Pharmacodiagnosics and Targeted Therapies—A Rational Approach for Individualizing Medical Anticancer Therapy in Breast Cancer

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:
1. Assess the predictive biomarkers currently used in breast cancer, including, for example, ER, HER-2, and TOP2A.
2. Discuss the predictive capability of biomarkers in relation to the mechanisms of action of the corresponding treatment.
3. Discuss the link between the targeted therapies currently used in breast cancer and the predictive biomarkers.
4. Evaluate the role of TOP2A testing in relation to treatment with anthracyclines.

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ABSTRACT
The selection of therapy for a particular breast cancer patient is traditionally based on average results from randomized clinical trials. Rational pharmacotherapy is in essence about selecting the right drug(s) for the right patient, and in order to guide this selection process pharmacodiagnostic tests are indispensable. A number of tests have been developed or are under development for targeted therapies, such as antiestrogens, human epidermal growth factor receptor 2 inhibitors, and topoisoasemerase inhibitors. Based on a biopsy from the tumor, the tests are able to identify patients with a high probability to benefit from these therapies. The detection of the predictive biomarkers is based on different technologies, such as immunohistochemistry, fluorescence in situ hybridization, and chromogenic in situ hybridization. Pharmacodiagnostic tests will play an important role in the further development of targeted therapies and may be seen as a prerequisite for the introduction of individualized medicine in oncology. The Oncologist 2007;12:397–405

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION
Within medical oncology there is a long tradition of the use of evidence-based medicine, and generally new medical treatments are introduced into clinical practice following positive results from well-conducted randomized clinical trials. Disagreements between different randomized trials have in early breast cancer often been resolved through the systematic overviews or meta-analyses conducted by the Early Breast Cancer

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Trialists’ Collaborative Group (EBCTCG). Analyzing individual patient data jointly from all or the vast majority of the trials conducted on a specific question, the EBCTCG has provided evidence to be used for clinical guidelines and international consensus reports. In early breast cancer, this approach has led to a considerable increase in survival over the last 20–25 years [1]. But we may now be at a crossroads. Continuation of this approach, where treatment strategy is solitarily based on estimation of average efficacy according to tumor type, will possibly only lead to small additional improvements in survival. In the future, the selection of therapy may, to a much greater extent, be based on the knowledge we can get from predictive biomarkers by analyzing the tumor cells from individual cancer patients.

Rational pharmacotherapy or rational use of drugs is in essence about selecting the right drug for the right patient. The individual patient should receive a medical treatment that addresses his or her specific clinical needs [2]. During recent years, a number of slide-based biomarker assays with predictive potential have been developed [3–9]. Based on a biopsy from the tumor, it is possible, with a certain probability, to determine whether a patient is likely to respond to a specific medical treatment or not. These biomarker assays detect either if the tumor overexpresses a given protein, often the target that the anticancer drugs interact with, or if there are aberrations in the tumor DNA. The genes analyzed often encode for one or more tumor proteins like human epidermal growth factor receptor 2 (HER-2) or topoisomerase IIα. The method used to detect overexpression of tumor proteins is immunohistochemistry (IHC), and for gene aberrations, the methods of choice are fluorescence in situ hybridization (FISH) and chromogenic in situ hybridization (CISH) [10–12]. Predictive biomarkers or pharmacodiagnostics are expected to play an important role in the further development of targeted therapies and are seen as a prerequisite for the introduction of individualized medicine in oncology [13, 14].

The intention of this short review is to give an overview of the pharmacodiagnostic possibilities currently being studied with respect to therapy selection in breast cancer. For the medical treatment of this disease, there has been a long tradition of the use of pharmacodiagnostic tests, as a result of the introduction of endocrine therapy in hormone receptor–positive patients with tamoxifen (Nolvadex®; AstraZeneca Pharmaceuticals, Wilmington, DE) and amenoglutethimide (Orimeten®; Novartis AG, Basel, Switzerland) in the 1970s [15]. Further, breast cancer is a very research-intensive area and several new pharmacodiagnostic tests have been developed or are under development. In particular, data on the use of the TOP2A FISH assay are described in this review.

