomassen C, Lohrisch C, Suter TM, Rueschhoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD: Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659-1672.
- 13 Romond EP, Perez EA, Bryant J, Suman VJ, Geyer CE, Davidson N, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisanski TM, Fehrenbacher L, Kutteh LA, Vogel G, Visscher DW, Yothers G, Jenkins RB, Brown A, Dakhil SR, Mamounas E, Lingle WL, Klein PM, Ingle JN, Wolmark N: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673-1684.
- 14 Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Tam AW, Lee J, Yarden Y, Libermann TA, Schlessinger J: Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 1984;309:418-425.
- 15 Tanner M, Ganchberg D, Di Leo A, Larsimont D, Rouas G, Piccart MJ, Isola J: Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples. *Am J Pathol* 2000;157:1467-1472.
- 16 Di Leo A, Dowsett M, Horten B, Penault-Llorca F: Current status of HER2 testing. *Oncology* 2002;63 (suppl 1):25-32.
- 17 De Laurentiis M, Arpino G, Massarelli E, Ruggiero A, Carloni C, Ciardiello F, Tortora G, D'Agostino D, Caputo F, Montagna E, Malorni L, Zinno L, Lauria R, Bianco A, De Placido S: A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. *Clin Cancer Res* 2005;11:4741-4748.
- 18 Osborne CK, Schiff R: Estrogen-receptor biology: continuing progress and therapeutic implications. *J Clin Oncol* 2005;23:1616-1622.
- 19 Pinto AE, Andre S, Pereira T, Nobrega S, Soares J: C-erbB-2 oncoprotein overexpression identifies a subgroup of estrogen receptor positive (ER+) breast cancer patients. *Ann Oncol* 2001;12:525-533.
- 20 Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney DW, Leahy DJ: Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 2003;421:756-760.
- 21 Baselga J, Norton L, Albanell J, Kin YM, Mendelsohn J: Recombinant humanized anti-HER2 antibody (Herceptin™) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res* 1998;58:2825-2831.
- 22 Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F, Slamon DJ: Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene* 1999;18:2241-2251.
- 23 Pietras RJ, Pegram MD, Finn RS, Maneval DA, Slamon DJ: Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene* 1998;17:2235-2249.
- 24 Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM: Humanization of an anti-p185 HER2 antibody for human cancer therapy. *Proc Natl Acad Sci* 1992;89:4285-4289.
- 25 Hancock MC, Langton BC, Chan T, Toy P, Monahan JJ, Mischak RP, Shawver LK: A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cisplatin against human breast and ovarian tumor cell lines. *Cancer Res* 1991;51:4575-4580.
- 26 Clynes RA, Towers TL, Presta LG, Ravetch JV: Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med* 2000;6:443-446.
- 27 Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA: Nonclinical studies addressing the mechanisms of action of trastuzumab (Herceptin). *Semin Oncol* 1999;26:60-70.
- 28 Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J: Trastuzumab (Herceptin), a humanized anti-HER2 receptor monoclonal antibody, inhibits basal and activated HER2 ectodomain cleavage in breast cancer cells. *Cancer Res* 2001;61:4744-4749.
- 29 Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A: p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol* 1989;9:1165-1172.
- 30 Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL: Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res* 2002;62:4132-4141.
- 31 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783-792.
- 32 Seidman AD, Fornier MN, Esteva FJ, Tan L, Kapitan S, Bach A, Panageas KS, Arroyo C, Valero V, Currie V, Gilewski T, Theodoulou M, Moynahan ME, Moasser M, Sklarin N, Dickler M, D'Andrea G, Cristofanilli M, Rivera E, Hortobagyi GN, Norton L, Hudis CA: Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. *J Clin Oncol* 2001;19:2587-2595.
- 33 Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter J, Paton V, Shak S, Lieberman G, Slamon DJ: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999;17:2639-2648.
- 34 Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002;20:719-726.
- 35 Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin A, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004;6:127.
- 36 Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thurlimann B, Senn HJ; Panel Members: Meeting highlights: international expert consensus on the primary therapy of early breast cancer. *Ann Oncol* 2005;16:1569-1583.
- 37 Mass R, Press M, Anderson S, Murphy M, Slamon DJ: Improved survival benefit from Herceptin (trastuzumab) in patients selected by fluorescence in situ hybridization (FISH). *Proceedings of the American Society of Clinical Oncology* 20, 20a, 2001.
- 38 Lebeau A, Deimling D, Kaltz C, Sendelhofert A, Iff A, Luthardt B, Untch M, Löhrs U: HER-2/neu analysis in archival tissue samples of human breast cancer: Comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol* 2001;19:354-363.
- 39 Mass R, Sanders C, Kasián C, Everett T, Johnson L, Anderson S: The concordance between the clinical trials assay (CTA) and fluorescence in situ hybridization in the Herceptin pivotal trials. *Proceedings of the American Society of Clinical Oncology* 19, 75a, 2000.
- 40 Bilous M, Dowsett M, Hanna W, Isola J, Lebeau A, Moreno A, Penault-Llorca F, Ruschhoff J, Tomasic G, van de Vijver M: Current perspectives on HER2 testing: a review of national testing guidelines. *Mod Pathol* 2003;16:173-182.
- 41 Arnould L, Denoux Y, MacGrogan G, Penault-Llorca F, Fiche M, Treilleux I, Mathieu MC, Vincent-Salomon A, Vilain MO, Couturier J: Agreement between chromogenic in situ hybridisation (CISH) and FISH in the determination of HER2 status in breast cancer. *Br J Cancer* 2003;88:1587-1591.

