Pharmaceutical Research, Vol. 23, No. 9, Septembet 2006 (© 2006)
DOI: 10.1007/s11095-006-9075-5

Expert Review

Heterogeneity of Breast Cancer among Patients and Implications for Patient Selection for Adjuvant Chemotherapy

Fabrice Andre[1,2] and Lajos Pusztai[1]

Received March 9, 2006; accepted May 24, 2006; published online August 12, 2006

Abstract. Although the benefits of adjuvant chemotherapy are not controversial, the absolute effect of such therapy is small. Therefore, there is a need to identify biomarkers that can help select patients with localized breast cancer for treatment. Despite intense research in this field, no biomarker has been shown to be useful to predict benefit of adjuvant chemotherapy in daily practice. This can partially be explained by the fact that breast cancer is composed of several distinct subclasses, as shown by large-scale genomic analyses. In this review, we discuss why the current research approach based on a single biomarker is limited by the heterogeneity of cancer among patients. We then propose three solutions to improve the research strategies in this field: investigate one biomarker in a single homogeneous subclass to improve its predictive value; study the predictive value of multibiomarker assays in larger populations; and use functional pathways to predict the efficacy of a given drug.

KEY WORDS: biomarker; breast cancer; chemotherapy; DNA microarrays; prognosis.

INTRODUCTION

Three generations of drugs have been undisputedly shown to improve breast cancer outcome, and several other drugs are being considered. The three generations of chemotherapy regimen that have been evaluated are the cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) regimen, the anthracycline-based regimens, and the taxane-based regimens. The CMF regimen was initially evaluated during the 1980s. Meta-analysis of randomized trials has shown that this regimen decreased the annual breast cancer death rate by 34% in women younger than 50 years and by 10% in women aged 50–69 years (1). Anthracycline-based regimens were evaluated during the 1990s. Seventeen trials with a combined patient population of 14,470 patients directly compared a CMF-based regimen with anthracycline-based chemotherapy. A meta-analysis of these trials showed that anthracycline-based regimens decreased the breast cancer death rate by 16%. Long-term toxicities included secondary leukemia and left ventricular dysfunction.

More recently, taxane-based regimens have reportedly improved breast cancer outcome (2,3). Although long-term toxicities are not well established, neurotoxicity could be a limiting toxicity (2,3).

Several other drugs will be evaluated soon in an adjuvant setting. Of these, platinum-based chemotherapy regimens and 5-fluorouracil oral derivatives are the most promising (4,5).

When discussing the optimal drug regimen for each patient, two important points need to be emphasized. First, although some trials have investigated the combination of drugs, most of the regimens use drugs sequentially to deliver optimal drug dosage and to limit toxicities. Second, it has been shown that each drug exhibits antitumor activity in patients who are refractory for another drug. For example, paclitaxel offers an approximate 20% response rate in patients whose breast cancer is refractory to anthracycline treatment (6).

These data suggest that each chemotherapeutic drug provides a small benefit to the overall population with early breast cancer but induces long-term toxicity and potentially delays the administration of effective drugs for refractory tumors. We need to be able to identify patients who benefit most from each chemotherapy regimen, to deliver the effective drug at the right time and for an optimal duration, and to limit long-term toxicity.

Before to go forward in the discussion about how to discover relevant predictive biomarkers, some considerations related to methodology should be reported. One commonly used approach to predictive marker discovery is to identify candidate biomarkers in prospective neoadjuvant trials when chemotherapy is given preoperatively and the pathologic response rates can be directly measured. In this clinical setting, it is usual to compare the pathologic complete response rates according to the biomarker status using a chi-square test. When several studies report concordant results, for example significantly higher pathologic complete response rates in a marker-positive subset of patients, it is reasonable to assume that the same biomarker will also predict benefit in the

[1] Department of Breast Medical Oncology, Unit 1354, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA.
[2] To whom correspondence should be addressed. (e-mail: fandre@mdanderson.org)

ABBREVIATIONS: CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; ER, estrogen receptor.

adjuvant treatment setting when (the same) chemotherapy is given postoperatively. Another approach to biomarker discovery relies on retrospective analysis of tissues obtained from adjuvant treatment trials. In this process, the candidate biomarker is evaluated using data from randomized trials and its predictive value is assessed by a statistical test for interaction (comparison of hazard ratios). Although this interaction test helps to determine which biomarker is "significantly predictive," it does not address the issue of clinical usefulness. A biomarker may be clinically useful by identifying patients who can avoid unnecessary treatment. This involves showing statistical equivalency of outcome between treated and untreated groups of marker-positive patients. This is best proven through an adequately designed prospective randomized clinical trial. Alternatively, one could perform pooled retrospective analysis of randomized trial data. However, it should be noted that validation of "lack of benefit" markers is challenging on two accounts. Falsely ascertaining lack of benefit from therapy for a subset of patients, while a in fact there is a small benefit, could have a significant impact in terms of public health given the high incidence of breast cancer. Also, patients often have low threshold to accept potentially curative adjuvant chemotherapy. Another potential clinical use of a biomarker is to identify a subgroup of patients with significantly higher than average benefit from treatment.

Until recently, the research strategy was to identify a single biomarker that could be used for the overall population. In this review, we discuss how this approach is limited by the heterogeneity of breast cancer and suggest three ways to improve the research strategies in this field: (a) investigate a biomarker only in a single homogeneous subclass of patients to improve predictive value; (b) study the predictive value of multi-gene markers in larger populations; and (c) use a priory defined functional pathways to predict the efficacy of pathway-specific drugs.