**Endocrine Treatment**

It has been known for many years that a large portion of breast cancer tumors are hormone sensitive, and testing for the presence of estrogen receptors (ERs) or progesterone receptors (PgRs) has been performed for several decades [16]. The correlations between the presence of hormone receptors and the effect of endocrine therapies, such as selective estrogen receptor modulators and aromatase inhibitors, have been established in a large number of clinical studies [17]. The measurement of ER in tumor tissue is routinely performed in order to select patients for endocrine treatment with tamoxifen or an aromatase inhibitor such as letrozole (Femar®, Novartis Pharmaceutical, Summit, NJ), anastrozole (Arimidex®, AstraZeneca Pharmaceuticals, Wilmington, DE), or exemestane (Aromasin®, Pfizer Pharmaceuticals, New York). Today, the preferred method for measurement of ER and PgR in tumor tissue is IHC.

In order to define targeted therapy, several elements need to be taken into consideration: the molecular target, the biomarker assay to identify the target, the anticancer drug that interacts with the target, and the treatment outcome. The first requirement that needs to be fulfilled is that the molecular target must be measurable and that the drug must interact specifically with this target. Secondly, treatment outcome, such as disease-free survival or overall survival, must correlate with the measurable target, for example, the degree of overexpression or the level of gene aberration [13, 14]. Tamoxifen has been used for nearly 30 years and must be characterized as the first targeted cancer therapy for which the requirements of a measurable target (ER and/or PgR) and a correlation with treatment outcome seem to be fulfilled. The use of tamoxifen in the adjuvant setting for the treatment of women with ER- and/or PgR-positive breast cancer is probably among the most well-documented cancer treatments available today [1].

**HER-2 Inhibitors**

In 20%–25% of women with breast cancer, tumors have an amplification of the HER-2 gene (alias of ERBB2). That the gene is amplified means that instead of having two copies of the HER-2 gene per cell, as under normal circumstances, there may be up to 50 or 100 gene copies per cell [18]. The HER-2 gene has been localized to chromosome 17, where it codes for the HER-2 protein, which is found at the cell surface. An amplification of the HER-2 gene will often lead to an overexpression of the receptor protein. The number of HER-2 receptors usually ranges from 20,000 to 50,000, but in cases of overexpression, the number may increase up to 2 million per cell [18]. HER-2 overexpression is known to be involved in the oncogenic process of the cell, and it has
been shown that it is associated with a more aggressive disease with a poorer prognosis [3].

HER-2 overexpression or HER-2 gene amplification is predictive for the use of the humanized monoclonal antibody trastuzumab (Herceptin®; Genentech, South San Francisco, CA), and before initiation of treatment a pharmacodiagnostic test with the use of either IHC and/or FISH must be performed [19]. The degree of overexpression and/or gene amplification is decisive for the initiation of treatment. According to the recently issued guidelines for HER-2 testing in breast cancer, a tumor is considered HER-2 positive if the IHC score is 3+, the average HER-2 gene/chromosome 17 ratio is >2.2 by FISH, or the average number of HER-2 gene copies/cell is six or greater [20, 21]. Examples of HER-2 overexpression in tumor tissue from breast cancer patients (as assessed by HercepTest™; Dako, Glostrup, Denmark) are given in Figures 1 and 2. Trastuzumab has been shown to be effective for the treatment of both primary and metastatic breast cancer [5, 22, 23]. In particular, the results that have been achieved with trastuzumab in the adjuvant setting must be regarded as a major step forward in the treatment of this type of breast cancer. Several large, phase III studies have recently been published in which trastuzumab has been given in combination with or following traditional chemotherapeutic drugs, such as anthracyclines and taxanes. The results from these studies showed a proportional risk reduction of approximately 50% with respect to recurrence of the disease [22–24].

