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Neoadjuvant comparisons of aromatase inhibitors and tamoxifen: pretreatment determinants of response and on-treatment effect☆

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Abstract

Adjuvant endocrine therapy reduces the risk of relapse and death from early stage hormone receptor positive breast cancer. However, tamoxifen is only partially effective because of the development of tumor resistance. Aromatase inhibitors (letrozole, anastrozole and exemestane) are also prone to the development of resistance but the pharmacologic action (estrogen deprivation) is distinct and so different mechanisms may be responsible. The problem of endocrine resistance can be directly studied in patients by examining the relationship between predictive tumor biomarkers and clinical outcome. In an example of a prospectively planned biomarker study, tumor samples were examined from a randomized trial of neoadjuvant endocrine treatment in which letrozole proved more effective than tamoxifen in terms of the rate of breast conservation and tumor regression. Interestingly letrozole was more effective at all levels of ER expression and was particularly more efficacious than tamoxifen for tumors that expressed HER1 and/or HER2 (with ER). This suggests that HER1/2 predicts primary tamoxifen resistance and relative sensitivity to potent estrogen deprivation, perhaps because HER1/2 signaling promotes the partial agonist effects of tamoxifen. A Phase 2 study of neoadjuvant letrozole is now underway to focus on gene expression profiling as a mechanism to further investigate the transcriptional programs that underlie resistance and sensitivity to estrogen deprivation. Expression profiles taken at baseline and after 1 month of therapy reveal dramatic reductions in the expression from genes responsible for DNA replication and synthesis, cell cycle progression, suppression of apoptosis and tissue invasion. When enough profiles have been generated it should be possible to detect complex interaction patterns that correctly reclassify ER+ disease into treatment responsive and resistant categories with high probability.

Keywords: Aromatase inhibitors; Letrozole; Tamoxifen

1. Neoadjuvant endocrine therapy—general considerations

Neoadjuvant endocrine therapy is an effective neoadjuvant treatment for postmenopausal women with locally advanced strongly ER+ breast cancer ineligible for breast-conserving surgery. For example, the oral aromatase inhibitor letrozole, 2.5 mg daily, administered for 4 months before surgery, was more effective than tamoxifen in increasing the rate of breast-conserving surgery [1,2]. Similar efficacy has been reported with anastrozole and exemestane in Phase 2 studies [3,4]. These findings are an important breakthrough for research on the endocrine treatment of early stage breast cancer because data on primary tumor responsiveness are available after only a short period of treatment. Predictive biomarker studies can therefore be carried out prospectively and efficiently with a great potential for insights into the molecular basis for endocrine treatment [5]. The use of neoadjuvant endocrine therapy in place of neoadjuvant chemotherapy can be further justified on the basis that the patients under study (older postmenopausal women with estrogen receptor positive tumors) are receiving a form of systemic treatment that is at least twice as effective as chemotherapy in providing protection from relapse and death from the disease [6,7]. While a randomized clinical trial of neoadjuvant chemotherapy versus neoadjuvant endocrine therapy will be required to unequivocally establish a role for neoadjuvant endocrine therapy as a routine treatment option, well conducted Phases 2 and 3 studies of this treatment modality are justified on the basis of the precedent set by the numerous Phase 2 studies of preoperative chemotherapy that were conducted before it was definitively demonstrated that relatively short delays in surgery to administer preoperative chemotherapy did not affect long-term outcomes [8]. The focus on older patients in the application of neoadjuvant endocrine therapy is another relevant consideration. Older patients are a poorly studied

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population and neoadjuvant chemotherapy is frequently not an option for these individuals on the basis of poor performance status, co-morbid conditions or patient refusal [9].

