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# Lower-Dose vs High-Dose Oral Estradiol Therapy of Hormone Receptor–Positive, Aromatase Inhibitor–Resistant Advanced Breast Cancer

## A Phase 2 Randomized Study

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**T**HE EFFICACY OF A SYNTHETIC estrogen, diethylstilbestrol (DES),<sup>1</sup> in the treatment of breast cancer was first described by Had-dow et al,<sup>2</sup> who discussed DES in terms of the wider paradox that certain organic compounds induce cancer but can be used in cancer treatment. Efficacy was restricted to postmenopausal women, suggesting that the decline in estrogen levels associated with menopause may sensitize breast cancer cells to DES.<sup>3</sup> Some patients could even be treated with intermittent therapy, with repeated regressions at reintroduction of DES.<sup>4</sup>

In the early 1980s tamoxifen was shown to be less toxic, although not more effective,<sup>5</sup> and DES was eventually withdrawn from human use in the United States. As an alternative to DES, estradiol

**Context** Estrogen deprivation therapy with aromatase inhibitors has been hypothesized to paradoxically sensitize hormone-receptor–positive breast cancer tumor cells to low-dose estradiol therapy.

**Objective** To determine whether 6 mg of estradiol (daily) is a viable therapy for postmenopausal women with advanced aromatase inhibitor–resistant hormone receptor–positive breast cancer.

**Design, Setting, and Patients** A phase 2 randomized trial of 6 mg vs 30 mg of oral estradiol used daily (April 2004–February 2008 [enrollment closed]). Eligible patients (66 randomized) had metastatic breast cancer treated with an aromatase inhibitor with progression-free survival ( $\geq 24$  wk) or relapse (after  $\geq 2$  y) of adjuvant aromatase inhibitor use. Patients at high risk of estradiol-related adverse events were excluded. Patients were examined after 1 and 2 weeks for clinical and laboratory toxicities and flare reactions and thereafter every 4 weeks. Tumor radiological assessment occurred every 12 weeks. At least 1 measurable lesion or 4 measurable lesions (bone-only disease) were evaluated for tumor response.

**Intervention** Randomization to receive 1 oral 2-mg generic estradiol tablet 3 times daily or five 2-mg tablets 3 times daily.

**Main Outcome Measures** Primary end point: clinical benefit rate (response plus stable disease at 24 weeks). Secondary outcomes: toxicity, progression-free survival, time to treatment failure, quality of life, and the predictive properties of the metabolic flare reaction detected by positron emission tomography/computed tomography with fluorodeoxyglucose F 18.

**Results** The adverse event rate ( $\geq$  grade 3) in the 30-mg group (11/32 [34%]; 95% confidence interval [CI], 23%–47%) was higher than in the 6-mg group (4/34 [18%]; 95% CI, 5%–22%;  $P = .03$ ). Clinical benefit rates were 9 of 32 (28%; 95% CI, 18%–41%) in the 30-mg group and 10 of 34 (29%; 95% CI, 19%–42%) in the 6-mg group. An estradiol-stimulated increase in fluorodeoxyglucose F 18 uptake ( $\geq 12\%$  prospectively defined) was predictive of response (positive predictive value, 80%; 95% CI, 61%–92%). Seven patients with estradiol-sensitive disease were re-treated with aromatase inhibitors at estradiol progression, among which 2 had partial response and 1 had stable disease, suggesting resensitization to estrogen deprivation.

**Conclusions** In women with advanced breast cancer and acquired resistance to aromatase inhibitors, a daily dose of 6 mg of estradiol provided a similar clinical benefit rate as 30 mg, with fewer serious adverse events. The efficacy of treatment with the lower dose should be further examined in phase 3 clinical trials.