Lapatinib (Tykerb®; GlaxoSmithKline, Research Triangle Park, NC), a novel small molecule tyrosine kinase inhibitor that targets both HER-2 and the epidermal growth factor receptor, has shown promising results in preclinical and early clinical studies [25, 26]. Studies in HER-2–positive breast cancer cell lines resistant to trastuzumab have shown that they are sensitive to lapatinib [27]. The results from recently presented clinical phase II and III studies in metastatic breast cancer indicate that preselection of patients based on HER-2 status, using either IHC or FISH, is important for the outcome of the treatment, as is known for trastuzumab [28, 29].

The predictive value has not been fully explored for other biomarkers in relation to HER-2–targeted therapies. The gene PTEN on chromosome 10q23 coding for phosphatase and tension homolog is often deleted. Loss of PTEN expression was put forward as a marker of resistance for trastuzumab in patients with HER-2–positive metastatic breast cancer in a small retrospective study [30], but this could not be confirmed in another small study with lapatinib [31]. Unconvincing results have also been obtained regarding the positive predictive value of insulin-like growth factor-1 receptor overexpression [31, 32]. HER-2 and MYC gene amplifications have both been established as prognostic factors in breast cancer, and a recent study suggests that their prognostic as well as predictive values may be additive [33]. Patients with coamplification of HER-2 and MYC benefited the most from the combined therapy with trastuzumab and chemotherapy, and had the worst prognosis if treated with chemotherapy alone.

The importance of using a pharmacodiagnostic test in clinical drug development has been illustrated using sample size calculations on alternative study designs. In a pivotal trial published by Slamon et al. [5], patients with advanced breast cancer were randomized to chemotherapy with and without trastuzumab [5, 34]. Only patients with HER-2–positive tumors, that is, overexpression of HER-2 with a score of 2+ or 3+, were eligible. Altogether, 469 patients were enrolled, and the result was highly statistically significant in favor of the trastuzumab arm with regard to 1-year survival [5, 34]. Without the use of HER-2 as a selection...
criterion, a much larger trial would have been needed to detect the survival gain observed in the Slamon et al. [5] trial. Assuming that the effect of trastuzumab in HER-2–negative patients is half the effect observed in HER-2–positive patients, the number of patients needed in order to show a significant difference in 1-year survival was calculated as 1,256. A second alternative was calculated, and here again an untargeted design was used with no HER-2 testing and the assumption that the assay-negative patients had no benefit from treatment with trastuzumab. The sample size needed for this alternative was 23,586 patients, which is 50 times more patients than were enrolled in the original study [34]. If an assay had not existed that could identify the patient population likely to respond, trastuzumab most certainly would have been discarded during clinical development because of insufficient activity in an unselected patient population, and we would today have been without an important anticancer drug [14].

TOPOISOMERASE INHIBITORS

Anthracyclines are among the most widely used chemotherapeutic drugs. These compounds have demonstrated antitumor activity against a number of different types of cancer, both solid tumors and hematological malignancies [35]. The first anthracycline was discovered in the late 1950s, but more than two decades would pass before the mechanism of action was explained. In the 1980s, it was shown that anthracyclines inhibit the topoisomerase IIα enzyme [36]. Interaction with the topoisomerase IIα–DNA complex results in double-stranded DNA breaks and subsequently apoptosis. Another important mechanism of action of the anthracyclines is the formation of free radicals, which occur through membrane lipid peroxidation. However, this mechanism seems to be not only associated with an antitumor effect, but also with an unpleasant cardiotoxic effect [35].

The anthracyclines, doxorubicin (Adriamycin®; Bedford Laboratories; Bedford, OH) and epirubicin (Ellence®; Pfizer Pharmaceuticals, New York) are used for treatment of both primary and metastatic breast cancer. Most frequently, the compounds are combined with other chemotherapeutics such as cyclophosphamide and 5-fluorouracil. In the adjuvant setting, clinical data have shown that chemotherapy regimens that include an anthracycline result in a statistically significant longer survival time than with non–anthracycline containing regimens [1].

