INTERNATIONAL BREAST CANCER STUDY GROUP
IBCSG 35-07
BIG 1–07

SOLE
Study of Letrozole Extension

A phase III trial evaluating the role of continuous letrozole versus intermittent letrozole following 4 to 6 years of prior adjuvant endocrine therapy for postmenopausal women with hormone-receptor positive, node positive early stage breast cancer

Coordinating Group: **International Breast Cancer Study Group (IBCSG)**

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Protocol Signature Page

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Study of Letrozole Extension - SOLE

Approved by:
CEO, International Breast Cancer Study Group
Prof. Dr. med. M. Castiglione

_____ _____

Date

Approved by:
Novartis representative(s)

_____ _____

Date

Approved by:
Group Statistician, International Breast Cancer Study Group
Prof. R.D. Gelber

_____ _____

Date

Principal Investigator Protocol Signature Page

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Study of Letrozole Extension - SOLE

I have read the protocol and agree that it contains all necessary details for conducting this study. I will conduct the study as outlined in the following protocol and in compliance with GCP. I will provide copies of the protocol and all drug information relating to pre-clinical and prior clinical experience furnished to me by IBCSG, **to all physicians responsible to me who participate in this study. I will discuss this material with them to assure that they are fully informed** regarding the drug and the conduct of the study. I agree to keep records on all patient information (Case Report Forms and patient's informed consent statement), drug shipment and return forms, and all other information collected during the study for a minimum period of 15 years.

Name of Principal Investigator:_____

_____ _____

Signature Date

Protocol Summary and Schema
SOLE
Study of Letrozole Extension

A phase III trial evaluating the role of continuous letrozole versus intermittent letrozole following 4 to 6 years of prior adjuvant endocrine therapy for postmenopausal women with hormone-receptor positive, node positive early stage breast cancer

Patient population

Postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with selective estrogen receptor modulator(s) (SERM) and/or aromatase inhibitor(s) (AI) for endocrine-responsive, node-positive operable breast cancer.

Rationale

In 2006, the standard duration of adjuvant endocrine therapy for breast cancer (either SERMs or AIs) is five years. Patients who receive extended adjuvant letrozole for five years following approximately five years of tamoxifen obtain further benefit compared with the five years of tamoxifen alone. Similarly, benefit has been demonstrated for switching from tamoxifen to an AI after 2 to 3 years of tamoxifen to complete five years of endocrine therapy, as well as initiating therapy with AI following surgery and administering the AI for five years.

Questions remain about the optimal duration and best schedule of AIs in the extended adjuvant setting. This trial tests the hypothesis that introducing 3-month treatment-free intervals during the course of five years of extended adjuvant letrozole will improve disease-free survival. This hypothesis is based on the theoretical principle that letrozole withdrawal for 3 months will permit some estrogenic stimulation which makes residual resistant disease susceptible to letrozole reintroduction.

Objective

To compare continuous letrozole for five years with intermittent letrozole over a five year period for postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive, node-positive, operable breast cancer.

Trial end points

Primary end point: Disease-free survival (DFS): time from randomization to local (including invasive recurrence restricted to the breast after breast conserving treatment), regional or distant relapse, contralateral breast cancer, appearance of a second (non-breast) malignancy, or death from any cause, whichever occurs first.

Secondary end points: overall survival (OS), distant disease-free survival (DDFS), breast cancer free interval (BCFI), sites of first failure, second (non-breast) malignancies, deaths without prior cancer events, and adverse events.

Statistical analysis

The randomization will be stratified according to participating center and prior SERM/AI endocrine therapy (SERM(s) alone, AI(s) alone, both SERM(s) and AI(s).

The primary analysis will be undertaken with the intention-to-treat population of all randomized patients. The primary endpoint is disease-free survival (DFS) and will be compared between treatment arms using a two-sided stratified logrank test with an overall experiment-wise alpha level equal to at most 0.05. Kaplan-Meier estimates of the DFS distributions will be calculated for each of the two treatment arms. Cox proportional hazards regression models will be used to investigate whether the treatment comparison is modified by adjustments for various covariates.

Sample size and anticipated trial duration

The sample size was determined to provide 80% power to detect a 20% reduction in the risk of an event defining DFS associated with intermittent letrozole compared with continuous letrozole (hazard ratio = 0.80; 25% increase in 4-year DFS from 90% to 91.917%) using a two-sided 0.05 level test of significance.

