

Growth factor signalling and response to endocrine therapy: the Royal Marsden Experience

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Abstract

De novo resistance to endocrine therapy is a near-universal feature of oestrogen receptor (ER)-negative breast cancer. Although many ER-positive breast cancers also show no response to tamoxifen or aromatase inhibitors on objective clinical grounds the large majority show reduced proliferation indicating that some oestrogen dependence is present in almost all ER-positive breast cancer. In neoadjuvant studies HER2 positivity is associated with poor response rates to tamoxifen but not aromatase inhibitors, consistent with preclinical models. Acquired resistance to tamoxifen is associated with decreases in ER positivity but most recurrent lesions remain ER-positive. A small proportion of these show increased HER2 expression and in these patients increased phospho-p38 may contribute to the tamoxifen-resistant phenotype. There is an unfortunate paucity of clinical and biological data on acquired resistance to aromatase inhibitors.

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Introduction

Over the last 10 years we have been pursuing an integrated programme of clinical and laboratory research aimed at a better understanding of the determinants of response and resistance to endocrine therapy. In this paper we describe the data derived from our studies of tissues derived from patients before and during treatment with the main endocrine agents used in post-menopausal breast cancer, tamoxifen and aromatase inhibitors. In pursuing this we kept in mind observations that approximately 50% of advanced breast cancer patients show a clinical response or clinical benefit to the tamoxifen. These patients eventually develop acquired resistance but have a good chance

of responding to subsequent treatment with an aromatase inhibitor. It would therefore appear that cross-resistance between these agents is not absolute, particularly in the setting of acquired resistance. This implies that the molecular mechanisms underpinning resistance to these agents must differ in many but not all cases.

De novo/intrinsic resistance

Over recent years we and others have made increasing use of the biological opportunities provided by neoadjuvant studies. Most recently we have completed a series of biomarker studies in the IMPACT trial, within which 330 patients were treated with anastrozole or tamoxifen or a combination of the two in a double-blind, randomized, 3-month neoadjuvant study (Dowsett *et al.* 2005a, Smith *et al.* 2005). The primary marker of biological efficacy in this study was the

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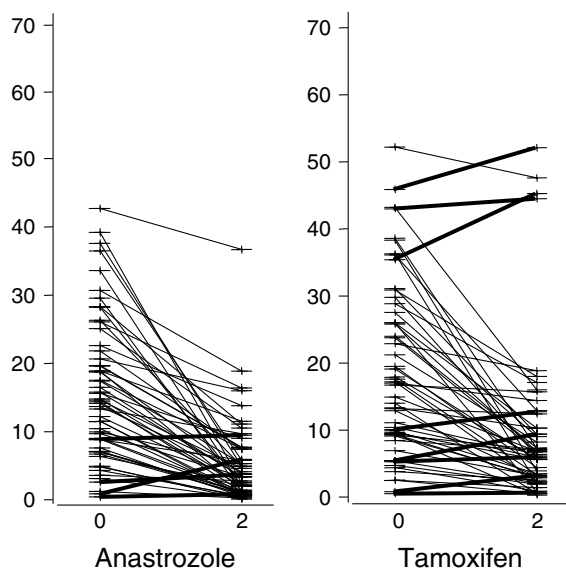


Figure 1 Changes in (%) Ki67 (y-axes) expression in individual ER-positive breast cancers during 2 weeks of treatment with anastrozole or tamoxifen. The bold lines indicate increases in Ki67 (Dowsett *et al.* 2005a).

