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# The Need to Examine Metastatic Tissue at the Time of Progression of Breast Cancer: Is Re-biopsy a Necessity or a Luxury?

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**Abstract** Knowledge of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor-2 (HER2) status is necessary for determining the optimal treatment of breast cancer patients. At the same time, the discordance between marker profiles (ER/PR and HER2) of primary and metastatic breast cancer is well documented. Whether discordant cases are secondary to "clonal selection" in the face of targeted anti-estrogen or anti-HER2 therapy or whether they are a laboratory artifact is still debated; both scenarios are likely. This article outlines current modalities for ER, PR, and HER2 testing in primary breast carcinoma and its metastases and reviews prospective and retrospective studies that have addressed these issues, as well as recent advances in the field.

**Keywords** Tissue biopsy · Metastases · Breast cancer · Estrogen receptor · ER · Progesterone receptor · PgR · Human epidermal growth factor receptor-2 · HER2

## Introduction

Advances in treatment options have led to prolonged survival of patients with breast cancer. The choice of anticancer therapy depends on several factors, including the biologic characteristics of breast cancer [1]. Breast cancers are often considered as three major subtypes based on the expression of hormone (estrogen and progesterone) receptors and human epidermal growth factor receptor-2 (HER2) and categorized into hormone receptor (HR)-positive, HER2-amplified tumors, and triple-negative breast cancer (all tumors negative for HRs and HER2). A number of gene-expression signatures have been reported that aim to improve prognostication and treatment selection provided by traditional clinical and pathological information. Based on RNA expression profiling, breast cancers are subdivided into distinct molecular subtypes: luminal A, luminal B, normal-like, basal, and HER2-positive [2]. Tumors within these subtypes have similar gene-expression patterns, clinical outcomes, and response to therapy; nonetheless, much heterogeneity exists within these groups, especially among basal-like carcinoma. Despite advances in the molecular understanding of cancer, therapeutic choices rely on traditional pathological and immunohistochemical (IHC) evaluation of the primary tumor.

In addition to cytotoxic chemotherapy, therapies targeting HRs and HER2 play an important role. Endocrine therapy targeting estrogen receptors (ERs) and progesterone receptors (PgRs) remains the most effective form of targeted systemic therapy [3]. Profound benefits have been gained from therapies targeting HER2, such as trastuzumab and lapatinib, in women with HER2-positive breast cancer [4]. It is evident that women whose tumors express these markers can benefit substantially from targeted treatments, whereas those with tumors not expressing the same antigens will experience toxicity with little if any benefit.

According to published consensus guidelines that reflect decades of experience and results of clinical trials, the expression of ERs, PgRs, and HER2 is tested in early-stage breast cancer [5, 6••]. Evaluation of ERs, PRs, and HER2 is

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carried out on the primary breast tumor even if the carcinoma is already metastatic to locoregional lymph nodes. Although the choice of anticancer therapy and the success in the control of metastatic disease rest heavily on the ER/PR and HER2 status of metastatic disease, the current guidelines do not mandate assessment of ER, PgR, and HER2 status of distant metastases, and therapeutic decisions for metastatic breast cancer (MBC) are often based on the profile of the primary tumor. An increasing body of literature suggests that tumor characteristics can change over time, especially with regard to ER/PgR and HER2 status [7–11].

Published data report altered ER profile in MBC compared with primary breast cancer, with a range from 18% to 56% (Table 1). For HER2, at least 23 different studies have been reported, with a total of over 2,500 patients (Table 2). Of these, over 400 patients have an altered HER2 status between the primary cancer and metachronous metastatic site, with a range from 0% to 38% [12].

It is unclear whether these alterations result from "clonal selection" over time in the face of adjuvant antiestrogen or anti-HER2 therapy. These specific treatments have significantly improved survival for breast cancer patients, so that patients live longer and have more time to relapse, and the question of successful therapy leading to clonal selection is raised.

Alternatively, preanalytical and analytical variables can cause discrepancies in results at different times and in different settings. Another important confounder could be interobserver variability in analyzing biopsy results. In this review, we describe the relevant literature and discuss potential causes of discordance, including technical aspects, possible biologic "evolution" of tumors over time, and the combination of these factors.

