Clinical and genomic analysis of a randomised phase II study evaluating anastrozole and fulvestrant in postmenopausal patients treated for large operable or locally advanced hormone-receptor-positive breast cancer

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Background: The aim of this study was to assess the efficacy of neoadjuvant anastrozole and fulvestrant treatment of large operable or locally advanced hormone-receptor-positive breast cancer not eligible for initial breast-conserving surgery, and to identify genomic changes occurring after treatment.

Methods: One hundred and twenty post-menopausal patients were randomised to receive 1 mg anastrozole (61 patients) or 500 mg fulvestrant (59 patients) for 6 months. Genomic DNA copy number profiles were generated for a subgroup of 20 patients before and after treatment.

Results: A total of 108 patients were evaluable for efficacy and 118 for toxicity. The objective response rate determined by clinical palpation was 58.9% (95% CI = 45.0–71.9) in the anastrozole arm and 53.8% (95% CI = 39.5–67.8) in the fulvestrant arm. The breast-conserving surgery rate was 58.9% (95% CI = 45.0–71.9) in the anastrozole arm and 50.0% (95% CI = 35.8–64.2) in...
Endocrine therapy is widely used to treat hormone-receptor-positive breast cancer. Neoadjuvant endocrine therapy, where drug treatment is given before surgery, is commonly given to elderly patients with large operable or locally advanced hormone-receptor-positive breast cancer because it has a favourable toxicity profile and permits breast-conserving surgery in 40–50% of patients who would otherwise have required mastectomy (Semiglazov et al, 2007; Mathew et al, 2009; Chia et al, 2010; Debled et al, 2014). In this setting, treatment with aromatase inhibitors is considered as a standard in post-menopausal patients (Eiermann et al, 2001; Smith et al, 2005). Fulvestrant is an oestrogen receptor downregulator with a different mechanism of action. Several trials have assessed fulvestrant in women with metastatic breast cancer (Chia et al, 2010; Robertson et al, 2012; Robertson et al, 2014), but in the neoadjuvant setting, there are only a few small studies comparing 500 mg fulvestrant to an aromatase inhibitor (Massarweh et al, 2011).

Resistance to hormonal therapy can arise through multiple mechanisms (reviewed by Ali and Coombes, 2002). Mutation of the oestrogen receptor can confer estradiol-independent activation of transcription (Weis et al, 1996) and has been identified in metastatic breast cancers treated with hormonal therapy (reviewed by Segal and Dowsett, 2014). Activation of growth factor signalling pathways, leading to phosphorylation of Erz on serines 118 and 167 in the ligand-independent transactivation domain, activates Erz in the absence of estradiol (reviewed by Ali and Coombes, 2002). Increased expression of coactivators, in particular amplification of the NCOA3 and CCND1 genes, and decreased expression of corepressors, are additional mechanisms of resistance to hormonal therapy (reviewed by Ali and Coombes, 2002). Finally, conversion to a progenitor or stem-like state in which ESR1 is not required for growth, for example, through expression of ELF5 (Kalugya et al, 2012) or SOX2 (Piva et al, 2014), confers oestrogen independence. Given the wide range of mechanisms, it is possible that resistance to fulvestrant and aromatase inhibitors could arise through different mechanisms. An important difference between the drugs is that aromatase inhibitors deprive the cell of oestrogen but do not induce degradation of the receptor, whereas fulvestrant induces Erz degradation. An increase in Erz level, for example, by amplification of the gene, might offset a reduced availability of ligand, leading to resistance to aromatase inhibitors. Resistance to fulvestrant seems unlikely to arise through this mechanism. Instead, mutations interfering with Erz degradation or activating the pathway downstream of the receptor might be favoured with fulvestrant. Endocrine therapy typically causes tumours to regress over a period of several months, during which time clonal selection may gradually occur. Unlike chemotherapy, hormonal therapy is not clastogenic or mutagenic, so genetic changes arising during treatment are unlikely to be secondary events provoked by iatrogenic DNA damage. Hence, hormonal therapy given in a neoadjuvant trial provides an ideal opportunity to study resistance to endocrine therapy arising in vivo.

Given the lack of clinical data on fulvestrant in patients with large operable or locally advanced hormone-receptor-positive breast cancer, and the potential to identify differences in the mechanism of resistance using the neoadjuvant model, we decided to perform a multicentre randomised phase II clinical trial of anastrozole and fulvestrant. This manuscript reports both the clinical findings of this trial and the genomic changes arising during endocrine therapy in the subgroup of patients for whom the tumour cell content was at least 50% in the samples taken before and after treatment.

