

Endocrine Therapy, New Biologicals, and New Study Designs for Presurgical Studies in Breast Cancer

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The preoperative setting is increasingly popular for the clinical investigation of hormonal agents and new biological drugs. The effectiveness of endocrine agents is well established for estrogen receptor-positive disease, and the emphasis in preoperative studies is on their combination with agents targeted at resistance mechanisms over 3 or more months. New agents are also being assessed for early evidence of clinical efficacy in shorter-term window-of-opportunity studies. The establishment of Ki67 as an intermediate marker of treatment benefit and of long-term outcome, with endocrine drugs, provides the opportunity for new trial designs with Ki67 as the primary endpoint. The PeriOperative Endocrine Therapy for Individualizing Care (POETIC) trial is randomizing (2:1) 4000 estrogen receptor-positive patients to 2 weeks presurgical treatment with a nonsteroidal aromatase inhibitor or no presurgical treatment. It provides a unique opportunity for detailed study of the determinants of response and resistance to estrogen deprivation as well as testing the role of presurgical therapy for improved biomarker-based estimates of prognosis.

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The enormous number of new biological agents aligned with the large investment that is required for their registration is a major challenge for clinical oncology and the pharmaceutical industry. The neoadjuvant scenario is seen as a means of prioritizing drugs for clinical development and is increasingly being exploited for this purpose. For estrogen receptor (ER)-positive disease, which constitutes approximately 80% of breast cancer, integration of drug development with endocrine treatment is essential. Validation of the proliferation biomarker, Ki67, as an intermediate endpoint has enabled the investigation of breast cancer within this scenario using a biomarker rather than clinical response and has created new study designs to integrate novel agents targeting pathways of *de novo* or acquired resistance.

Ki67 as a Marker of Treatment Benefit and Predictor of Long-Term Outcome

The IMPACT (Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen) trial compared the aromatase inhibitor anastrozole vs tamoxifen vs the combination in a randomized double-blind design in 330 postmenopausal women with ER-positive breast cancer. This was parallel to the ATAC (Arimidex, Tamoxifen Alone or in Combination) adjuvant trial of these same three treatments in 9366 patients over a median 30-month follow-up, which showed that the aromatase inhibitor was more effective in restricting recurrence than tamoxifen or the combination. In IMPACT, Ki67 was selected as the primary biomarker endpoint since endocrine therapy was known to have a profound effect on proliferation—one of the two major determinants of tumor growth. In contrast, induction of apoptotic cell death, the other major determinant of growth, had been found to be decreased rather than increased as

expected by both tamoxifen and the aromatase inhibitor, vorozole, in earlier neoadjuvant studies (1). There was a highly significant difference between the treatments in Ki67 suppression following 2 and 12 weeks (ie, at surgery) endocrine treatment: anastrozole suppressed Ki67 by 76% and 82%, compared with tamoxifen by 60% and 62%, and the combination by 64% and 61%, respectively (2). Thus, differences in Ki67 suppression mirrored those in recurrence-free survival (RFS) in ATAC.

Further support for the clinical importance of Ki67 changes was provided by higher 2-week values of Ki67 predicting a significantly worse RFS (3). In a multivariable model along with standard clinical parameters, 2 week but not pretreatment Ki67 remained in the model. This implies that the 2-week value integrates the prognostic significance of baseline levels of Ki67 together with the improved outcome that is predicted by decreased Ki67.

Although there is no likelihood that such short-term studies could replace phase III adjuvant trials for registration purposes, they may play a role in excluding ineffective agents from further clinical development. For example, had the Ki67 and other biomarker data on IMPACT that showed consistent equivalence between tamoxifen and the combination of anastrozole and tamoxifen been available when designing the ATAC trial, it seems unlikely that the trial would have proceeded with the combination arm. Exclusion of that arm would have reduced necessary recruitment to approximately 5000 patients and probably approximately 18 months could have been cut off time to trial completion.

