Short-Term Changes in Ki-67 during Neoadjuvant Treatment of Primary Breast Cancer with Anastrozole or Tamoxifen Alone or Combined Correlate with Recurrence-Free Survival

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ABSTRACT

Purpose: Neoadjuvant (preoperative) therapy for breast cancer may allow for the development of intermediate markers of treatment benefit, thereby circumventing the need for efficacy trials of adjuvant therapy, which require much larger patient numbers and longer follow-up. The aim of this study—as part of the Immediate Preoperative “Arimidex” (anastrozole), Tamoxifen, or Arimidex Combined with Tamoxifen (IMPACT) trial (n = 330)—was to test the hypotheses that changes in Ki-67 after 2 weeks and/or 12 weeks: (i) differed between treatments, (ii) predicted clinical tumor response, and/or (iii) may predict long-term outcome differences between treatments in adjuvant therapy.

Experimental Design: The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial compared these same agents in the adjuvant setting. Biomarkers were measured in biopsy specimens taken before and after 2 and 12 weeks of treatment.

Results: Suppression of the proliferation marker Ki-67 after 2 and 12 weeks was significantly greater with anastrozole than with tamoxifen (P = 0.004 and P < 0.001) but was similar between tamoxifen and the combination (P = 0.600 and P = 0.912). This result closely parallels that compared the same three treatments in 330 patients with a median follow-up of 9,366 patients. Against expectations, apoptosis treatments after a median follow-up of 31 months in the treatment, biomarkers were measured in biopsy specimens taken before and after 2 and 12 weeks of treatment.

Conclusions: The data indicate that short-term changes in proliferation in the neoadjuvant setting may be able to predict outcome during adjuvant use of the same treatments. If this can be confirmed, these findings could lead to a profound change in approaches to drug development in breast cancer. The data indicate that estrogen is not an important survival factor for human breast cancer cells.

INTRODUCTION

The treatment of primary breast cancer patients with adjuvant medical therapy has led to substantial improvements in relapse-free survival and overall survival (1). The availability of increasing numbers of new medical therapies promises further improvements in outcome. At present, the assessment of these new treatments in early breast cancer requires the conduct of very large randomized adjuvant clinical trials involving thousands of patients, with follow-up extending over several years before first results emerge. The implications in terms of time and cost are self-evident. Reliable intermediate end points that allow more rapid evaluation would be of enormous value. The neoadjuvant (preoperative) setting in which medical therapies may be evaluated prior to surgery is being increasingly exploited with this in mind (2). Clinical response to neoadjuvant chemotherapy has been found to predict disease-free survival and overall survival (3, 4). There are no direct data to confirm this for endocrine therapy.

Although clinical response to neoadjuvant treatment may be a valuable intermediate marker, early changes in the biological determinants of tumor regression or progression, namely proliferation and apoptosis, might be able to provide earlier—and possibly more reliable—prediction of long-term outcome than response itself. An initial, but not prerequisite, consideration would be that these biomarker changes relate to clinical response in the neoadjuvant setting. We have published data from relatively small studies that support this latter possibility (5–7) and report here a large, randomized endocrine neoadjuvant trial in which these concepts have been rigorously assessed for the first time.

A randomized trial of the aromatase inhibitor, anastrozole (Arimidex) versus tamoxifen versus the combination as primary adjuvant therapy in postmenopausal women newly diagnosed with early breast cancer [the “Arimidex”, Tamoxifen, Alone or in Combination (ATAC) trial] reported that relapse-free survival was significantly better with anastrozole than with tamoxifen or the combination (8, 9). This was an important clinical advance, but it required a median 31 months of follow-up of 9,366 patients involving approximately 25,000 woman-years of treatment. We undertook a neoadjuvant trial that compared the same three treatments in 330 patients with...
primary breast cancer over a period of 12 weeks [the Immediate Preoperative Arimidex, Tamoxifen, or Arimidex Combined with Tamoxifen (IMPACT) trial]. The primary goal of the study was to compare the clinical response to the treatments over that period (10). However, we also collected biopsy materials before treatment, after 2 weeks of treatment, and at the time of surgery to allow us to test the hypotheses that changes in Ki-67 after 2 weeks and/or 12 weeks: (i) differed between treatments, (ii) predicted clinical tumor response, and/or (iii) may predict long-term outcome differences between treatments in adjuvant therapy. In addition, we assessed changes in apoptosis, because these could also influence tumor growth. Furthermore, we compared the changes in the ratio of Ki-67/apoptosis (%), known as the growth index or cell turnover index (7, 11, 12), which attempts to approximate the combined contribution of these factors to changes in growth.