METHODS

Protocol approval. The study was approved by the South-West France and Overseas Departments, Committee for Protection of Research Subjects (Comité de Protection des Personnes, Sud-Ouest et Outre-Mer III, 2007/57) and conducted according to the Declaration of Helsinki, EU clinical trials directives (2001/20/CE) and Good Clinical Practice guidelines. The clinicaltrials.gov registry number is NCT0087858. Patients gave signed informed consent to participate in the study and to allow the use of their tumour samples for research including genomic analysis. Samples from the trial were stored in the Bergonie Institute Tumour Bank in compliance with French laws on tumour banks (authorisation no. AC-2008–812). The translational study was approved by the French authorities (authorisation no. M70674–4201). The funders had no role in the study design, data analysis or manuscript drafting.

Study design. This was a non-comparative multicentre randomised phase II study in which patients from three French centres were randomly assigned in a 1:1 ratio to receive either 1 mg per day anastrozole administered orally for 6 months (control arm) or 500 mg of fulvestrant administered as an intramuscular infusion every 4 weeks for 6 months with a loading dose in the first month (experimental arm). Anastrozole (ARIMIDEX, Astra Zeneca, London, UK) was provided as a 1 mg film-coated tablets. Fulvestrant (FASLODEX, Astra Zeneca) was provided as a 250 mg/5 ml solution. Before starting treatment, three trucut biopsies were taken for research purposes; two were frozen and one was fixed in formalin. Before treatment, the decision for breast-conserving surgery or total mastectomy at the end of the trial was made according to each centre’s policy.

Eligibility. Patients were eligible if they met the following criteria: post-menopausal woman; histologically confirmed non-metastatic T2-T3-T4 invasive breast cancer; grade I or II; oestrogen and/or progesterone-receptor-positive; clinically measurable disease at
baseline; not eligible for breast-conserving surgery at baseline; HER2 positive or negative. Women with grade III tumours were eligible only if they were aged 65 years or over. Patients with inflammatory breast cancer, N2 disease, a history of cancer within the past 5 years, or a contra-indication to hormonal therapy were not eligible.

End points. The primary end point was the objective response rate (ORR) at 6 months determined by clinical palpation, defined as the rate of complete and partial responses (CR + PR) according to RECIST Criteria (Version 1.0). The same doctor was responsible for clinical assessment initially and at 6 months. Secondary end points included: ORR determined by ultrasound and mammography at 6 months based on RECIST Criteria; rate of breast-conserving surgery at 6 months; pathological response using the Sataloff classification (Sataloff et al, 1995); and safety profile examination using common terminology criteria for adverse events (CTCAE, version 3.0, DCTD, NCI, NIH; http://ctep.cancer.gov). Exploratory analyses were carried out for the preoperative endocrine prognostic index (PEPI score; Ellis et al, 2008) and changes in the fraction of tumour nuclei expressing Ki-67. Follow-up was planned for 5 years.

Statistical considerations. The sample size for this non-comparative phase II trial was based on the primary end point, ORR. According to the Fleming one-step method, for a null hypothesis of 40% ORR and an alternative hypothesis of 60% ORR, 52 assessable patients needed to be treated in each treatment arm (104 in total), with a one-sided alpha of 5% and a power of 90%. The experimental arm was deemed interesting for further research if at least 27 clinical responses out of 52 treated eligible patients were observed. This decision rule corresponded to rejecting the null hypothesis. After adjusting the sample size by 15% to allow for non-evaluable patients, 120 patients were required. The randomisation was stratified by centre. Two analysis populations were considered: (i) the safety population, defined as all patients who received at least one dose of the allocated study medication (a dose of anastrozole or an injection of fulvestrant), and (ii) the efficacy population (primary endpoint), defined as all eligible (no major eligibility deviations) and evaluable patients who started the allocated treatment and who were evaluated at 6 months. Patients discontinuing treatment due to progression or death from any cause were considered as treatment failures. Patients who missed the evaluation at 6 months due to treatment discontinuation for toxicity, refusal to participate or lost to follow-up were excluded from the efficacy population.

