Breast Cancer Res Treat (2012) 133:237–246
DOI 10.1007/s10549-011-1947-7

CLINICAL TRIAL

Dose-dependent change in biomarkers during neoadjuvant endocrine therapy with fulvestrant: results from NEWEST, a randomized Phase II study

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Received: 23 December 2011 / Accepted: 26 December 2011 / Published online: 28 January 2012

Abstract NEWEST (Neoadjuvant Endocrine Therapy for Women with Estrogen-Sensitive Tumors) is the first study to compare biological and clinical activity of fulvestrant 500 versus 250 mg in the neoadjuvant breast cancer setting. We hypothesized that fulvestrant 500 mg may be superior to 250 mg in blocking estrogen receptor (ER) signaling and growth. A multicenter, randomized, open-label, Phase II study was performed to compare fulvestrant 500 mg (500 mg/month plus 500 mg on day 14 of month 1) versus fulvestrant 250 mg/month for 16 weeks prior to surgery in postmenopausal women with ER+ locally advanced breast cancer. Core biopsies at baseline, week 4, and surgery were assessed for biomarker changes. Primary endpoint: change in Ki67 labeling index (LI) from baseline to week 4 determined by automated computer imaging system (ACIS). Secondary endpoints: ER protein expression and function; progesterone receptor (PgR) expression; tumor response; tolerability. ER and PgR were examined retrospectively using the *H* score method. A total of 211 patients were randomized (fulvestrant 500 mg: $n = 109$; 250 mg: $n = 102$). At week 4, fulvestrant 500 mg resulted in greater reduction of Ki67 LI and ER expression versus 250 mg (−78.8 vs. −47.4% [$p < 0.0001$] and −25.0 vs. −13.5% [$p = 0.0002$], respectively [ACIS]); PgR suppression was not significantly different (−22.7 vs. −17.6; $p = 0.5677$). However, *H* score detected even greater suppression of ER (−50.3 vs. −13.7%; $p < 0.0001$) and greater PgR suppression (−80.5 vs. −46.3%; $p = 0.0018$) for fulvestrant 500 versus 250 mg. At week 16, tumor response rates were 22.9 and 20.6% for fulvestrant 500 and 250 mg, respectively, with considerable decline in all markers by both ACIS and

On behalf of the NEWEST Investigators.
The details of the investigators participating in the study are given in Appendix.

Electronic supplementary material The online version of this article (doi:10.1007/s10549-011-1947-7) contains supplementary material, which is available to authorized users.

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H score. No detrimental effects on endometrial thickness or bone markers and no new safety concerns were identified. This provides the first evidence of greater biological activity for fulvestrant 500 versus 250 mg in depleting ER expression, function, and growth.

Keywords Estrogen receptor-positive breast cancer · Fulvestrant 500 mg · Faslodex® · Neoadjuvant · Biomarkers

Introduction

Endocrine therapy is commonly used in the neoadjuvant setting to attempt to downstage large primary tumors and permit breast conserving surgery [2]. This setting also enables assessment of tumor response in situ and allows for further tailoring of subsequent adjuvant therapy based on the biological characteristics of the individual tumor.

Fulvestrant is an estrogen receptor (ER) antagonist with no known agonist effects. Data from the recent COmparisoN of Fulvestrant In Recurrent or Metastatic breast cancer (CONFIRM) study showed that a high-dose regimen of fulvestrant 500 mg was associated with a significantly longer progression-free survival than the 250 mg regimen (hazard ratio [HR] = 0.80, 95% confidence interval [CI] 0.68–0.94; $p = 0.006$), corresponding to a 20% reduction in the risk of progression [5]. These data led to the approval of fulvestrant 500 mg (500 mg on day 0, 14, 28, and every 28 days thereafter) for the treatment of postmenopausal women with locally advanced or metastatic breast cancer who have progressed or recurred after prior anti-estrogen therapy. In the first-line setting, the randomized Phase II Fulvestrant fIRst-line Study comparing endocrine Treatments (FIRST) study demonstrated that fulvestrant 500 mg is at least as effective as anastrozole in terms of clinical benefit (odds ratio [OR] = 1.30, 95% CI 0.72–2.38; $p = 0.386$), and has a similar objective response rate (36.0 vs. 35.5%, respectively) [11]. In a preplanned follow-up analysis reporting mature data, time to progression was 23.4 months for fulvestrant 500 mg compared with 13.1 months for anastrozole (HR = 0.66, 95% CI 0.47–0.92; $p = 0.01$) [13].