2. The letrozole 024 trial of neoadjuvant endocrine therapy—clinical outcomes

To compare letrozole and tamoxifen in a treatment naïve setting, a double-blind randomized Phase 3 study was conducted that compared 4 months of neoadjuvant oral letrozole (2.5 mg daily) with tamoxifen (20 mg daily) in which 337 patients were enrolled (referred to hereafter as the "Letrozole 024 Study"). Eligibility criteria included hormone receptor positivity (ER and/or progesterone receptor (PgR) immunohistochemical staining of at least 10% of tumor nuclei) and ineligibility for breast-conserving surgery. An intent-to-treat analysis demonstrated that letrozole was more effective neoadjuvant therapy than tamoxifen, with a 55% clinical response rate on the letrozole arm versus 36% on the tamoxifen arm ($P = 0.001$ by Mantel–Haenzel Chi-squared test, adjusted for baseline tumor size and nodal status). Furthermore, the incidence of breast-conserving surgery was significantly higher on letrozole arm (45% versus 35%, $P = 0.022$) [2]. The safety of neoadjuvant endocrine therapy, in terms of late in breast recurrence and effects on relapse and overall survival has yet to be determined, although preliminary data on local control are encouraging (see Dixon et al., this volume), as long as patients routinely receive breast irradiation and adjuvant aromatase therapy.

3. Biomarker studies in the setting of neoadjuvant endocrine therapy

3.1. Estrogen receptor

The initial objective of the letrozole 024 neoadjuvant endocrine therapy study was confirm though a central laboratory analysis the ER and progesterone receptor (PgR) status of each tumor so that the crucial eligibility criteria of positive hormone receptor status could be confirmed. Baseline biopsies for a central analysis of ER and PgR status were received from 278 patients. This material allowed an adjusted analysis to be performed for study-biopsy confirmed ER and/or PgR positive cases. The original intent-to-treat analysis was found to have modestly underestimated the benefit of preoperative endocrine therapy because of the presence of a small number of ER and PgR negative cases. The results of this adjusted analysis are presented in Table 1. These results demonstrate that preoperative letrozole is a viable and non-toxic neoadjuvant regimen as long as a robust assay for ER is available to exclude hormone receptor negative cases. Interestingly a linear relationship between the degree of ER positivity (as determined by the Allred score) and the likelihood of response was observed, so by selecting patients with high levels of ER expression (Allred scores of 7 and 8) will ensure response rates are at least 60% and possibly higher (Fig. 1). Interestingly, at every level of ER expression, letrozole was more effective than tamoxifen indicating that ER cannot be used as a way of defining a group in which tamoxifen and letrozole are equivalent.

Table 1
Summary of the results for a comparison of letrozole and tamoxifen as 4 months preoperative endocrine therapy for patients with study-biopsy confirmed ER and/or PgR positive cases

Study-biopsy-confirmed ER and/or PgR positive	Letrozole no.	Tamoxifen no.	*P*-value
Total number in arm	124 (100)	126 (100)	
Overall response (CR plus PR)			
Clinical measurements	74 (60)	52 (41)	0.004
Ultrasound	48 (39)	31 (28)	0.119
Mammogram	47 (38)	25 (20)	0.002
Rate of breast-conserving surgery	60 (48)	45 (36)	0.036
Clinical progressive disease	10 (8)	15 (11)	

P-values were calculated by the Mantel–Haenzel Chi-squared test, adjusted for baseline tumor size (T2 versus >T2) and nodal status (N0 versus >N0) Reproduced with permission of the *Journal of Clinical Oncology*. The values in parentheses are in percent.

3.2. Progesterone receptor

A similar analysis was conducted to examine the relationship between response and PgR Allred expression category (Fig. 2). Unlike ER, the relationship between PgR Allred expression levels and log odds of response did not fit a linear model because maximal response rates for both drugs occurred at intermediate levels of expression, not at the highest levels of expression. If the absolute difference in log odds from the peak letrozole response rate associated with an Allred score of 5 is assessed by logistic regression, an inverse V shaped model was the best model that fit the data ($P = 0.0015$, Wald's test). This indicated that high as well as low PgR expression scores were associated with a lower chance of responding than intermediate scores. A similar model fit the tamoxifen data if the peak response rate was taken to be an Allred score of 4, although there was a ~10-fold lower level of statistical confidence than that seen for letrozole ($P = 0.0165$).