Pathological assessment. Ki-67 was scored centrally by a pathologist (GMG) in a blinded manner according to the recommendations of Dowsett et al (2011) by counting at least 1000 tumour nuclei per sample after staining with mib1 antibody. Ki-67 was scored before treatment on the core biopsy and after treatment on the surgical resection specimen. Tumours were defined as hormone-receptor positive or negative by local pathologists with a 10% cutoff for scoring as positive. To evaluate the pathological response, the Sataloff classification was used (Sataloff et al, 1995). The PEPI score was calculated as described by Ellis et al (2008).

Genomic study. Only samples with at least 50% tumour cells after treatment were selected for the genomic substudy (the tumour cell content was frequently below this cutoff after treatment). DNA was purified from frozen samples and analysed on a GAIIx DNA sequencer (Illumina, San Diego, CA, USA). Low-depth whole-genome sequencing data were converted to DNA copy number plots with the CNAnorm package (Gusnanto et al, 2012) in R statistical software (R Core Team, 2013) as described by Wood et al, 2010). Base quality, mapping quality, read depth and tumour cell content are summarised in Supplementary Table 6, and further details of the bioinformatics approaches are given in the Supplementary Methods file.

RESULTS

Baseline characteristics. A total of 120 patients were enrolled in three French comprehensive cancer centres between March 2008 and May 2012 and randomly assigned to anastrozole (61 patients, 51%) or fulvestrant (59 patients, 49%) arm. Two patients withdrew their consent. Ten patients were not assessable for the efficacy analysis: four in the standard arm and six in the experimental arm (see Figure 1 for details). A total of 118 patients and 108 patients were assessable for the safety and efficacy analyses, respectively (Figure 1). Patient and tumour characteristics are summarised in Table 1. Baseline characteristics were balanced between the two treatment arms, but there were more T2 N0 Grade 1 tumours in the anastrozole arm and more lobular carcinomas in the fulvestrant arm.

Clinical response and surgery. Of the 56 patients eligible and evaluable for efficacy in the anastrozole arm, 7 achieved a CR and 26 a PR, giving an ORR of 58.9% (95% CI = 45.0–71.9). Of the 52 patients eligible and evaluable for efficacy in the fulvestrant arm, 6 achieved a CR and 22 achieved a PR, giving an ORR of 53.8% (95% CI = 39.5–67.8; Table 2). Analysis including all eligible patients gave very similar results (Supplementary Table 1). Eight tumours progressed under therapy; six in the fulvestrant arm and two in the anastrozole arm. Five patients were HER2-positive at inclusion, of whom one progressed and four had stable disease. In the anastrozole arm, 33 patients underwent breast-conserving surgery (58.9%, 95% CI = 45.0–71.9). In the fulvestrant arm, 26 patients underwent breast-conserving surgery (50.0%, 95% CI = 35.8–64.2).

![Figure 1. Flow chart of patient enrolment and analysis populations.](image)
Two patients in the anastrozole arm and six in the fulvestrant arm did not undergo surgery (see the Supplementary Material for details); both patients in the anastrozole arm and three in the fulvestrant arm had a biopsy at 6 months.

**Pathological response, ER, PR, Ki67 and PEPI score.** Pathological response data at 6 months for the primary tumour were available for 96 patients. The percentage of ER-positive tumour nuclei in each arm before and after treatment was shown in Figure 2C and D, with summary statistics in Supplementary Table 2 and Allred scores in Supplementary Table 3. The figure shows that there was a large decrease in PR staining in both arms (fulvestrant arm, P = 0.046); no other correlations were observed between Ki-67 and receptor expression (Supplementary Figure 1). There was no significant difference between the reduction in Ki-67 in the two arms, or between the reduction in Ki-67 in clinical responders and non-responders (Figure 2E and F, Supplementary Figure 1; Wilcoxon test not significant).

The PEPI score (PEPI for relapse-free survival, Ellis et al, 2008) after 6 months of therapy was calculated for 76 patients. The proportion of patients in each of the three PEPI risk subgroups was broadly similar in both treatment arms (Table 2).

**Toxicity.** Both treatments were well tolerated. Grade 1/II adverse events occurring in ≥5% of patients are presented in Supplementary Table 1.