**Trial Registration** clinicaltrials.gov Identifier: NCT00324259

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**For editorial comment see p 797.**

became an uncommonly used therapy after the failure of more contemporary endocrine agents, with 30 mg (10 mg by mouth 3 times per day) on the prescribing label for proprietary formulations. More recently, high-dose DES (15 mg daily) was reported in a European study to be an effective, although relatively poorly tolerated, treatment after the development of resistance to aromatase inhibition.<sup>6</sup> Song et al<sup>7</sup> reported that prolonged estrogen deprivation primed MCF-7 cells for estradiol-induced apoptosis at concentrations more typical of hormone therapy. Furthermore, the unexpected decrease in breast cancer incidence observed in women receiving equine estrogens alone in the Women's Health Initiative Trial has stimulated interest in the possibilities of low-dose estrogen therapy for breast cancer.<sup>8</sup>

We therefore conducted a phase 2 randomized trial in postmenopausal women with hormone receptor–positive, aromatase inhibitor–resistant advanced disease comparing 30 mg estradiol (10 mg, 3 times per day) with 6 mg (2 mg, 3 times per day) to specifically address whether exposure to third-generation aromatase inhibition treatment sensitizes advanced estrogen receptor–positive breast cancer to lower, better tolerated, and safer doses of estradiol.

## METHODS

### Patients

The study was approved by the ethics committees at each of the participating institutions and registered with the National Cancer Institute. Between April 2004 and February 2008, 66 postmenopausal women with advanced estrogen receptor (ER)– and/or progesterone receptor (PgR)–positive breast cancer (defined as at least 10% of malignant cells with positive nuclear staining) and Eastern Cooperative Oncology Group performance status 0 to 2 were enrolled into the protocol after providing written consent (FIGURE 1). Eligible patients had received prior treatment with an aromatase inhibitor in the advanced disease setting, with at least 24 weeks of progression-free survival (PFS) before disease progression. A patient re-

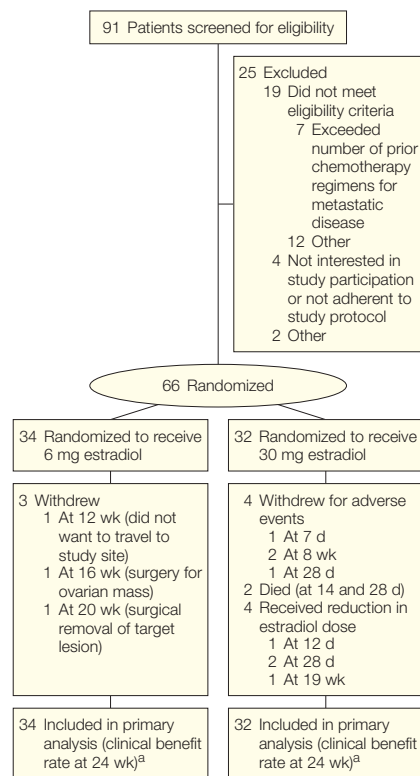
mained eligible even if further lines of endocrine therapy had been unsuccessfully used. Eligibility also included relapses at least 2 years after initiation of adjuvant aromatase inhibitor therapy. In this instance, estradiol therapy was offered as first-line endocrine treatment. Menopausal status was defined as age 50 years or older and amenorrhea for 1 year or bilateral oophorectomy, or serum follicle-stimulating hormone and estradiol levels in the postmenopausal range before the initiation of aromatase inhibitor therapy. One line of chemotherapy for advanced disease was permissible. Adequate hematological, renal, and hepatic function was required and treatment with an intravenous bisphosphonate was mandatory for all patients with bone metastasis.