**PATIENTS AND METHODS**

**Study Design**

The design of the clinical aspects of this trial are described in detail elsewhere (10). In summary, this was a randomized, double-blind, double-dummy, multicenter trial in which patients with primary breast cancer were randomized 1:1:1 to receive a preoperative daily dose of anastrozole (1 mg) plus tamoxifen placebo, or tamoxifen (20 mg) plus anastrozole placebo, or anastrozole (1 mg) plus tamoxifen (20 mg) for 12 weeks prior to surgery (Fig. 1). Eligible patients were postmenopausal women with previously untreated, core needle biopsy–proven, invasive, estrogen receptor–positive breast cancer. Tumors were operable or locally advanced but potentially operable (after medical downstaging) and without evidence of metastatic spread. Any women receiving hormone replacement therapy stopped this prior to the trial. To be evaluable for the biomarker end points, patients had to have ceased such therapy at least 4 weeks before the start of treatment.

The primary clinical objective of the trial was to compare the differences between the treatments in objective tumor response. Clinical measurements of tumor size (bidimensional by calipers) were made at baseline and prior to surgery at 12 weeks. Objective clinical response was calculated based on WHO criteria. Written informed consent was obtained from all patients prior to study entry, and a research ethics committee at all study sites approved the protocol.

Core-cut biopsies were taken prior to starting therapy and at 2 weeks (nonobligatory). Patients not proceeding to surgery for whatever reason were invited to have a further core biopsy at 12 weeks. Core biopsies and the excision biopsy were fixed in 10% neutral-buffered formalin for 24 to 28 hours prior to processing and embedding at local pathology centers in paraffin wax blocks. These blocks (or in a small proportion of cases unstained sections derived from them) were sent to the central laboratory (Academic Department of Biochemistry, Royal Marsden Hospital, London, United Kingdom).

Estrogen receptor analyses were conducted locally to determine initial eligibility for the study and repeated centrally by immunohistochemical analysis after treatment using the Novocastra 6F11 antibody as previously described (7). Those cases (n = 9) that were found to be negative (< 1% cells staining positive) by central analysis were excluded from the data analysis. Ki-67 was stained using the MIB-1 antibody (DAKO, Glostrup, Denmark), and apoptosis was assessed using the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling technique, both according to previously described methodologies. Ki-67 and apoptosis were scored as the percentage of positively stained cells among 1,000 and 3,000 malignant cells, respectively. In a small proportion of samples, there were < 3,000 cells scorable for apoptosis, and on these occasions total cell numbers of 1,500 or more were accepted, but samples with fewer cells than this were deemed unevaluable for apoptosis. The growth index was calculated as Ki-67/apoptosis (%).

**Statistical Analysis**

The study was powered for clinical response rate comparisons assuming an objective response rate (complete response + partial response) to tamoxifen of 40%. To detect an increase in response rate with anastrozole to 60% with 80% power and a two-sided 5% significance level required 102 patients per treatment arm. For comparative data, 102 patients were also required for the comparison between tamoxifen and the combination arm. To allow for missing data, 110 patients per arm were recruited.

For changes in Ki-67, powering was determined on the basis of previously published estimates of a reduction of 47% after a median of 21 days’ treatment with tamoxifen (13). Data from 50 patients in each of the study arms would enable the detection of a further reduction with anastrozole or the combination to 67%, with 80% power at a 5% level of significance.

Descriptive statistics for Ki-67, apoptosis, and growth index [Ki-67/apoptosis (%)] were expressed as geometric means because of the approximate lognormal distribution of the data. Values at 2 and 12 weeks were also expressed as geometric mean proportions of the baseline and transformed into percentage changes (henceforth termed geometric mean percentage change from baseline, a negative change denoting a reduction). ANOVAs were conducted at a two-sided 5% significance level for within-treatment and between-treatment comparisons....
Logistic regression was conducted to determine whether percentage change in the molecular parameters predicted for clinical response. Primary analyses were conducted based on the per protocol population using parametric statistics, and secondary nonparametric analyses were conducted for confirmation. Only the parametric analyses are presented. Similar to the ATAC trial and the IMPACT clinical analysis, statistical comparisons were made only for comparing anastrozole with tamoxifen and the combination with tamoxifen.