BREAST CANCER: A GROUP OF MULTIPLE SUBCLASSES

Evidence of Inter-tumor Heterogeneity of Breast Cancer

Breast cancer characteristics have been known for a long time to differ among patients (7) and these characteristics include estrogen receptor (ER) expression, tumor grade, patient age, and prognosis. Recent data derived from DNA microarray analyses have confirmed these findings by showing that breast cancer actually consists of at least 3–6 subclasses including Her2-overexpressing breast cancer; basal-like breast cancers; luminal-type A, B, and C breast cancers; and normal-like breast cancer (8–10).

Further, the clinical and pathological characteristics vary among these subclasses. For example, luminal-type breast cancer is characterized by ER expression and low- or intermediate-grade malignancy, whereas basal-like breast cancer is characterized by a lack of ER expression and high-grade malignancy. BRCA1-mutated and p53-mutated tumors usually have the same molecular and clinical characteristics as those seen in basal-like cancer (8,11). Luminal and basal-like cancers probably rise from different normal cells, i.e., luminal and myoepithelial cells (9).

Efforts have been made to distinguish among these tumor subclasses on the basis of immunohistochemical characteristics (12–14). Such analyses have shown that luminal-type breast cancers typically express ER and cytokeratin 18, whereas basal-like tumors express cytokeratin 5/6, c-kit, and EGFR but not ER.

Although the relevance of the proposed subclasses of breast cancer, as well as the ability to detect these molecular subclasses at the individual level, is controversial, there is a consensus that large scale gene expression differences exist among breast cancer.

In addition to between-patient heterogeneity, breast cancers may also show intratumoral molecular heterogeneity, meaning that the expression of particular genes could differ at different locations within the same tumor. DNA microarray experiments failed to show large scale gene expression differences within different regions of the same cancer or even between primary tumors and lymph node or distant metastasis. However, smaller scale differences that may affect dozens or hundreds of genes can and likely to exist. Discovery and validation of markers whose expression shows large regional variation within a cancer is difficult because of unavoidable sampling error that will affect the results of any such study. Not surprisingly, for most current biomarkers, even if variably expressed, a single representative biopsy or tissue section is sufficient to determine marker status.

Do Breast Cancer Molecular Subclasses Exhibit Differential Chemotherapy Sensitivity?

In this section, we provide answers to the following two questions: (1) Are the proteins involved in the cellular response to chemotherapy different among the breast cancer subclasses? and (2) Is the efficacy of chemotherapy different among the breast cancer subclasses?

In considering how the molecular heterogeneity of breast cancer could affect current research regarding predictive biomarkers, it is first important to determine whether the proteins involved in the cellular response to chemotherapy are different among the breast cancer subclasses. In this regard, Troester *et al.* (15) showed that the gene expression profile induced by cellular exposure to anthracyclines is clearly different according to cell origin (luminal- *versus* basal-like). Although the observations of these researchers were limited to four cell lines, their findings show that a single protein does not necessarily mediate resistance or sensitivity across all subclasses of breast cancer. Similarly, Rouzier *et al.* (16) observed in a series of patients with breast cancer treated with neoadjuvant chemotherapy that the biomarkers associated with pathological complete response were different between basal-like and Her2-overexpressing tumors. Although these data are limited, they support the hypothesis that the biomarkers responsible for resistance to a given drug differ among the breast cancer subclasses.

With regard to the differential efficacy of chemotherapy among the different subclasses, one study (16) showed that the rates of pathological complete response to neoadjuvant chemotherapy were indeed different according to the molecular subclass shown by genomic profiling. These rates were 45% in patients with Her2-overexpressing or basal-like tumors, 7% in patients with luminal-type tumors, and 0% in patients with normal-like tumors. Although no study specifically focused on the predictive value of breast cancer molecular subclassification regarding adjuvant chemothera-

py, some reports have suggested a differential effect of chemotherapy according to ER status (17,18). Furthermore, several reports have suggested that adjuvant anthracycline-based chemotherapy is more effective in patients with Her2-overexpressing breast cancer than in patients with other subclasses of breast cancer (19–21).

Although the concept of breast cancer molecular subclassification and cancer heterogeneity among patients is recent and controversial, data previously reported suggest that the mechanism of action and the efficacy of cytotoxic agents differ among the breast cancer molecular subclasses. These data would suggest that predictive biomarkers for the efficacy of chemotherapy probably differ among tumor subclasses. In the next sections we discuss how this latter finding has an impact on future research strategies for finding predictive biomarkers.

LIMITATIONS OF RESEARCH BASED ON A SINGLE PREDICTIVE BIOMARKER IN OVERALL BREAST CANCER CASES

It has been suggested that the cellular response to drugs differs among subclasses; thus, we can speculate that a single biomarker for drug resistance will have different predictive values depending on the breast cancer molecular subclass. Therefore, because of breast cancer heterogeneity, research that focuses on a single biomarker in the overall population is suboptimal for the following reasons: first, since we hypothesize that a single biomarker has predictive value in only a subset of patients, by extension, the biomarker has less predictive value in the other patient subsets. Second, since most biomarkers are associated with a particular cancer subclass, their predictive value usually reflects the differential efficacy of chemotherapy among breast tumor subclasses (Table I). Finally, this latter argument suggests a need for a reproducible percentage of breast cancer subclasses in order to be able to compare studies.