## Biopsy Techniques

The most widely used biopsy techniques for sampling of distant metastases include fine-needle aspiration biopsy (FNAB) and core needle biopsy (CNB). Both types of biopsy can be used to sample superficial and palpable lesions without radiologic assistance, or to target visceral or skeletal lesions under the guidance of ultrasound, CT, or both. Both FNAB and CNB can result in a false-negative diagnosis, and clinical and radiologic correlation is required to determine the need for repeat biopsy.

FNAB can help to distinguish benign from clearly malignant processes. It is suitable for primary breast cancer diagnosis and is useful for evaluating possible distant metastases in organs such as lung, liver, or bone. FNAB is easy to perform in the outpatient setting with minimal risk of complications and can be applied for sampling of metastatic disease. In oncology practice, FNAB is a useful tool to confirm the diagnosis of a suspicious lesion, and it can be used to characterize biologic features and changes in relation to the primary tumor.

FNAB yields limited material consisting of cell aggregates; its interpretation requires expertise in cytologic diagnosis. The rate of satisfactory FNAB is higher if a cytopathologist or cytotechnologist is available to assess the adequacy of the aspirate and triage the use of FNAB material for special studies [13].

The tissue cores obtained with CNB allow evaluation of cytologic atypia and tissue architecture. Examination of breast cores can assess whether carcinoma is present and whether it consists of in situ or invasive disease. The presence of biopsy site changes in an excisional biopsy specimen following CNB is proof that the area of interest has been removed by the surgeon, whereas tissue damage following FNAB is usually

**Table 1** Discordant estrogen receptor status in primary breast cancers compared with corresponding relapses

| Author/Publication | Patients, *N* | Discordant patients | |
|---|---|---|---|
| | | n | % |
| Klarsson et al., 2010 ASCO abstract | 486 | 170 | 35 |
| Locatelli et al., 2010 ASCO abstract | 255 | 37 | 14.5 |
| Simmons et al., 2009 Ann Oncol | 29 | 12 | 40 |
| Liedtke et al., 2009 Ann Oncol | 228 | 42 | 18.4 |
| Broom et al., 2009 Anticancer Res | 62 | 11 | 17.7 |
| Simmons et al., 2009 Ann Oncol | 25 | 10 | 40 |
| Amir et al., 2008 Clin Oncol | 9 | 5 | 55.6 |
| Guarneri et al., 2008 Oncologist | 75 | 17 | 22.7 |
| Wu et al., 2008 Clin Cancer Res | 10 | 2 | 20 |
| Lower et al., 2005 Breast Cancer Res Treat | 200 | 60 | 30 |
| Wang et al., 2004 Ai Zheng | 65 | 23 | 35.4 |
| Nedergaard et al., 1995 APMIS | 101 | 21 | 20.8 |
| Kamby et al., 1989 Br J Cancer | 62 | 23 | 37.1 |

*APMIS* Acta Pathologica, Microbiologica et Immunologica Scandinavica, *ASCO* American Society of Clinical Oncology

(*Adapted from* Lindström et al. [12]. Reprinted with permission. )

**Table 2** Discordant HER2/neu status in primary breast cancers compared with corresponding relapses

| Author/Publication | Patients, *N* | Discordant patients | |
|---|---|---|---|
| | | n | % |
| Amir et al., 2010 ASCO abstract | 258 | 14 | 5 |
| Locatelli et al., 2010 ASCO abstract | 172 | 24 | 13.9 |
| Simmons et al., 2009 Ann Oncol | 29 | 12 | 40 |
| Liedtke et al., 2009 Ann Oncol | 528 | 72 | 14 |
| Lower et al., 2008 Breast Cancer Res Treat | 382 | 127 | 33 |
| MacFarlane et al., 2008 ASCO abstract 1000 | 160 | 9 | 6 |
| Wilking et al., 2008 SABCS No. 6033 | 155 | 18 | 12 |
| Tapia et al., 2007 Breast Cancer Res | 105 | 8 | 8 |
| Pectasides et al., 2006 Anticancer Res | 16 | 6 | 38 |
| Solomayer et al., 2006 Breast Cancer Res Treat | 45 | 17 | 38 |
| Gong et al., 2005 Cancer | 60 | 2 | 3 |
| Lipton et al., 2005 Cancer | 240 | 61 | 25 |
| Zidan et al., 2005 Br J Cancer | 58 | 8 | 14 |
| Luftner et al., 2004 Proc Am Soc Clin Oncol | 80 | 10 | 13 |
| Meng et al., 2004 PNAS | 24 | 9 | 38 |
| Edgerton et al., 2003 Appl IMM | 113 | 19 | 17 |
| Sekido et al., 2003 Int J Oncol | 44 | 2 | 5 |
| Gancberg et al., 2002 Ann Oncol | 107 | 10 | 9 |
| Tanner et al., 2001 Cancer Res | 45 | 0 | 0 |
| Shimizu et al., 2000 J Surg Oncol | 21 | 0 | 0 |
| Masood et al., 2000 Ann Clin Lab Sci | 56 | 1 | 2 |
| Neihans et al., 1993 JNCI | 30 | 1 | 3 |