nuclear proliferation antigen Ki67. A remarkable observation was that in the anastrozole arm there were only four of 56 patients that did not show a numerical decrease in the percentage of cells staining positive for Ki67 (Dowsett *et al.* 2005a; Fig. 1). This is in contrast to the much lower response rates judged on clinical criteria (Smith *et al.* 2005). It indicates that nearly all oestrogen receptor (ER)-positive breast carcinomas have some dependence on oestrogen for continued proliferation and that withdrawal of this oestrogen results in decreased proliferation. Although some samples showed a near-complete loss of Ki67 staining, which may indicate a complete biological response, it is clear that the changes in Ki67 varied very markedly between patients such that the biological response/resistance to oestrogen deprivation appears to be a continuum. Data from the tamoxifen arm were similar (Fig. 1) in many respects but eight of 54 patients did not show a decrease in Ki67 in this group and overall the suppression of Ki67 was significantly poorer than with anastrozole. Of particular importance, the greater suppression of Ki67 at 2 and 12 weeks for anastrozole than either tamoxifen or the combination in the IMPACT trial was parallel to the improved efficacy of anastrozole over these two arms in the large adjuvant ATAC trial (Baum *et al.* 2002). It therefore appears that analysis of Ki67 is a useful marker of early response and resistance to

hormonal agents that may be correlated with possible determinants of response and resistance.

Data from a similar neoadjuvant study of letrozole versus tamoxifen in ER-positive patients indicated that those that were epidermal growth factor receptor (EGFR)- and/or HER2-positive had a poorer chance of response to tamoxifen than patients that were negative for both receptors (Ellis *et al.* 2001). In contrast, patients positive for one or other of these growth factor receptors showed an 88% response rate to letrozole; the response rate was only 54% to letrozole in patients negative for both growth factor receptors. In the IMPACT study, only four patients were EGFR-positive and ER-positive. Thus we have confined our analysis to the 34 patients that were both HER2- and ER-positive. Seven of 12 (58%) HER2-positive patients responded to anastrozole compared with two out of nine (22%, $P=0.09$) to tamoxifen and four out of 13 (31%) to the combination (Smith *et al.* 2005). The response rates in the HER2-negative population were 28 out of 68 (41%) for anastrozole, 31 out of 73 (43%) for tamoxifen and 33 out of 64 (52%) for the combination. Thus, although the numbers of patients are small in this subgroup analysis, in the HER2-positive group the data tend to support those of the letrozole study. So far as Ki67 is concerned, in the overall population there was marginally greater Ki67 suppression in the HER2-negative group than in the HER2-positive group after 2 weeks, and this difference seemed to be confined to the group treated with tamoxifen (Dowsett *et al.* 2005b). At 12 weeks there was a significantly greater suppression of Ki67 in the HER2-negative group than in the HER2-positive group overall ($P=0.002$). At this time point there was a statistically significant greater suppression by anastrozole of Ki67 in the HER2-negative group compared with the HER2-positive group: the geometric mean suppression in the HER2-positive group was 45%; that in the HER2-negative group was 85%. This contrasts with the good clinical response in this small HER2-positive group. The individual Ki67 changes in the HER2-positive group (Fig. 2) reveal an apparent recovery in Ki67 levels in many of the anastrozole-treated patients by week 12. A potential explanation for the discordance between clinical and biological responses may be that the clinical response is underpinned by reduced proliferation throughout the 12 weeks but that towards the end of this time a recovery of Ki67 may occur that has not yet had an effect on tumour size.

In the adjuvant setting several studies suggest that patients overexpressing HER2 may derive relatively less benefit from adjuvant tamoxifen than HER2-negative

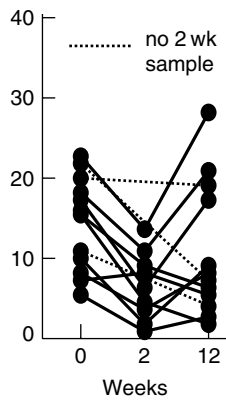


Figure 2 Changes in (%) Ki67 (y-axis) expression in individual ER-positive, HER2-positive breast cancers during 12 weeks of treatment with anastrozole (Dowsett *et al.* 2005b).

patients (reviewed in Ring & Dowsett 2003). However, this is not a universal finding. Studies in both adjuvant and metastatic settings have methodological limitations, since they are retrospective, have small numbers of patients and use inconsistent methods to assess HER2 status. The difficulty in making a conclusive judgment is illustrated by data from the collection of samples from the NATO and CRC adjuvant breast cancer trials with 2 years of tamoxifen versus no treatment (Dowsett *et al.* 1999). In this study the relative risk of recurrence for patients negative for both HER2 and EGFR between tamoxifen and no treatment was 0.54 and for