To achieve this goal requires 647 events defining DFS, assuming 4800 patients are accrued (1600 patients per year for 3 years), 5% non-assessability at 4 years, and approximately 5 years of additional follow-up. One year of start-up time, as participating centers obtain ethics committee approval and complete regulatory processes, is anticipated.

Procedures

All patients will be followed every 6 months for years 1 to 5, and thereafter yearly for assessment of disease status and for survival data collection.

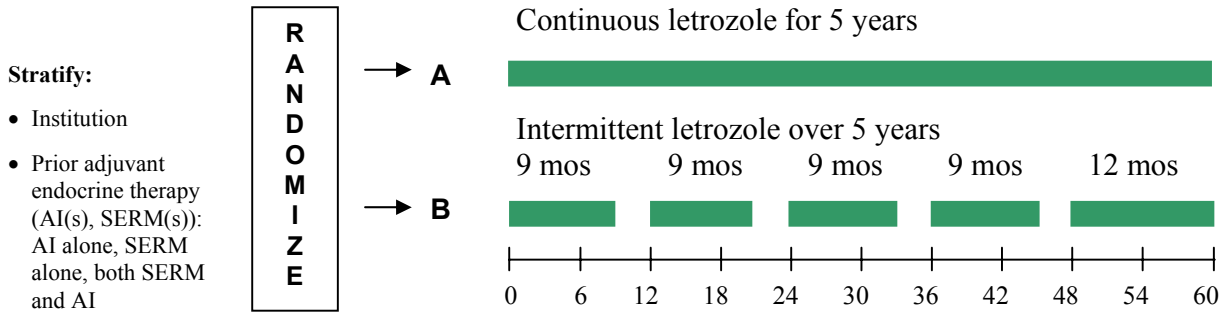
Risks and benefits

Adjuvant treatment with letrozole has been shown to have a favorable effect on time without recurrence of breast cancer. The adjuvant treatment with letrozole is generally well tolerated.

Trial Design

At completion of 4 to 6 years of prior adjuvant SERM/AI endocrine therapy, patients will be randomized to one of two treatment groups:

Schema



Extended Adjuvant Endocrine Therapy

Letrozole: A: Continuous letrozole 2.5 mg daily for 5 years
B: Intermittent letrozole 2.5 mg daily for the first 9 months of years 1 through 4, followed by 12 months in year 5

Randomization Timing

In principle, patients should start trial treatment as soon as possible after randomization. Trial treatment should begin no later than 6 weeks from the date of randomization.

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1. Introduction

1.1. Adjuvant treatment in breast cancer

Breast cancer is the most common malignant disease in women; and the most common form of cancer death in women in Europe with an estimated 370,100 new cases diagnosed and 129,900 deaths [1]. At diagnosis, 90% of the patients appear to have an operable breast cancer, that is, disease confined to the breast and to the ipsilateral axilla. More than 50% of these patients, however, die of metastatic disease. In fact, once metastases become overt, the disease is considered, with very few exceptions, incurable. Since the late nineteen-forties randomized trials of adjuvant systemic therapy (either endocrine or cytotoxic) have been conducted in an effort to reduce the number of relapses and to prolong the survival of patients with operable disease. The Oxford Overview summarizing the available results of all such trials indicated that adjuvant systemic treatments with chemotherapy, endocrine therapy, and combinations of both improved prognosis of patients with breast cancer [2].

1.2. Letrozole

There are two classes of third generation AI(s). Agents such as anastrozole and letrozole act by reversibly binding to the aromatase enzyme, which is responsible for the production of estrogens in post menopausal women. Exemestane is an oral irreversible inactivator of aromatase that depletes plasma estrogen by more than 90% and whole body aromatization by 98%. Unlike reversible AI(s), it cannot be displaced from the aromatase enzyme. At clinically administered doses, the plasma half-lives of anastrozole (1 mg once daily), letrozole (2.5 mg once daily), and exemestane (25 mg once daily) were 41-48 hours, 2-4 days, and 27 hours, respectively. The time to steady-state plasma levels was 7 days for both anastrozole and exemestane and 60 days for letrozole. Androgenic side effects have been reported only with exemestane [3]. There is evidence in postmenopausal women with metastatic disease that AI(s) produce response rates and survival equivalent to or superior to those seen with tamoxifen [4, 5]. Letrozole is up to 150-250 times more potent than the first generation aromatase inhibitor aminoglutethimide (AG), *in vitro* and more than 10,000 times as potent as AG in inhibiting aromatase *in vivo* [6].