### Table 1. Patient and tumour characteristics

|                          | Anastrozole arm | Fulvestrant arm |
|--------------------------|-----------------|-----------------|
| **Age (years)**          |                 |                 |
| Median (range)           | 69 (54–86)      | 71 (51–91)      |
| **ECOG PS**              |                 |                 |
| 0                        | 53 (86.9)       | 49 (83.1)       |
| 1                        | 8 (13.1)        | 10 (16.9)       |
| **Tumour size (mm)**     |                 |                 |
| Median (range)           | 45 (25–90)      | 50 (30–110)     |
| **T classification**     |                 |                 |
| T2                       | 43 (70.5)       | 32 (54.2)       |
| T3                       | 9 (14.8)        | 13 (22.0)       |
| T4                       | 2 (3.3)         | 8 (13.6)        |
| T4a                      | 0 (0)           | 1 (1.7)         |
| T4b                      | 6 (9.8)         | 4 (6.8)         |
| T4c                      | 1 (1.6)         | 1 (1.7)         |
| **N classification**     |                 |                 |
| N0                       | 39 (63.9)       | 29 (49.2)       |
| N1                       | 22 (36.1)       | 29 (49.2)       |
| NX                       | 0 (0)           | 1 (1.6)         |
| **Histology**            |                 |                 |
| Ductal invasive carcinoma| 45 (73.8)       | 39 (66.1)       |
| Lobular invasive carcinoma| 14 (23.0)     | 19 (32.2)       |
| Mucinous adenocarcinoma   | 2 (3.3)         | 1 (1.7)         |
| **Histologic grades**    |                 |                 |
| I                        | 11 (18.0)       | 8 (13.6)        |
| II                       | 45 (73.8)       | 44 (74.6)       |
| III                      | 3 (4.9)         | 5 (8.5)         |
| Not available            | 2 (3.3)         | 2 (3.4)         |
| **Receptor status**      |                 |                 |
| ER+                      | 61 (100)        | 59 (100)        |
| PR+                      | 54 (88.5)       | 55 (93.2)       |
| PR –                     | 6 (9.8)         | 3 (5.1)         |
| PR not available         | 1 (1.6)         | 1 (1.7)         |
| **HER2**                 |                 |                 |
| Positive                 | 4 (6.6)         | 3 (5.1)         |
| Negative                 | 55 (90.2)       | 55 (93.2)       |
| Missing                  | 2 (3.3)         | 1 (1.7)         |

**Abbreviations:** ECOG PS = performance status; ER = oestrogen receptor; PR = progesterone receptor. HER2 positive if immunohistochemistry 3+ or if FISH amplified.

### Table 2. Clinical and pathological response at 6 months

|                          | Anastrozole arm | Fulvestrant arm |
|--------------------------|-----------------|-----------------|
| Clinical response        |                 |                 |
| N                        | 56              | 52              |
| CR                       | 7 (12.5)        | 6 (11.5)        |
| PR                       | 26 (46.4)       | 22 (42.3)       |
| SD                       | 21 (37.5)       | 18 (34.6)       |
| PD                       | 2 (3.6)         | 6 (11.5)        |
| ORR (CR + PR)            | 33 (58.9)       | 28 (53.8)       |
| Surgery                  | N = 56          | N = 52          |
| Breast-conserving surgery| 33 (58.9)       | 26 (50)         |
| Mastectomy               | 21 (37.5)       | 20 (38.5)       |
| No surgery               | 2 (3.6)         | 6 (11.5)        |
| **Pathological response**|                 |                 |
| Sataloff classification T|                 |                 |
| TB (Pathological response ≥50%) | 24 (42.9) | 13 (25.0) |
| TC                       | 23 (41.1)       | 24 (46.2)       |
| TD                       | 3 (5.4)         | 5 (9.6)         |
| Missing                  | 6 (10.7)        | 10 (19.3)       |
| Sataloff classification N|                 |                 |
| NA                       | 3 (5.4)         | 6 (11.5)        |
| NB                       | 16 (28.6)       | 8 (15.4)        |
| NC                       | 20 (35.7)       | 12 (23.1)       |
| ND                       | 7 (12.5)        | 15 (28.8)       |
| Missing                  | 10 (17.9)       | 11 (21.2)       |
| **PEPI score**           |                 |                 |
| ≤1                       | 41              | N = 41          |
| >1                       | 8 (19.5)        | 4 (11.4)        |
| >3                       | 17 (41.5)       | 19 (54.3)       |
| Missing                  | 16 (39.0)       | 12 (34.3)       |