The therapeutic index of the anthracyclines is low, with frequent acute and subacute side effects. In addition to the traditional chemotherapeutic side effects, such as nausea/vomiting, alopecia, leukopenia, and stomatitis, long-term treatment-related cardiomyopathy and secondary leukemia have been observed [37–39]. To give a potential toxic drug to a patient is always a balance between the expected benefit and the side effects, and for the use of the anthracyclines in the adjuvant treatment of breast cancer it would be im-

Figure 3. Tumor cells with amplification of the TOP2A gene as assessed by TOP2A FISH pharmDx™ (Dako, Glostrup, Denmark). The red signal comes from the TOP2A gene and the green signals come from the reference probe (centromere 17). Notice that the TOP2A signals constitute the majority.

Figure 4. Tumor cell with deletion of the TOP2A gene as assessed by TOP2A FISH pharmDx™ (Dako, Glostrup, Denmark). The red signal comes from the TOP2A gene and the green signals come from the reference probe (centromere 17). Notice that the reference signals constitute the majority.
important to be able to preselect the individuals that may benefit most from this specific treatment.

The gene that codes for topoisomerase IIα, which is the primary target for the anthracyclines, is TOP2A. This gene is located at chromosome 17 close to the HER-2 gene. The TOP2A gene can be detected in tumor tissue by FISH. Figures 3 and 4 show fluorescence microscopic images of tumor tissue with the use of FISH technology to detect aberrations in the TOP2A gene (assessed by TOP2A FISH pharmDx™; Dako, Glostrup, Denmark). A number of clinical studies have shown that patients who have tumors with TOP2A gene aberrations, especially amplifications, experience a significantly better effect from anthracycline-based chemotherapy than patients with a normal TOP2A gene status [8, 9, 40–48]. Several of these studies have included a control group that has been treated with a non–anthracycline-containing chemotherapy regimen, enabling them to draw valid conclusions with respect to the predictive properties of TOP2A gene aberrations [9, 24, 47, 48].

In a large, adjuvant trial coordinated by the Danish Breast Cancer Cooperative Group (DBCG 89D) 980 pre- and postmenopausal high-risk patients were randomized to receive either CEF (cyclophosphamide, epirubicin, and 5-fluorouracil) or CMF (cyclophosphamide, methotrexate, and 5-fluorouracil). The study results were originally reported in the Journal of Clinical Oncology in 2005 [9], but at the 2006 Annual Meeting of the American Society of Clinical Oncology an updated analysis was presented with longer follow-up time. The results reported in this publication are based on the recently presented data [48]. The primary endpoint of the study was recurrence-free survival (RFS). TOP2A gene status was evaluated by FISH in 773 (79%) of the 980 patients included in the trial. Tumor tissue from the patients was collected retrospectively and analyzed for TOP2A gene aberrations. Close to 25% of the patients showed aberrations in the TOP2A gene, with an approximately equal distribution of amplifications and deletions. The gene aberrations were mainly found among the HER-2–positive patients, but a relative large proportion (22%) was found among HER-2–negative patients. For the primary study endpoint, it was shown that patients with TOP2A-amplified tumors had a significantly better RFS with anthracycline-based chemotherapy than patients with a normal status. Using both univariate and multivariate statistics for analyzing the interaction between TOP2A gene status and treatment outcome, a significant predictive value was found with respect to TOP2A amplification. TOP2A gene deletion showed a similar but nonsignificant trend. Kaplan-Meier plots for treatment with CMF and CEF, with respect to TOP2A gene status and RFS, are shown in Figure 5. Multivariate Cox analyses showed a significant risk reduction of 61% for patients with TOP2A-amplified tumors treated with CEF, and again a similar nonsignificant trend was observed with respect to TOP2A deletions. A Forrest plot for TOP2A and
HER-2 status with respect to RFS is shown in Figure 6. From this study it was concluded that *TOP2A* amplification is associated with a favorable outcome of adjuvant treatment with epirubicin in primary breast cancer.