The high potency of letrozole is not accompanied by any significant effect on adrenal steroidogenesis *in vitro* or *in vivo* over its maximally effective dose range [7, 8]. Inhibition of adrenal steroidogenesis resulting in adrenal hypertrophy does occur with therapeutic doses of AG. The high potency and selectivity of letrozole explains its pharmacological profile and high therapeutic index. In postmenopausal patients with advanced breast cancer, daily doses of 0.1 mg to 5 mg letrozole suppressed plasma levels of estradiol, estrone and estrone sulfate to 75-95% from baseline [9]. Estrogen suppression was maintained throughout the treatment period of 28 days in all patients.

Letrozole is a highly selective inhibitor of the aromatase enzyme. No clinically relevant changes in the plasma levels of cortisol, aldosterone, 11-deoxycortisol, 17-

hydroxyprogesterone, ACTH or plasma renin activity were found in postmenopausal patients treated with a daily dose of letrozole ranging from 0.1 to 5 mg [10, 11].

For postmenopausal women with endocrine responsive disease, the use of letrozole has been shown to yield some advantage in terms of treatment outcome as compared to tamoxifen in advanced disease [3] and preoperative setting where a double-blinded, randomized phase III trial of primary endocrine therapy was recently reported [12]. Letrozole 2.5 mg or tamoxifen 20 mg were given daily for 4 months to postmenopausal women with hormone receptor–positive breast cancer who were ineligible for breast-conserving surgery [12]. Among the 250 patients who received letrozole, 60% responded and 48% underwent successful breast-conserving surgery. The response to tamoxifen was significantly lower in terms of response rate (41%), and proportion of patients eligible for breast saving surgery. Differences in response rates between letrozole and tamoxifen were most striking for tumors that overexpressed ErbB-1 and/or ErbB-2 and were ER positive (88% *v*s 21%, p= .0004). In this study, the incidence of adverse events (AEs) was the same (57%) for the letrozole and tamoxifen groups. The most commonly reported AEs in both groups were hot flushes, headache, and nausea. The frequency of AEs suspected to be related to the study drugs was comparable for both groups (38% and 34% in the letrozole and tamoxifen groups, respectively). No other treatment-related effects with either of the study drugs were seen. Four patients discontinued study medication because of AEs (one patient in the letrozole group for a pulmonary embolism and 3 patients in the tamoxifen group for hepatitis C, erythema multiforme or cholestasis) [12].

1.3. Aromatase inhibitors in the adjuvant setting

Trials comparing AIs to tamoxifen for postmenopausal women in the adjuvant setting are mature. AIs have been studied in the adjuvant setting either as alternatives to tamoxifen or as sequential therapy after tamoxifen among postmenopausal women. The Arimidex, Tamoxifen Alone or in Combination (ATAC) trial compared primary use of an aromatase inhibitor, anastrozole, with either tamoxifen alone or the combination of the two as adjuvant therapy for early-stage breast cancer. The ATAC trial demonstrated improvements in disease-free survival (hazard ratio = 0.82) with use of anastrozole as monotherapy [13-15]. There was no benefit to combining the estrogen-deprivation effects of anastrozole with the antiestrogen effects of tamoxifen. Patients who were treated with anastrozole had significantly lower incidences of the following predefined adverse events compared with those treated with tamoxifen: hot flushes, vaginal bleeding, vaginal discharge, endometrial cancer, ischemic cerebrovascular events, venous thromboembolic events, and deep venous thromboembolic events. Women who were treated with anastrozole had significantly higher incidences of arthralgia and fractures than those treated with the bone-sparing agent tamoxifen; however, the risk ratio for fractures relative to tamoxifen has been shown to stabilize with treatment duration beyond two years.

The Breast International Group (BIG) 1-98 trial has recently reported on the use of the aromatase inhibitor letrozole compared with tamoxifen as primary therapy for early-stage breast cancer [16, 17]. 8028 women were randomized between March 1998 and May 2003 to receive five years of adjuvant endocrine therapy with letrozole, tamoxifen, or a sequence of these agents. Of these, the 4922 patients allocated continuous therapy with either letrozole or tamoxifen were recently analyzed [16]. At a median follow-up of 51 months, 352 DFS events

among 2463 patients allocated letrozole and 418 events among 2459 patients allocated tamoxifen were observed. This reflected an 18% reduction in the risk of an event (hazard ratio 0.82; 95 percent confidence interval 0.71 to 0.95; *P*= 0.007). Hazard ratios for the other defined endpoints were similar, though those for overall survival and systemic disease-free survival were not statistically significant. No subgroups showed significantly different relative efficacy; in particular, no significant heterogeneity was observed by nodal involvement status or progesterone receptor status.