Ki67 as a Marker of Acquired Resistance

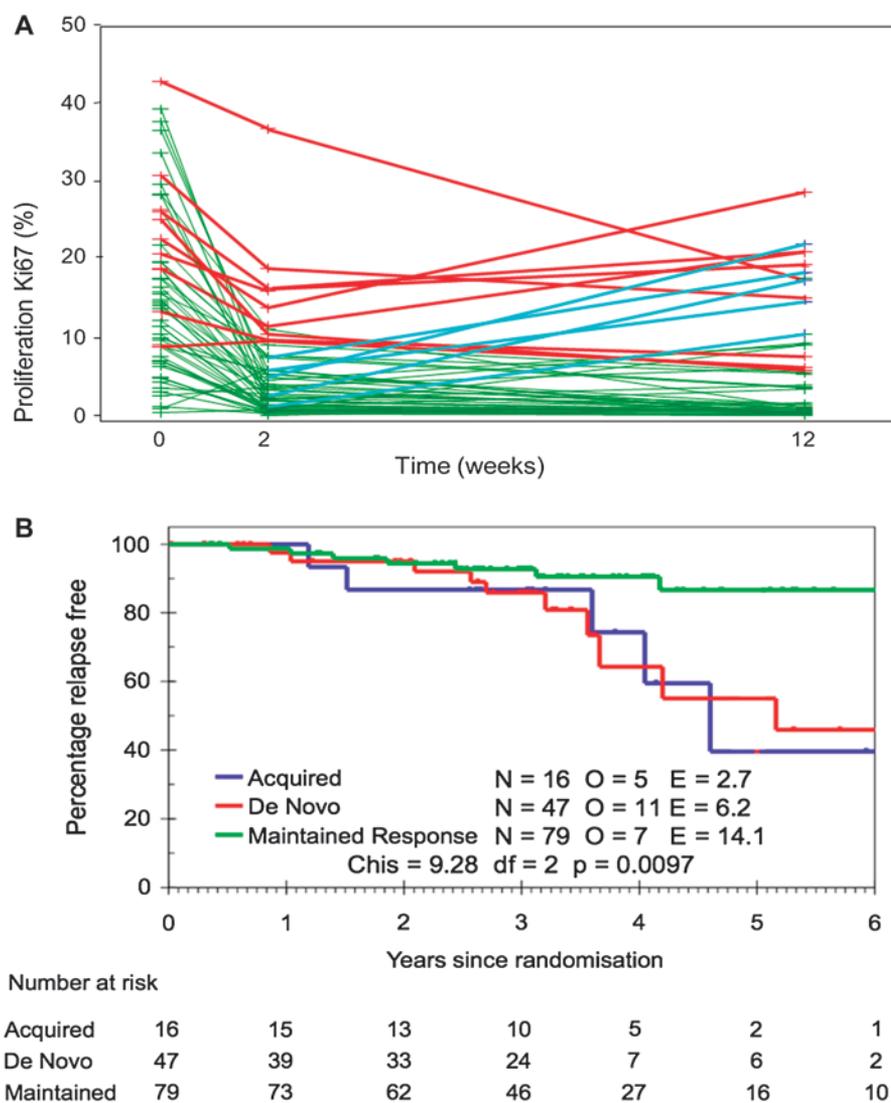
In IMPACT, 52 out of 56 patients on anastrozole showed some suppression of proliferation over the 2-week period, suggesting that

nearly all ER-positive patients derive some benefit from therapy with an aromatase inhibitor. The majority showed similar suppression at 12 weeks to that at 2 weeks, but approximately 15% showed a recovery in Ki67 by that time (Figure 1, A), providing very early biological evidence of acquired treatment resistance. This was supported clinically by RFS in this group of patients with acquired Ki67 resistance (see Figure legend for definition) being no better than that in the de novo Ki67 resistance group and significantly worse than that in patients with persistent Ki67 response (Figure 1, B). This provides an opportunity for biomarker-led study of agents targeting acquired resistance to hormonal therapy in the neoadjuvant setting, even though clinical endpoints are unavailable because surgery is generally timed to avoid the overt appearance of resistance. Studies of clonality based, for example, on mutation frequency and/or DNA losses and gains alongside the measurement of Ki67 may provide evidence for whether the mechanism of this escape from treatment control is via selection of resistant cell populations as opposed to epigenetic changes.

We reported the first such study in which patients received an aromatase inhibitor for the first 2 weeks of their neoadjuvant therapy and were then randomized to addition or not to the tyrosine kinase inhibitor (TKI), gefitinib, with Ki67 as the primary endpoint (4). Gefitinib was wholly ineffective in that trial. Together with other data on the modest effectiveness of gefitinib in ER-positive breast cancer, this contributed to the decision to not proceed to phase III developments.

