Short communication

Integration of endocrine therapy with targeted agents

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Introduction

Combining endocrine therapies for breast cancer with various targeted biological therapies has become a very active area of clinical research aimed at overcoming or preventing endocrine resistance. Several theories, each supported by preclinical and - in some instances - clinical data, have been proposed to explain both the acquired and *de novo* endocrine resistant phenotype that is observed in oestrogen receptor (ER)-positive breast cancer [1]. These include mechanisms whereby there is a sustained dependence on ER-mediated signalling despite resistance to an individual endocrine agent, and various gene silencing mechanisms that cause reversible suppression of ER activity. Still other mechanisms involve acquired changes in peptide growth factor mediated mitogenic signalling pathways, which may or may not crosstalk with existing ER-signalling pathways. For example, it is known that activation of signalling via the human epidermal growth factor receptor (HER) family (namely epidermal growth factor receptor [EGFR] and HER2) can amplify existing endocrine signalling within ER-positive breast cancer cells, thus bypassing the inhibitory effects of any anti-oestrogen such as tamoxifen [2] or oestrogen deprivation therapy [3]. This in turn manifests clinically as endocrine resistance. However, in clinical practice the strong likelihood is that, for ER-positive breast cancer at least, no single unifying mechanism for endocrine resistance will be discovered. Therefore, identifying which resistance mechanism is operational in an individual patient could become clinically relevant to tailoring the subsequent therapy.

Current clinical trials have investigated three approaches to overcoming endocrine resistance, including maximal blockade of ER signalling, combinations of endocrine therapy with novel therapies that target the HER family of growth factor receptors, and combinations with drugs that target relevant downstream signalling pathways. Not all approaches have been successful to date, despite often very encouraging preclinical data. As discussed below, various issues in appropriate clinical trial design and patient selection must be addressed in order to maximize the potential of this new integrated approach.

Maximal blockade of oestrogen receptor signalling

Given the published evidence for retention of a functional ER pathway after acquired resistance to tamoxifen/oestrogen deprivation therapy, one strategy has been to develop endocrine therapies that deliver maximal ER signalling blockade. Fulvestrant is a novel type of ER antagonist that prevents ER dimerization and leads to rapid degradation of the fulvestrant-ER complex, producing loss of cellular ER [4]. It has been shown that, because of its unique mechanism of action, fulvestrant delays the emergence of acquired resistance compared with tamoxifen in an MCF-7 hormone-sensitive xenograft model [5]. Clinical data from phase II studies in postmenopausal women with advanced breast cancer suggested some modest efficacy for fulvestrant in a second/third-line setting [6-8]. This was confirmed in the large randomized phase III EFECT (Evaluation of Faslodex versus Exemestane Clinical Trial) study [9], which demonstrated similar efficacy for fulvestrant versus exemestane in patients who have progressed on treatment with nonsteroidal aromatase inhibitors (AIs) [9].

Laboratory evidence has suggested that the efficacy of fulvestrant - especially in the setting of endocrine resistance, where activated ER signalling may still be dominant - could critically depend on the background oestrogen environment in which the cells exist. This has led to the concept that ER-positive endocrine resistant cells may need maximal ER signalling blockade.

Recent experiments with tamoxifen-stimulated breast cancer xenografts demonstrated paradoxical effects on tumour growth,

AI = aromatase inhibitor; EGFR = epidermal growth factor receptor; ER = oestrogen receptor; HER = human epidermal growth factor receptor; mTOR = mammalian target of rapamycin; PFS = progression-free survival; PI3K = phosphoinositide-3 kinase.

which depended on whether fulvestrant was administered in the presence or absence of oestradiol [10]. Similar findings have been reported in cells resistant to long-term oestrogen deprivation, in which maximal growth inhibition of cells was observed with a dose of 10^{-8} mol/l fulvestrant, but the titration back of increasing amounts of oestradiol resulted in re-growth of cells that fulvestrant was no longer able to antagonize effectively [11]. In addition, in a xenograft model, combined therapy with letrozole plus fulvestrant was significantly more effective than either agent alone, delaying emergence of resistance [12]. On the basis of these findings, an ongoing phase III trial (SoFEA [Study of Faslodex versus Exemestane with/without Arimidex]) will compare progression-free survival in patients who have progressed on a nonsteroidal AI, and who are subsequently treated with either fulvestrant plus continued anastrozole or with fulvestrant alone. A further first-line phase III study (FACT [Fulvestrant and Anastrozole Clinical Trial]) has compared anastrozole plus fulvestrant versus anastrozole alone in endocrine sensitive advanced breast cancer. These trials will hopefully address the issue of whether maximal hormonal blockade (total ligand deprivation plus complete ER downregulation) will better treat or prevent endocrine resistance.