*Appl IMM* Applied Immunohistochemistry and Molecular Morphology, *ASCO* American Society of Clinical Oncology, *JNCI* Journal of the National Cancer Institute, *PNAS* Proceedings of the National Academy of Science, *SABCS* San Antonio Breast Cancer Symposium

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very limited and cannot be identified with certainty. Both FNAB and CNB cause tissue disruption that leads to artifactual displacement of neoplastic cells along the biopsy tract [14]. A recent review based on three prospective and 12 retrospective studies did not document an increased risk of tumor recurrence secondary to biopsy tumor seeding, although this possibility cannot be ruled out [15].

No prospective study has compared the techniques of CNB and FNAB from metastatic lesions, but prospective and retrospective comparisons of the two techniques have been performed in primary breast cancers. A study from M. D. Anderson Cancer Center (MDACC) suggests that a proportion of results discordant between primary and metastatic sites could be secondary to suboptimal reproducibility of measurement methods [16]. In addition, potential intratumoral heterogeneity of biomarker expression may affect the validity of CNB interpretation in small biopsy specimens [17].

Discordant results in ER, PgR, or HER2 status are observed in different biopsies of the same primary carcinoma, or when comparing results for the primary tumor and its metastases.

Incomplete fixation can account for some discordant cases. Formalin, the most commonly used fixative, penetrates tissue at a rate of 1 mm per hour, but tissue penetration is different from fixation, which requires at least 6 h for sufficient protein crosslinking to occur for adequate tissue and antigen preservation. The time required for fixation ranges between 6 and 72 h and is independent of biopsy technique and tissue type, although fatty tissue, such as the breast parenchyma, can fix at a slower rate [18]. Shorter fixation time markedly reduces antigen immunoreactivity, with absent signal or markedly reduced staining intensity [16]. The current guidelines for ER/PgR and HER2 testing have been specifically formulated for evaluation of formalin-fixed tissue. Other fixatives could be used, but their results need to be validated.

To avoid artifacts secondary to "cold ischemia," it is recommended not to delay formalin fixation for more than 1 h after the biopsy is performed. Storing unfixed specimens overnight in a refrigerator will be associated with poor antigen preservation [19]. Refrigeration of a specimen already placed in formalin also is not recommended, as cold temperature delays formalin fixation. Tissue obtained by CNB of skeletal lesions usually requires decalcification. The acid-based solution used to decalcify bone reduces the immunoreactivity of most antigens and limits accurate assessment of ER, PgR, and HER2 [20].

Special considerations regarding optimal fixation apply to evaluation of FNAB material [6••], as most fixatives

used are alcohol-based and often the FNAB material is fixed at first in an alcohol (methanol)–based fixative; then a cell pellet is prepared, which is further fixed in formalin. The effects of alcohol fixation and of the two-step fixation process on ER, PgR, and HER2 stains have not been rigorously evaluated and could account for some discrepant results in FNAB material compared with CNB material. In one study, results of ER staining on FNAB cell block material were comparable to those of CNB or excisional biopsies, but less staining was observed for PgR. In contrast, HER2 staining of FNAB cell block material yielded a higher rate of 3+ or 2+ results than the corresponding CNB or surgical excision specimens [21].

Alcohol fixation causes cell autofluorescence that could impair detection of *HER2* gene amplification using fluorescent in situ hybridization (FISH). In general, it is prudent to avoid alcohol-based fixatives (even as an intermediate step) for FNAB material that may require FISH testing.