Compared to patients receiving tamoxifen, more patients receiving letrozole reported at least one adverse event (AE) of any grade (2292 patients *vs* 2165 patients). Patients on tamoxifen experienced significantly more thromboembolic events, endometrial pathology, hot flushes, night sweats and vaginal bleeding. Patients on letrozole experienced significantly more bone fractures, arthralgia, low-grade cholesterol elevation, and cardiovascular events other than ischemic heart disease and cardiac failure. The relatively higher recording of low-grade cholesterol elevation on letrozole may be largely an artifact reflecting a cholesterol-lowering effect of tamoxifen. The overall incidence of cardiac failure did not differ significantly between the two arms.

Different trials have reported that the use of tamoxifen followed by aromatase inhibitor therapy may be clinically advantageous. The Intergroup Exemestane Study (IES) compared sequential treatment strategies. Patients in the IES trial had received 2 to 3 years of tamoxifen without evidence of tumor recurrence before random assignment to either ongoing tamoxifen treatment or to the aromatase inhibitor exemestane. Cross over from tamoxifen to exemestane yielded improved disease-free survival. Switching to exemestane was associated with a significantly lower incidence of gynecological symptoms, vaginal bleeding, muscle cramps and thromboembolic events compared with continued tamoxifen. However, switching to exemestane was associated with a significantly higher incidence of arthralgia, diarrhea and visual disturbances compared with continued tamoxifen. There was a suggestion towards increased osteoporosis (7.4% *vs* 5.7%; *P*=0.02) and higher fracture rates in the exemestane group than in the tamoxifen group (3.1% *vs* 2.3%; *P*=0.08) [18]. The updated safety data confirmed that patients switching to exemestane experienced fewer gynecological symptoms, vaginal bleeding, muscle cramps and thromboembolic events compared with continued tamoxifen. Switching to exemestane continued to be associated with a significantly higher incidence of diarrhea and arthralgia compared with continued tamoxifen. In addition, other musculoskeletal adverse events were more common with exemestane (with the exception of muscle cramps). Importantly, exemestane was associated with a higher incidence of myocardial infarction compared with tamoxifen, although it remains a possibility that this is attributable to the protective effects of tamoxifen, reducing the atherogenic risk profile. The Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 8/Arimidex-Nolvadex (ARNO) 95 trial also examined sequential therapy, either switching patients from tamoxifen to anastrozole after 2 years or leaving patients on tamoxifen for a total of 5 years. The cross-over improvement resulting from sequential switching of treatment at 2 years in the ABCSG/ARNO trial was slightly more favorable than the improvement seen in the IES trial (hazard ratio, 0.60 *vs* 0.67, respectively) [19]. In the related but much smaller Italian Tamoxifen Anastrozole (ITA) trial among women with lymph node–positive breast cancer who were free of recurrence after 2 to 3 years of tamoxifen treatment, patients were randomly assigned to either ongoing tamoxifen or

cross over to anastrozole resulting in significantly improved disease free survival with the introduction of anastrozole [20].

1.4. Extended treatment with letrozole after 5 years of tamoxifen

The MA.17 trial, led by the National Cancer Institute of Canada, was open to women who had completed 5 years of tamoxifen as primary adjuvant therapy for early-stage breast cancer and who were without clinical evidence of recurrence. These patients were randomly assigned to either extended adjuvant therapy with the aromatase inhibitor, letrozole, or placebo. After 2 to 3 years of follow-up, extended treatment with letrozole after 5 years of tamoxifen demonstrated a reduction in the risk of both locoregional and distant breast cancer recurrence compared with placebo. For the sequential strategy of 5 years of tamoxifen followed by an aromatase inhibitor, the recurrence rate associated with cross over to an aromatase inhibitor was 43% lower than the recurrence rate of tamoxifen alone (hazard ratio = 0.57) [21]. Letrozole was associated with a significantly higher incidence of hot flushes, arthritis, arthralgia and myalgia, but a significantly lower incidence of vaginal bleeding compared with placebo. There were no significant differences between letrozole and placebo in the incidence of osteoporosis (5.8% *vs* 4.5%; P=0.07) or fracture rates (3.6% *vs* 2.9%; P=0.24). Efficacy and safety data from an update of the MA.17 trial (median follow-up of 2.5 years) were consistent with the initial analysis. In addition, newly diagnosed osteoporosis was higher in the letrozole group compared with placebo (P=0.003), although the incidence of bone fractures was similar for both groups (P=0.25) [22].