Two presurgical studies have previously shown that treatment with fulvestrant leads to a dose-dependent downregulation of ER, depletion of the ER-regulated protein progesterone receptor (PgR), and reduction in proliferative activity as indicated by the Ki67 labeling index (LI) with doses up to 250 mg [4, 12]. It was expected, therefore, that neoadjuvant therapy with a high-dose regimen of fulvestrant 500 mg would further increase biological activity on ER expression, function, and growth.

Against this background, the current study was designed to evaluate the effects of neoadjuvant endocrine therapy with fulvestrant 500 mg and fulvestrant 250 mg in terms of biological activity (Ki67 LI, ER, and PgR), tumor response, and tolerability in postmenopausal women with locally advanced breast cancer.

The ChromaVision™ Automated Cellular Imaging System (ACIS) used in this study is an image analysis system that can detect and count individual pixels of two chromogen colors used to stain histological sections. The use of automated image analysis systems has become more frequent over recent years, although it has never previously been used for biomarker measurement in a fulvestrant trial setting. Therefore, we also used an established manual scoring method (*H* score). Since the *H* score method has been used effectively in previous fulvestrant studies and those of other endocrine agents [12], its use in the present study enabled subsequent cross-study comparisons to be made when considering the effects of fulvestrant observed here in the neoadjuvant setting.

Patients and methods

Study design and patients

NEWEST (Neoadjuvant Endocrine Therapy for Women with Estrogen-Sensitive Tumors; 9238IL/0065) was a randomized, open-label, multicenter, Phase II study involving postmenopausal women with newly diagnosed, ER-positive, locally advanced breast cancer who had received no prior breast cancer treatment (NCT0093002). Eligible patients (intent-to-treat [ITT] population) were randomly assigned to receive neoadjuvant treatment with either fulvestrant 500 mg/month (plus 500 mg on day 14 of month 1) or fulvestrant 250 mg/month for 16 weeks immediately before surgery.

Women had to be postmenopausal (≥60 years old, or age ≥45 years with amenorrhea for ≥12 months, or follicle-stimulating hormone and estradiol levels within postmenopausal range, or prior bilateral oophorectomy). Other key inclusion criteria were: histologically or cytologically confirmed invasive breast cancer; ER-positive disease as determined locally; operable or potentially operable locally advanced tumor ($T_{2, 3, 4b}$, N_{0-3}, M_0); tumor size ≥2 cm; willingness to undergo biopsy procedures and surgery; and World Health Organization performance status 0–2. Key exclusion criteria were: any previous treatment for breast cancer; inoperability; multifocal disease (>2 major tumor nodules); presence of metastatic disease; other current malignancy or prior malignancy within the previous 3 years; abnormal laboratory values; any severe concurrent condition; history of bleeding diathesis or need for long-term anticoagulant therapy; or treatment with a non-approved or experimental drug within 4 weeks of randomization.

All patients provided written informed consent prior to registration. The study was conducted in accordance with the Declaration of Helsinki and with local ethics committee approval at each participating center (36 centers in Austria, Brazil, Germany, India, the United Kingdom, and the United States).

Treatment

Eligible patients were randomized 1:1 to receive either fulvestrant 500 mg or fulvestrant 250 mg for 16 weeks preceding the surgery. Fulvestrant 500 mg was given as two 5-mL intramuscular (IM) injections, one in each buttock, on days 0, 14, 28, and every 28 days thereafter for 16 weeks. Fulvestrant 250 mg was given as one 5-mL IM injection, in the buttock, on days 0, 28, and every 28 days thereafter for 16 weeks. Patients in the fulvestrant 250 mg arm did not receive additional (fulvestrant placebo) injections. At the completion of 16 weeks of treatment, patients underwent definitive surgery (lumpectomy or mastectomy).

Study objectives

The primary objective of the study was to compare the effects of fulvestrant 500 and 250 mg on expression of the proliferation marker Ki67 after 4 weeks of treatment. Secondary objectives included: effects on ER and PgR expression, tumor response, and tolerability; effects on endometrial thickness and uterine dimensions; effects on serum markers of bone turnover (bone-specific alkaline phosphatase [ALP], C-terminal telopeptides of type-1 collagen [CTX-1], and procollagen type 1 N propeptide [PINP]); and downstaging assessed by a comparison of the actual surgery performed at 16 weeks with the likely surgery predicted at study entry. Ki67 index and ER and PgR expression were also assessed at 16 weeks to monitor for sustained fulvestrant activity.