**Abbreviations:** CR = complete response; NA = evidence of therapeutic effect, no residual disease; NB = no evidence of therapeutic effect, no axillary metastasis; NC = evidence of therapeutic effect, axillary metastasis present; ND = no evidence of therapeutic effect, axillary metastasis present; ORR = objective response rate; PD = progressive disease; PEPI = preoperative endocrine prognostic index; PR = partial response, SD = stable disease; TA = complete or nearly complete response; TB = therapeutic response over 50%; TC = therapeutic response under 50%; TD = no evidence of therapeutic effect.
was the availability of material, in particular, the presence of at least 50% tumour cells in the post-treatment sample. In five cases, two independent biopsies were tested before treatment and in four cases two different parts of the surgical specimen were tested after treatment. In the remaining cases, single samples were tested before and after treatment. Next-generation sequencing was performed at low depth and reads were counted in moving windows to estimate DNA copy number. Hierarchical clustering was used to detect broad patterns in the data (Figure 3; the Treeview data files are provided as Supplementary Data to permit examination of the heatmap at high resolution). As expected for ER+ breast cancer, many tumours had minimally rearranged ‘simplex’ copy number profiles with gains of 1q/16p and loss of 16q. Apart from H06 and H13, all samples from the same tumour clustered together. Overall, the clustering shows that the differences between tumours were in general much greater than the differences before and after treatment of the same tumour.

### Table 3. Ki-67 before and after treatment

|                      | Before treatment (%) | After treatment (%) |
|----------------------|----------------------|---------------------|
| **Both arms (n = 81)** |                      |                     |
| Mean                 | 16.9                 | 3.2                 |
| 95% CI               | 14.3–20.1            | 2.2–4.7             |
| **Anastrozole arm (n = 43)** |                 |                     |
| Mean                 | 16.7                 | 3.2                 |
| 95% CI               | 13.3–21.0            | 1.9–5.5             |
| **Fulvestrant arm (n = 38)** |             |                     |
| Mean                 | 17.1                 | 3.2                 |
| 95% CI               | 13.1–22.5            | 1.8–5.7             |

Abbreviation: CI = confidence interval. Geometric mean and CIs based on logged data because Ki-67 is log-normal.

**Focal differences in tumour profiles before and after treatment.**

Eight tumours contained classic amplicons previously reported in ER+ breast tumours (Supplementary Figure 2). Most amplicons were present before and after treatment but three tumours (H09, H14 and H15) showed focal changes in the copy number profile after treatment. Figure 4B shows the copy number profiles for tumour H09. Potential driver genes in six amplicons are highlighted with arrows. The ratio of copy number before and after treatment for these genes is shown in Figure 4A (the blue dots are for H09, the other tumours are given black dots). The ratios in Figure 4A confirm the impression from the profiles in Figure 4B that new amplicons containing ESR1, ATG5 and MSH2 appeared after treatment. FISH was used to confirm the presence of the ESR1 amplicon. The gene was highly amplified after treatment (Figure 4D, green dots), but, unexpectedly, it was also amplified before treatment (Figure 4C). The green dots are more diffuse before treatment because an organic fixative was used instead of formaldehyde, which precludes precise quantification of the difference. There was no increase in ER staining by IHC after treatment in H09, perhaps because the signal was already so strong (Supplementary Table 5). Progesterone receptor was undetectable by IHC in H09 both before and after treatment (Supplementary Table 5), as commonly occurs in luminal B tumours. Tumour H14 showed focal changes after treatment, including a copy number increase after treatment on chr14q in a region containing the FOXA1 gene (Supplementary Figure 3). Both H09 and H14 were in the anastrozole arm. In summary, copy number profiles showed the presence of focal differences in three tumours after treatment, including two showing changes affecting genes implicated in oestrogenic signalling (ESR1 and FOXA1).