The current guidelines for HER2 FISH interpretation on tissue sections require counting of the signal in at least 20 cells in the area of strongest signal intensity [22], but neoplastic cells can be very limited in FNAB material, even though sufficient for diagnosis. Therefore, negative HER2 IHC and/or FISH results in material obtained by FNAB should always be interpreted with caution if the material is scant, as the nature of the specimen does not allow field selection.

With regard to discrepant results in ER, PgR, and HER2 testing in material obtained with different procedures from the same tumor, Mann et al. [23] examined the results in paired CNB and surgical excision specimens from 100 patients. They found that ER and PgR were positive in a number of CNB specimens but negative in the surgical specimens in 9% of those patients. They concluded that it is better to test ER and PgR in CNB specimens than in excision specimens. However, sampling error and inadequate fixation of surgical specimens left uncut in formalin overnight could account for false-negative results in the excisional biopsies. A pilot study of tissue samples from 10 invasive breast cancer cases after fixation for 1, 3, 6, and 9 to 10 h found significant differences in the intensity of the stain or the percentage of cells stained depending on the time in fixation [24], emphasizing the importance of optimal formalin fixation for optimal results.

In addition to discordance in biopsy results at different time points, interinstitutional pathology review may reveal discordance with potential clinical relevance. A Canadian group conducted a retrospective review of 100 randomly selected interinstitutional pathology consultations for breast cancer. Of 93 eligible cases, 10 (11%) underwent a change in diagnosis considered to have medium or high impact for a change in clinical management [25].

## Assessment of HER2 Status

Approximately 15% to 20% of human MBCs overexpress HER2 [26]. Amplification of the *HER2* gene is a significant predictor of survival and response to HER2-directed therapies. These include trastuzumab (Herceptin; Genentech, South San Francisco, CA), a recombinant humanized monoclonal antibody targeted against an extracellular region of the HER2 receptor, and lapatinib (Tykerb; GlaxoSmithKline, Research Triangle Park, NC), a dual inhibitor of the epidermal growth factor receptor 1 (EGFR1) and HER2 tyrosine kinases [4]. Both are approved by the United States Food and Drug Administration (FDA) for treatment of HER2-overexpressing MBC. Primary HER2-positive breast cancers tend to recur in visceral organs such as the liver and the brain, while sparing bone [1, 27]. Historically, retrospective testing of tumors that showed positive staining with monoclonal antibodies to HER2 was used to validate a polyclonal assay and later FISH testing [4]. Newer tests were correlated with the assays used in clinical trials and appeared to select subgroups of patients with greater likelihood of response; they were considered alternatives to the use of monoclonal stains. Positivity for these tests was required to qualify patients for trial enrollment [4]. HER2 status of all newly diagnosed breast cancers is routinely assessed using the testing algorithm standardized by the American Society of Clinical Oncology and the College of American Pathologists, including IHC analysis of HER2 protein expression and FISH analysis of *HER2* gene copy number [28•].

The most widely used testing strategy evaluates HER2 immunoreactivity on all tumors, followed by FISH only for those tumors with 2+ IHC staining intensity. Cases that are HER2 3+ by IHC or 2+ by IHC and FISH amplified are reported as HER2 positive. *HER2* gene amplification is evaluated as a ratio between copies of the *HER2* gene, which is located on chromosome 17, and copies of a reference gene, *CEP17*, representing the same chromosome (number of copies of the *HER2* gene / number of copies of *CEP17* >2.2) or an absolute volume number of gene copies per cell (>6). Current guidelines for HER2 assessment and reporting have standardized patient selection but are far from being predictive of patient response to available HER2-directed agents. HER2 status discordance between the primary tumor and distant breast cancer metastases has become particularly important because therapies targeting HER2 have established efficacy in treating breast cancer. Overall, HER2 status seems to be highly conserved as breast cancers metastasize [5].

A study reported by Tapia et al. [29] showed discordance between the primary tumor and distant metastasis in 7.6% of 105 patients, none of whom had received trastuzumab

therapy. Reevaluation of the HER2 FISH status by rescoring the specimens or by hybridizing routine tissue sections revealed that in five patients (4.7%), discrepancies were due to interpretational difficulties. In two of these patients, focal amplification due to intratumoral heterogeneity had been overlooked. Three patients had borderline amplification, with a ratio close to 2. Discrepancy remained unexplained in three patients (2.9%) [29]. Changes in HER2 status are supported by data obtained by measuring HER2 in serum and in circulating tumor cells; 25% to 37% of the patients studied converted from HER2 negative to a positive HER2 phenotype [30].