Although biomarkers of drug resistance have been evaluated in patients with breast cancer, to date, none have been adopted into clinical use because of their ultimate inability to predict response to treatment. To illustrate how tumor heterogeneity is responsible for this, we focus on two frequently investigated biomarkers: p53 and topoisomerase IIα.

p53 is a protein involved in the cellular response to anthracyclines (22). Although *in vitro* studies have clearly shown that the mutated form of p53 confers resistance to anthracyclines, translational research studies in humans have generated large amounts of non-concordant data. When discussing about predictive value of p53 mutations, it is important to emphasize that (a) p53 mutations are associated with a basal-like phenotype (11), a subclass highly sensitive to anthracyclines (16), (b) molecular pathways for cell death have been reported to be p53-dependant in BRCA1 wild type cell lines and p53-independent in BRCA1-mutated cell lines (27). From these two considerations emerges the hypothesis that p53 mutations could have a differential predictive value between basal-like tumors (BRCA1-mutated) and non-basal tumors.

Several reports have shown that mutated p53 correlated with resistance to anthracyclines (23–25), whereas other data have suggested that the mutated form correlated closely with the efficacy of anthracyclines-based chemotherapy (26). A more in-depth analysis of the findings of Geisler *et al.* (25) and Bertheau *et al*. (26), however, showed that the distribution of breast molecular subclasses was different between the two studies. That is, in the Geisler *et al*. study, most patients (72%) presented with low- or intermediate-grade tumors, whereas in the Bertheau *et al*. study, most patients had ER− and high-grade tumors. Putting these clinical data into the current knowledge of breast cancer subclasses suggest that p53 could be associated with resistance in luminal like tumor (low grade) and is associated with efficacy in basal-like tumors (ER− and high grade).

These findings, while only used as an illustration and not yet validated in large studies, could illustrate how a single biomarker that is usually linked to a single breast cancer subclass could have a differential predictive value in each cancer subclass; and thus investigates how the predictive value of a single biomarker in the overall population of breast cancer is suboptimal. Recent advances in the field of molecular classification further underscore the importance of considering the predictive value of a single biomarker in single homogeneous subclasses instead of the overall population.

Topoisomerase IIα, a cellular target of anthracyclines, could also illustrate this point. As with studies of p53, studies of preoperative chemotherapy, although more reproducible than those focused on p53, have generated contradictory results regarding the predictive value of topoisomerase IIα (28–30). Thus it is not clear whether topoisomerase IIa has predictive value in the overall population of patients with breast cancer. However, in considering the expression of topoIIα in each molecular subclass, it appears that the TOPO2A gene is coamplified with the *Her2* gene (31) and that the topoIIα subunit is primarily expressed in patients with Her2-overexpressing breast cancer (32).

These data could give rise to the hypothesis that the predictive value of topoIIα in the overall population is actually linked to the Her2 status and that topoIIα could have very good predictive value in the Her2-overexpressing subgroup, while its predictive value is less in overall breast cancer.

We have highlighted the fact that the expression of most of the previously reported biomarkers for drug resistance is actually associated with a specific subclass of breast cancer

Table I. Correlation Between Biomarkers for Drug Resistance and Breast Tumor Subclasses

Biomarker	Predicted effect	Molecular subclasses (percent of expression for each biomarker)		
p53 mutations (11)	Anthracycline resistance or sensitivity according to studies	Her2-overexpressing subclass 71%	Basal-like subclass 81%	Luminal-like subclass 13% (luminal A)
Topoisomerase IIα gene amplification (35)	Anthracycline sensitivity	Her2-gene amplified 50%	Her2-gene non amplified 0%	
Bcl2 expression (49)	Taxane resistance	ER-negative disease 59%		ER-positive disease 85%
Tau expression (50)	Paclitaxel resistance	Correlates with ER+ status (multiple regression analyses, $p = 0.06$)		

and could present a differential predictive value among subclasses. This consideration limits the power of a single biomarker to predict, with a high level of accuracy, those patients who will benefit from adjuvant chemotherapy in the overall population of patients with breast cancer. Although the investigation related to single biomarkers in the overall population is underpowered by the heterogeneity of cancer, it must be emphasized that these researches have the major advantage to be statistically feasible. Indeed, the populations are large and the probabilities of false-positive results are low.

In the next section we discuss three possible ways to circumvent the difficulties inherent to investigating biomarkers in a disease composed by multiple entities.

WHAT RESEARCH STRATEGIES COULD BE PROPOSED FACING AN INTER-TUMOR HETEROGENEITY

As the foregoing discussion has shown, research to identify biomarkers that can predict the benefit of adjuvant chemotherapy should focus on one or more of the following: investigating one biomarker in a single homogeneous subclass; studying the predictive value of multibiomarkers in larger populations; and using functional pathways to predict the efficacy of a given drug.

Research Focusing on Specific Molecular Subclasses

The fact that most biomarkers of drug resistance are linked to a specific molecular subclass of breast cancer provides the rationale for studies investigating a given biomarker only in a specific subclass. This would improve the biomarker's predictive value and decrease the "background noise" from nonrelevant subclasses. This approach requires a priori definition of both the molecular subclass and the biomarker. As illustration of what kind of study design can be done to address this hypothesis, Di Leo *et al.* (33) investigated the predictive value of the combination of Her2 and the topoIIα subunit. An interesting finding was that *Her2* and *TOPO2A* gene coamplification was associated with beneficial anthracycline-based chemotherapy, compared with the amplification of the *Her2* gene alone. Since these data were generated in a small subset of patients, this study can just be considered as an illustration for the proposed research strategy, and cannot validate the hypothesis that this research approach works better than the conventional one. Further studies have confirmed the link between topoII and *Her2* gene amplifications (31,32). On the basis of these data, the BCIR group (34) retrospectively looked at the predictive value of topoisomerase IIα gene amplification in patients with Her2-overexpressing breast cancer in a randomized trial that compared anthracycline-based chemotherapy with taxane-based chemotherapy. The analysis, although preliminary, trend to report a higher benefit for anthracyclines in Her2+/TOPO2A amplified gene.