Novel Agents

The short-term presurgical window-of-opportunity scenario has been used to gather early evidence of clinical benefit or not of novel agents, either in whole populations or in specific subgroups. This includes data on the epidermal growth factor receptor (EGFR)-TKI, erlotinib, that indicate its activity in ER-positive breast cancer outside of the EGFR-overexpressing group (5). We have reported that the cyclooxygenase-2 inhibitor, celecoxib, has very modest, if



Early acquired Ki67 resistance: target for treatment

any, significant effect on Ki67 over a 2 week period in ER-positive disease, providing evidence against its acting as an aromatase suppressant (6). In addition, we are currently evaluating the effectiveness of the dual EGFR/HER2) TKI, lapatinib, in HER2-negative tumors to determine whether there is any clinical effect outside of its now established HER2-positive target population. It should also be noted that Ki67 may not be the most appropriate endpoint for the evaluation of agents that are not directly antiproliferative. For example, antiangiogenic agents may have limited effects on proliferation, but reduced vascular density or increased cell death adjacent to collapsed vessels are pharmacodynamic endpoints that may with extended study be useful as markers of antitumor efficacy (7). In contrast, the association of early decreases in Ki67 with clinical response to cytotoxic agents in the neoadjuvant setting may be by virtue of their selective killing of more highly proliferative cells (8).

Ethical Issues

Neoadjuvant therapy with endocrine agents aims to downstage tumors, and the imperative is therefore to have firm evidence for therapeutic benefit of the agent(s) used. For new agents with limited evidence of single-agent activity, strategies that combine the agent with established agents such as aromatase inhibitors are generally necessary; it may however be possible to create an early window in which the added agent is used alone for a short period that is not disadvantageous to the patient.

The ethical use of new agents in pure window-of-opportunity studies where there is no implied therapeutic advantage demands substantial evidence of safety given that most patients in this group are potentially curable by surgery alone. However, if short pretreatment assessment of parameters like Ki67 was shown to have general applicability in predicting outcome, the impact on trial design could be enormous, and ineffective treatment to thousands of patients could be avoided. Consistent decision making on ethical acceptability would be aided by the development of international guidelines for the level of safety data required for nontherapeutic presurgical studies.

Studies of Mechanisms of Action

The variable degree of suppression of Ki67 with aromatase inhibitor treatment concurs well with the clinical heterogeneity of ER-positive breast cancer. To understand the molecular aberrations that underpin this heterogeneity, we have conducted genome-wide expression analysis on samples taken before and after treatment with an aromatase inhibitor (9). Profound changes in transcription occurred over the 2-week period, including that of many classical estrogen-dependent genes, such as *TFF1*, *CCND1*, *PDZK1*, and *AGR2*, but also of many other downstream genes that are not directly regulated by estrogen. For example, we have recently found that the decreased expression of a hypoxia metagene that occurs in many tumors on neoadjuvant treatment with an aromatase inhibitor is strongly correlated with that of a proliferation metagene but is not correlated with changes in expression of classical estrogen-dependent genes (10). The evidence points to the changes in hypoxia resulting from the profound reduction in proliferation, and therefore, reduced oxygen needs rather than being due directly to decreased estrogenic stimulation.

With respect to predictors of responsiveness to estrogen deprivation, a global index of dependence on estrogen that summated the overall estrogen dependence of transcription was lower in tumors with low levels of ER and/or amplified HER2 (9). Further study in this area with an expanded cohort of samples indicated that many genes associated with inflammation/immune response are also associated with poor Ki67 change (11).

The POETIC (PeriOperative Endocrine Therapy for Individualizing Care) Trial

A number of the data described above are now under much more detailed study in a unique perioperative trial. The POETIC trial has, as its primary endpoint, the evaluation of a long-standing hypothesis that endocrine therapy given before breast cancer surgery might improve outcome. This hypothesis was developed by Fisher et al. (12) from the observation that in rats, both chemotherapy and endocrine therapy were associated with improved survival when given before surgical removal of a mammary tumor. This observation has never been tested clinically with endocrine agents.