1.5. Optimal sequence of endocrine therapy in a low estrogen environment

Recent evidence suggests that estradiol is capable of inducing programmed cell death (i.e., apoptosis) in breast cancer cells that have developed resistance following extensive antihormonal therapy. In particular, cells that are maintained estrogen-free for years initially start to grow spontaneously; in this case, even minimal concentrations of estrogen produce a cytocidal effect on cells that are exhaustively deprived of estrogen [23-25]. An antitumor action was observed also for physiological levels of estradiol on breast tumors grown in athymic mice [26].

Clinical observations also indicate an antitumor activity of estradiol, supporting a role for intermittent treatment with antiaromatase therapy. In fact, a small study of high-dose estrogen therapy following exhaustive antihormonal therapy was recently reported. Evaluation of response was performed after 3 months on therapy. On 26 evaluable patients, 4 patients obtained complete response and 6 patients partial response. In addition, two patients had stable disease for ≥6 months duration. [27].

These new data suggest a rational approach for the treatment of patients with ER-positive breast cancer during extensive antihormonal therapy. Low-dose estrogen levels (achievable through interruptions of treatment with AIs) could be used to induce apoptosis in breast cancer cells that might have developed resistance following extensive antihormonal therapy.

The 3 months interruption after 9 months of letrozole is based on the prolonged estradiol and estrone suppression observed after a single administration of letrozole in healthy postmenopausal women. In these subjects estradiol was maintained suppressed 2 weeks after a single dose of letrozole [28]. Moreover, the maximal response to estradiol of breast tumors transplanted into athymic mice was observed after 4 weeks of treatment [29]. Finally, as mentioned above, a clinical effect of high-dose estrogen therapy following exhaustive antihormonal therapy was observed after 3 months of treatment.

1.6. Cost-effectiveness of intermittent delayed letrozole

Modelled analyses from the UK and the US suggest that, in postmenopausal women with hormone-receptor-positive early-stage breast cancer, letrozole as extended adjuvant therapy after tamoxifen, rather than no further treatment, is a cost-effective treatment strategy. Sensitivity analyses have shown these results to be robust [30]. However, a recent study compared the efficiency of adjuvant therapy with AIs or with tamoxifen in postmenopausal women with operable breast cancer and positive estrogen receptors. The follow-up of a hypothetical cohort of women starting treatment at 63 years of age was simulated during 10 and 20 years. The probabilities and costs of transition between health states and quality adjusted life years (QALYs) were evaluated. The cost of gaining one QALY was lower with the introduction of exemestane after tamoxifen than with letrozole after 5 years of tamoxifen, indicating that the latter option might be less cost-effective [31]. The introduction of a regimen that decreases to 75% the yearly amount of letrozole may improve the cost-effectiveness of extended letrozole administration.

2. Trial objectives

This trial will compare continuous letrozole for five years with intermittent letrozole over a five year period for postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive node-positive operable breast cancer.

2.1. Primary endpoint

Disease-free survival (includes second (non-breast) malignancies and deaths)

2.2. Secondary endpoints

2.2.1. Overall survival

2.2.2. Distant disease-free survival

2.2.3. Breast cancer free interval (events are reappearance of invasive breast cancer at any site including contralateral disease)

2.2.4. Sites of first DFS failure

2.2.5. Second (non-breast) malignancies

2.2.6. Deaths without prior cancer event

2.2.7. Adverse events

3. Patient selection: criteria for patient eligibility/ineligibility

3.1. Patient characteristics

3.1.1. Patients must be postmenopausal using any one of the following criteria. Because letrozole is not effective in pre- or perimenopausal patients, and may stimulate ovarian function, definitive confirmation of postmenopausal status is required.

- Patients of any age who have had a bilateral oophorectomy (including radiation castration AND amenorrheic for > 3 months)
- Patients 56 years old or older. If the patient has any evidence of ovarian function, biochemical evidence of definite postmenopausal status (defined as estradiol, LH, and FSH in the postmenopausal range) is required.
- Patients 55 years old or younger must have biochemical evidence of definite postmenopausal status (defined as estradiol, LH, and FSH in the postmenopausal range. Patients who have received prior LHRH analogue within the last year are eligible if they have definite evidence of postmenopausal status as defined above.