Although the predictive value of topoIIα gene amplification in the context of adjuvant anthracycline-based chemotherapy in patients with Her2-overexpressing tumors needs to be confirmed and although reported studies are considered only as hypothesis-generating studies, there are converging arguments suggesting that the predictive value of topoIIα is more relevant to the Her2-overexpressing subclass of tumors than to the other subclasses.

Although looking at the predictive value of a single biomarker in a homogeneous subclass is biologically relevant, this approach presents has two major limitations: first, since each molecular subclass is represented relatively infrequently, focusing on each subclass would require that several thousand patients be screened. Second, even in a homogeneous subclass, a single biomarker may not accurately predict the entire benefit of a given drug. Therefore, although research focusing on the predictive value of a single biomarker in the context of homogeneous subclasses can show the biomarker has greater predictive value than in other subclasses, this research approach is limited by the number of patients and by the relatively poor power of such a study. For these reasons, investigating a combination of several biomarkers has been proposed.

Multigene Assay to Select Patients for Chemotherapy

Because of the limitations of research focusing on a single biomarker assay (Table II), multigene assays have been developed. A multi-marker assay may have a greater ability to predict a clinical outcome because it uses information from a large number of genes each with some but limited predictive value. It is assumed that the combination will perform better than any individual marker. Large-scale genomic analyses that make use of DNA microarray technology illustrate how multibiomarker assays work. In microarray studies, a very large number of genes are compared individually between two groups of patients using some form of signal-to-noise ranking, usually a *t*-test. A drawback to this approach is that *t*-tests primarily evaluate differences in averages of expression between two groups and the *P* value depends on both the between group and within group variation. This is an important consideration particularly if one accepts the hypothesis that breast cancer is not a single disease but a collection of molecularly distinct neoplastic diseases. One could assume that in some instances a predictive marker is restricted to only one particular molecular subset of cancers, whereas in another molecular subset within the same outcome group a different predictor may be useful. Under such circumstances global *t*-tests may be of limited power to identify truly valuable but molecular class-specific predictors. However, in theory combination of several genes in a single assay could measure both molecular class as well as class-specific marker status.

Several gene signatures that predict the efficacy of neoadjuvant chemotherapy have been identified during the past few years in small pilot studies (35–37). For example, we developed a multigene signature (35) that included 74 genes associated with pathological complete response after neoadjuvant paclitaxel/FAC. This signature was generated from a 24-patient discovery set and assessed in an 18-patient validation set. An extension of this study was recently completed which included 133 patients (38). Predictive signatures for other regimens have also been reported (36,37). The clinical validation of these signatures in independent studies has not yet been performed. However, a recurring criticism is that many different prognostic and predictive signatures were proposed often for the same purpose but with very little overlap in the predictive genes (39,40). In fact several different but equally good predictive signatures can be defined from the same data set for the same

Table II. Theoretical Advantages and Limitations of Research Strategies for Predictive Biomarkers

Research approach	Advantages	Limitations	Proposed solutions for improvements	Illustration
Single biomarker in overall patients	•Low false-discovery rate	•Suboptimal results due to tumor heterogeneity	•Go to approaches 2, 3, and 4	Predictive value of p53 mutations
	•Results and techniques reproducible if studies are performed in similar populations	•Usually reflects imbalance of expression between subclasses		
	•Current size of tissue collections adapted	•Need preexisting rationale and identification		
Single biomarker in homogeneous disease subclasses	•Low false-discovery rate	•Current tissue collections not adapted for this purpose (need increased number of patients)	•Combine tissue collections	Predictive value of topoIIα gene amplification in the Her2- overexpressing subclass
	•Results and techniques reproducible	•Need preexisting rationale and identification		
	•Results more optimal than approach 1 (homogeneous disease)			
Multibiomarker assay	•Results more optimal than approach 1: a. Combination of biomarkers predictive for each subclass b. No need for previous rationale or identification	•Higher rate of false-discovery rates than approaches 1 and 2	•Combine tissue collections	Gene signatures
		•Strategy for gene selection not adapted to heterogeneous tumors (*t*-test)	•Select biomarkers based on frequency and not average of expression	
		• Available tissue collections underpowered for validation purpose (gene arrays)	•Select biomarkers in homogeneous disease	
Functional pathway	•Results more optimal than approach 1 (combination of biomarkers with common pathway) •Low false-discovery rates	•Very early step of development		SBIME software, gene set enrichment analysis

purpose. This is due to the large number of genes whose expression is correlated with each other and the large number of genes whose expression is associated with a particular outcome. Within- and between-laboratory reproducibility of gene expression data has extensively been studied and recent reports indicate high reproducibility in well-trained laboratories particularly when the same platform and standard operating procedures are used (41–43 also http://www.fda.gov/nctr/science/centers/toxicoinformatics/maqc). One of the most important limitations of this approach is false-positive results. Indeed, the investigation of numerous genes with conservative *P* values generates some false-positive results due to hazard. For example, using an alpha level at 0.05, when 1,000 genes are studied, 50 are expected to predict events which they are not associated with (false positive). It must be emphasized that other methods like, as example, Metagene analysis are sometimes used to report cDNA array data (44).