The predictive value of *TOP2A* amplification was evaluated in another large adjuvant study (the Breast Cancer International Research Group [BCIRG] 006 trial) recently presented [24]. In total, 3,222 patients with *HER-2*-amplified breast cancer tumors were randomized to one of the following three regimens: (a) doxorubicin and cyclophosphamide followed by docetaxel (ACT), (b) doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (ACTH), and (c) docetaxel, carboplatin, and trastuzumab (TCH). In a subset of the patients (*n* = 2,120) *TOP2A* amplification was evaluated by FISH. Thirty-five percent of the patients were found to have *TOP2A*-amplified tumors. As shown in Figure 7, patients who did not receive anthracyclines (TCH) had similar outcomes irrespective of *TOP2A* status. However, treatment with an anthracycline-containing regime (ACT and ACTH) was associated with a higher disease-free survival rate in patients with *TOP2A* amplification. Based on the study results, it was concluded that patients with coamplification of the *TOP2A* and *HER-2* genes received a therapeutic advantage from the anthracycline–trastuzumab combination regimens, and that patients who are not *TOP2A* amplified may be ideal candidates for non-anthracycline based regimens, thus avoiding potential cardiac toxicity [24].

The predictive value of *TOP2A* gene aberrations has been investigated in a number of clinical studies, which, in total, include >5,000 patients. All these studies seem to confirm the predictive value of *TOP2A* gene amplification [40–48]. In order to further validate the use of *TOP2A* as a predictive biomarker, a meta-analysis is currently being performed comprising approximately 4,600 women who have been treated with either anthracycline-based chemotherapy or CMF. The data from this analysis originate from adjuvant clinical studies performed in the United Kingdom, Denmark, Belgium, and Canada, and the preliminary results are expected to be published in 2007 [49].
macodiagnostic predictive tests for treatment with endocrine agents and HER2 inhibitors.

The Future

The predictive biomarkers developed so far cover only a minor part of the anticancer drugs that today are used routinely in medical oncology. In the years to come, it is expected that a number of new tests will emerge in connection with the targeted and selective anticancer drugs that are under development. These tests will be based on both single and multiple proteins or genes and will use technologies such as IHC, FISH, CISH and real-time reverse transcription-polymerase chain reaction. The development of these biomarker assays should be seen as an integral part of the drug development process [50]. An assay that is able to select the patient population who will most likely benefit from the targeted therapy will need to be developed in parallel with the anticancer drug. In the absence of such an assay, there is no targeted therapy [13].

For a number of the existing anticancer drugs, we will also see predictive biomarker assays being developed, and the first example of this is the use of TOP2A for selection of patients for anthracycline-based chemotherapy. As we learn more about the mechanism of action of these drugs, it will create the basis for the development of new biomarker assays, which most likely will lead to a redefinition of their role in the treatment of cancer patients. For some of the existing anticancer drugs, the use of pharmacodiagnostic tests will probably also change our perception of what is targeted therapy or not.

That patients with apparently the same cancer respond differently to a given anticancer drug is a challenge that medical oncology has been dealing with for years, and it is only now that we are starting to understand part of this variability. Pharmacodiagnostic testing has given us the understanding that breast cancer and other cancers can be divided into different biological subgroups that respond differently to a given medical treatment. One of the explanations for this interpatient variation is likely to be found in the genes that express the target of the anticancer drug in the tumor cell, such as receptor proteins, enzymes, or signal peptides. Pharmacodiagnostic testing has given us a rational tool that will lead to the recognition that the individual cancer patient is unique and that the treatment to a much greater extent than today needs to be personalized or individually tailored.

Pharmacodiagnostic assays are so far only available for a small number of drugs, and if the goal of individualized medicine is to become a reality, a considerable number of predictive biomarkers needs to be developed. These biomarkers should cover a broad spectrum of the most frequently used anticancer drugs. Despite the fact that it will take some time before this goal is reached, it is today impossible to talk about rational anticancer pharmacotherapy without discussing pharmacodiagnostic testing.

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Disclosure of Potential Conflicts of Interest

K.V.N. and B.E. own stock in Dako Denmark A/S.

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