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Breast Cancer

Molecular Alterations Between the Primary Breast Cancer and the Subsequent Locoregional/Metastatic Tumor

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Learning Objectives:

After completing this course, the reader will be able to:

1. Describe the rate of discordance of predictive marker phenotype (i.e., ER/PR, HER2) between the primary and the relapsed/metastatic breast cancer lesion.
2. Explain the impact of a change in predictive marker phenotype between the primary and relapsed/metastatic lesion on treatment options for these patients.

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Abstract

Background. **Metastatic breast cancers have historically been presumed to have the same predictive biomarkers as the initial primary tumor. We compared the expression of these biomarkers in a large paired tissue microarray (TMA) series of primary and subsequent relapsed tumors.**

Methods. **Using the British Columbia Cancer Agency Breast Cancer Outcomes Unit database, patients with biopsy-proven relapses were identified and linked to a large TMA series of primary breast cancers from 1986–1992. Charts were reviewed, and tissue blocks of the metastatic cancer were collected to create a separate TMA. Immunohistochemical assessment with the same antibodies and conditions was performed for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER)-2 on both the primary and relapsed tumors.**

Results. **One hundred sixty cases were received that had tumor adequate for analyses. Of these, 71.9% had no changes in either the ER or PR status or HER-2 status. Of the 45 (28.1%; 95% confidence interval [CI], 21.2%–35.1%) tumors that did have changes in receptor status, 7.5% were in-breast recurrences or new breast primaries, 4.4% had changes in PR status only and were therefore deemed clinically irrelevant, and 19.4% (95% CI, 13.3%–25.5%) had changes in either the ER or HER-2 status from regional or distant relapses. Five percent of tumors had a receptor status change going from ER^+ or PR^+ to ER^- or**

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PR^-; 9.4% went from ER^- or PR^- to ER^+ or PR^+. With regard to HER-2 status, 3.8% of tumors went from positive to negative and 1.3% went from negative to positive. For all discordant cases, biopsies of the relapsed lesion were obtained prior to initiation of first-line treatment for metastatic disease. In the primary tumors that were ER^+, time to relapse was significantly shorter in the discordant relapsed cases than in the concordant ones ($p = .0002$). Changes in loss or gain of either biomarker were seen across the discordant cases.

Conclusions. **A significant proportion of relapsed tumors had changes in either ER or HER-2 status, which would dramatically alter treatment recommendations and clinical behavior. This study suggests that biopsies of relapsed and metastatic breast cancers should be performed routinely in clinical practice.** ***The Oncologist*** 2012;17:172–178

Introduction

Breast cancer is the most prevalent cancer diagnosis in women in industrialized nations. Systemic treatment is delivered in the vast majority of patients with either early or advanced stage disease. Despite numerous advances in adjuvant treatments for localized breast cancer, a significant proportion of patients suffer systemic relapse [1]. Although we have seen increasingly efficacious treatments for metastatic breast cancer over time [2], it is still considered an incurable disease today.

Currently, the only predictive factors generally used to guide the systemic treatment of patients with breast cancer are the estrogen receptor (ER) and progesterone receptor (PR) status, and the human epidermal growth factor receptor (HER)-2 status from the initial breast cancer lesion. Despite historical data suggesting the potential for ER [3] and PR [4] discordance, in the majority of patients who suffer a relapse, the assumption has been that these predictive factors are unchanged. As such, there are currently no clinical practice guidelines advising physicians to rebiopsy at the time of relapse.

This assumption was recently called into question by the publication of several small studies that suggested that relapsed or metastatic lesions may have a different hormone receptor or HER-2 status from that of the primary tumor [5]. While adding to the growing body of evidence suggesting discordance in the molecular phenotype between primary and relapsed breast cancer, the clinical impact of these studies has been limited by small sample sizes, differences in detection methodology between the primary and relapsed lesions, and the retrospective nature of the studies.

We sought to compare the hormone receptor and HER-2 status of relapsed or metastatic breast cancer with those of the original tumor in a relatively large paired series with identical contemporaneous methodology for detection and scoring for both the primary and relapsed lesions. Once we had established that, indeed, discordance in the molecular phenotype did exist, we attempted to determine whether there was a pattern seen in the discordant cases that could be attributed to the systemic treatments received.