3.1.2. Patients must be accessible for follow-up.

3.2. Disease characteristics

3.2.1. At diagnosis, patients must have had operable, non-inflammatory breast cancer.

3.2.2. Patients must be clinically disease-free at randomization. (Note: It is recommended but not required that disease-free status be verified by abdominal ultrasound, chest x-ray, and bone scan (if symptomatic). A mammogram within one year prior to randomization is recommended.)

3.2.3. Patients must have had steroid hormone receptor positive tumors (ER and/or PgR), determined by immunohistochemistry, after primary surgery and before commencement of prior endocrine therapy.

3.2.4. Following primary surgery, eligible patients must have had evidence of lymph node involvement either in the axillary or internal mammary nodes, but not supraclavicular nodes.

3.2.5. There must have been no evidence of recurrent disease or distant metastatic disease at any time prior to randomization.

3.2.6. Not eligible: Patients who have had bilateral breast cancer.

3.3. Prior surgery and radiotherapy

3.3.1. Patients must have had proper local treatment including surgery with or without radiotherapy for primary breast cancer with no known clinical residual loco-regional disease.

3.4. Prior/concurrent disease and conditions

3.4.1. Patients must have clinically adequate hepatic function.

3.4.2. Not eligible: Patients who have had a bone fracture due to osteoporosis at any time during the 4-6 years of prior endocrine SERM/AI therapy.

3.4.3. Not Eligible: Patients who have had any previous or concomitant malignancy EXCEPT adequately treated: basal or squamous cell carcinoma of the skin, in situ carcinoma of the cervix or bladder, contra- or ipsilateral in situ breast carcinoma.

3.4.4. Not eligible: Patients who have had any other non-malignant systemic diseases (cardiovascular, renal, lung, etc.) that would prevent prolonged follow-up.

3.4.5. Not eligible: Patients with psychiatric, addictive, or any disorder which compromises compliance with protocol requirements.

3.5. Prior treatment

3.5.1. Patients must have completed 4 to 6 years of prior adjuvant endocrine therapy with SERM(s), aromatase inhibitor(s), or a sequential combination of both. When calculating 4-6 years, neoadjuvant endocrine therapy should not be included.

3.5.2. Patients must have stopped prior endocrine SERM/AI therapy, and must be randomized within 12 months (1 year) of the last dose of prior endocrine SERM/AI therapy.

3.5.3. Patients may have received any type of prior adjuvant therapy, including but not limited to neoadjuvant chemotherapy, neoadjuvant endocrine therapy, adjuvant chemotherapy, trastuzumab, ovarian ablation, GnRH analogues, lapatinib.

3.6. Concurrent treatment

3.6.1. Patients must have stopped hormone replacement therapy (HRT), bisphosphonates

(except for treatment of bone loss), or any investigational agent at randomization. (Note: These agents are also not permitted during trial treatment.)

3.7. Protocol requirements before randomization

3.7.1. Pathology material from the primary tumor must be available for submission for central review as part of the quality control measures for this protocol.

3.7.2. Written Informed Consent (IC) must be signed and dated by the patient and the investigator prior to randomization.

3.7.3. Written consent to pathology material submission, indicating the patient has been informed of and agrees to tissue material use, transfer and handling, must be signed and dated by the patient and the investigator prior to randomization.

4. Randomization and stratification

This trial will use a web-based randomization system. Each Participating Group will determine how its Participating Centers will access the randomization system, either through a Group Randomization Center, or directly from the Participating Center. The following procedures should be used in either case. Specific details for randomizing are in the "IBCSG Registration/Randomization Procedures Manual," which is available on the IBCSG website (www.ibcsg.org).

4.1. Randomization timing

In principle, patients should start trial treatment as soon as possible after randomization. Trial treatment should begin no later than 6 weeks from the date of randomization.

4.2. Registration procedures

Complete the following steps to randomize a patient on this trial.

4.2.1. Verify eligibility.

4.2.2. Obtain written informed consent both for the clinical trial and the pathology material submission, signed and dated by the patient and physician

4.2.3. Complete Confirmation of Registration Form (A). The date the Informed Consent Form and the consent to pathology material submission section of the Informed Consent Form were signed by the patient and the date signed by the investigator are both required to complete randomization.

4.2.4. Depending on your Group's choice, either

- Telephone or fax your Randomization Center to review the eligibility and randomization information. Your Randomization Center will access the IBCSG Registration/Randomization System.
- Directly access the IBCSG Registration/Randomization System.