An inverse V shaped relationship between PgR expression and response to letrozole was not anticipated from prior information on the predictive properties of PgR in breast cancer [10]. It is generally accepted that expression of PgR is a biomarker for estrogen-dependent cancers with a "functional ER" because PgR requires activated ER for expression [11]. Furthermore it is also assumed that like ER, the relationship between PgR level and response is linear with the most responsive tumors expressing the highest levels of expression. While this hypothesis explains the initial increase in response rates associated with Allred scores of 0–5, i.e. the initial rise in response rates associated with the appearance

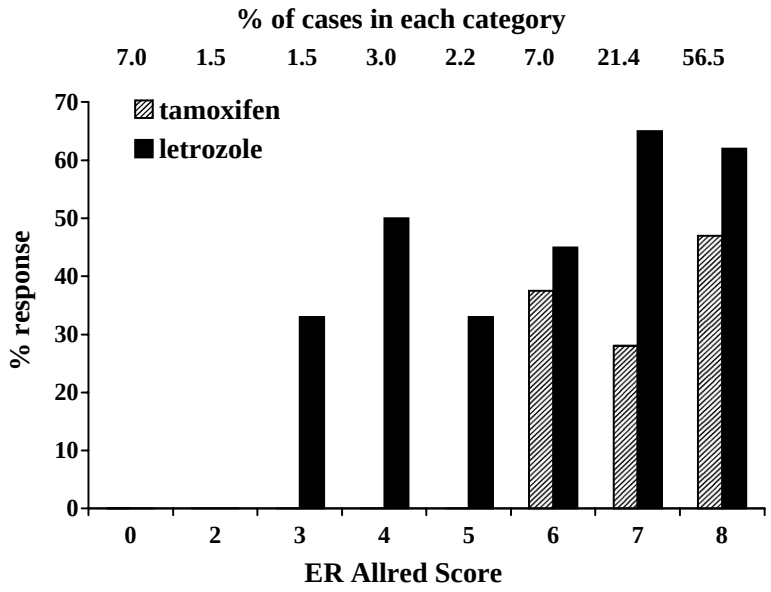

Fig. 1. The relationship between ER Allred score and response to preoperative letrozole and tamoxifen (excluding six ER−, PgR+ cases). The relationship between ER expression level and log odds of response fit a linear model that was significant by logistic regression within treatment groups (letrozole $P = 0.0013$ and tamoxifen $P = 0.0061$, Wald's test). Reproduced with permission from the *Journal of Clinical Oncology*.

of PgR expression, it does not predict the subsequent decline in response rates associated with PgR Allred scores of 6–8. In order to explain this novel finding, we have postulated that PgR expression levels also reflects tumor estrogen content and aromatase activity and PgR rich tumors have such high levels of estrogen production (or perhaps estrogen hypersensitivity) that they exhibit relative resistance to aromatase inhibitor therapy. This hypothesis, if correct, has important implications for breast cancer treatment. Tumors associated with the highest levels of PgR expression may require more potent estrogen deprivation or additional endocrine manipulations, such as the co-administration of a potent antiestrogen, to achieve more optimal clinical outcomes.

3.3. HER1 and HER2

Samples from the letrozole 024 trial were also used to try to resolve a long-standing hypothesis concerning the

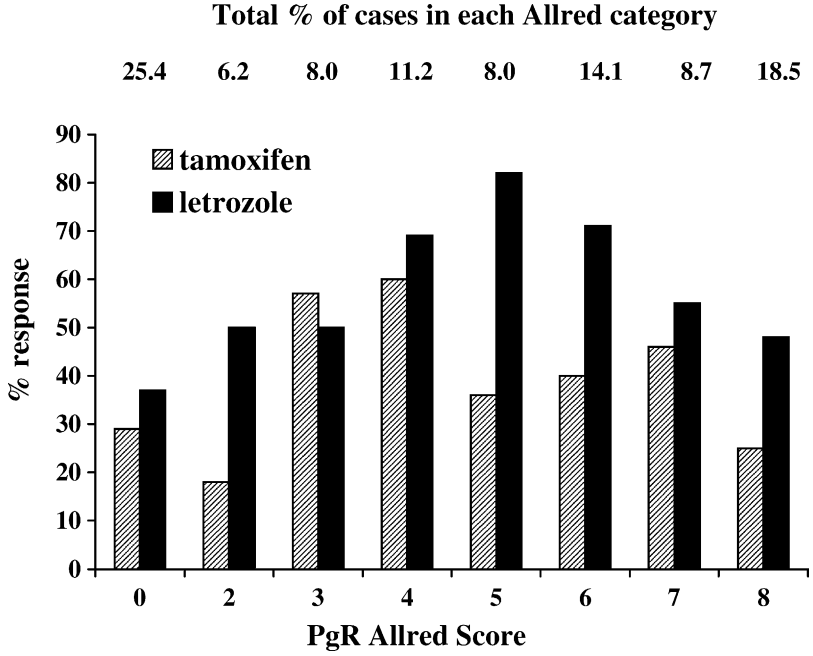

Fig. 2. The relationship between PgR Allred score and response to letrozole and tamoxifen.