As previously reported, a specific problem linked to intertumor heterogeneity is the fact that genes with high predictive values that are expressed in a very small subset of cases will not be readily detected by *t*-statistics. To circumvent this limitation, therefore, some investigators have selected genes separately from different relatively homogeneous molecular groups and have combined these into a single signature for the entire population. For example, Wang *et al*. (45) generated a signature for breast cancer prognosis by selecting 76 genes associated with ER− (16 genes) or ER+ (60 genes) phenotype. By using this approach, they found that the molecular signature-based prognosis had a hazard ratio of 5.5 (range, 2.46–12.5) for all cases (ER−/+) combined. Other multibiomarker scoring systems are being investigated, primarily as a means of predicting prognosis. The most popular is the Oncotype DX (46). This combination of 16 genes has been strongly associated with breast cancer prognosis in ER+ cases treated with tamoxifen. In the context of new molecular classification, this multigene assay is interesting because it integrates both biomarkers associated with breast cancer molecular heterogeneity (Her2, ER) and biomarkers previously reported to be associated with prognosis (e.g., survivin, Ki67, MMP11).

Although a multibiomarker assay is probably a more interesting approach than a single-biomarker assay in the context of heterogeneous diseases such as breast cancer, its power remains hampered by the fact that optimal gene signatures probably differ among the various breast cancer subclasses and because biomarkers are initially filtered on the basis of a *P* value for the overall population, which do not represent the combined results from highly relevant genes with infrequent expression.

Other strategies that combine the multigene assay approach and decrease the limitations due to tumor heterogeneity could improve the results. These approaches would focus not on the genes by themselves but on their functional pathways. Indeed, although genes involved with the cellular response to chemotherapy are different among subclasses, we can speculate that only a few functional pathways are involved in the bioactivity of a given drug.

Functional Pathways to Predict Treatment Efficacy: A Promising Tool in the Context of Heterogeneous Disease

As previously reported, the specific genes involved in the cellular response to a given drug are different among the breast cancer molecular subclasses. Although multibiomarker assays could partially reverse this problem by combining genes, this approach has limitations, as previously reported. The major limitations are related to the difficulties to extract relevant information at a single gene level due to the technology-related noise. In addition, looking at single or a limited number of genes miss important information regarding the physiological effect of genes-network. Based on these considerations, several teams have developed functional pathways analyses to overcome these limitations. Considering that the functional pathways involved in the cellular response to a given drug could be common to different molecular subclasses, analysis of such pathways to predict the efficacy of a given drug is of particular interest. The principle of this approach is to cluster genes into a single functional pathway to predict the benefit of a given drug. The approach consists in clustering the genes according to molecular functions or biological processes, as it refers for example in the Gene Ontology database (47). Several distinct approaches have been developed to analyze functional pathways. The gene set enrichment analyses (GSEA) (48) is an example. In this analysis, the gene sets are defined based on prior biological knowledge, e.g., published information about biochemical pathways or coexpression in previous experiments. The goal of GSEA is to determine to which extent members of a determined gene set are differentially expressed between two distinct outcome groups. This approach is currently being used to analyze what molecular pathways may be involved in determining response or resistance to chemotherapy. A similar concept, while different in the methodology, is provided by SBIME analysis. The main difference between this and GSEA is that SBIME analyze the whole dataset without initial filtration of genes. This approach could therefore take into account genes with moderate variations that could be of importance in a functional pathway but not at single gene level. This software has been recently shown to detect functional pathways associated with docetaxel resistance in patients with locally advanced breast cancer (Kauffman *et al.*, submitted). Although conventional analysis has failed to detect such a pathway, the authors used the SBIME software to identify the oxidoreduction pathway as a major pathway for predicting docetaxel resistance. Considering our incomplete understanding of molecular pathways, analysis of the whole unfiltered data set is of interest and may allow one to discover previously unsuspected associations.

CONCLUSIONS

In this review, we have discussed how the heterogeneity of breast cancer among patients could affect research strategies focused on predicting drug efficacy. First, knowledge of the breast cancer molecular subclasses, along with use of biomarkers, could help us determine breast cancer prognosis and thereby help select patients to avoid adjuvant chemotherapy. Second, considering that proteins involved in the cellular response to a given drug are different among breast cancer subclasses, and given the fact that most of the biomarkers for drug resistance are linked to a specific subclass, research focusing on a single biomarker in the overall population may not generate optimal results. To circumvent the problems posed by this heterogeneity, we propose looking at biomarkers in homogeneous subclasses. Finally, functional approaches that

aim at clustering unfiltered genes into a single pathway are of particular interest in the context of heterogeneous disease.

ACKNOWLEDGMENTS

Fabrice Andre was supported by a fellowship from Fondation de France and Lilly Foundation.