It is generally believed that discrepant results are more likely to occur because of heterogeneity of HER2 amplification or interpretational difficulties.

## Concordance in the Adjuvant and Neoadjuvant Setting

With the increasing use of neoadjuvant therapies, clinicians use information on biomarkers obtained at the time of CNB before and after neoadjuvant chemotherapy. A study ($n$=100) analyzed concordance rates between HER2 FISH results on CNB and on subsequent excisional biopsies of the same tumor [31]. Comparison was made with special consideration for patients with HER2 2+ tumors treated with neoadjuvant chemotherapy to determine the constancy of HER2 status before and after treatment; the authors state that it is unknown if therapy included trastuzumab. The concordance rate between FISH results determined on the CNB and subsequent excisional biopsy of the same tumor after neoadjuvant chemotherapy was 86%. Of patients receiving neoadjuvant chemotherapy ($n$=15), 93% had no change in HER2 status as determined by IHC, and 87% had no change as determined by FISH. Of 2+IHC staining CNB cases ($n$=14), 79% showed concordant FISH results in the CNB and subsequent excisional biopsy specimens. Larger cohorts must be studied to verify this finding [31].

Another study reviewed HER2-overexpressing samples of patients receiving neoadjuvant chemotherapy and trastuzumab. FISH done on pretreatment specimens confirmed HER2 amplification before therapy. A pathologic complete response was achieved in 72 (50.7%) of the 142 patients. After treatment, tumor was available in 25 patients; 8 (32.0%) of the 25 were HER2-negative by FISH. One third of patients with significant residual disease lost HER2 amplification, and this change was associated with poor relapse-free survival. The authors advised that residual tumor identified at the time of surgery should be reassessed for HER2 status [32]. It is possible that a change in HER2 status could reflect heterogeneity of HER2 expression within the tumor, suggesting that trastuzumab successfully treated a HER2-overexpressing component but residual HER2-negative tumor cells survived after treatment (ie, clonal selection).

## Recent Reports on Rebiopsy of Metastatic Breast Cancer

### Prospective Data

One recent report prospectively pooled patient data from two studies (the Breast Recurrence In Tissues Study [BRITS] in the UK and the Canadian DESTINY study) [33]. In pooled analysis, biopsies of recurrent lesions were analyzed for ER and PgR status by IHC and for HER2 by IHC and FISH. Receptor status of recurrent disease was compared with that of the primary tumor in 258 patients. Discordance rates between the primary and recurrent tumors were 13% for ER, 28% for PgR, and 5% for HER2. Reevaluation for receptor status was performed in all cases, in contrast to other studies, which used data extracted from original pathology reports. Gain and loss of receptor expression were similar for ER and for HER2, but loss of PgR was more common than gain (76% vs 8%). There was no significant receptor profile discordance among triple negative primary tumors. Biopsy results altered management in 15.9% of patients, and the number of biopsies needed to alter immediate patient management was 6.3 [33].

Another prospective study enrolled 40 individuals with distant metastases [34]. Of 35 patients who underwent biopsy of the metastases, 29 had samples with sufficient material for analysis. The authors demonstrated a change in hormone receptor status in 40% of patients and a change in HER2 status in 8% [34], changing clinical management in 20% of patients. Thus, the number of biopsies needed to alter at least one patient's management was five.

Ongoing prospective efforts include a study to evaluate changes in molecular biomarkers in HER2-positive MBC during trastuzumab therapy (SHERsig study: a prospective study to evaluate alterations in molecular biomarkers in HER2-positive metastatic breast cancer together with assessment of trastuzumab use beyond progression after initial exposure to trastuzumab-taxane–based treatment). The SHERsig trial evaluates how patients respond to trastuzumab-based treatment and observes what molecular alterations occur in patients who respond to a second trastuzumab-based regimen. Identification of altered molecular biomarker signatures during HER2 targeted therapy could pinpoint resistant cases through serial biopsies of metastatic disease. An ongoing prospective study at Memorial Sloan-Kettering Cancer Center investigates patients with HER2 positive MBC and progression on trastuzumab or lapatinib or recurrence after adjuvant

trastuzumab whenever biopsy is feasible to characterize molecular signatures of recurrence (Chandarlapaty, personal communication).