Table 2
Calculation of odds ratio of clinical response, letrozole vs. tamoxifen, in subgroups of patients with tumors that were either ErbB1 and/or ErbB2 positive (ErbB1/2+) and ER+ or ErbB1 and ErbB2 negative (ErbB1/2−) and ER+

Category	Letrozole	Tamoxifen	Odds ratio (letrozole vs. tamoxifen)	*P*-value
ErbB1/2 + ER+	15/17 (88%)	4/19 (21%)	28 (4.5–177)	0.0004
ErbB1/2 − ER+	55/101 (54%)	42/100 (42%)	1.7 (0.9–2.9)	0.0780

Biomarker status was defined by IHC using published definitions of positive and negative status. This analysis ignored PgR status (none of the ErbB1/2+ cases were ER−, PgR+).

relationship between HER1 (ErbB1 or EGFR) and HER2 (HER2/neu or ErbB2) expression and resistance to endocrine therapy [12–15]. For tumors that were true double positives, i.e. HER1+ and/or HER2+ and ER+, a positive HER1 and/or HER2 status became a significant sensitivity marker for letrozole treatment (RR 88% for HER1+ and/or HER2+ and ER+ versus 54% for HER1− and HER2− and ER+, $P = 0.02$). This surprising finding suggests that in early stage disease, HER1+ and/or HER2+ are sensitivity markers for estrogen deprivation therapy. This finding lead to a logistic regression analysis to assess the level of significance associated with the difference in efficacy between letrozole and tamoxifen within the subset of tumors that were HER1+ and/or HER2+ and ER+ (Table 2). Letrozole was found to be considerably more active than tamoxifen in this tumor subset (RR 88% versus 21%, odds ratio for response 28, $P = 0.0004$). These data suggest that the majority of ER+ primary tumors that express HER1 and HER2 still exhibit estrogen-dependent growth and can be effectively treated with an aromatase inhibitor. One interpretation of these data is that HER1 and HER2 signaling promotes the partial agonist effects of tamoxifen [1]. There remains some controversy over these findings and certainly these results, while intriguing need to be confirmed by other investigators, particularly in the adjuvant setting. A subsequent abstract reported that letrozole is more effective than tamoxifen in the suppression of cell cycling, particularly in the HER1/2+, ER+ subset. These data support the case that aromatase inhibitors should be favored in this biomarker subtype [16].

4. Current neoadjuvant endocrine therapy studies

4.1. A Phase 2 investigation incorporating gene expression profiling

With the completion of the first maps of the human genome, together with new technologies to screen tumors for gene expression and somatic mutations, the number of biomarkers and therapeutic targets to translate into clinical practice is increasing dramatically. Recent publications on gene expression profiling in breast cancer have underscored the considerable potential of this technology [17]. There are, in essence, two approaches to the statistical analysis of the huge data sets generated by gene expression profiling termed “unsupervised” analysis and “supervised” analysis. An unsupervised analysis aims to compare the profile of each tumor to identify “relatedness” to other tumors to create a “molecular” classification of the disease that is distinct from traditional clinical classification systems. The objective of this analysis is to identify tumors with similar molecular etiologies that may exhibit characteristic clinical behaviors and also share therapeutic targets. On this basis Sorlie et al. have recently suggested that breast cancer can be sub-classified into five groups of tumors with clinical implications. Of note, they propose that ER+ breast cancers are composed of three subgroups, a luminal ER+ subtype A with a relatively good prognosis and luminal subtypes B and C that carry a worse prognosis and express a novel set of genes whose coordinated functions are unknown, a feature they share with the poor prognosis (ER−) basal subtype and the HER2+ subtype [18]. In contrast to the unsupervised approach, a “supervised” analysis compares the gene expression profiles of tumors divided on the basis of a clinical characteristic, for example alive or dead from disease, response to treatment versus no response, ER+ versus ER− or node positive and node negative. Once a gene expression “cluster” that cleanly distinguishes one group from the other has been identified (usually a set of genes that numbers between 10 and 100) its predictive properties must be subsequently validated in a prospective manner in an independent data set. The supervised approach has been successful in, for example, identifying a signature composed of 75 genes whose absence is characteristic of tumors from women who are free from recurrence after local treatment of breast cancer [17]. Similarly several groups, including our own, have identified clusters of genes that associate with ER alpha in breast cancer gene expression profiles (Table 3) [19,20]. Interestingly a careful examination of the expression levels of genes within these ER clusters shows a considerable degree of tumor-to-tumor variation in the degree to which any particular member is expressed. This suggests that expression from at least some of these genes in combination with ER could be more predictive for response than examination of ER alone.