Patients were excluded on the basis of central nervous system involvement, a history of deep venous thrombosis, pulmonary embolism, stroke, acute myocardial infarction, congestive cardiac failure, untreated hypertension, ischemic changes on a baseline electrocardiogram, undiagnosed abnormal vaginal bleeding, untreated cholelithiasis, previous malignancy not treated with curative intent or with an estimated recurrence risk of greater than 30%, and untreated metabolic disturbances (glucose  $\geq$ 200 mg/dL and triglycerides  $>$ 400 mg/dL or an elevated calcium level [local laboratory limit]). Treatment with fulvestrant within 12 months of study initiation was also an exclusion criterion because this agent had been shown to antagonize estrogen-induced apoptosis *in vitro*.<sup>7</sup>

### Procedures and Definitions

A randomization table was created using the SAS program PROC PLAN (SAS Institute, Cary, North Carolina). To better ensure the balance of potential risk factors in 2 groups, treatment assignment was implemented in small blocks of 4 to 6 patients. Patients were randomized to receive either 1 oral 2-mg generic estradiol tablet (commercial stock) 3 times daily (total daily dose, 6 mg) or five 2-mg tablets (10 mg)

**Figure 1.** Patient Enrollment, Randomization, and Treatment Flow



<sup>a</sup>Clinical benefit rate denotes response plus stable disease at 24 weeks.

3 times daily (total daily dose, 30 mg). Patients were examined after 1 and 2 weeks for clinical and laboratory toxicities and flare reactions and thereafter every 4 weeks.

Tumor radiological assessment occurred every 12 weeks. At least 1 measurable lesion defined by response evaluation criteria in solid tumors (RECIST) was followed-up or, in the case of bone-only disease, at least 4 measurable lesions on computed tomography (CT) scan bone windows were assessed by World Health Organization response criteria and an elevation in a baseline tumor marker greater than 2 times the upper limit was required to assist in the evaluation of tumor response.<sup>9</sup> No evidence of disease progression at the 24-week evaluation defined stable disease. The clinical benefit rate (CBR) defined the fraction of patients with either RECIST response or stable disease.

### Fluorodeoxyglucose F 18 Positron Emission Tomography/CT Imaging and Analysis

Patients underwent baseline clinical imaging by positron emission tomography/CT with fluorodeoxyglucose F 18 (FDG-PET)/CT as many as 4 weeks before study initiation; FDG-PET/CT was repeated on the same scanner 24 hours after initiation of the assigned dose of estradiol. The third dose was taken typically 1 to 3 hours before the expected time of injection of FDG. The fasting glucose level was required to be less than 200 mg/dL immediately prior to injection of 10 to 15 mCi (370-555 MBq) of FDG. After 1 hour, a spiral CT scan (typically 95-111 effective mAs, 130 kVp, and 5-mm slice thickness) was performed followed by pelvis-to-skull emission images.

The PET emission images were corrected for measured attenuation and reconstructed using an ordered-subset estimation-maximization iterative algorithm. All PET images were evaluated semiquantitatively by determining the standardized uptake value. The percent changes in maximum standardized uptake value for FDG were determined. Baseline FDG-PET/CT studies were reviewed to select metastatic lesion(s) for analysis. In patients with multiple lesions, the average standardized uptake value of 6 or fewer lesions was determined. An increase in tumor standardized uptake value of 12% or greater was prospectively defined as the threshold for a positive estradiol stimulation test.<sup>10</sup>

### Quality-of-Life Analysis

Participants were surveyed at baseline and at 28 days using the multidimensional Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire<sup>11</sup> and a 6-item estrogen adverse effect questionnaire (headaches, bloating, breast tenderness, retention of fluid, nausea, and vomiting). Both the FACT-B and estrogen adverse effects questionnaires used a 5-point scale ranging from 0 (not at all) to 4 (very much). Cronbach  $\alpha$  was reported to be 0.90 on the FACT-B, indicating high internal con-

sistency of items on this measure.<sup>11</sup> Some of the items on the FACT-B (measuring physical, social, functional, and emotional well-being [as separate items] and additional breast cancer concerns) were summed to create a total FACT-B score—with higher scores indicating better quality of life (QOL).