### Retrospective Series

Most studies looking at discordance rates between primary and metastatic breast cancer are retrospective and could be inherently biased because of inclusion of selected patient cohorts. These studies rely on data retrieved from pathology reports obtained using older pathology techniques and reagents different for primary and secondary tumors, and there is a possibility of heterogeneous groupings that include those with local recurrences.

One of the largest series is a Swedish study detailing results in patients who underwent biopsy for a suspicious recurrence with at least one radiologic lesion. ER and PgR information was available from the primary tumor and one or more recurrent sites in 486 and 456 patients respectively, resulting in 679 and 630 pairs [35]. For ER, 27% of patients changed from positive in the primary tumor to negative in the relapsed tumor, and 8% changed from negative to positive. PgR status changed from positive to negative in 38%, and 5% changed from negative to positive. Patients with concordant ER status (whether positive or negative) showed overall survival significantly longer than the discordant group. Stable ER-positive patients had the same outcome as primary ER-negative breast cancer with ER-positive relapse, and patients who had ER-negative relapse had a shorter survival regardless of the ER status in the primary tumor. A new primary cancer or a metastasis from another cancer such as chondroma, primary lung cancer, carcinoid metastasis, metastatic colorectal carcinoma, high-grade lymphoma, or a benign lesion were also found, further underscoring the clinical value of rebiopsy for appropriate patient management [12].

A retrospective study from MDACC reported ER, PgR, and HER2 discordance of 18.4%, 40.3%, and 13.6%, respectively. Findings were correlated with clinical and pathological parameters. Immunohistochemistry scores for ER and PgR showed weak concordance between primary and recurrent tumors. Concordance of HER2-FISH scores was higher [16]. Concordant ER/PgR status was associated with better postrecurrence survival than discordant cases, for whom survival was similar to that of patients with triple-negative breast cancer [16].

A study reported ER/PgR and HER2 status in 255 patients with matched primary breast and hepatic metastases. HER2 discordance between the matched primary breast and liver metastasis samples was 13.9%. Of patients with HER2-positive primary cancer considered for trastuzumab therapy, 31.5% had a negative HER2 liver biopsy result, whereas 5.9% of those initially negative for HER2 were HER2 positive on the metastasis biopsy. For ER status, 14.5% of tumors were discordant: 25.9% of those with a negative primary had a positive secondary, and 11.2% of those with a positive primary had a negative liver metastasis [36]. ER/PgR and HER2 were manually scored. Systemic therapy changed for 31 (12.1%) of 255 patients; thus, the number needed to biopsy to alter immediate patient management was 8.2.

A study of 50 patients naive for endocrine therapy who had paired tissue samples found that loss of ER in the secondary tumor was a significant predictor of poor response to endocrine therapy [37]. Data derived mainly from Karolinska showed a discordance rate of 12% for HER2 status [12]. This was mostly derived using IHC with two to three monoclonal antibodies, and FISH verifications. FISH was used on the cytologic aspirates from the same laboratory. The discordance rate is similar to the 13.6% rate described by MDACC [16].

## Conclusions

Effective therapy of breast cancer requires accurate diagnosis confirming the relevant molecular markers. Changes in receptor expression may be apparent because of the techniques used or interobserver variability. Rather than accepting a dichotomized laboratory result, clinicians need to familiarize themselves with testing methods such as IHC versus FISH.

A study showed that up to 82% of patients with suspected metastatic lesions agree to undergo a biopsy [34]. Lack of resources, technical difficulties in obtaining metastatic tissue, and the reluctance to undertake an invasive procedure in a patient who has advanced disease are all factors contributing to lack of rebiopsy. Decisions need to be balanced with the more rational use of therapy based on biopsy findings. Of the three markers—ER, PgR, and HER2—results from PgR, particularly from core biopsy, should be interpreted with caution [38]. Fortunately, compared with ER, PgR has a less robust correlation with response to hormonal therapy [3]. Endocrine-responsive cancers have less visceral involvement and a longer disease-free interval [1]. Loss of ER is associated with distant metastasis compared with locoregional recurrence, as well as de novo endocrine resistance. Therefore, the development of metastatic disease and early failure of endocrine therapy have been suggested as indications for biopsy [39].