Until recently it was not clear how many patients were required for supervised analysis. The obvious concern was that, like “traditional” single predictive biomarker studies, hundreds of patient samples would be required in order to develop robust predictive models. However, the power generated by the large number of genes examined (and

Table 3
Genes that cocluster with ER alpha in gene expression profiles of breast cancer

Gene	GenBank accession
Sodium channel, nonvoltage-gated 1-alpha (SCNN1A)	X76180
Serine or cysteine proteinase inhibitor, member 3	X68733
N-Acylsphingosine amidohydrolase (acid ceramidase)	U70063
Lipocalin 1 (LCN1)	L14927
Transforming growth factor-beta type Ill receptor	L07594
Glutamate receptor precursor 2 (GRIA2)	L20814
Cytochrome P450-IIB, phenobarbital-inducible (CYP2B)	M29 874
Carcinoembryonic antigen mRNA (CEACAM5)	M29540
Mammaglobin 1 (MGB1)	U33147
Estrogen-regulated LIV-1 protein (LIV1)	U41060
Prolactin-induced protein (PIP)	HG1763
Matrix Gla protein (MGP)	X53331
Trefoil factor 3 (TFF3)	L08044
Trefoil factor 1 (TFF1)	X52003
Hepatocyte nuclear factor-3-alpha (HNF3A)	U39840
Serine protease hepsin (HPN)	X07732
X box binding protein-1 (XBP1)	M31627
Zn-alpha2-glycoprotein (AZGP1)	X59766
Estrogen receptor-alpha (ESR1)	X03635

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presumably their complex interactions) appears to overcome statistical weaknesses associated with relatively small patient data sets. Recent investigations in lymphoma and breast cancer suggest that studies that focus on a binary supervised analysis can generate significant prognostic models in studies of between 50 and 100 patients [17,18,21].

On the basis of these preliminary studies a Phase 2 neoadjuvant letrozole study has been funded by the National Cancer Institute to examine gene expression profiles at baseline, 1 month and at surgery (Fig. 3). Each tumor will be defined as responsive or non-responsive on the basis of clinical measurements, radiological measurements and changes in tumor proliferation. mRNA expression profiling will be employed to identify a gene expression cluster that predicts responsiveness to neoadjuvant aromatase inhibitor therapy at diagnosis. RNA extracted from a baseline tumor biopsy from each case will be profiled with an Affymetrix

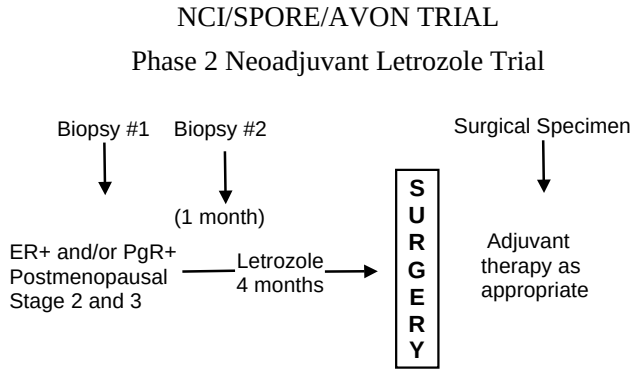

Fig. 3. Schema for a Phase 2 study of neoadjuvant letrozole incorporating serial tumor sampling for gene expression analysis.