### Estradiol and Insulinlike Growth Factor 1 Levels

Estradiol levels were quantified using an ultra-sensitive radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, Texas) that measures estradiol concentrations with a 5 pg/mL lower detection limit. Serum total insulinlike growth factor 1 (IGF-1) was measured using kits for the Siemens Immulite 1000 (Siemens Healthcare Diagnostics, Deerfield, Illinois), which provides chemiluminescent immunometric detection of IGF-1 levels with a 25 ng/mL lower detection limit.

### Statistical Analysis

Therapeutic efficacy and safety were assessed based on an intention-to-treat principle. The primary outcome for this study was the CBR. The secondary outcomes included the incidence of grade 3 and higher toxicities or serious adverse events, PFS, time to treatment failure, serum IGF-1 and estradiol levels, tumor FDG uptake within 24 hours of treatment initiation (metabolic flare), and quality of life.

The study was designed using the Simon minimax 2-stage design to detect, with 80% power at a 1-sided .05 significance level, a minimum rate of interest in each group, and a 20% CBR—with a maximum expected rate of 40%. If both doses achieved this level of activity, the best-tolerated dose would be recommended for further study (defined as the group with the lowest frequency of all grade 3 or higher toxicities or serious adverse events, regardless of type).

Demographic and clinical characteristics of the 2 groups were compared using the *t* test or Fisher exact test as appropriate. The CBR for each group and the 95% exact binomial confidence intervals (CIs) were calculated.

The difference of grade 3 and higher toxicities or serious adverse events between 2 groups was compared by Cochran-Armitage 2-sided trend test.

PFS was defined as the time from treatment initiation to disease progression or death. Time of last observation for patients remaining in the study and the time at which dose reductions, study drug termination, and withdrawal of consent occurred were treated as censored data. Time to treatment failure treated all events that led to termination of the assigned treatment as events, and time of last observation for patients remaining in the study and time of withdrawal of consent as censored data. PFS and time to treatment failure were estimated using the Kaplan-Meier product-limit method and the differences between the 2 groups were compared by the log-rank test.

To assess the ability of FDG-PET metabolic flare to predict response, positive predictive value (the proportion with clinical benefit among patients with metabolic flare) and negative predictive value (the proportion with no clinical benefit among patients without metabolic flare) were also calculated. Analysis of covariance (ANCOVA) was used to test the effect of response to therapy on total FACT-B scores at 28-day follow-up, controlling for baseline FACT-B.

For analysis of the estrogen adverse effects as a grouping variable, the level of estrogen adverse effects at 28 days was reduced to a dichotomous variable (high or low) using the median value. Using a factorial ANCOVA controlling for baseline total FACT-B scores and response to therapy, we tested the main and interaction effects of the treatment group (6 mg vs 30 mg) and severity of estrogen adverse effects at follow-up on 28-day follow-up FACT-B scores. Repeated-measures ANCOVA (RM-ANCOVA) was used to measure the significance of change in estrogen adverse effects after 28 days, grouping by treatment group and change in total FACT-B scores after 28 days, controlling for response to therapy, and sorting by treatment group and the dichoto-

mous estrogen adverse effects variable.

A *P* value of less than .05 was taken to indicate significance and all statistical tests were 2-sided. All the analyses were performed using the SAS statistical package version 9 (SAS Institute Inc) or SPSS version 16.0 (SPSS Inc, Chicago, Illinois).

## RESULTS

### Study Population and Toxicity

Ninety-one patients were screened for the study (Figure 1). The trial accrued 66 patients (self-reported, 80% (53) white, 15.0% (10) black, and 5% (3) other as required by the National Institutes of Health funding mechanism; mean age 58.9 years, range, 36.4-83.9), with 32 patients in the 30-mg group and 34 in the 6-mg group. There were no statistically significant differences in baseline patient and tumor characteristics in the 2 study groups (TABLE 1). The study population was dominated by patients with a late relapse pattern since the average time from diagnosis to relapse was more than 7 years.