RESULTS

A total of 330 patients were recruited into the IMPACT study (Fig. 1): 113 received anastrozole, 108 received tamoxifen, and 109 received combined therapy. Objective response rates based on caliper measurements were seen in 37.2%, 36.1%, and 39.4% of patients in the anastrozole, tamoxifen, and combination arms, respectively (population of all randomized patients). Biopsies were available for 292 patients: 98 treated with anastrozole, 98 with tamoxifen, and 96 with the combination. Major violations or deviations from the trial protocol occurred for 12, 10, and 11 of these patients, leaving 86, 88, and 85 per protocol patients, respectively. Data are presented only for patients treated per protocol. The demographics of this population are shown in Table 1.

Changes in Ki-67

Ki-67 data were available on all per protocol patients. Figure 2 shows the changes in Ki-67 for individual patients in whom samples were available according to treatment received. In all three treatment groups, the large majority of patients showed a suppression of Ki-67 by 2 weeks that was largely maintained after 12 weeks. After 2 weeks, 4/56 (7%), 8/56 (14%), and 8/45 (18%) showed a numerical increase in Ki-67 in the anastrozole, tamoxifen, and combination groups, respectively. Although Ki-67 increased for some patients between 2 and 12 weeks, overall, the reduction in Ki-67 levels was marginally greater after 12 weeks than after 2 weeks.

Geometric mean percentage change in Ki-67 at 2 and 12 weeks is shown for the three treatment arms in Fig. 3 and Table 2. For each treatment, the reduction in geometric mean Ki-67 levels was statistically significant at both 2 and 12 weeks. The reduction was significantly higher for anastrozole than for tamoxifen at both 2 and 12 weeks (P = 0.004, P = 0.001, respectively). In contrast, the reduction was similar for tamoxifen compared with the combination at 2 or 12 weeks (P = 0.600, P = 0.912).

Tumor response was assessed at 3 months. Overall, Ki-67 change was greater in responders than nonresponders at 2 weeks (geometric mean change = −75.3% versus −61.7%) and 12 weeks (geometric mean change = −73.2% versus −67.3%), although this difference was not significant (2 weeks, P = 0.188; 12 weeks, P = 0.106; Fig. 4). There were no significant differences for change in Ki-67 between responders and nonresponders in the individual treatment groups at either 2 or 12 weeks except after 2 weeks in the tamoxifen arm (geometric mean change = −78.3% versus −44.1% for responders and nonresponders, respectively, P = 0.013).

Changes in Apoptosis

There was a highly significant positive correlation between pretreatment scores for Ki-67 and apoptosis (ρ = 0.57, P < 0.0001). After 2 and 12 weeks’ treatment with anastrozole, apoptosis was reduced significantly by 25.8% and 20.5%, respectively [geometric means; 95% confidence intervals (CI), 12.0-37.4 and 4.8-33.6, respectively; Fig. 5]. In the tamoxifen and combination arms, reductions in apoptosis of 14.8% and 17.3% at 2 weeks did not reach statistical significance. After 12 weeks, there was very little difference from baseline apoptosis levels in these two arms.

Change in Ki-67: Apoptosis

Geometric mean percentage change in growth index [Ki-67/ apoptosis (%)] at 2 and 12 weeks is shown for the three treatment arms in Fig. 6 and Table 3. For each treatment, the reduction in growth index was statistically significant at both 2 and 12 weeks. The reduction was higher for anastrozole than for tamoxifen at both 2 and 12 weeks, but only significantly so after 12 weeks (P = 0.094, P = 0.003, respectively; the nonparametric test was significant for anastrozole versus tamoxifen at 2 weeks, P = 0.036). The reduction was similar for tamoxifen compared with the combination at 2 and 12 weeks (P = 0.96, P = 0.79).

DISCUSSION

The IMPACT trial was designed as the neoadjuvant equivalent of the ATAC trial. IMPACT was initiated before analysis of ATAC with a view to assessing whether the clinical or biological end point data from the neoadjuvant trial would have predicted disease-free survival data from the adjuvant ATAC trial.