Study assessments

Assessment of biomarkers

Core biopsies, using an 11- to 14-gauge needle, were taken at baseline, at week 4, and at surgery (week 16). These tumor cores were routinely formalin-fixed and paraffin-embedded locally, with central immunohistochemical assessment of changes in Ki67, ER, and PgR expression at each time point using well-established methods [7]. Briefly, 5-μm sections of pre- and post-treatment tissue samples were dewaxed in xylene and rehydrated through graded alcohols after which endogenous peroxidase was blocked. Following heat-mediated antigen retrieval and blocking of non-specific binding, the sections were incubated with the MIB-1 anti-Ki67 antibody, the 1D5 anti-ER antibody or the PgR 636 anti-PgR antibody (all supplied by Dako, Ely, UK). Binding of the primary antibodies was visualized using an avidin–biotin complex and the chromogen 3,3′-diaminobenzide. The sections were lightly counterstained with hematoxylin before being dehydrated and mounted. Quality control slides were included in all assays to ensure consistency. In the first instance, the immunohistochemical staining of the tissues was assessed using the ChromaVision™ ACIS. This system detects and determines the intensity and counts individual pixels of the two chromogen colors used in the immunohistochemical procedures (in this case, brown = positive; blue = negative). Wherever possible, ten representative fields across each tumor specimen were scored; in cases where ten fields could not be obtained, every available tumor cell was included in the analysis. The Ki67 LI was defined as the percentage of tumor cell nuclei positively stained with intensity above a predetermined threshold. In the case of ER and PgR, the mean intensity as well as the percentage of positively stained nuclei was calculated and combined to produce a proprietary histoscore. ER and PgR expression were also assessed retrospectively on the same stained tissue samples using the *H* score method which is derived by microscopic assessment of the percentage of tumor cells in each of five staining categories (negative, very weak, weak, moderate and strong) to give an *H* score ranging from 0 to 300 [8, 12]. This assessment was performed at the Tenovus Centre for Cancer Research by two experienced observers (JMWG and PF) who were blinded to the ACIS and clinical outcome data and reached a consensus for each slide. Sequential samples from each patient were evaluated at the same time to ensure comparative assessment of tumor histology wherever possible. To ensure the analysis was robust, only paired samples for both the ACIS and *H* score methods were included. Any samples with non-specific staining or unacceptably low cellularity were eliminated from analysis.

Assessment of clinical response

During the 16-week treatment phase, patients underwent clinical breast examination every 4 weeks. Tumor volume was measured by 3D ultrasound at baseline, week 4, and after 16 weeks of treatment before definitive surgery. Optional tumor measurements by magnetic resonance imaging (MRI) were obtained at baseline and 16 weeks. Tumor response was defined as complete response (disappearance of all lesions), partial response (≥65% reduction in tumor volume by 3D ultrasound), disease progression (≥73% increase in tumor volume), or stable disease (neither partial response nor disease progression) [17]. Objective responders were those patients with a complete response or partial response.

Statistical analysis

Sample size calculation was based on the primary endpoint. Based on a 5.36% (±0.616) reduction in Ki67 values following treatment with fulvestrant 250 mg in Study 018 (which compared the short-term biological effects of fulvestrant vs. tamoxifen) [12], a sample size of 80 patients per group would provide 80% power to detect a difference of 0.274 in log-transformed Ki67 values at 4 weeks for fulvestrant 500 mg relative to 250 mg at the two-sided, $p = 0.05$ significance level. Data for the efficacy endpoints were analyzed and summarized on an ITT basis. Treatment differences in Ki67 LI between fulvestrant 500 and 250 mg were assessed using analysis of variance (ANOVA), modeling natural log-transformed changes from baseline Ki67 LI to Ki67 LI at week 4. Also, a post hoc ANOVA was used to assess the effects of fulvestrant 500 mg and fulvestrant 250 mg on ER and PgR expression derived by the ACIS method. For easier interpretation of the data, treatment effects (least squares mean and CIs) were back-transformed and expressed as percentages. Mean percentage changes in *H* scores were calculated from baseline to weeks 4 and 16 using the manually derived score data. Differences in tumor response were analyzed using logistic regression. The safety population consisted of all patients who received at least one dose of study drug. Only patients with a baseline endometrial thickness ≤5 mm were included in the statistical analysis of this safety endpoint.