The grade 3 or higher adverse events are summarized in TABLE 2. Adverse effects were generally characteristic of estradiol therapy. Most notably, there were fewer patients with high-grade nausea and vomiting, electrolyte disturbance, and problems with pleural effusion in the 6-mg group. Consistent with these toxicity differences, the mean (SD) trough levels of estradiol at 1 month were 302 (519) pg/mL in the 6-mg group and 2403 (2268) pg/mL in the 30-mg group (*P* < .001; TABLE 3). Only 1 grade 3 tumor flare occurred (pain in a retro-orbital metastasis with diplopia in the 30-mg group) and was managed by interruption of therapy, followed by re-treatment at the 6-mg dose after flare symptoms subsided. Grade 1 or 2 vaginal bleeding was observed in 17 patients, was associated with younger age (mean age, 54 [9] vs 61 [11] years; *P* = .04), and was well-controlled with progestin therapy either orally or as an intrauterine device. There was no evidence that the use of a progestin interacted with response. The

rate of thrombosis was low with 1 event in each study group. Overall, there were significantly fewer grade 3 or higher adverse events in the 6-mg group with 4 of 34 (11%; 95% CI, 5%-22%) vs 11 of 32 in the 30-mg group (34%; 95% CI, 23%-47%; *P* = .03; Table 2).

### Response

The slight imbalance in numbers assigned to the 2 groups (32 in the 30-mg group and 34 in the 6-mg group) was a consequence of yearly data and safety monitoring, which led to early closure of the 30-mg group for toxicity concerns after 32 patients had been enrolled, after which the study was completed by enrolling the remaining 2 patients into the 6-mg group. The primary end point (CBR) was 9 of 32 (28%; 95% CI, 18%-41%) in the 30-mg group and 10 of 34 (29%; 95% CI, 19%-42%) in the 6-mg group (TABLE 4). There were relatively few RECIST responses (1 in the 30-mg group and 3 in the 6-mg group). Two of the stable disease patients in the 30-mg group were identified after a dose reduction to 6 mg because of a grade 3 or 4 adverse event. Only 7 patients entered the study who had relapsed while receiving adjuvant aromatase inhibitor therapy (with 1 partial response and 1 stable disease both in the 6-mg group). There was no difference between the 2 groups in PFS (*P* = .46; FIGURE 2A) or time to treatment failure (*P* = .09; Figure 2B).

After noting a significant number of patients responding to estradiol, the study was extended to address the hypothesis that the acquired aromatase inhibitor resistance exhibited by the trial population might, in some instances, be reversed by an extended period of estradiol therapy. The protocol was therefore amended in 2005 to allow data collection on response to re-treatment with the last aromatase inhibition received, ie, avoiding a change in the type of aromatase inhibitor so that true reversal of resistance could be assessed. This approach was only offered to patients experiencing clinical benefit on estradiol. To date, 7 patients have been

**Table 1.** Patient and Tumor Characteristics by Study Group<sup>a</sup>

Characteristic	No. (%)	
	6 mg (n = 34)	30 mg (n = 32)
Age, median (range), y	54.7 (36.3-83.8)	59.5 (39.4-77.7)
Diagnosis, median (range), mo	90.2 (16.6-381.5)	82.4 (12.9-327.2)
Race		
Black	4 (12)	6 (19)
White	29 (85)	24 (75)
Other	1 (3)	2 (6)
Site		
Bone/soft tissue	18 (53)	13 (40)
Visceral	5 (15)	5 (16)
Both	11 (32)	14 (44)
Estrogen receptor status		
Negative	1 (3)	0 (0)
Positive	33 (97)	32 (100)
Progesterone receptor status		
Negative	8 (24)	6 (19)
Positive	26 (76)	26 (81)
Prior systemic therapy		
Aromatase inhibitor (adjuvant)	3 (9)	4 (13)
Aromatase inhibitor (metastatic)	32 (94)	29 (91)
Tamoxifen (adjuvant)	18 (53)	14 (44)
Tamoxifen (metastatic)	6 (18)	3 (9)
Chemotherapy (adjuvant)	20 (59)	16 (50)
Chemotherapy (metastatic)	4 (12)	5 (16)

<sup>a</sup>No listed parameters showed statistically significant differences.

re-treated with an aromatase inhibitor (eTable available at <http://www.jama.com>). Three patients have experienced clinical benefit (2 partial responses and 1 stable disease lasting 36, 36, and ≥28 weeks, respectively).