An important question is whether confirming or verifying receptor status in new metastases and management changes would ultimately lead to improvements in patient quality of life and survival. Though the results of rebiopsy have clearly had an impact on management decisions and it

is intuitive that "tissue-informed" systemic treatment should improve outcomes, this belief has yet to be proven in clinical trials.

Theoretically, exposing the tumor to a prolonged period of treatment targeting one pathway may upregulate other pathways and affect which cells survive to travel to distant locations. Molecular differences could be due to treatment or biologic differences between the primary and metastatic tumor cells. Tumor clone selection during treatment could explain the discordant molecular signatures of primary and metastatic lesions, but one study found that discordance in molecular profiles occurred regardless of chemotherapy [40].

The mechanism by which metastases arise with a different profile from the primary tumor is still unclear, but heterogeneous tumor populations, genetic drift, or clonal selection of tumor clones are all possible. It is believed that cancers evolve by a series of discrete events, so finding heterogeneity is not unexpected. Cancer could gradually evolve in the metastatic site, acquiring a dramatically altered profile, and then return to the primary site and expand its mass [41]. The process of "tumor seeding" has been demonstrated experimentally [42•]. Gene loss and/or additional mutations raise questions about the clonal nature of tumors. It is possible that primary tumors are heterogeneous, with different clones giving rise to disseminated tumor cells with molecular profiles different from most of the primary tumor. Discordance between primary and metastatic sites is not unique to breast cancer and has been reported in other tumor types [43]; the idea of heterogeneity within tumors has been theorized as one possible cause for different mutational profiles in primary and metastatic tumors in colorectal cancer [44] and lung carcinogenesis [45]. Intratumoral heterogeneity was analyzed in 44 breast carcinomas and five normal breast tissues, using tissue microarray (TMA) for ER, PgR, HER2, E-cadherin, EGFR, p53, and MIB-1. Intratumoral heterogeneity was seen with ER, PgR, HER2, p53, and MIB-1. E-cadherin and EGFR failed to show intratumoral heterogeneity. This finding was thought to indicate problems with interpretation of small biopsy specimens [46]. Another study with genome-wide expression profiling of 50 biopsies from 18 individual patients demonstrated that variation between a primary cancer and its metastasis was less than the total variation observed across the patient population. Nevertheless, a fraction of genes exhibited significant intratumoral heterogeneity. A high degree of reproducibility was observed in single-gene predictors of ER and PgR expression, with high IHC concordance [47•].

Discordance between different metastases has been observed with upregulation of genes involved in DNA replication and signal transduction [48]. However, these studies do not detect epigenetic changes, postgenomic changes, phosphorylation events, expression of growth factor receptors or their associated downstream kinases, or differences in the microenvironment. Furthermore, alterations in downstream molecules can influence responsiveness to therapeutic interventions regardless of confirmation of membrane receptor status; for example, the loss of phosphatase and tensin homologue (PTEN) reduces responsiveness to trastuzumab [49].

It is expected that future clinical trials will increasingly require obtaining metastatic tissue to assess molecular differences, not only at the receptor level but also at the functional pathway level. Target-driven therapeutic interventions will be developed to be molecular profile specific.

An increasingly rational drug development effort has resulted in agents against new molecular targets that are active against only those tumors with specific molecular alteration or phenotype. Potential agents for the future include receptor and nonreceptor tyrosine kinase inhibitors including HER1 (EGFR) , HER 2 and 3, insulin-like growth factor receptor, c-met, fibroblast growth factor receptor, and HSP 90 inhibitors; intracellular signaling molecules such as PI3 kinase, AKT, and mTOR; antiangiogenic agents; and other agents that interfere with DNA repair, such as poly-ADP-ribose polymerase (PARP) inhibitors [50]. Tailored therapies will be required based on individual molecular signatures, allowing maximal benefit and avoidance of toxicities. Diagnostic tissue analysis and its accurate interpretation are becoming increasingly vital as such endeavors continue to individualize and improve treatment modalities in breast cancer.

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- •• Of major importance

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