After a median of 31 months follow-up, the ATAC trial reported that there was a 22% improvement in recurrence-free survival for anastrozole compared with tamoxifen (8). There was no significant difference between tamoxifen and the combination in recurrence-free survival or of any other trial end points. The pattern of changes in Ki-67 after both 2 and 12 weeks in the IMPACT trial parallels the pattern seen with disease-free survival in the ATAC trial after both 2 and 12 weeks: anastrozole showed a significantly improved suppression of Ki-67 compared with tamoxifen but no significant difference between tamoxifen and the combination. Thus, after exposure of only 159 patients to 2 weeks of treatment, a total of 6 patient-years of exposure, data were obtained that would have predicted the efficacy results of the ATAC trial, which required ~25,000 patient-years of exposure. If confirmed by similar trials, these findings could lead to a substantial change in the approach to development of new adjuvant treatments.

However, disease outcome is only one of the end points of an adjuvant trial, and acceptance of a new treatment such as anastrozole requires data on long-term safety as well as efficacy. The adverse effect data for IMPACT also reflected those seen in ATAC, (10) and biological indices such as lipid and bone metabolite profiles were collected. However, by definition, long-term data cannot be acquired rapidly. Thus, for the moment, short-term studies of the type described here could be viewed as indicative of whether a treatment actually warrants evaluation in the adjuvant setting with the very large resources in patient and
investigator commitment and pharmaceutical and/or public funds that this requires. Indeed, we can speculate that had the data from IMPACT been available prior to the initiation of ATAC, the combination arm in ATAC might not have been initiated. For short-term adjuvant therapies, however, in particular those including chemotherapy, treatment duration would be similar for adjuvant and neoadjuvant treatment, making reservations on long-term safety data much less relevant. If similar results were consistently obtained for predictive biological indices in this latter setting, it is possible that the neoadjuvant approach could begin to replace long-term adjuvant chemotherapy trials for evaluating different drug combinations.

In contrast to the Ki-67 data, the overall clinical response data from IMPACT did not show a similar pattern to ATAC. There was no significant difference between tamoxifen and either anastrozole or the combination, although anastrozole showed a significantly greater response in terms of surgery downstaging than tamoxifen in the 38% of patients who were not considered eligible for breast-conserving surgery at study entry, (10) this group being the target population for preoperative therapy. It was also notable that clinical response and change in Ki-67 were not significantly correlated other than with the tamoxifen arm after 2 weeks of treatment. Interestingly, we have previously found that clinical response and change in Ki-67 were significantly, but not closely, correlated after 2 or 3 weeks of tamoxifen (14, 15). It is difficult to explain why a significant relationship should occur with tamoxifen at this early time point but not the other two treatments. It is notable that there were trends to a greater effect with the other treatments at 2 weeks, and it may be by chance that the boundary of statistical significance was crossed with tamoxifen. Greater reductions of Ki-67 with the aromatase inhibitors vorozole and letrozole compared with tamoxifen after 12 weeks of treatment have also been reported from randomized

**Table 1** Patient demographics for the IMPACT study

<table>
<thead>
<tr>
<th></th>
<th>Anastrozole ($n = 86$)</th>
<th>Tamoxifen ($n = 88$)</th>
<th>Combination ($n = 85$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean (SD)</td>
<td>72.3 (8.9)</td>
<td>71.8 (8.5)</td>
<td>71.6 (8.0)</td>
</tr>
<tr>
<td>Tumor diameter, mean (SD)</td>
<td>Caliper</td>
<td>3.9 (1.2)</td>
<td>4.1 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Ultrasound</td>
<td>2.7 (0.9)</td>
<td>2.7 (1.3)</td>
</tr>
<tr>
<td>Previous HRT, $n$ (%)</td>
<td>Yes</td>
<td>23 (26.7)</td>
<td>21 (23.9)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>58 (67.4)</td>
<td>64 (72.7)</td>
</tr>
<tr>
<td></td>
<td>Not reported</td>
<td>5 (5.8)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Hysterectomy, $n$ (%)</td>
<td>Yes</td>
<td>15 (17.4)</td>
<td>14 (15.9)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>53 (61.6)</td>
<td>61 (69.3)</td>
</tr>
<tr>
<td></td>
<td>Not reported</td>
<td>18 (20.9)</td>
<td>13 (14.8)</td>
</tr>
</tbody>
</table>

**Fig. 2** Individual patient plots for percentage of Ki-67 staining at baseline, 2 weeks, and 12 weeks for the three treatment arms.
neoadjuvant trials (11, 16), but there are no comparable adjuvant treatment data at present with these agents.