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**(9472):1687–1717, May 14–20 (2005).
2. M. Trudeau, F. Charbonneau, K. Gelmon, K. Laing, J. Latreille, J. Mackey, D. McLeod, K. Pritchard, L. Provencher, and S. Verma. Selection of adjuvant chemotherapy for treatment of node-positive breast cancer. *Lancet Oncol.* **11**:886–898, Nov 6 (2005).
3. A. K. Nowak, N. R. Wilcken, M. R. Stockler, A. Hamilton, and D. Ghersi. Systematic review of taxane-containing *versus* non-taxane-containing regimens for adjuvant and neoadjuvant treatment of early breast cancer. *Lancet Oncol.* **6**:372–380, Jun 5 (2004).
4. M. D. Pegram, T. Pienkowski, D. W. Northfelt, W. Eiermann, R. Patel, P. Fumoleau, E. Quan, J. Crown, D. Toppmeyer, M. Smylie, A. Riva, S. Blitz, M. F. Press, D. Reese, M. A. Lindsay, and D. J. Slamon. Results of two open-label, multicenter phase II studies of docetaxel, platinum salts, and trastuzumab in HER2-positive advanced breast cancer. *J. Natl. Cancer Inst.* **96** (10): 759–769, May 19 (2004).
5. J. A. O'Shaughnessy. The evolving role of capecitabine in breast cancer. *Clin. Breast Cancer* **4**(Suppl 1):S20–S25, Apr (2003).
6. E. Rivera, F. A. Holmes, D. Frye, V. Valero, R. L. Theriault, D. Booser, R. Walters, U. Buzdar, K. Dhingra, G. Dhingra, and G. N. Hortobagyi. Phase II study of paclitaxel in patients with metastatic breast carcinoma refractory to standard chemotherapy. *Cancer* **89**(11):2195–2201, Dec 1 (2000).
7. G. Contesso, H. Mouriesse, S. Friedman, J. Genin, D. Sarrazin, and J. Rouesse. The importance of histologic grade in long-term prognosis of breast cancer: a study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. *J. Clin. Oncol.* **5**(9): 1378–1386, Sep (1987).
8. T. Sorlie, C. M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M. B. Eisen, M. Rijnvan de, S. S. Jeffrey, T. Thorsen, H. Quist, J. C. Matese, P. O. Brown, D. Botstein, P. Eystein Lonning, and A. L. Borresen-Dale. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. USA* **98**(19): 10869–10874, Sep 11 (2001).
9. C. M. Perou, T. Sorlie, M. B. Eisen, M. Rijnvan de, S. S. Jeffrey, C. A. Rees, J. R. Pollack, D. T. Ross, H. Johnsen, L. A. Akslen, O. Fluge, A. Pergamenschikov, C. Williams, S. X. Zhu, P. E. Lonning, A. L. Borresen-Dale, P. O. Brown, and D. Botstein. Molecular portraits of human breast tumours. *Nature* **406**(6797): 747–752, Aug 17 (2000).
10. C. Sotiriou, S. Y. Neo, L. M. McShane, E. L. Korn, P. M. Long, A. Jazaeri, P. Martiat, S. B. Fox, A. L. Harris, and E. T. Liu. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc. Natl. Acad. Sci. USA* **100**(18):10393–10398, Sep 2 (2003).
11. T. Sorlie, R. Tibshirani, J. Parker, T. Hastie, J. S. Marron, A. Nobel, S. Deng, H. Johnsen, R. Pesich, S. Geisler, J. Demeter, C. M. Perou, P. E. Lonning, P. O. Brown, A. L. Borresen-Dale, and D. Botstein. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc. Natl. Acad. Sci. USA* **100**(14):8418–8423, Jul 8 (2003).
12. W. D. Foulkes, J. S. Brunet, I. M. Stefansson, O. Straume, P. O. Chappuis, L. R. Begin, N. Hamel, J. R. Goffin, N. Wong, M. Trudel, L. Kapusta, P. Porter, and L. A. Akslen. The prognostic implication of the basal-like (cyclin E high/p27 low/p53+/glomeruloid-microvascular-proliferation+) phenotype of BRCA1-related breast cancer. *Cancer Res.* **64**(3):830–835, Feb 1 (2004).
13. T. O. Nielsen, F. D. Hsu, K. Jensen, M. Cheang, G. Karaca, Z. Hu, T. Hernandez-Boussard, C. Livasy, D. Cowan, L. Dressler, L. A. Akslen, J. Ragaz, A. M. Gown, C. B. Gilks, M. van de Rijn, and C. M. Perou. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin. Cancer Res.* **10**(16):5367–5374, Aug 15 (2004).