Methods

The British Columbia Cancer Agency (BCCA) has a mandate of cancer control in the entire province of British Columbia. The BCCA Breast Cancer Outcomes Unit (BCOU) database maintains a prospective database with detailed baseline demographic, pathologic, adjuvant therapy, and initial relapse data from all breast cancer patients diagnosed since 1989 and referred to the BCCA regional cancer centers. Using the BCCA BCOU database, we were able to identify all patients who had a biopsy-proven local, regional, or distant relapse. We excluded women diagnosed with an interval contralateral new breast primary and women with a prior nonbreast cancer malignancy or a synchronous presentation of bilateral breast cancer. We then linked this identified cohort to a current large (n = 4,444) tissue microarray (TMA) series of primary breast cancers diagnosed in 1986–1992 already in a TMA [18]. This primary breast cancer TMA is also fully annotated with baseline clinical, pathologic, and outcome data.

We reviewed the charts of those patients who were linked to the primary breast cancer TMA to determine whether adequate tissue samples were available for collection. A secondary chart review was also done to confirm information regarding the site of relapse and systemic treatments delivered both prior to and following the relapse biopsy to assess for a potential impact on discordant cases. Baseline demographic information, including age, date of initial diagnosis, date of relapse, primary surgery, and adjuvant systemic and locoregional treatment, was also collected.

We requested that all available tissue blocks be sent from the originating hospital and created a second microarray of the metastatic tumors. Duplicate 0.6-mm cores were obtained from the tumor blocks. The tumors were graded and immunohistochemistry (IHC) was performed for ER (Lab Vision SP 1 antibody; Lab Vision IHC System Solutions, Freemont, CA) [18], PR (Ventana 1E2 antibody; Ventana Medical Systems, Inc., Tucson, AZ), and HER-2 (Lab Vision SP 3 antibody, Lab Vision IHC System Solutions) [19] as previously described in detail. Both the ER and PR status were considered positive if $\geq$1% of the cells stained positive. HER-2 was considered positive if it was 3+ on IHC. If the tumor was 2+, fluorescence in situ hybridization for HER-2 was performed (on the primary TMA only) as per standard conditions and scoring criteria. The same conditions and antibodies were used to evaluate the receptor status on both the primary tumor and relapsed TMAs. Appropriate internal (both positive and negative) controls were used on both the primary and metastatic TMAs. We compared the ER, PR, and HER-2 status of the primary breast cancer with that of the relapsed lesion. The pathologist scoring the relapsed TMA was blinded to the IHC results from the primary TMA series.

Statistical Analysis

Patient characteristics, treatment variables, and relapse information were summarized using descriptive statistics such as the mean, median, and range for continuous variables and

Table 1. Baseline demographic and clinical characteristics

Characteristic	*n* (%)
Median age (range), yrs	60 (23–89)
Systemic treatment	
No systemic treatment	71 (44%)
Hormones	44 (27%)
Chemotherapy	33 (21%)
Chemotherapy and hormones	12 (8%)
Site of relapse	
Local	34 (21%)
Regional	99 (62%)
Distant	27 (17%)
Type of surgery	
Lumpectomy	7 (6%)
BCS and XRT	45 (28%)
Mastectomy with or without XRT	107 (66%)
ER status (primary)	
Positive	97 (61%)
Negative	56 (35%)
Unknown	4 (4%)
PR status (primary)	
Positive	69 (43%)
Negative	71 (44%)
Unknown	20 (13%)
HER-2 status (primary)	
Positive	29 (18%)
Negative	125 (78%)
Unknown	6 (4%)
Stage	
1	51 (32%)
2	90 (56%)
3	15 (9%)
Unknown	4 (3%)

Abbreviations: BCS, breast-conserving surgery; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; PR, progesterone receptor; XRT, radiation therapy.

counts and percentages for categorical data. The discordance rate for the primary tumor and relapse receptor status was calculated as a percentage of all patients included in the TMA and the 95% confidence interval (CI) was calculated using the normal approximation method. Time to first relapse in patients who were ER^+ on primary samples was analyzed using the Kaplan–Meier method; the log-rank test was used to test for differences in time to relapse (TTR) between those with primary–relapse ER status discordance and concordance.