In the former case, the Randomization Center will provide the Participating Center with the following information. In the latter case the Randomization System will provide this information via e-mail.

- Randomization number (patient ID)
- Treatment assignment
- Date of randomization

4.2.5. When randomization is complete, fill in Confirmation of Registration Form (A) with the information above and fax Confirmation of Registration Form (A) and Pathology Material Consent Form (PMC) to an IBCSG DataFax number. These forms are considered the essential documents for regulatory purposes. They should be filed at your institution.

4.2.6. File original of all forms.

4.3. Randomization help desk

The IBCSG Data Management Center (located at FSTRF) is responsible for developing and maintaining the IBCSG Registration/Randomization System. The Randomization Help Desk includes technical personnel and administrators of the randomization programs at the Data Management Center in Amherst, NY, USA.

Business Hours: 7:30-18:00 US Eastern Time

FSTRF Randomization Help Desk
Frontier Science & Technology Research Foundation (FSTRF)
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716 834 0900 ext. 301
Fax: +1 716 834 8432
Email: bc.helpdesk@fstrf.org

4.4. Randomized groups

Randomization (1:1) to 2 groups:

Arm A: Continuous letrozole for 5 years

Arm B: Intermittent letrozole over a 5 year period

4.5. Stratification

4.5.1. Institution

4.5.2. Prior endocrine SERM/AI therapy (SERM(s) and aromatase inhibitor(s) only)

- SERM(s) alone (without AI(s))
- AI(s) alone (without SERM(s))
- Both SERM(s) and AI(s), each for at least 1 month

5. Treatment details

5.1. Trial treatments

Arm A: Continuous letrozole 2.5 mg daily for 5 years

Arm B: Intermittent letrozole 2.5 mg daily for the first 9 months of years 1 through 4, followed by 12 months in year 5

Compliance: At each visit, patients should return the last dispensed drug container. The investigator or designee will count the remaining pills and record the information on the CRF.

5.2. Side effects of letrozole

5.2.1. Adverse effects [6]

Safety data of letrozole are available from a wide range of clinical trials in first-line and second-line advanced breast cancer, with adjuvant and extended adjuvant treatment as well as from post-marketing experience.

Approximately one third of the patients with metastatic breast cancer treated with 2.5 mg letrozole, 40% of the patients under adjuvant letrozole and 70-75% of the patients under letrozole following standard adjuvant tamoxifen (extended adjuvant therapy) experienced adverse events.

The most frequent adverse experiences reported during the course of clinical trials irrespective of causality were hot flushes, musculoskeletal disorders (bone pain, back pain, arthralgia), nausea, dyspnea, and fatigue.

The following adverse reactions were observed during clinical trials and in the postmarketing phase:

Very common (≥ 10%): Arthralgia, hot flushes.

Common (1 – 10%): Myalgia, bone pain, osteoporosis, bone fractures, fatigue, peripheral edema, elevated serum cholesterol, increased appetite, weight gain, anorexia, depression,

headache, dizziness, nausea, vomiting, dyspepsia, constipation, diarrhea, alopecia, increased sweating, rash.

Uncommon (0.1 – 1%): Arthritis, thrombophlebitis (including superficial and deep vein thrombosis), hypertension, ischemic cardiac events (including angina pectoris, myocardial infarction, cardiac failure), palpitations, tachycardia, cerebrovascular accident, somnolence, insomnia, memory impairment, dysesthesia, taste disturbance, general edema, cataract, eye irritation, blurred vision, anxiety, dyspnea, increased hepatic enzymes, weight loss, abdominal pain, stomatitis, dry mouth, vaginal bleeding, vaginal discharge, vaginal dryness, breast pain, pruritus, dry skin, urticaria.

Rare (0.01 – 0.1%): Pulmonary embolism, arterial thrombosis, transient ischemic attack (cerebrovascular infarction).

For the extended adjuvant therapy after standard adjuvant tamoxifen no significant qualitative differences with respect to the general safety profile were found. The most frequently observed adverse events were hot flushes (49.7%), fatigue (33.6%), arthralgia/arthritis (28.7%) night sweats (24.2%), edema (18.4%), headache (20.1%) hypercholesterolemia (15.5%), dizziness (14.2%), constipation (11.3%), nausea (8.6%), and myalgia (6.7%). Of these common adverse events, hot flashes (49.7 % vs. 43.3%), arthralgia/arthritis (27.7 % vs. 22.2 %) and myalgia (9.5% vs. 6.7 %) occurred at a significantly higher incidence under letrozole than placebo.