U 133 subA Gene Chip. The gene expression profiles of responders and non-responders will be compared by structured factor regression modeling and by class membership predictor algorithms. A list of predictive biomarkers will be generated to take into account a priori consideration of biomarker properties and concordance between single gene assays and gene array analysis. This list of predictive genes will be further refined after consideration of how frequently each transcript is expressed, the inter-tumor variation in expression, the degree to which each mRNA is up-regulated with respect to normal breast epithelial tissue and the robustness with which a single gene mRNA in situ assay correlates with the microarray data. In addition the intent is also to investigate mechanisms of aromatase inhibitor resistance based on an analysis of post-treatment samples. Changes in gene expression at 1 month will be compared between responding and non-responding tumors to address a series of a priori models that explain intrinsic resistance of ER+ breast cancer to estrogen deprivation therapy.

4.2. Preliminary gene expression data

To date only a small number of tumors have been subjected to array analysis. Table 4 illustrates the types of profiles that have been generated by comparing the baseline sample with a second sample taken at 1 month. In this particular example of a letrozole responsive locally advanced breast cancer (Fig. 4), remarkable shifts in gene

Table 4
A list of genes exhibiting a marked decrease in mRNA expression with neoadjuvant aromatase inhibitor therapy from samples taken at baseline and 1 month from the tumor illustrated in Fig. 4

Log base 2 scale	Genes decreased 1 month
−4.5	Topoisomerase (DNA) II alpha (l70 kD)
−3.3	Ataxin 2 related protein
−4.1	Ribonucleotide reductase M2 polypeptide
−3.5	Baculoviral IAP repeat-containing 5 (survivin)
−3.8	Forkhead box M1
−3.6	Interferon-induced protein with tetratricopeptide repeats 1
−3.3	5-Methyltetrahydrofolate-homocysteine metnyltransferase reductase
−3.7	Cell division cycle 2: G1 to S and G2 to M
−4.0	S100 calcium-binding protein P
−6.5	Matrix metalloproteinase 1 (interstitial collagenase)
−3.8	Orosomucoid 1
−4.4	Carboxypeptidase B1 (tissue)
−3.5	CD36 antigen (collagen type I receptor, thrombospondin receptor)
−4.5	Prolactin-induced protein
−3.7	CGI-142
−3.5	H2A histone family, member A
−4.1	Protein regulator of cytokinesis 1
−4.2	Hypothetical protein MGC4309
−4.1	Nucleolar protein 3 (apoptosis repressor with CARD domain)
−4.1	WD40 repeat domain 11 protein
−4.6	Hemoglobin, alpha 1