Results

The BCCA BCOU database identified 1,593 cases in which relapse was confirmed with a biopsy. It was determined that 281 of these cases were linked to the primary breast cancer TMA from 1986–1992. Thirty of the 281 cases linked to the TMA were excluded based on inadequate tissue sample as determined by chart review; 251 blocks were, therefore, requested. Of these, 184 blocks were received, and 160 had tissue adequate for creation of a TMA series.

Table 2. Summary of receptor status differences between the primary and relapsed or metastatic lesion

Receptor status	*n* of changes, *n* (%)
ER or PR positive to negative	8 (5.0%)
ER or PR negative to positive	15 (9.4%)
HER-2 negative to positive	2 (1.2%)
HER-2 positive to negative	6 (3.8%)

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Table 1 shows baseline patient demographics for the 160 blocks received and scored on the relapses. The mean age was 60 years (range, 23–89 years). Thirty-two percent of patients had stage I disease at diagnosis, whereas 56% had stage II disease, 9% had stage III disease, and the initial disease stage was unknown in 3%. Forty-four percent of patients had no adjuvant systemic therapy, 27% received hormonal therapy alone, 21% received chemotherapy, and 8% had both chemotherapy and hormonal therapy. With regard to initial surgery, the majority of patients had a modified mastectomy with or without radiation (67%). Twenty-eight percent had breast-conserving surgery and radiation therapy, and only 5% had lumpectomy alone. The patterns of relapse available for the TMA were as follows: 21% ($n = 34$) with local relapse, 64% ($n = 99$) with regional relapse, and 15% ($n = 27$) with distant relapse. The median time to first relapse (local, regional, or distant) was 35 months (range, 4–149 months). Secondary chart review confirmed that all the biopsies that exhibited a change in ER or PR status and/or HER-2 status were acquired from patients before first-line treatment in the metastatic setting was initiated.

Of the 160 blocks that were scored, 45 (28.1%; 95% CI, 21.2%–35.1%) had changes in ER or PR status or HER-2 status. Of those, 12 (7.5%) were deemed to be potentially new ipsilateral breast primaries or local recurrences based on review of the charts and pathologic reports; two additional cases had a change in PR status in the absence of a change in ER status and were not considered clinically relevant. Excluding those, 31 (19.4%; 95% CI, 13.3%–25.5%) had changes in the predictive marker phenotype in the context of a locoregional or distant relapse.

Table 2 illustrates the breakdown of the specific receptor status changes. Eight patients (5.0%; 95% CI, 1.6%–8.4%) had a receptor status change going from ER^+ or PR^+ to ER^- or PR^-, and 15 patients (9.4%; 95% CI, 4.9%–13.9%) went from ER^- or PR^- to ER^+ or PR^+. Six patients (3.8%; 95% CI, 0.8%–6.7%) who were originally HER-2^+ became HER-2^-, whereas two patients (1.3%; 95% CI, 0.0%–3.0%) went from HER-2^- to HER-2^+. There did not appear to be any pattern of change in molecular phenotype associated with a particular systemic adjuvant treatment modality.

Delving further into the clinical behavior of the discordant cases, we looked at TTR based on the change in biomarker status and type of adjuvant systemic therapy delivered. Figure 1a shows the Kaplan–Meier curves for TTR comparing all patients who were ER^+ concordant between initial and metastatic lesions with discordant cases (those who went from ER^+ to ER^-). It should be noted that the initial analysis of receptor discordance excluded in-breast recurrences; however, the TTR analysis included all patients with either a local, regional, or distant relapse and did not exclude in-breast recurrences. There was a statistically significant difference in TTR between the two groups ($p = .0002$). The median TTRs were 4.2 years (95% CI, 3.3–5.2 years) and 1.9 years (95% CI, 1.3 years to not applicable) in the ER concordant ($ER^+ \rightarrow ER^+$) and discordant ($ER^+ \rightarrow ER^-$) groups, respectively. Among those patients who were ER^+ and who received hormonal therapy following initial diagnosis, there was a statistically significant longer TTR in the receptor concordant ($ER^+ \rightarrow ER^+$) group than in the receptor discordant group ($ER^+ \rightarrow ER^-$) ($p = .0021$) (Fig. 1B). Less than half of the patients who were ER^+ at the time of initial diagnosis received adjuvant hormonal treatment, and this may be in part a result of the fact that many of them were treated prior to the era when premenopausal ER^+ patients were universally recommended treatment with adjuvant tamoxifen.