In the POETIC trial, postmenopausal patients with ER-positive primary breast cancer are randomized to a nonsteroidal aromatase inhibitor (either anastrozole or letrozole, according to local preference) or to no treatment (2:1) for 2 weeks before and 2 weeks subsequent to excision (Figure 2). The 2-week presurgical window is approximately the time generally taken between diagnosis and surgery in the United Kingdom. The presurgical treatment essentially brings forward into that window treatment that the patients will otherwise have started postsurgically. Postsurgical therapy is not specified in the protocol but should not be influenced according to the randomization or not to an aromatase inhibitor. Four thousand patients are required to evaluate this question to detect a 3% improvement in recurrence from 10% to 7% with 91% power (two-sided $\alpha = 5\%$) and will therefore be more than 10-fold larger than any other short-term presurgical study. If the intervention offers no advantage, but also no disadvantage, in terms of RFS, it will still provide important safety data for the conduct of mechanistic studies in this scenario.

POETIC: PeriOperative Endocrine Treatment for Individualized Care

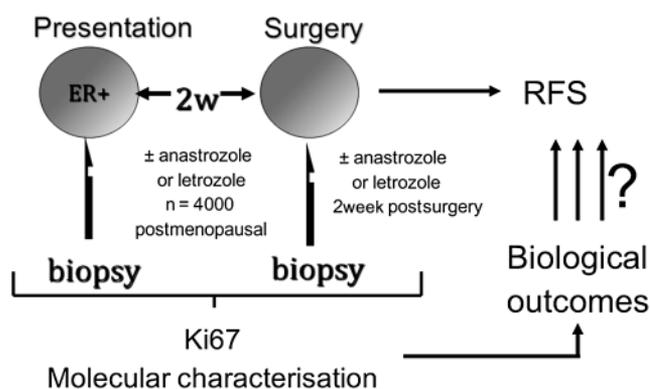


Figure 2. Graphical representation of the POETIC trial.

A secondary aim is to determine the accuracy with which Ki67, 2 weeks after starting the aromatase inhibitor, predicts for RFS compared with pretreatment Ki67. This will establish whether presurgical therapy to elicit an improvement in the prognostic value of Ki67 merits widespread application.

Furthermore, secondary analyses will extend the observation described above that there are profound and variable gene expression changes within endocrine therapy in breast cancers over the 2-week period. The trial will provide biopsy material that will allow, in a large number of patients, 1) the degree of changes to be related to eventual clinical outcome and 2) biomarker determinants of these changes to be assessed in pretreatment samples. Measurement of the gene expression changes will also allow testing of the hypothesis that evaluation of gene expression on treatment may be more accurate in terms of prognosis and predictive information than when conducted before treatment, such as with Oncotype Dx (Genomic Health Institute, Redwood City, CA) and MammaPrint (Agendia, Irvine, CA).

The POETIC trial is also collecting blood samples for germline DNA evaluation of polymorphisms that may affect the biological response to estrogen deprivation therapy and for plasma estrogen measurement to test compliance and systemic response. It is known that aromatase inhibition and estrogen suppression with letrozole are more complete than with anastrozole (13,14). While preliminary study based on small patient numbers found no significant differences in Ki67 expression or in changes in gene expression between the two agents (9), the POETIC study will have sufficient statistical power to characterize any differences much more fully.

A trial of this size, which requires biological material to be collected at diagnosis and at surgery, requires large numbers of centers and personnel from clinical teams that would not normally have a major role in clinical trials. For example, in POETIC, radiologists take further biopsies for specific research purposes after the patient has been asked to provide prediagnostic consent. In addition, core-cut biopsies, at the time of surgery to avoid any biological or artifactual bias that might occur by comparing cores with sections of the whole excision, involve surgical team members whose focus at that time is entirely on the operation. Consultation with patients on this unusual study design was also important to maximize recruitment.

Over 80 centers in the United Kingdom are actively recruiting to POETIC. Recruitment by the end of August 2011 was around 2100 patients and 100 patients per month. Thus, the major challenges in recruiting large numbers of patients to a perioperative trial have been overcome. The study will have sufficient power to answer clinical and biological questions that will give a unique insight into mechanisms of response and resistance to endocrine therapy.

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