14. D. M. Abd El-Rehim, G. Ball, S. E. Pinder, E. Rakha, C. Paish, J. F. Robertson, D. Macmillan, R. W. Blamey, and I. O. Ellis. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *Int. J. Cancer* **116**(3):340–350, Sep 1 (2005).
15. M. A. Troester, K. A. Hoadley, T. Sorlie, B. S. Herbert, A. L. Borresen-Dale, P. E. Lonning, J. W. Shay, W. K. Kaufmann, and C. M. Perou. Cell-type-specific responses to chemotherapeutics in breast cancer. *Cancer Res.* **64**(12):4218–4226, Jun 15 (2004).
16. R. Rouzier, C. M. Perou, W. F. Symmans, N. Ibrahim, M. Cristofanilli, K. Anderson, K. R. Hess, J. Stec, M. Ayers, P. Wagner, P. Morandi, C. Fan, I. Rabiul, J. S. Ross, G. N. Hortobagyi, and L. Pusztai. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin. Cancer Res.* **11**(16):5678–5685, Aug 15 (2005).
17. R. Arriagada, M. Spielmann, S. Koscielny, T. ChevalierLe, T. Delozier, M. Reme-Saumon, M. Ducourtieux, T. Tursz, and C. Hill. Results of two randomized trials evaluating adjuvant anthracycline-based chemotherapy in 1146 patients with early breast cancer. *Acta Oncol.* **44**(5):458–466,1431–854 (2005).
18. I. C. Henderson, D. A. Berry, G. D. Demetri, C. T. Cirrincione, L. J. Goldstein, S. Martino, J. N. Ingle, M. R. Cooper, D. F. Hayes, K. H. Tkaczuk, G. Fleming, J. F. Holland, D. B. Duggan, J. T. Carpenter, E. Frei 3rd, R. L. Schilsky, W. C. Wood, H. B. Muss, and L. Norton. Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. *J. Clin. Oncol.* **21**(6):976–983, Mar 15 (2003).
19. M. Colozza, A. Sidoni, A. M. Mosconi, A. Cavaliere, G. Bisagni, S. Gori, V. AngelisDe, A. Frassoldati, R. Cherubini, A. R. Bian, C. Rodino, B. Mazzocchi, Z. Mihailova, E. Bucciarelli, and M. TonatoItalian Oncology Group for Clinical ResearchHER2 overexpression as a predictive marker in a randomized trial comparing adjuvant cyclophosphamide/methotrexate/5-fluorouracil with epirubicin in patients with stage I/II breast cancer: long-term results. *Clin. Breast Cancer* **6**(3):253–259, Aug (2005).
20. P. M. Ravdin. Is Her2 of value in identifying patients who particularly benefit from anthracyclines during adjuvant therapy? A qualified yes. *J. Natl. Cancer Inst. Monographs* (30):80–84 (2001).
21. S. Paik, J. Bryant, E. Tan-Chiu, G. Yothers, C. Park, D. L. Wickerham, and N. Wolmark. HER2 and choice of adjuvant chemotherapy for invasive breast cancer: national surgical adjuvant breast and bowel project protocol B-15. *J. Natl. Cancer Inst.* **92**(24):1991–1998, Dec 20 (2000).
22. C. G. Ferreira, C. Tolis, and G. Giaccone. p53 and chemosensitivity. *Ann. Oncol.* **10**(9):1011–1021, Sep (1999).
23. E. Rahko, G. Blanco, Y. Soini, R. Bloigu, and A. Jukkola. A mutant TP53 gene status is associated with a poor prognosis and anthracycline-resistance in breast cancer patients. *Eur. J. Cancer* **39**(4):447–453, Mar (2003).
24. A. Anelli, R. R. Brentani, A. P. Gadelha, A. Amorim De Albuquerque, and F. Soares. Correlation of p53 status with outcome of neoadjuvant chemotherapy using paclitaxel and doxorubicin in stage IIIB breast cancer. *Ann. Oncol.* **14**(3): 428–432, Mar (2003).
25. S. Geisler, P. E. Lonning, T. Aas, H. Johnsen, O. Fluge, D. F. Haugen, J. R. Lillehaug, L. A. Akslen, and A. L. Borresen-Dale. Influence of TP53 gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. *Cancer Res.* **61**(6):2505–2512, Mar 15 (2001).
26. P. Bertheau, F. Plassa, M. Espie, E. Turpin, A. de Roquancourt,