Figure 2A shows the Kaplan–Meier curves for TTR comparing all patients who did not receive adjuvant systemic therapy who were ER^- concordant between initial and metastatic lesions with discordant cases (i.e., those who went from ER^- to ER^+). The median TTRs were 2.0 years (95% CI, 2.5–6.0 years) and 3.1 years (95% CI, 2.4–5.4 years) for the ER^- concordant ($ER^- \rightarrow ER^-$) and discordant ($ER^- \rightarrow ER^+$) cases, respectively ($p = .006$). There was no statistically significant difference in TTR for concordant ($ER^- \rightarrow ER^-$) versus discordant ($ER^- \rightarrow ER^+$) cases among those patients who were initially ER^- who received adjuvant hormonal therapy (Fig. 2B).

We did not perform TTR analysis in the context of HER-2 status changes in part because of the small number of patients available for analysis. Further, the patient population evaluated in this study predated any anti–HER-2 therapies so no comment can be made about the impact of treatment on TTR in concordant versus discordant cases.

Discussion

This is one of the largest series published to date demonstrating discordance in molecular phenotype between the primary breast cancer and relapsed or metastatic lesion. The strength of this study lies in the acquisition of tissue from both the primary tumor and the relapsed lesion, the identical treatment of the primary and relapsed lesions with regard to specific antibody staining conditions and scoring criteria, the blinding of the pathologist, and the assessment of both ER or PR status and HER-2 status. We demonstrated that a discordance rate of ~20% exists between the predictive marker phenotype of the primary and metastatic tumor in the context of locoregional or distant disease.

Our results parallel those of other smaller recent studies. A paper by Gutierrez et al. [20] demonstrated rates of discordance with regard to ER and HER-2 status of 17% and 11%, respectively, in a cohort of patients with locoregional recurrence who had previously been treated with tamoxifen. This served as an important proof of principle, but was limited by sample size and the fact that the majority of recurrences were in the ipsilateral breast or chest wall. Our study included a larger sample size and excluded ipsilateral or in-breast recurrences as part of our analyses. A paper by Liedtke et al. [11] added more to the discussion by correlating outcome with receptor discordance. That retrospective study demonstrated that patients who were found to have receptor discordance fared poorly compared with those who maintained receptor concordance, presumably because of inappropriate use of hormonal and/or targeted therapies (although those data were not reported). Further, that study was limited by the fact that the primary tumor receptor status was based on written pathology reports.

Despite the strengths of our study, there are some limitations that warrant discussion. Despite the relatively large sample size, the number of patients with true distant metastases was limited, so the conclusions may not be generalizable to all patients presenting with distant metastases. Every attempt was made to distinguish recurrent or metastatic disease from new ipsilateral breast primaries, and those samples that were deemed to possibly be new primaries based on review of clinical notes and pathological reports were considered separately. The retrospective nature of the study does not limit applicability to modern breast cancer populations because the creation of both the primary tumor TMA and the relapsed tumor TMA occurred within the last few years. Thus, one potential limitation is assessment of the impact of adjuvant hormonal or chemotherapeutic treatments (e.g., exposure to adjuvant taxanes, trastuzumab, aromatase inhibitors), because the adjuvant treatment received by the study population is not consistent with today's standard. With a prospectively conducted study, patterns of predictive marker phenotype discordance in the context of current adjuvant therapies may emerge, although to date this has not been demonstrated [21].