### Large-scale differences in tumour profiles before and after treatment.

We corrected for differences in normal tissue...
contamination then calculated a Z score for the presence of large-scale differences between samples, as described in the Supplementary Methods file and Supplementary Figure 4. Seven pairs of tumours stood out as being significantly different (H08, H09, H10, H13, H14, H15 and H19; Supplementary Table 6). Intriguingly, the commonest change after treatment was not the appearance of new changes but the disappearance of old ones. Specifically, in four tumours the profile became simpler through the disappearance of gains and losses of whole chromosomes or chromosome arms. We can exclude dilution of the signal by normal tissue because the 1q gain was still clearly visible in all the affected tumours. Figure 5A shows tumour H13 in which a gain of chr7 and losses of chr4, 6, 11, 12, 17 and 18 disappeared, leaving only the changes on chr1 and 16. Figure 5B shows tumour H08 in which changes on chr2, 7 and 15 became more normal; the overall profile shows many other rearrangements that persisted. Figure 5C shows tumour H10 in which changes on chr3, 13 and 18 disappeared, loss of chr10 and 15 appeared, whereas changes on chr1, 7, 11, 16 and 22 remained. Figure 5D shows tumour H19 in which changes on chr3 and 10 disappeared, 20q was gained and changes on many other chromosomes remained. The differences after treatment of tumour H06 did not reach significance in our Z test (Supplementary Table 6), probably because of the low tumour cell content after treatment, but if chr1 is used as the reference chromosome in the linear model, chr3, 7, 8, 10, 13 and 18 appear to have reverted to a more normal profile (Supplementary Figure 2). Three of the tumours that became simpler showed stable disease and two a PR; three were in the fulvestrant arm and two were in the anastrozole arm (Supplementary Table 6). In summary, genomic profiles showed differences after treatment in one-third of cases, and the commonest change was a simplification of the profiles.

**DISCUSSION**

The main conclusion from this study is that anastrozole and fulvestrant are effective neoadjuvant treatments for post-menopausal patients with large operable or locally advanced ER+ breast cancers. Both drugs made breast-conserving surgery a viable option for some women who were not eligible for it initially. The main conclusion from the genomic study is that breast tumours undergoing neoadjuvant endocrine therapy show clonal variation that could be explained by clonal selection.

The primary clinical end point, the ORR, was 58.9% in the anastrozole arm and 53.8% in the fulvestrant arm. These figures are consistent with rates around 50% observed in previous studies (Eiermann et al, 2001; Mathew et al, 2009; Ellis et al, 2011). The percentage of women receiving breast-conserving surgery (58.9% for anastrozole and 50% for fulvestrant) was also similar to that previously reported after letrozole, anastrozole or tamoxifen (40 and 50%, Eiermann et al, 2001; Smith et al, 2005). In conclusion, our results are at least as good as those with 3–4 months of aromatase inhibitors and therefore are consistent with longer therapy (e.g., 6 months) being better than shorter.

We chose to compare anastrozole with fulvestrant because the literature does not provide clear guidance on their use in the neoadjuvant setting (Howell et al, 2004; Smith et al, 2005; Massarweh et al, 2011; Kuter et al, 2012; Ciruelos et al, 2014). Comparative studies have demonstrated that both treatments are effective in the metastatic setting (Mauriac et al, 2003; Robertson et al, 2012). Aside from the question of efficacy, parenteral administration of fulvestrant has potential clinical advantages in patients with swallowing problems or neurological problems, such as Alzheimer’s disease. Our decision to treat for 6 months was based on our previous study in elderly or frail women (Mauriac et al, 2002). It is longer than was initially recommended, but other groups have now reported favourable outcomes after neoadjuvant endocrine treatment for up to 7.5 months (Dixon et al, 2009; Carpenter et al, 2014).

The trend towards prolonging adjuvant hormonal therapy out to 10 years means there is considerable interest in using neoadjuvant therapy to provide early evidence of drug efficacy. Reassessment of drug efficacy after 6 months of neoadjuvant hormonal therapy provides reassurance that a therapeutic opportunity is not being lost and could potentially save many
lives. In our study, more tumours had a pathological response over 50% in the anastrozole arm (43%) than in the fulvestrant arm (25%) but the size of the study and the slight imbalance between the arms in tumour size, node status and grade at enrolment mean differences of this size could easily arise by chance. Moreover, the Sataloff grading system was not originally designed to assess pathological response to neoadjuvant endocrine therapy and may not be a good surrogate marker for long-term survival (Dowsett et al, 2006; Chia et al, 2010). In contrast, changes in Ki67 expression have been widely studied to evaluate the response to neoadjuvant endocrine therapy (Dowsett et al, 2005; Dowsett et al, 2007; Dowsett et al, 2009; De Censi et al, 2011; Jonat and Arnold, 2011). Most tumours in both arms of our study showed a fall in Ki-67 after therapy (Figure 2), and the magnitude of the changes was similar to that associated with favourable outcomes in previous studies. The similarity of the PEPI scores in the two arms likewise suggests that patients in the two treatment arms may have similar long-term outcomes (Dowsett et al, 2005; Ellis et al, 2008). However, this requires confirmation, especially in the light of recent data from the FIRST trial, indicating a PFS and OS advantage for fulvestrant in the metastatic setting (Robertson et al, 2014). Currently, the risk of relapse based on Ki-67 expression or the PEPI score strongly influences the decision whether to start adjuvant chemotherapy, but they will become less important if the ASTER study provides clear-cut evidence that patients over 70 years with high-grade tumours benefit from the addition of chemotherapy to hormonal therapy.