Co-targeting ER and HER family signalling: prevention of acquired resistance

Based on the preclinical evidence and rationale for co-targeting ER and HER family signalling, a number of trials have been conducted with either the HER2 monoclonal antibody trastuzumab or the EGFR/HER2 tyrosine kinase inhibitors gefitinib, erlotinib or lapatinib in combination with endocrine therapy [13]. Some of these trials were conducted in patients with established hormonal resistance, in which activated growth factor pathways may be operative. However, many were conducted in the first-line setting in ER-positive hormone-sensitive patients, and treatment was combined with an AI (clinical and experimental data have shown that tyrosine kinase inhibitors alone may have limited activity in this setting). Therefore, the primary end-point for these trials was to investigate whether time to disease progression can be significantly prolonged by the addition to endocrine treatment of a targeted therapy, thus delaying the emergence of resistance, as demonstrated in the various preclinical models described above.

Three key randomized studies in advanced breast cancer have been reported to date. A double-blind, placebo-controlled phase II trial of tamoxifen with/without gefitinib as first-line endocrine therapy was conducted in 290 postmenopausal women with ER-positive metastatic breast cancer [14]. This study set out to test the preclinical concept that combination therapy could delay the onset of acquired resistance to endocrine therapy, as demonstrated both *in vitro* and in xenograft models *in vivo* [2,3]. Disease was either endocrine naïve or had developed more than a year after completion of adjuvant tamoxifen (stratum 1; $n = 206$), or it had developed during or after AI therapy (stratum 2; $n = 84$). In stratum 1 (endocrine naïve) there was a numerical increase in progression-free survival (PFS) from 10.9 to 8.8 months (hazard ratio = 0.84, 95% confidence interval = 0.59 to 1.18; $P = 0.31$), which met the predefined criterion of a 5% improvement in PFS. Patients who had been pre-exposed to AIs did not gain any benefit from the combination, suggesting that patient characteristics are crucial in selecting an appropriate population in which to test these therapies.

The first results of a second randomized trial of gefitinib and anastrozole versus anastrazole alone, conducted in a similar first-line patient population of women with ER-positive advanced breast cancer, were reported at the American Society of Clinical Oncology meeting in June 2008 [15]. There was a significant prolongation of PFS from a median of 8.2 months with anastrozole to 14.6 months with the combination (hazard ratio = 0.55, 95% confidence interval = 0.32 to 0.94). The number of patients in this second study was only 93, and no information was available on how many patients had received prior adjuvant endocrine therapy, and therefore which patients derived benefit from the combination. This will be important information, especially given that in both gefitinib studies there was no improvement in objective response rates (initial tumour shrinkage was no greater for the combination). However, a numerical improvement in clinical benefit rate seen in both studies (inclusion of patients with stable disease) implies that any clinical gain exists by delaying resistance, probably in those with initial endocrine-sensitive ER-positive disease, as first demonstrated in both *in vitro* and xenograft models for the addition of gefitinib to endocrine therapy [2,3].

Likewise, targeting HER2 in hormone-receptor positive breast cancer has been explored as a means of improving endocrine responsiveness. This may involve re-expression of silenced ER, as outlined in the preclinical data [16]. Indeed, there is clinical evidence - from a series of 10 patients with ER-negative/HER2-positive advanced breast cancer who had serial biopsies during trastuzumab therapy [17] - that trastuzumab can restore both ER expression and endocrine responsiveness. A phase II clinical trial of letrozole and the monoclonal antibody trastuzumab in patients with ER-positive/HER2-positive metastatic breast cancer revealed that the combination was well tolerated and had a clinical benefit rate (partial response + stable disease) of 50% [18]. Subsequently, a randomized phase II trial conducted in 207 patients with known ER-positive/HER2-positive metastatic breast cancer recently reported a doubling of PFS with the addition of trastuzumab over anastrozole alone (4.8 months versus 2.4 months; $P = 0.0016$) [19]. Lapatinib, a potent oral tyrosine kinase inhibitor of both EGFR and HER2, has since been explored in combination with endocrine therapy based on *in vitro* data demonstrating that oestrogen deprivation significantly enhances the anti-proliferative effects of lapatinib in HER2-amplified breast cancer cell lines [20,21]. Similarly,

preclinical evidence suggests that lapatinib can significantly enhance sensitivity to tamoxifen in cell lines with acquired tamoxifen resistance [22].