It is important to consider why the Ki-67 changes in IMPACT were well aligned with the adjuvant outcome data, but clinical response was not, and that there was no close relationship between clinical response and change in Ki-67. It is possible that the difficulty of reliably and reproducibly measuring tumor size by calipers, particularly in a trial such as this involving many investigators on different sites, explains these discordances. This may also be an explanation for the observation of a greater clinical response for anastrozole than are generally considered to be influential in recurrence-free survival. However, although inhibiting proliferation may be sufficient to delay the growth of a tumor (and be of likely benefit to the patient), this would not be reflected as a clinical response unless it was sufficient to overcome the initial positive growth rate of the tumor. Thus, inhibiting tumor proliferation is expected to be detected by Ki-67 and to be influential in recurrence-free survival but would not necessarily result in tumor regression.

This relationship between lowered Ki-67 and inhibition of tumor growth in the absence of objective tumor regression is demonstrable in xenograft studies because the initial growth rate of the tumor is also measurable (19).

It was also notable that very few patients did not show a reduction in Ki-67 on any of the three treatments. For anastrozole, the number showing a reduction was 52/56 (93%). This biological response rate to estrogen deprivation is much greater than the clinical response rate recorded in this neoadjuvant trial or elsewhere (17). This suggests that on clinical grounds, many more patients benefit from endocrine therapy than are generally considered to. This observation also has implications for mechanistic studies of endocrine resistance that may be better conducted with biological rather than clinical end points.

There were increases in Ki-67 for some patients between 2 weeks and 12 weeks. It is conceivable that this may be an early sign of the development of treatment resistance, but from the present data, it is difficult to distinguish this from analytic imprecision.

Ki-67 is a widely used marker of proliferation. It is a nuclear antigen that is expressed at all points of the cell cycle in cells that are undergoing mitosis (20). Other indices such as S phase and newer markers such as Mcm2 (21) might be better discriminants of the antiproliferative effects of endocrine therapy and deserve assessment in this context.

However, in considering changes in the growth dynamics of tumors, it is important to consider the contribution of cell death as well as proliferation. As expected in the present study, we

![Fig. 3 Percentage change in Ki-67 expression (geometric mean, 95% CI) from baseline during treatment (2 and 12 weeks).](image_url)

### Table 2  Geometric mean percentage change in Ki-67 expression from baseline at 2 and 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Anastrozole (n = 86)</th>
<th>Tamoxifen (n = 88)</th>
<th>Combination (n = 85)</th>
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</thead>
<tbody>
<tr>
<td>Baseline to 2 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>56</td>
<td>56</td>
<td>46</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>−76.0</td>
<td>−59.5</td>
<td>−63.9</td>
</tr>
<tr>
<td>95% CI</td>
<td>−81.9 to −68.2</td>
<td>−68.5 to −47.9</td>
<td>−73.3 to −51.4</td>
</tr>
<tr>
<td>Baseline to 12 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>80</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>−81.6</td>
<td>−61.9</td>
<td>−61.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>−86.3 to −74.8</td>
<td>−69.6 to −52.2</td>
<td>−69.3 to −50.8</td>
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</table>
observed the previously reported significant correlation of apoptosis with proliferation before treatment (22). We, and others, have described the substantial increases in apoptosis that accompany decreased proliferation in estrogen-dependent cell lines (23) and xenografts (24, 25) with antiestrogen treatment or estrogen deprivation. Thus, estrogen has been considered an important cell survival factor for hormone receptor–positive breast carcinomas. The data from the IMPACT trial are in major contrast with the results from model systems. Estrogen deprivation with anastrozole was associated with a significant decrease in apoptosis after 2 weeks that was maintained after 12 weeks. Similar but nonsignificant trends to a decrease were shown by tamoxifen and the combination after 2 weeks. These were absent after 12 weeks yet no increase was seen. The data confirm those previously observed by our group in a smaller neoadjuvant trial of vorozole and tamoxifen (11). We have described significant early increases in apoptosis during cytotoxic therapy (26, 27) where, in general, clinical responses occur much more quickly than with endocrine therapy. The lack of increase in apoptosis with endocrine therapy is consistent with this. It is possible that the capacity of breast cancer cells to pass into apoptosis is retarded by the profound antiproliferative effects of antiestrogenic therapy: it has been observed that c-myc is a determinant of both proliferation and apoptosis (28), and its expression is enhanced by estrogen and suppressed by antiestrogens. However, as indicated above, it is clear that decreased proliferation is not a constraint on the induction of apoptosis by endocrine treatment in experimental systems. These data therefore indicate that, in contrast to its role in these model systems, estrogen does not seem to be an important cell survival agent for human breast cancer cells.

