Introduction
Oestrogen may maintain the growth of some breast cancers, and oestrogen deprivation therapy can cause tumour regression. However, even in breast cancers that possess oestrogen receptors (the key transcription factor in oestrogen signalling), response rates to endocrine therapy are only 50% to 60%, hence the interest in identifying additional factors that might predict response/resistance to hormone therapy. In this respect, it had been hoped that molecules that are regulated by oestrogen would be excellent candidates. However, this review makes the case that oestrogen-regulated markers are unlikely to be robust indicators of hormone dependence in breast cancers.

Evidence is based on correlating oestrogen regulated markers (identified on account of having an oestrogen response element [ERE] in the promoter region of their genes and being classically oestrogen regulated in many hormone-sensitive tissues [the example used is the progesterone receptor (PR)], expression being regulated by oestrogen in breast cancer cell lines, and expression being regulated by oestrogen deprivation therapy in primary breast cancers) with clinical response to endocrine therapy either in patients with advanced breast cancer or in the neoadjuvant setting.

Progesterone receptor and clinical response to neoadjuvant hormone therapy
Two major randomized trials of neoadjuvant endocrine therapy (P024 and IMPACT [Immediate Preoperative Arimidex Compared to Tamoxifen]) and one other large study assessed the relationship of PR status to clinical response to either tamoxifen or an aromatase inhibitor. All found a higher incidence of PR positivity in clinically responding tumours [1-3] as compared with nonresponders. However, it is clear that a substantial number of PR-negative tumours respond to endocrine therapy, and conversely that many clinical nonresponding tumours possess PR. Consequently, PR is a poor marker of clinical response to endocrine therapy.

It could be argued that rather than measure pretreatment PR expression, it might be more informative to relate change in PR level with clinical response. Treatment with aromatase inhibitors is associated with a reduction in immunohistochemical staining for PR [1-3]. However, this occurs in about 90% of cases irrespective of clinical response. It is clear that downregulation of PR occurs in most clinically nonresponsive tumours.

Genes regulated by oestrogen in breast cancer cell lines
Many studies have been conducted to determine the effects of oestrogen addition/withdrawal in breast cancer cell lines maintained in culture or as xenografts in immunosuppressed animals; most have used the MCF7 cell line. Five studies published since 2000 [4-8] have been used as a database in which to identify oestrogen-regulated genes. No single gene was listed in all five publications, but two were quoted in four publications and nine in at least three journals. A total of 141 genes were confirmed by publication in two journals. The general lack of consensus probably reflects variables such as the amount of oestrogen used, duration of exposure, different sources of cell lines, conditions of growth and different platforms of assays. These 141 genes were compared with the gene signatures based on a pretreatment tumour analysis from patients with advanced breast cancer and predicting response to tamoxifen, as defined by Janssen and coworkers [9]. The overlap between the genes was minimal. However, such a comparison is based upon change in expression for the cell line gene set and pretreatment expression for the clinical response signature.

To avoid this confounder, the 141 gene list has been compared with genes that most significantly changed on letrozole neoadjuvant treatment but differentially expressed between responders and nonresponders [10]. No correlation was found between the two sets of genes. Although the identity of these genes remains unreported, none were found among the 141 regulated by oestrogen in the MCF7 cell line. There are two major reasons for this. First, some genes change in both nonresponding and responding cases. Second, some genes change in culture following oestrogen...
exposure/withdrawal but are relatively unaffected by letrozole treatment in primary tumours. This can be illustrated, as is shown in Figure 1, by comparing the change in expression of the nine most commonly quoted oestrogen-regulated genes in MCF7 in responding and nonresponding tumours. It can be seen that genes such as NRIP1, STC2, CCND1, MYB and TFF1 are frequently downregulated by letrozole treatment in clinically responding tumours, but this also occurs in many of the nonresponding cases. Other genes such as AURKA, IGFBP4, SLC7A5 and TPD52-1 are relatively unaffected by treatment in both responders and nonresponders.

### Genes that are influenced by endocrine therapy in breast cancer

A list of genes most significantly influenced by neoadjuvant treatment (at 14 days) with letrozole has been published [10]. However, this has no overlap with genes whose expression is differentially changed between clinical responders and nonresponders; and, therefore, that most oestrogen-regulated genes are unlikely to be robust markers of clinical response to endocrine therapy.

It is concluded that oestrogen-regulated genes are molecular markers of oestrogen sensitivity (not dependence); that expression profiles and molecular responses to endocrine therapy in clinically resistant tumours may be similar to clinical responders; and, therefore, that most oestrogen-regulated genes are unlikely to be robust markers of clinical response to endocrine therapy.

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