### Pharmacodynamic Analysis

To compare the systemic endocrine effects of the 2 doses of estradiol, serum IGF-1 levels were assessed. IGF-1 decreased from baseline in the 6-mg group by a mean (SD) of 61 (32) ng/mL and in the 30-mg group by 61 (41) ng/mL (Table 3). These decreases from baseline were highly significant (*P* < .001), but did not differ between the 2 groups (*P* = .96). The FDG-PET/CT data allowed a direct comparison of the 2 doses of estradiol at the level of the

metastatic tumor. No differences in the change in FDG uptake were detected in the 2 treatment groups in responding patients (with mean changes of 20.9% [21.7%] in 6-mg group and 22.1% [11.7%] in the 30-mg group;  $P = .92$ ), indicating 6 mg daily stimulated glucose uptake to a similar degree as the higher dose (Table 3).

**The Predictive Value of FDG-PET Metabolic Flare**

The relationship between metabolic flare assessed by FDG-PET/CT and response, combining the 2 groups, could be conducted in 46 patients (Table 4). Ten patients were not evaluable for response because early toxicity prevented response assessment (Figure 1); the PET data

were considered technically inadequate or were not available in another 8 patients. The presence of a metabolic flare was a highly significant predictor of response ( $P < .001$ ). With at least a 12% increase in FDG uptake prospectively defined as a metabolic flare, the positive predictive value for response was 12 of 15 (80%; 95% CI, 61%-92%) and the negative predictive value for nonresponse was 27 of 31 (87%; 95% CI, 76%-94%). PFS was significantly longer for patients with a metabolic flare (log-rank  $P = .02$ ).

**Table 2.** Grade 3 and Higher Toxicities and the Incidence of Serious Adverse Events by Study Group

Toxicity Class	No. (%)			
	6 mg (n = 34)		30 mg (n = 32)	
	Grade 3	Grade 4-5	Grade 3	Grade 4-5
Nausea/vomiting	0	0	5 (16)	0
Low sodium	1 (3)	0	5 (16)	0
Pleural effusion	0	0	4 (13)	0
Pain	6 (18)	0	4 (13)	0
Thrombosis/embolism	0	1 (3)	0	1 (3)
Central nervous system ischemia	1 (3)	0	0	0
Infection	2 (6)	0	1 (3)	2 (6)
Hypercalcemia	0	0	2 (6)	0
Other	6 (18)	0	10 (31)	0
	<b>Grade 1-2 /Grade 3</b>	<b>Grade 4 /Grade 5</b>	<b>Grade 1-2 /Grade 3</b>	<b>Grade 4 /Grade 5</b>
Serious adverse event incidence	30 (88)/3 (9)	1 (3)/0	21 (66)/8 (25)	3 (9)

**Table 3.** Biomarker Comparisons Between Study Groups<sup>a</sup>

Outcome	6 mg		30 mg		P Value
	No.	Mean (SD)	No.	Mean (SD)	
Insulinlike growth factor 1, ng/mL	29	-61 (32)	23	-61 (41)	.96
Estradiol, pg/mL	29	302 (519)	23	2403 (2268)	<.001
Fluorodeoxyglucose-positron emission tomography standardized uptake value, %	8	20.9 (21.7)	7	22.1 (11.7)	.92

<sup>a</sup>Mean difference is between 28 days and baseline in insulinlike growth factor 1, estradiol levels, and estradiol-induced changes in fluorodeoxyglucose-positron emission tomography standardized uptake value uptake in responding patients.