The observation that apoptosis was affected by the three treatments to differing degrees may also be important as a contributory influence on the relationship of proliferation with recurrence-free survival and clinical response. We have previously attempted a first approximation of this by calculating the Ki-67/apoptosis ratio [the growth index (11) / cell turnover index (12)], although it cannot be expected to closely reflect the true dynamics of these processes in tumor growth dynamics. In this study, the differences in the growth index between the treatments were largely similar to those with Ki-67 alone, although, as expected, the differences were marginally smaller and at 2 weeks, the difference between anastrozole and tamoxifen was not statistically significant. Comparison of drugs (or combinations of drugs) that vary more substantially than these endocrine agents in the relative importance of changes in proliferation and apoptosis to their mechanism of antitumor efficacy is likely to require greater reliance on, and sophistication in, the combined analysis of both determinants of growth.

In summary, in the IMPACT trial, changes in Ki-67 but not clinical response were markedly similar to recurrence-free survival in the ATAC adjuvant trial. If confirmed, these data could radically alter the process of drug development in breast cancer. More patients are biologically responsive to hormonal agents than are recorded as clinically responsive. The data indicate that estrogen is not an important survival factor for breast cancer cells.

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Fig. 6 Percentage change in growth index [Ki-67/apoptosis (%); (geometric mean, 95% CI)] at 2 and 12 weeks for the three treatment arms.

Table 3  Geometric mean percentage change in growth index from baseline at 2 and 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Anastrozole (n = 86)</th>
<th>Tamoxifen (n = 88)</th>
<th>Combination (n = 85)</th>
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<tbody>
<tr>
<td>Baseline to 2 weeks</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n</td>
<td>46</td>
<td>49</td>
<td>43</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>−66.1</td>
<td>−50.6</td>
<td>−53.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>−75.9 to −52.3</td>
<td>−63.5 to −33.1</td>
<td>−66.4 to −34.5</td>
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<td>Baseline to 12 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>69</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>−77.0</td>
<td>−61.0</td>
<td>−64.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>−84.5 to −66.0</td>
<td>−70.8 to −47.9</td>
<td>−73.9 to −52.7</td>
</tr>
</tbody>
</table>
Dr. James Ingle: With all the experience you have had in doing this research, what lessons have you learned for applying to the next generation of neoadjuvant studies? Different time points? Multiple earlier time points? Smaller, more intensive studies? What is your conclusion?

Dr. Johnston: We have just started a study on gefitinib and anastrozole versus anastrozole alone to look at the combination of growth inhibitor and endocrine therapy versus endocrine alone. We are still going to biopsy at 2 weeks because it is clinically acceptable to do so. I think biopsying at an earlier time point would require a lot of work to work out whether it should be 1 day or 3 days or so forth. Chemotherapy studies have shown massive induction of apoptosis with biopsying 24 hours post-treatment. We never thought that we would see that quick a change in apoptosis in the endocrine scenario to do an early biopsy time point. I think there would be a whole host of sequential time point studies you would have to do to get a better handle on the timing, and I don’t think it is practical or feasible to do them. In the gefitinib plus anastrozole study we are going to apply some gene expression profiling to see if we can learn a bit more about patterns of changes. We are saving that material for subsequent analysis, but the study will be powered with Ki-67 as the end point.

Acknowledgments

We thank all of the investigators who participated in the biological markers component of the IMPACT trial, and the contribution of Steve Crosier who trimmed the specimens.

References

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