A phase III trial has completed recruitment of 1,200 patients with metastatic ER-positive breast cancer who were randomly assigned to receive either letrozole alone or letrozole combined with lapatinib. This large study may offer an important insight into the subgroups of patients who are most likely to benefit from a lapatinib-endocrine combination, such as known ER-positive/HER2-positive breast cancer with potential *de novo* endocrine resistance (at least 200 such patients were included in the study), or ER-positive/HER2-negative tumours that might develop acquired resistance to letrozole during treatment because of adaptive EGFR or HER2 upregulation. To identify the latter, all patients had serum taken at baseline entry for assessment of circulating extracellular domain HER2, which has been reported to be a predictor of poorer outcome with endocrine therapy, with seroconversion occurring during endocrine therapy in up to 25% of patients with ER-positive metastatic disease treated with either letrozole or tamoxifen [23]. Thus, correlative biomarker studies will be crucial to the interpretation of which patients with ER-positive tumors may derive benefit from combined targeted therapy with endocrine treatment.

Targeting downstream signalling

Other intracellular pathways downstream from cell surface growth factor receptors may become operative in endocrine resistance and may thus be appropriate targets for combination therapy strategies. The phosphoinositide-3 kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is activated by a number of growth factors, including insulin, insulin-like growth factor I, basic fibroblast growth factor, epidermal growth factor and vascular endothelial growth factor. Mutations in the catalytic domain of PI3K have been identified in 20% to 25% of breast cancers [24,25]. Although PI3K inhibitors are still in the early stages of development, mTOR inhibitors have been tested in breast cancer in combination with endocrine therapies. A randomized phase II study of letrozole alone or in combination with the mTOR inhibitor temsirolimus suggested a modest benefit from the combination in terms of median PFS (13.2 months versus 11.6 months) [26]. Unfortunately, the resulting large phase III randomized trial of letrozole alone or in combination with temsirolimus in 992 postmenopausal women was terminated early after an interim analysis demonstrated a lack of benefit from the combination [27]. It is probable that inability to identify patients in whom the tumours exhibit dependence on PI3K/mTOR activation severely limited the likelihood of demonstrating benefit in this large phase III trial. Likewise, concern has been expressed that mTOR inhibition may induce a feedback loop via S6kinase and insulin-like growth factor receptor, which enhances further Akt activation, thus overcoming the effects of mTOR inhibition. Thus, an understanding of adapative escape pathways is also critical to utilizing targeted therapies effectively, because - ultimately - vertical combinations of more than one key signal transduction inhibitors in combination with endocrine treatments may prove to be more effective.

More recent studies conducted in the neoadjuvant setting have evaluated the benefit of adding the mTOR inhibitor everolimus to letrozole. In a randomized phase II study conducted in 270 postmenopausal women with ER-positive primary operable breast cancer (>2 cm in size), the combination of letrozole and everolimus for 4 months before surgery resulted in significantly greater tumour shrinkage, as judged by ultrasound (58% versus 47%; $P = 0.03$), and a greater reduction in cell proliferation, as measured by changes in Ki-67 after 15 days therapy [28]. In associated biomarker studies to identify those tumours that are most likely to respond to combined mTOR antagonists and AI, elevated levels of one of the downstream biomarkers of mTOR activation (pS6240 kinase) was associated with a greater likelihood of response to the combination (odds ratio = 2.1) [29]. These types of clinical studies in primary breast cancer can yield informative biomarker data on those tumours that are more likely to respond better 'up front' to the combination, but they may be more limited in predicting which tumours then utilize the given downstream signalling pathway during prolonged endocrine therapy as a means of developing acquired endocrine resistance.

Future challenges

There is an increasing body of evidence that suggests that ER signalling survives, and that growth factor receptor and downstream kinases often operate in conjunction with ER through bidirectional crosstalk to account for both *de novo* and acquired endocrine resistance. The nature of the interaction between ER and mitogenic signalling probably varies over time and from one patient to another. Despite the strong preclinical data and rationale, translation of these hormone-resistance hypotheses into successful clinical studies of combined targeted therapies and endocrine treatments have yielded varied results to date. This may in part be attributable to poor selection of patients, lack of stratification for prior endocrine responsiveness, inappropriate clinical end-points and an incomplete understanding of whether the given target is an 'addictive' component in the cell. In other words, it is unlikely that patients will respond to combinations with specific inhibitors unless the intended target is a significant driver of endocrine-resistant growth. Thus, biological analyses are still required to shed further light on the clinically relevant mechanisms of endocrine resistance that operate in individual patients, because these may ultimately show us the most intelligent way to combat the various hormone-resistance pathways that ER-positive breast cancer cells utilize to survive.

Competing interests

The author declares that they have no competing interests.

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