**Table 4.** Treatment Response by Study Group and Contingency for Interaction Between the Presence of a Positive FDG-PET/CT Estradiol Stimulation Test and Response to Estradiol Treatment

	Response		Metabolic Flare on FDG-PET/CT <sup>a</sup>		
	6 mg (n = 34)	30 mg (n = 32)	Yes <sup>b</sup>	No <sup>b</sup>	Total
	Complete remission	0	0	NA	NA
Partial response	3 (9)	1 (3)	3	0	3
Stable disease	7 (20)	8 (25)	9	4	13
Progression disease	21 (62)	16 (50)	3	27	30
Not assessable	3 (9)	7 (22)	NA	NA	NA

Abbreviation: FDG-PET/CT, fluorodeoxyglucose-positron emission tomography/computed tomography; NA, not applicable.

<sup>a</sup>Combined data from both groups,  $P < .001$ .

<sup>b</sup>Total yes responses to metabolic flare on FDG-PET/CT, 15; total no responses to metabolic flare on FDG-PET/CT, 31; overall responses, 46.

**QOL Analysis**

The scores from the 6 estrogen adverse effect items were combined to produce a single score (Cronbach  $\alpha$ , 0.61 at baseline and 0.72 at 28-day follow-up). A significant increase in severity of adverse effects from baseline to follow-up was observed overall (0.47-0.80;  $P < .001$ ), but the change was not significantly different by treatment group (0.47-0.70 in 6-mg group vs 0.46-0.92 in 30-mg group;  $P = .10$ ). However, the study underestimated the negative effect of treatment on QOL in the 30-mg group because patients with the most severe adverse effects were dose-reduced or withdrew before the 28-day QOL follow-up (Figure 1). In the factorial ANCOVA, FACT-B scores at follow-up differed significantly by the dichotomous estrogen adverse effects measure (low adverse effects, 114.8 vs high adverse effects, 99.8;  $P = .003$ ) but not by treatment group (6 mg, 109.5 vs 30 mg, 106.9;  $P = .52$ ) after controlling for baseline FACT-B and response to therapy. The difference in QOL by estradiol adverse effects intensity met the criterion of a minimally important difference of 7 to 8 points.<sup>12</sup> A significant interaction between estrogen adverse effects and treatment group on FACT-B at follow-up ( $P = .03$ ) indicated that the poorest QOL was reported by patients in the 30-mg group who had more severe adverse effects.

**COMMENT**

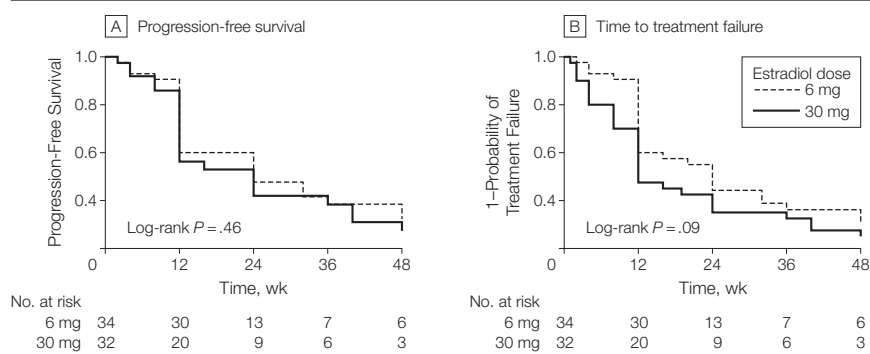
The CBR rates of 28% (30 mg) and 29% (6 mg) reported in this study were just below our prespecified expectations for clinical activity because the lower

boundaries of the 95% CIs crossed 20% (Table 4). However, at the time the study was powered, there was only limited information on the activity of further endocrine therapy in patients who had progressed while using an aromatase inhibitor. Recent data from a large phase 3 double-blind randomized clinical trial that compared fulvestrant and exemestane in patients with disease progression after a nonsteroidal aromatase inhibitor produced outcomes very similar to our experience with estradiol (CBR of 32.2% and 31.5%, respectively).<sup>13</sup> On this basis, it is reasonable to conclude that the activity of estradiol is sufficient to warrant further investigation.