Another potential limitation of the current study is the possibility for antigen loss over time in formalin-fixed paraffin-embedded tissue blocks. The tissue samples used to create both the primary and relapsed TMAs were, in the majority of cases, >15 years old. This is a potential explanation for ER^+, PR^+, or $HER\text{-}2^+$ tumors becoming negative; however, it does not explain the reverse. Of the 31 cases in which molecular differences between the primary and metastatic lesions were observed, 17 (54.8%) exhibited a gain in receptor status.

The issue of intratumoral heterogeneity is one hypothesis as a reason for the rate of discordance seen between the primary and relapsed lesion. There is a large amount of data in the literature surrounding heterogeneity in the assessment of both ER and HER-2 status. Estimated rates of HER-2 heterogeneity within a tumor sample are in the range of 5%–30% in the published literature [22], and are less for ER [23]. Moreover, in a recently published study in which a lobular cancer primary and subsequent metastasis genome and transcriptome were sequenced to a single nucleotide resolution, the authors found that six somatic mutations were present in the primary at low frequencies (1%–15%), again supporting the existence of tu-

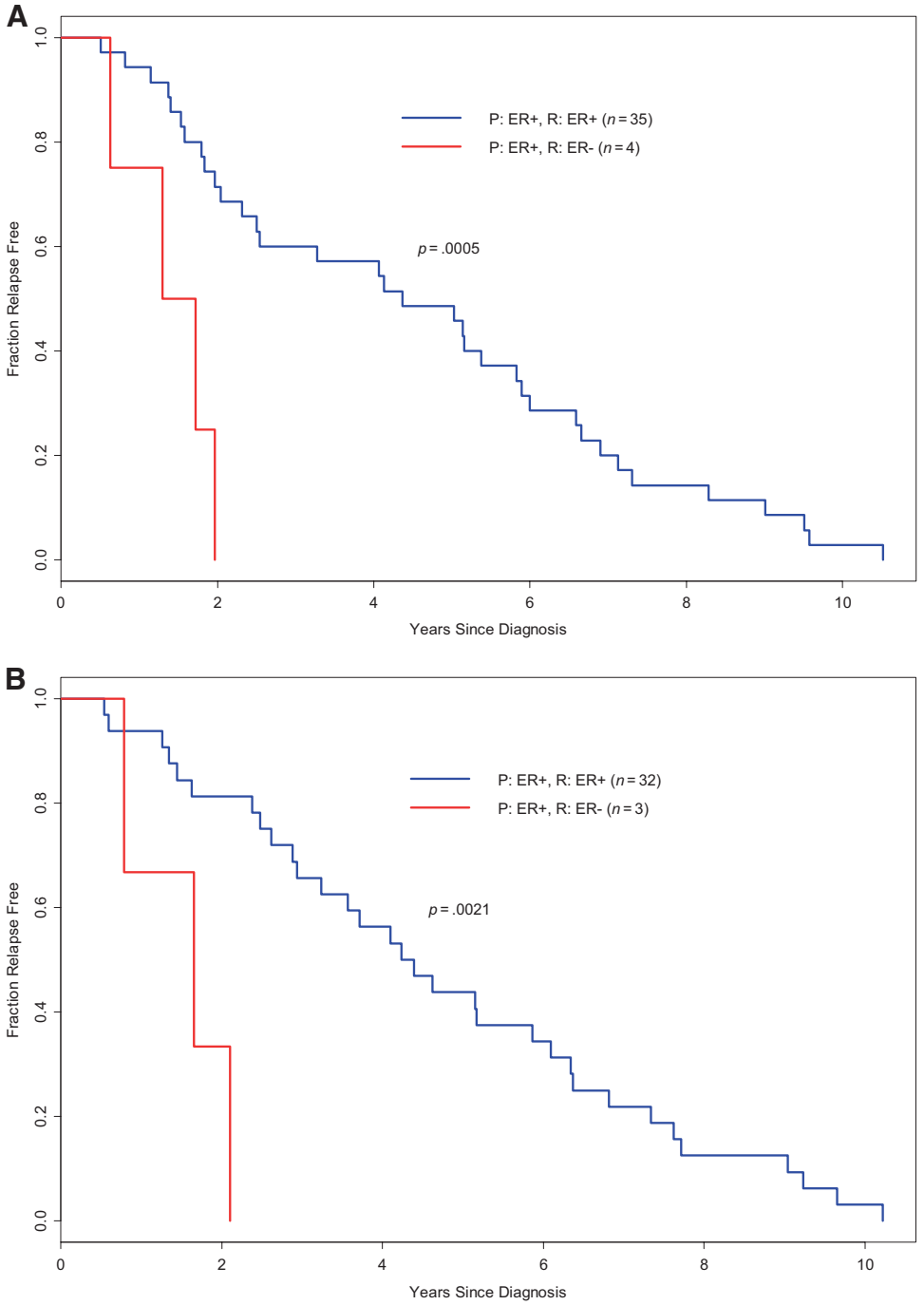

Figure 1. Kaplan–Meier plot of time to relapse in primary ER^+ cases by ER status at the time of relapse in patients who did not receive adjuvant systemic therapy **(A)** and in patients who received adjuvant tamoxifen **(B)**.

Abbreviations: ER, estrogen receptor; P, primary; R, relapse.

mor heterogeneity [24]. What remains unclear is to what degree, if any, prior systemic therapy exposure alters the emergence of different subpopulations of heterogeneous clones. We were unable to detect a clear pattern that would implicate adjuvant systemic treatment as a clear contributing factor for receptor status discordance. It is unclear if such a pattern would emerge with evaluation of primary and metastatic tumors that were collected prospectively and within the context of a larger sample size.

The issue of heterogeneity is not limited to that which potentially exists within a single site of metastasis. Indeed, Wu and colleagues reported on a small study ($n = 10$) in which the authors examined multiple metastatic tumors at autopsy for ER or PR and HER-2, among other markers. They found significant heterogeneity of predictive markers among tumors from different sites in the same patient assessed at the same point in time [25]. They did not, however, correlate their results with any antecedent systemic treatments the patients received.