42 Van de Vijver MJ, Rueschoff J, Penault-Llorca F, Bilous M, Hanna W: Chromogenic in-situ hybridisation (CISH) compared with FISH and IHC for detection of HER2 gene amplification: an international validation ring study. *Breast Cancer Res Treat* 2003;82(suppl 1).

43 Hofmann M, Gaiser T, Kneitz H, Weng LP, Schmidtgen C, Gutjahrs TS, Gross C, Henken T, Rueschoff J: HER2 status assessment by chromogenic in situ-hybridization (CISH) demonstrates high sensitivity for predicting response to Herceptin. 2004. San Antonio Breast Cancer Conference, 2004.

44 Dandachi N, Dietze O, Hauser-Kronberger C: Chromogenic in situ hybridization: a novel approach to a practical and sensitive method for the detection of HER2 oncogene in archival human breast carcinoma. *Lab Invest* 2002;82:1007–1014.

45 Kobayashi M, Ooi A, Oda Y, Nakanishi I: Protein overexpression and gene amplification of c-erbB-2 in breast carcinomas: a comparative study of immunohistochemistry and fluorescence in situ hybridization of formalin-fixed, paraffin-embedded tissues. *Hum Pathol* 2002;33:21–28.

46 Crowder RJ, Lombardi DP, Ellis MJ: Successfull targeting of erbB2 receptors-is PTEN the key? *Cancer Cell* 2004;6:103–104.

47 Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG, Somerfield MR: 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2001;19:1865–1878.

48 Hortobagyi GN: Trastuzumab in the treatment of breast cancer. *N Engl J Med* 2005;353:1734–1736.

49 Jacobs TW, Siziopikou K, Prioleau J, Raza S, Baum J, Hayes DF, Schnitt SJ: Do prognostic marker studies on core needle biopsy specimens of breast carcinoma accurately reflect the marker status of the tumor? *Mod Pathol* 1998;11:259–264.

50 Mueller-Holzner E, Fink V, Frede T, Marth C: Immunohistochemical determination of HER2 expression in breast cancer from core biopsy specimens: a reliable predictor of HER2 status of the whole tumor. *Breast Cancer Res Treat* 2001;69: 13–19.

51 Di Loreto C, Puglisi F, Rimondi G, Zuiani C, Annia G, Della Mea V, Beltrami CA: Large core biopsy for diagnostic and prognostic evaluation of invasive breast carcinomas. *Eur J Cancer* 1996;32A: 1693–1700.

52 Connor CS, Tawfik OW, Joyce AJ, Davis MK, Mayo MS, Jewell WR: A comparison of prognostic tumor markers obtained on image-guided breast biopsies and final surgical specimens. *Am J Surg* 2002;184:322–324.

53 Taucher S, Rudas M, Mader RM, Gnant M, Dubsky P, Roka S, Bachleitner T, Kandioler D, Steger G, Mittlböck M, Jakesz R: Prognostic markers in breast cancer: the reliability of HER2/neu status in core needle biopsy of 325 patients with primary breast cancer. *Wien Klin Wochenschr* 2004;116: 26–31.

54 Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, Brown A, Yothers G, Anderson S, Smith R, Wickerham DL, Wolmark N: Real-world performance of HER2 testing – National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 2002;94:852–854.