HER2- and/or EGFR-positive patients was 1.17. However, despite there being over 800 patients in the study overall, the small size of the HER2-/EGFR-positive population resulted in error estimates that completely overlapped with those for the negative population. At present there are no outcome data related to HER2 status from adjuvant trials of aromatase inhibitors. However, this is an issue of substantial contemporary importance and studies are currently underway in our laboratories to determine the significance of HER2 status in the ATAC trial (Dowsett 2004).

Although there are no direct data on growth factor receptor positivity in adjuvant trials of aromatase inhibitors, there is related interest in outcome data on progesterone receptor since about 30% of ER-positive, progesterone receptor (PgR)-negative agents are HER2- and/or EGFR-positive compared with only 10% of ER-positive, PgR-positive patients. The overview data from 2000 (Early Breast Cancer Trialist's Collaborative Group 2005) show a 41% reduction in recurrence in ER-positive, PgR-poor patients receiving about 5 years of tamoxifen compared to a 40% reduction in the ER-positive, PgR-positive patients. This indicates that there is no under-performance of tamoxifen in ER-positive, PgR-negative patients although these data are in conflict with those from a large non-randomized series (Bardou *et al.* 2003). In the ATAC trial after 47-month mean follow-up there were 18% fewer recurrences with anastrozole and tamoxifen in the ER-positive, PgR-positive group in

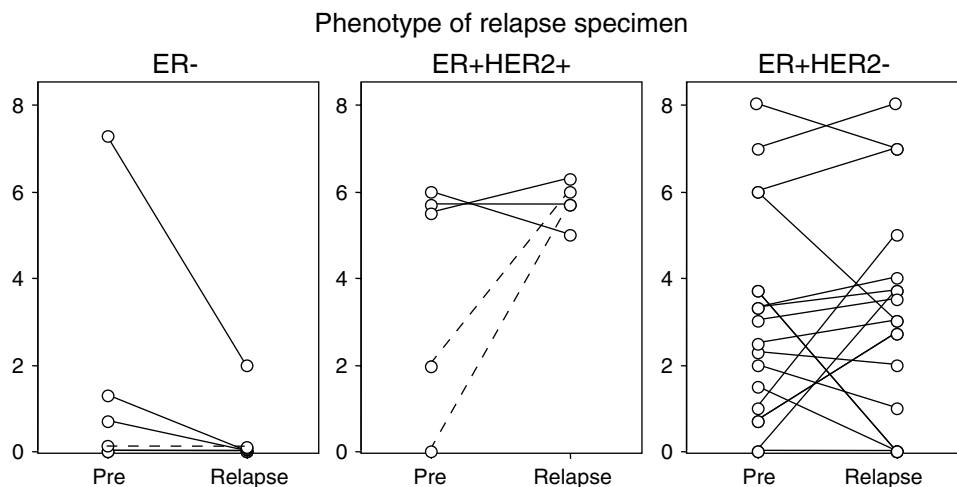


Figure 3 Changes in p-p38 expression (IHC staining for p-p38 was interpreted using the Allred score (y-axes), Allred *et al.* 1998) in 29 ER-positive tamoxifen-treated breast cancers between presentation and relapse according to the ER/HER2 phenotype of the relapse specimen (Gutierrez *et al.* 2005). The dashed lines indicate the three patients whose phenotype changed from HER2-negative to HER2-positive.

the overall hormone receptor-positive population (Dowsett *et al.* 2005c). This was similar to that in the overall hormone receptor-positive group where the reduction was 22%, but it was much less than that seen in the ER-positive, PgR-negative group where the reduction was 52%. It is important to note however, that this is a retrospective sub-group analysis which requires examination in other adjuvant trials.