The most intriguing result from the genomic study was the simplification of copy number profiles after treatment. It is possible that these changes were simply a consequence of sampling from different sites within a clonally heterogeneous tumour. To rule this out would require high-depth analysis of samples taken from multiple sites within the surgical specimen. The alternative, and much more interesting, possibility is that the differences reflect clonal selection by the treatment. As it is not possible for a tumour to correct large-scale rearrangements, the most plausible explanation under the clonal-selection hypothesis is that rearranged clones were eliminated by the treatment, leading to expansion of an ancestral clone with a related, but simpler, profile. Residual tumour after neoadjuvant hormonal therapy has previously been noted to show histological evidence of increased differentiation (Samarthai et al, 2012). Transformation is accompanied by subtle changes in the spectrum of target genes regulated by ERz, leading to a shift from transcription of tumour-suppressive, differentiation-linked genes towards the expression of oncogenic genes (Ross-Innes et al, 2012). A possible interpretation of our results is that cells cope with a reduction in ER-dependent survival signals by reducing the transcriptional stress associated with chromosomal imbalances. Clones emerging from such a selection would be more normal: better able to enter G0, better able to differentiate and more responsive to oestrogen. This builds on so-called ‘intrinsic tumour suppression’, a concept that has been extensively characterised in model systems, in particular in studies on the MYC oncogene (Lowe et al, 2004). This model is also compatible with the increase in copy number of ESR1 in H09, which could be viewed as an adaptive response to offset the reduced availability of oestrogen in anastrozole-treated cells. That said, the clinical data point rather to a tumour that is indifferent to oestrogen: there was no change in tumour size, grade or Ki-67, and PR started at zero and stayed there. The numbers are too small to meaningfully correlate the genomic changes we observed with response to therapy, but they provide no immediate support for the idea that genomic simplification of heterogeneous tumours is a major resistance mechanism. In contrast with the variability of chromosomal arm copy numbers, the classic amplicons known to harbour driver oncogenes in breast cancer were almost invariably present both before and after treatment, consistent with the widely accepted view that breast tumours are addicted to the oncogenes on these

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**Figure 4. Genomic analysis before and after treatment of tumour H09.**

(A) Plots showing the copy number ratio before vs after treatment of the indicated genes within amplified regions (H09, blue dot; other tumours, black dots). The copy number of ESR1, ATG5 and MSH2 increases after treatment, whereas that of PPM1D, PAK1 and NCOA3 is unchanged. The grey horizontal lines show the 0.999th quantiles of the ratios. (B) Genomic profiles showing the genes in A marked with arrows. (C) and (D) FISH for ESR1 before treatment (C) and after treatment (D). The red probe detects the centromere of chromosome 6, the green probe detects the ESR1 gene.
amplicons. Amplification of ESR1 has a chequered history (Holst et al, 2007). Given the importance of oestrogen in the biology of breast cancer cells, ESR1 is an obvious candidate for amplification, but decades of research point instead to lineage choice as the main factor leading to ERa expression in breast cancer. Our FISH results indicate that the appearance of the ESR1 amplicon in the copy number profile after treatment reflects clonal variation rather than de novo amplification. This clonal variation could be due to clonal selection by the treatment or sampling from different regions within a heterogeneous tumour (a ‘needle placement artefact’), but whatever the mechanism, we can exclude ESR1 amplification as the initiating oncogenic event responsible for the ERa positivity of this tumour.

In conclusion, the good response rates and low toxicity observed in this study show that both anastrozole and fulvestrant are effective and well-tolerated neoadjuvant hormonal treatments for post-menopausal women with large operable or locally advanced hormone-receptor-positive breast cancer. The results of the genomic study point to the re-emergence of tumour clones with less rearranged genomes as a potential new mechanism of resistance to endocrine therapy, but, given the potential for sampling effects, larger studies testing multiple biopsies from each tumour will be required to clarify whether this a reproducible, clinically important phenomenon.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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