In further studies of estradiol treatment, the 6-mg dose should be favored because it was significantly safer with a lower serious adverse event rate. We also observed that intense estradiol adverse effects have a negative effect on QOL, which is mitigated by lowering the estradiol dose. Thus, in women with advanced breast cancer and acquired resistance to aromatase inhibition, a daily estradiol dose of 6 mg provided a similar CBR as 30 mg daily, with fewer adverse events that affect QOL. We express caution regarding safety and emphasize that patients must continue to be excluded from further investigations on the basis of risk of serious adverse effects from estrogen. These exclusion criteria probably accounted for the low rate of thrombosis in the study. The low rate of hypercalcemia (no cases in the 6-mg group), historically a major problem with estrogen treatment,<sup>5</sup> almost certainly reflects the uniform use of an intravenous bisphosphonate in patients with bone metastasis. The enhanced tolerability of the 6-mg dose in terms of nausea and vomiting is reflected in the serum estradiol measurements, which achieved the goal of an average concentration typical for the first trimester of pregnancy with the 30-mg dose, and the preovulatory phase of the menstrual cycle with the 6-mg dose.

Biomarker analysis contributed evidence that 6 mg of estradiol is a bio-

**Figure 2.** Progression-Free Survival and Probability of Treatment Failure by Study Group



Median (range) follow-up time was 83 (37-215) weeks for the 6-mg group and 86 (6-212) weeks for the 30-mg group.

logically effective dose in the postaromatase inhibitor setting. Serum IGF-1 suppression was equivalent in the 2 groups of the study and, more directly, so was estradiol stimulation of tumor FDG uptake in responding patients (Table 3). Thus the 6-mg dose should be also favored when conducting the FDG-PET estradiol stimulation test.

Given that only a minority of patients will respond, the validation of the FDG-PET estradiol stimulation test as a predictive biomarker for estradiol therapy is a major finding of this study (Table 4). The 12% increase in FDG uptake as the threshold for a “positive estradiol stimulation test” was prespecified on the basis of an earlier study.<sup>10</sup> We have therefore validated the 12% threshold and broadened the spectrum of agents for which the test predicts activity, ie, a positive PET-based estradiol stimulation test predicts sensitivity to fulvestrant, aromatase inhibitors, and estradiol. Additionally, in our original study,<sup>14</sup> we demonstrated that metabolic flare early during treatment with tamoxifen predicted sensitivity to tamoxifen itself. The estradiol stimulation test therefore differentiates between hormone receptor-positive patients in whom serial endocrine therapy with a number of different agents is likely to be an effective approach and patients in whom a change to nonendocrine treatment approaches is likely to be necessary earlier in the treat-

ment course. In the group with a positive estradiol stimulation test, the order with which endocrine therapies are applied is an important consideration. For example, patients with a positive test result would be a reasonable population to further investigate re-treatment with an aromatase inhibitor after estradiol progression because our limited experience suggests that estradiol therapy may, in some cases, resensitize metastatic estrogen receptor-positive breast cancer to estrogen deprivation therapy.

In conclusion, 6 mg of estradiol daily, which produces estradiol levels similar to those in ovulating premenopausal women, is an active low-cost treatment for postmenopausal women with advanced breast cancer and acquired resistance to aromatase inhibitor treatment and should be further investigated. The activity of other endocrine agents after successful treatment with estradiol, including aromatase inhibitor re-treatment, should be explored further. Finally, investigation of the mechanism of estradiol efficacy is critical for progress since the use of this treatment in earlier disease settings will require a robust tissue-based predictive biomarker that identifies the subset of tumors susceptible to this paradoxical treatment.

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