M. Marty, F. Lerebours, Y. Beuzard, A. Janin, and H. Thede. Effect of mutated TP53 on response of advanced breast cancers to high-dose chemotherapy. *Lancet* **360**(9336):852–854, Sep 14 (2002).
27. A. Fedier, A. Moawad, U. Haller, and D. Fink. p53-deficient cells display increased sensitivity to anthracyclines after loss of the catalytic subunit of the DNA-dependent protein kinase. *Int. J. Oncol.* **23**(5):1431–1437, Nov (2003).
28. A. M. Minisini, C. LoretoDi, M. Mansutti, D. Artico, S. Pizzolitto, A. Piga, and F. Puglisi. Topoisomerase IIalpha and APE/ref-1 are associated with pathologic response to primary anthracycline-based chemotherapy for breast cancer. *Cancer Lett.* **224**(1):133–139, Jun 16 (2005).
29. F. Cardoso, V. Durbecq, D. Larsimont, M. Paesmans, J. Y. Leroy, G. Rouas, C. Sotiriou, N. Renard, V. Richard, M. J. Piccart, and A. LeoDi. Correlation between complete response to anthracycline-based chemotherapy and topoisomerase II-alpha gene amplification and protein overexpression in locally advanced/metastatic breast cancer. *Int. J. Oncol.* **24**(1):201–209, Jan (2004).
30. T. Petit, M. Wilt, M. Velten, R. Millon, J. F. Rodier, C. Borel, R. Mors, P. Haegele, M. Eber, and J. P. Ghnassia. Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Eur. J. Cancer* **40**(2):205–211, Jan (2004).
31. D. G. Hicks, B. J. Yoder, J. Pettay, E. Swain, S. Tarr, M. Hartke, M. Skacel, J. P. Crowe, G. T. Budd, and R. R. Tubbs. The incidence of topoisomerase II-alpha genomic alterations in adenocarcinoma of the breast and their relationship to human epidermal growth factor receptor-2 gene amplification: a fluorescence *in situ* hybridization study. *Human Pathol.* **36**(4):348–356, Apr (2005).
32. R. Bhargava, P. Lal, and B. Chen. HER-2/neu and topoisomerase IIa gene amplification and protein expression in invasive breast carcinomas: chromogenic *in situ* hybridization and immunohistochemical analyses. *Am. J. Clin. Pathol.* **123**(6):889–895, Jun (2005).
33. A. Di Leo, D. Gancberg, D. Larsimont, M. Tanner, T. Jarvinen, G. Rouas, S. Dolci, J. Y. Leroy, M. Paesmans, J. Isola, and M. J. Piccart. HER-2 amplification and topoisomerase IIalpha gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. *Clin. Cancer Res.* **8**(5):1107–1116, May (2002).
34. M. F. Press, L. Bernstein, G. Sauter et al. Topoisomerase II-alpha gene amplification as a predictor of responsiveness to anthracycline-containing chemotherapy in the Cancer International Research Group 006 clinical trial of trastuzumab (herceptin) in the adjuvant setting. San Antonio Breast Cancer Symposium, Abs 1045 (2005).
35. M. Ayers, W. F. Symmans, J. Stec, A. I. Damokosh, E. Clark, K. Hess, M. Lecocke, J. Metivier, D. Booser, N. Ibrahim, V. Valero, M. Royce, B. Arun, G. Whitman, J. Ross, N. Sneige, G. N. Hortobagyi, and L. Pusztai. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *J. Clin. Oncol.* **22**(12):2284–2293, Jun 15 (2004).
36. L. Gianni, M. Zambetti, K. Clark, J. Baker, M. Cronin, J. Wu, G. Mariani, J. Rodriguez, M. Carcangiu, D. Watson, P. Valagussa, R. Rouzier, W. F. Symmans, J. S. Ross, G. N. Hortobagyi, L. Pusztai, and S. Shak. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J. Clin. Oncol.* **23**(29):7265–7277, Oct 10 (2005).
37. J. C. Chang, E. C. Wooten, A. Tsimelzon, S. G. Hilsenbeck, M. C. Gutierrez, R. Elledge, S. Mohsin, C. K. Osborne, G. C. Chamness, D. C. Allred, and P. O'Connell. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* **362**(9381):362–369, Aug 2 (2003).
38. K. R. Hess, K. Anderson, W. Symmans, V. Valero, N. Ibrahim, J. A. Mejia, D. Booser, R. L. Theriault, A. U. Buzdar, P. J. Dempsey, R. Rouzier, N. Sneige, J. S. Ross, T. Vidaurre, H. L. Gomez, G. N. Hortobagyi, and L. Pusztai. Pharmacogenomic predictor of sensitivity to preoperative paclitaxel and 5-fluorouracil, doxorubicin, cyclophosphamide chemotherapy in breast cancer. *J. Clin. Oncol.* (2006) in press.
39. S. Michiels, S. Koscielny, and C. Hill. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* **365**(9458):488–492, Feb 5 (2005).
40. L. Ein-Dor, I. Kela, G. Getz, D. Givol, and E. Domany. Outcome signature genes in breast cancer: is there a unique set?. *Bioinformatics* **21** (2):171–178, Jan 15 (2005).
41. K. Anderson, K. R. Hess, M. Kapoor, S. Tirrell, J. Courtemanche, B. Wang, Y. Wu, Y. Gong, G. N. Hortobagyi, W. F. Symmans, and L. Pusztai. Reproducibility of gene expression signature-based predictions in replicate experiments. *Clin. Cancer Res.* **12**(6):1721–1727, Mar 5 (2006).
42. The Toxicogenomics Research ConsortiumStandardizing global gene expression analysis between laboratories and across platforms. *Nat. Methods* **2**(5):351–356, May (2005).
43. R. A. Irizarry, D. Warren, F. Spencer, I. F. Kim, S. Biswal, B. C. Frank, E. Gabrielson, J. G. Garcia, J. Geoghegan, G. Germino, C. Griffin, S. C. Hilmer, E. Hoffman, A. E. Jedlicka, E. Kawasaki, F. Martinez-Murillo, L. Morsberger, H. Lee, D. Petersen, J. Quackenbush, A. Scott, M. Wilson, Y. Yang, S. Q. Ye, and W. Yu. Multiple-laboratory comparison of microarray platforms. *Nat. Methods* **2**(5):345–350, May (2005).
44. E. Huang, S. H. Cheng, H. Dressman, J. Pittman, M. H. Tsou, C. F. Horng, A. Bild, E. S. Iversen, M. Liao, C. M. Chen, M. West, J. R. Nevins, and A. T. Huang. Gene expression predictors of breast cancer outcomes. *Lancet* **361** (9369):1590–1596, May 10 (2003).
45. Y. Wang, J. G. Klijn, Y. Zhang, A. M. Sieuwerts, M. P. Look, F. Yang, D. Talantov, M. Timmermans, M. E. Meijer-van Gelder, J. Yu, T. Jatkoe, E. M. Berns, D. Atkins, and J. A. Foekens. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* **365**(9460):671–679, Feb 19–25 (2005).
46. S. Paik, S. Shak, G. Tang, C. Kim, J. Baker, M. Cronin, F. L. Baehner, M. G. Walker, D. Watson, T. Park, W. Hiller, E. R. Fisher, D. L. Wickerham, J. Bryant, and N. Wolmark. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N. Engl. J. Med.* **351**(27):2817–2826, Dec 30 (2004).
47. M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, S. S. Dwight, K. Dolinski, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock. Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat. Genet.* **25**(1):25–29, May (2000).
48. A. Subramanian, P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, and J. P. Mesirov. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. USA* **102**(43):15545–15550, Oct 25 (2005).
49. A. Linjawi, M. Kontogiannea, F. Halwani, M. Edwardes, and S. Meterissian. Prognostic significance of p53, bcl-2, and Bax expression in early breast cancer. *J. Am. Coll. Surg.* **198**(1):83–90, Jan (2004).
50. R. Rouzier, R. Rajan, P. Wagner, K. R. Hess, D. L. Gold, J. Stec, M. Ayers, J. S. Ross, P. Zhang, T. A. Buchholz, H. Kuerer, M. Green, B. Arun, G. N. Hortobagyi, W. F. Symmans, and L. Pusztai. Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proc. Natl. Acad. Sci. USA* **102**(23):8315–8320, Jun 7 (2005).