Tolerability

The frequency and severity of adverse events (AEs) were recorded throughout the study and up to 8 weeks after the last injection. Changes from baseline in endometrial thickness and uterine dimensions were assessed at 16 weeks using transvaginal ultrasound (in all patients with an intact uterus). Patients with apparent thickening of the endometrium (>5 mm) or with suspicious ovarian findings were referred to a gynecologist for advice, but were allowed to continue the study unless the investigator decided otherwise. Serum was collected for analysis of bone CTX-1 (a marker of bone resorption) and of both ALP and PINP (markers of bone formation), which were assessed twice at baseline (before randomized treatment), then every 4 weeks until surgery.

Results

Patients

A total of 211 women were included in the study; 109 were randomized to receive fulvestrant 500 mg and 102 to receive fulvestrant 250 mg. The first subject was enrolled on 7 February 2005 and the last subject completed the study on 9 July 2007. Patient disposition throughout the study is shown in Supplemental Fig. 1. Overall, 99.0% of patients had ER-positive disease and only one patient in each group had unknown ER status. Patient demographics and characteristics at baseline were similar between groups, as outlined in Table 1. The mean age of patients enrolled was 67 years and 85.3% were Caucasian.

Biological activity

Fulvestrant 500 mg reduced mean Ki67 LI to a significantly greater extent than fulvestrant 250 mg (mean

Table 1 Patient demographics and characteristics at baseline

	Fulvestrant, 500 mg ($n = 109$)	Fulvestrant, 250 mg ($n = 102$)
Mean age, years (range)	66.9 (47–94)	66.8 (47–87)
Age category, *n* (%)		
<65 years	46 (42.2)	44 (43.1)
≥65 years	63 (57.8)	58 (56.9)
Race (%)		
Caucasian	92 (84.4)	88 (86.3)
Black	5 (4.6)	3 (2.9)
Oriental	1 (0.9)	3 (2.9)
Other	11 (10.1)	8 (7.8)
WHO performance status (%)		
Unknown	2 (1.9)	2 (2.0)
0	19 (17.4)	16 (15.7)
1 or 2	88 (80.7)	84 (82.4)
ER/PgR status (%)		
ER+/PgR+	76 (69.7)	72 (70.6)
ER+/PgR−	23 (21.1)	20 (19.6)
ER or PgR unknown	10 (9.2)	10 (9.8)
Primary tumor stage (%)		
T2	53 (48.6)	51 (50.0)
T3/T4b	55 (50.5)	50 (49.0)
Unknown	1 (0.9)	1 (1.0)
Tumor grade (%)		
1	12 (11.0)	9 (8.8)
2	56 (51.4)	52 (51.0)
3	18 (16.5)	21 (20.6)
Unassessable, missing or not done	23 (21.1)	20 (19.6)
Intact uterus, (%)		
Yes	87 (79.8)	82 (80.4)
No	16 (14.7)	14 (13.7)
Unknown	6 (5.5)	6 (5.9)

ER estrogen receptor, *PgR* progesterone receptor, *WHO* World Health Organization

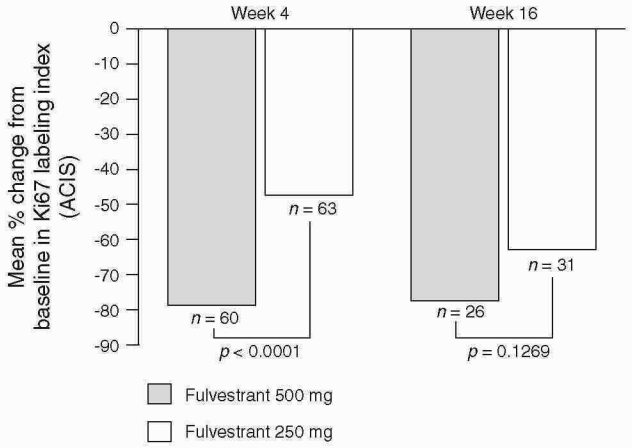

Fig. 1 Effects of fulvestrant 500 mg and fulvestrant 250 mg on Ki67 labeling index after 4 and 16 weeks of treatment (intent-to-treat). *ACIS* Automated Cellular Imaging System