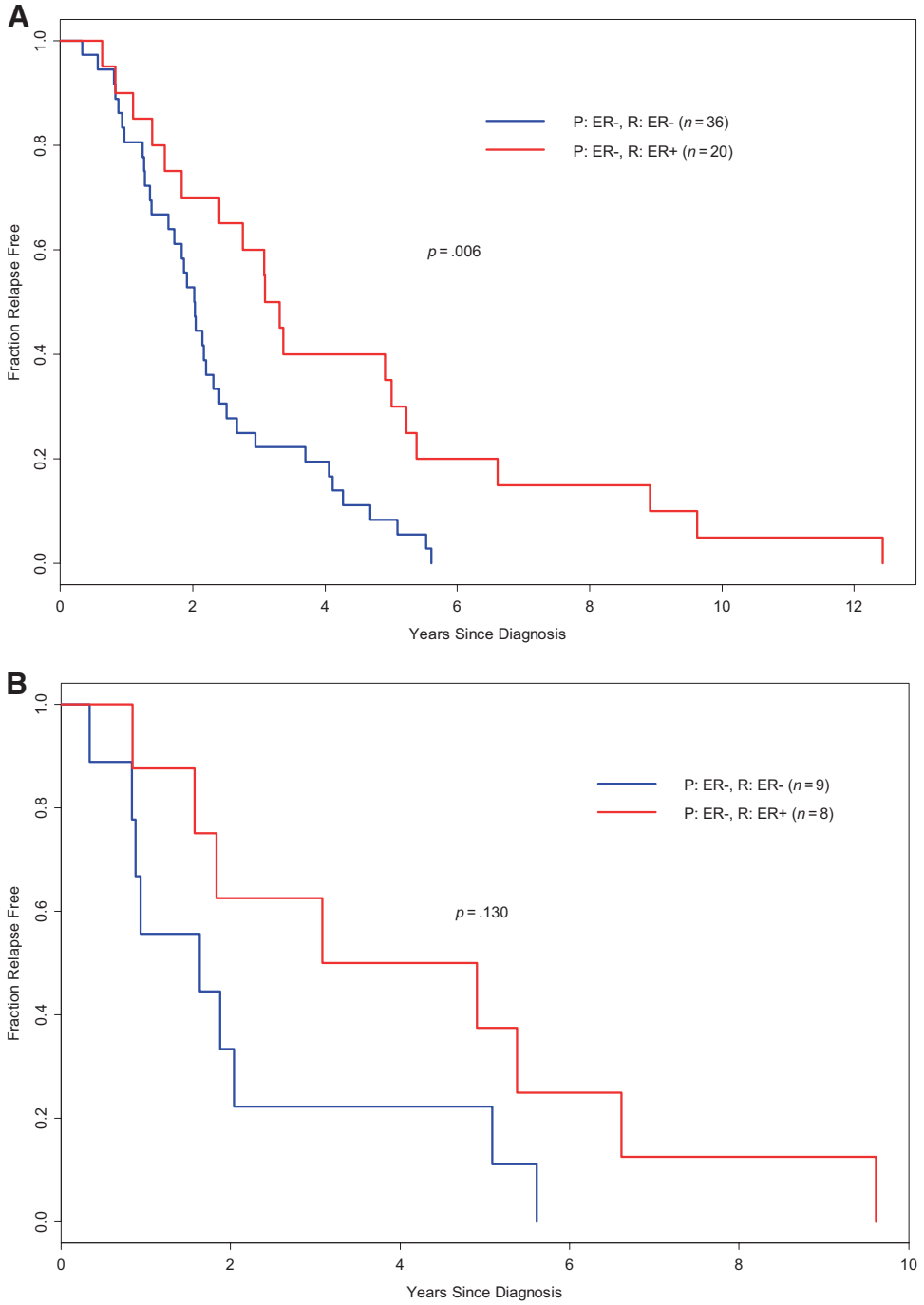

Figure 2. Kaplan–Meier plot of time to relapse in primary ER$^-$ cases by ER status at the time of relapse in patients who did not receive adjuvant systemic therapy **(A)** and in patients who received adjuvant tamoxifen **(B)**.

Abbreviations: ER, estrogen receptor; P, primary; R, relapse.

An alternative theory is that changes in predictive markers are the result of survival of a breast cancer stem cell that subsequently redifferentiates to express alternative molecular phenotypes. Though this appears to be an attractive hypothesis, much work remains to be done to definitively demonstrate that breast cancer stem cells (or tumor-initiating cells) exist in clinical tumors and can be reproducibly and readily identified from collected specimens.

Attempts that have been made to correlate discordance in receptor status with interval treatment have yielded no obvious pattern [16, 20, 26] that would predict in which patients we may see these molecular differences between primary and metastatic lesions. Similarly, we observed no such pattern.

At the 2010 Annual American Society of Clinical Oncology meeting, Amir and colleagues reported the results of a prospective study looking at rates of hormone receptor and HER-2 discor-

dance [21]. Their protocol mandated that patients be rebiopsied at the time of relapse. The investigators observed a rate of discordance of 38.8% and determined that, for 15.1% of patients, the treatment was changed based on the results of the biopsy. Unfortunately, a large number of these patients had ipsilateral breast recurrences, so it is unclear if these cases represent new breast primaries or relapsed disease. A Spanish-led observational study prospectively enrolled >200 patients and will assess the rates of discordance in HER-2 status between primary and metastatic tumors (ClinicalTrials.gov identifier, NCT01377363).

In summary, this is one of the largest studies to date exhibiting changes in the ER or PR status and HER-2 status between the primary and relapsed or metastatic breast cancer in which both the primary and metastatic tumors were collected and evaluated under similar conditions. These findings have significant implications for the selection of treatment options of relapsed breast cancer and subsequent response to therapy, and they add to the growing body of evidence illustrating the need for rebiopsy at the time of relapse or recurrence whenever feasible. Future studies will need to be done with sophisticated molecular interrogation from the genome to functional proteomics to understand the biology of this discordance.

Author Contributions

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See the accompanying commentary on pages 151–153 of this issue.