Acquired resistance

We have previously reported no significant change in ER levels in 18 patients during their acquisition of resistance to primary tamoxifen therapy (Johnston *et al.* 1995). In this same group there was also no significant change in PgR status although the overall trend was downwards. HER2 was measured on 15 of these pairs of samples and was found to be negative in all patients at both presentation and relapse (Newby *et al.* 1997). One of these responders was positive for EGFR at presentation but negative at relapse, but the others were all EGFR-negative in both samples. Thus, so far as acquired resistance is concerned, in primary breast cancer there is little evidence for increased HER2 or EGF receptor expression using conventional cut-offs for positivity.

However, in patients relapsing during adjuvant tamoxifen treatment, although the majority of patients remain positive in the relapse sample, there is a significant loss of ER and PgR (Johnston *et al.* 1995). Kuukasjarvi and colleagues reported the loss of ER expression in metastatic lesions in the absence of intervening treatment (Kuukasjarvi *et al.* 1996). This loss of ER may therefore be independent of tamoxifen. The loss may nonetheless be highly relevant clinically.

We have recently extended these studies to a wider analysis of growth factor receptors and some of their associated downstream kinases (Gutierrez *et al.* 2005). A tissue microarray was constructed of pairs of samples from the same patient, firstly at presentation and secondly at the development of tamoxifen resistance during adjuvant therapy. A total of 54 patients had material available and 39 of these had sufficient tissue in both samples on the arrays to provide near-complete sets of data. 29 of these were ER-positive and 10 were ER-negative, since the samples were from patients that started treatment as far back as 1982 when ER was not used regularly for directing adjuvant therapy. In the overall population of 39 patients there was a highly significant positive correlation between ER and both PgR and Bcl2 expression and a significant negative correlation with HER2 expression. These are well-established relationships and provide evidence for this

group being a representative population. There were no significant relationships between ER and phospho-(p-)p38, p-Akt or p-ERK (phospho-extracellular-signal-regulated kinase). At relapse the significant relationships of ER with PgR, HER2 and Bcl2 were all lost but relationships between ER, p-p38 and p-ERK then emerged. Quantitative analysis of the relationship between these phosphorylated kinases and ER in the ER-positive population revealed retention of the relationship with p-p38 but loss of that with ERK.

Of the 29 ER-positive patients, five showed no ER staining in the relapsed sample, similar to our earlier observations outlined above (Johnston *et al.* 1995). Only three of the 29 ER-positive patients were also HER2-positive (in contrast with six of the 10 ER-negative). At relapse, a further three previously HER2-negative patients showed positive HER2 staining. In two of these it was in the presence of maintained ER staining, but in one ER positivity was lost. Fluorescence *in situ* hybridization (FISH) analysis of these samples revealed that none of them were HER2-amplified prior to treatment but that two were amplified in the post-treatment sample, one of these being the patient who was now ER-negative.

No significant changes were seen for p-Akt or p-ERK between the pre-treatment and the relapse specimens. However, for p-p38 there were some apparently important associations (Fig. 3). For those patients who were ER-negative at relapse only one of five showed any p-p38 staining and this was at a low score of 2 in the relapse specimen. This contrasts with the five patients who were ER-positive and HER2-positive at relapse. All of these showed scores of 5 or above in the relapse sample. For the three patients who were ER-positive and HER2-positive initially, high p-p38 levels were already present at presentation but in those who gained HER2 expression, p-p38 expression was also enhanced in the relapsed specimen. These data indicate that further attention should be paid to the expression of p-p38 and its possible role in tamoxifen resistance, particularly since there are data from model systems that support such a role (Gutierrez *et al.* 2005).

At present there are no biological data on samples derived at relapse from patients on aromatase inhibitors. This is an area of substantial clinical importance over the next few years as these agents are likely to become more frequently used in the adjuvant setting. For aromatase inhibitors as with other agents the collection of biopsy tissue from relapsed disease prior to or at subsequent secondary relapse could markedly enhance our understanding of mechanisms of response and resistance (Dowsett 2004).

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