Table 2 Effects of fulvestrant 500 mg and fulvestrant 250 mg on Ki67 labeling index after 4 weeks of treatment (ITT)

	Fulvestrant 500 mg ($n = 109$)	Fulvestrant 250 mg ($n = 102$)
Evaluable patients[a], *n*	60	63
Mean percent reduction from baseline	−78.8	−47.4
95% CI	−70.8 to −84.6	−28.6 to −61.3
Absolute reduction from baseline	−17.5	−10.5
95% CI	−15.7 to −18.8	−6.3 to −13.6
p value[b]	<0.0001	

Ki67 labeling index was determined by ChromaVision™ Automated Cellular Imaging System (ACIS)

CI confidence interval, *ITT* intent-to-treat

[a] Patients for whom data were available at both baseline and 4-week time points

[b] From ANOVA, modeled on the natural log-transformed change from baseline with treatment as a model term

percent change from baseline: −78.8 vs. −47.4%, $p < 0.0001$) after 4 weeks of treatment (Fig. 1; Table 2). This corresponded with a significantly greater reduction in mean ER expression at week 4 for fulvestrant 500 mg compared with fulvestrant 250 mg using both ACIS and *H* scoring methods (Fig. 2a, b; Table 3). However, the magnitude of reduction caused by fulvestrant 500 mg detected by *H* score (−50.3%) was greater than that detected by ACIS (−25.0%). At week 16, reductions in mean Ki67 LI (−77.4 vs. −62.8%; Fig. 1) as well as mean ER expression by ACIS (−36.5 vs. −31.3%; Fig. 2a) and *H* score (−45.2 vs. −56.1%; Fig. 2b) were observed for both fulvestrant 500 mg and fulvestrant 250 mg, but the differences between the doses were not significant at this longer treatment time point. Fulvestrant 500 mg reduced mean PgR expression to a greater extent than fulvestrant 250 mg at week 4 (Fig. 2c, d; Table 3). These differences reached statistical significance using the *H* score method (−80.5 vs. −46.3%; $p = 0.0018$; Table 3; Fig. 2d) but were not statistically significant according to ACIS data (−22.7 vs. −17.6%; Table 3; Fig. 2c). At week 16, decreases in PgR were observed relative to baseline, but there was no significant difference in PgR expression for fulvestrant 500 mg compared with fulvestrant 250 mg using either ACIS (−29.2 vs. −30.5%; Fig. 2c) or *H* score methods (−88.0 vs. −84.5%; $p = 0.6445$; Fig. 2d).

Clinical activity

At weeks 4 and 16, tumor response rates in the ITT population were numerically higher with fulvestrant 500 mg than with fulvestrant 250 mg (17.4 vs. 11.8% at week 4 and 22.9 vs. 20.6% at week 16, respectively) (Table 4). In a post hoc analysis of evaluable patients with a baseline and a 16-week assessment ($n = 69$ in both arms), tumor response rates were 36.2 and 30.4% for fulvestrant 500 mg and fulvestrant 250 mg, respectively (Table 4). Overall, only 13% of evaluable patients progressed during the 16 weeks of therapy (fulvestrant 500 mg $n = 8$; fulvestrant 250 mg $n = 10$).

Tolerability

In total, 208 patients were eligible for assessment of tolerability. Both treatments were well tolerated over the 16-week treatment period. Treatment-related AEs were experienced by 37.4 and 30.7% of patients and treatment-related serious AEs by 0.9 and 3.0% of patients in the fulvestrant 500 mg and fulvestrant 250 mg groups, respectively. Only two AEs (one per group) led to withdrawal; neither was thought to be treatment-related (one transient ischemic attack; one pulmonary embolism). One patient randomized to fulvestrant 250 mg experienced an AE leading to death during the posttreatment follow-up period that was also not considered to be treatment-related (cause of death unknown, possibly cardiac-related). The most common AEs are described in Table 5.

Both doses of fulvestrant reduced endometrial thickness, with changes after 16 weeks of treatment similar between fulvestrant 500 mg and fulvestrant 250 mg groups (Supplemental Table 1). Serum bone marker levels were similar within and between the two groups throughout the study, with neither dose producing substantial changes in any of the three bone markers assessed (ALP, CTX, and PINP) (Supplemental Fig. 2). Few patients reported receiving prior medications (bisphosphonates, corticosteroids, hormone replacement therapy) that might confound interpretation of bone or endometrial data (fulvestrant 500 mg: 8 patients;

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