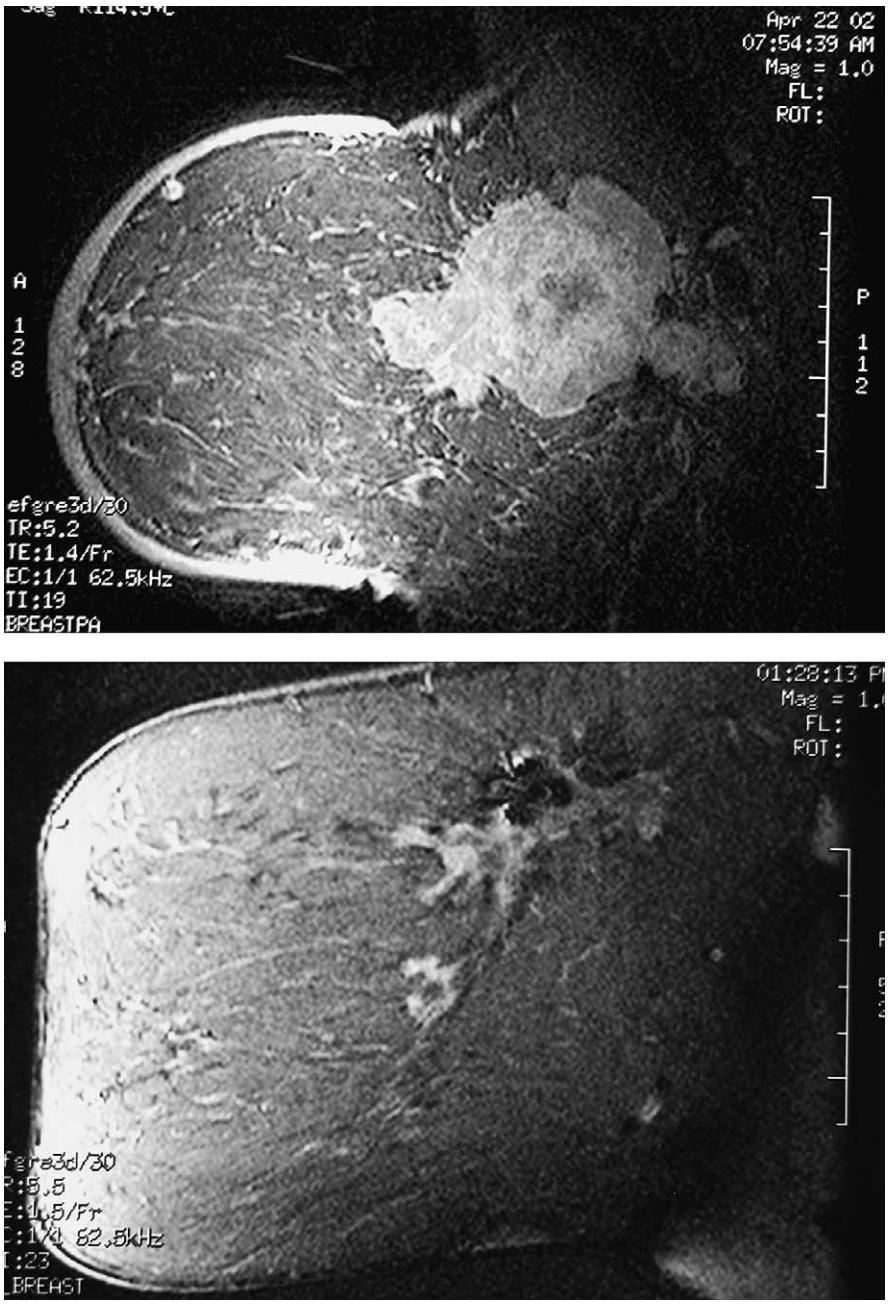

Fig. 4. Breast MRI taken at diagnosis and after 4 months neoadjuvant therapy with letrozole 2.5 mg daily. The patient's surgical outcome improved from inoperable to successful mastectomy with negative surgical margins. Note the resolution of breast skin thickening, a sign of inflammatory breast cancer.

expression were documented that illustrate the broad molecular basis for the efficacy of estrogen deprivation therapy. For example we observed marked down-regulation in expression from cell cycle genes (e.g. cell division cycle 2, G1 to S and G2 to M), cell survival genes (e.g. apoptosis repressor with CARD domain), DNA synthesis genes (5-methyltetrahydrofolate-homocysteine methyltransferase reductase), genes for DNA replication enzymes (topoisomerase (DNA) II alpha (170 kD), tissue invasion genes (matrix metalloproteinase 1 (interstitial collagenase) and cell motility factors (protein regulator of cytokinesis 1). Clearly these are all early indices of the effectiveness of endocrine therapy and the absence of these changes early on in therapy can easily be imagined to be indicative of primary resistance to therapy.

5. Conclusion

Clearly the field of endocrine therapy for breast cancer is on a new investigative path. The power of gene expression profiling will be brought to bear to resolve one of the

most critical questions in breast cancer management—why do ER+ tumors develop resistance to endocrine treatment? A key future goal of these investigations is to extend these studies beyond the narrow focus of the neoadjuvant setting because the short-term treatment of primary breast cancers for a few months does not allow studies that address the critical question of acquired or secondary resistance which may take years to develop. To examine secondary resistance we must examine the gene expression profile of advanced disease, overcoming the formidable technical difficulties inherent in tumor analysis in this clinical setting. Ideally one would want to generate longitudinal gene expression profiles so that the genotype of the tumor can be monitored at multiple points throughout the patient's clinical course. We hope that these profiles would provide clues on the nature of resistance and open up opportunities to prevent or reverse resistance by inhibiting the switch to transcriptional programs responsible for the onset of estrogen-independent tumor growth.

Acknowledgements

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