In the adjuvant setting, hot flashes (33.7%), arthralgia/arthritis (21.2%), night sweating (13.9%), weight increase (10.7%), nausea (8.8%), bone fractures (5.7%) and fatigue (5.3%) were the most common reported adverse events. Compared to tamoxifen, bone fractures (5.7% vs 4%), arthralgia (21.2% vs 13.5%) and – although a rare event – osteoporosis (2.0% vs 1.1%) were significantly more frequent under letrozole. Conversely, the incidence of hot flashes, night sweats, thromboembolic events (1.2% vs 2.8%), endometrial cancer (0.2% vs 0.4%) and endometrial proliferative disorders (0.3% vs 1.8%) was higher for tamoxifen. Myocardial infarctions were seen at similar rates (0.6% vs 0.4%).

Patients receiving letrozole had less secondary malignancies reported at any time after randomization (1.9% vs 2.4%) with endometrial cancer being the most common (0.4 vs 0.2%).

Of the non-breast cancer related deaths, deaths related to other second (non-breast) malignancy and cardiovascular cause were most frequently reported.

5.2.2. Drug Interactions

Letrozole inhibits in vitro the cytochrome P450-isoenzymes 2A6 and moderately 2C19, however, CYP2A6 does not play a major role in drug metabolism. In in vitro experiments letrozole did not substantially inhibit the metabolism of diazepam (a substrate of CYP2C19) at concentrations approximately 100-fold higher than those observed in plasma at steady-state. Thus, clinically relevant interactions with CYP2C19 are unlikely to occur. Nevertheless, caution should be used in the concomitant administration of drugs whose disposition is mainly dependent on these isoenzymes and whose therapeutic index is narrow.

There was no evidence of other clinically relevant interaction in patients receiving other commonly prescribed drugs (e.g. benzodiazepines; barbiturates; NSAIDs such as diclofenac sodium, ibuprofen; paracetamol; furosemide; omeprazole).

Clinical interaction studies with cimetidine and warfarin indicated that the coadministration of letrozole with these drugs does not result in clinically significant drug interactions, even though cimetidine is a known inhibitor of one of the cytochrome P450 isoenzymes capable of metabolising letrozole in vitro.

There is no clinical experience to date on the use of letrozole in combination with other anti-cancer agents.

5.3. Concomitant treatments

5.3.1. Patients may not receive HRT, bisphosphonates (except for the treatment of bone loss) or any other investigational agent during trial treatment.

5.3.2. Patients may not receive any SERMs or AIs except for protocol-specified letrozole during trial treatment.

5.4. Study drug supply

Study drug will be supplied by Novartis. Details of drug supply, drug accountability and compliance are described in Appendix V.

6. End points and definitions of treatment failure

6.1. Trial end points

6.1.1. Primary Endpoint:

Disease-free survival (DFS) is defined as the time from randomization to local (including invasive recurrence restricted to the breast after breast conserving treatment), regional or distant relapse, contralateral breast cancer, appearance of a second (non-breast) malignancy, or death from any cause, whichever occurs first. Appearance of DCIS or LCIS either in the ipsilateral or in the contralateral breast will not be considered as an event for DFS (but must be reported on the Follow-Up Form (E)). See Section 6.3 for other exceptions.

6.1.2. Secondary end points:

- Overall survival (OS) is defined as the time from randomization to death from any cause.
- Distant disease-free survival (DDFS) is defined as the time from randomization to any recurrent or metastatic disease in distant sites (i.e., other than the local mastectomy scar/chest wall/skin, the ipsilateral breast in case of breast

conservation, or the ipsilateral axilla and internal mammary lymph nodes), second (non-breast) malignancy, or death from any cause, whichever occurs first.

- Breast cancer free interval (BCFI) is defined as the time from randomization to local (including recurrence restricted to the breast after breast conserving treatment), regional, or distant relapse, or contralateral breast cancer. In calculating BCFI, second (non breast) malignancies are ignored and deaths without cancer event are censored at the time of death as a competing event. Appearance of DCIS or LCIS either in the ipsilateral or in the contralateral breast is not considered a BCFI event, but should be recorded on the Follow-up Form (E).
- Sites of first DFS failure.
- Second (non-breast) malignancies
- Deaths without prior cancer event
- Adverse events

6.2. Diagnosis of Events

The diagnosis of failure event depends on evidence of recurrent disease which can be classified as either suspicious or acceptable. In either case, this should be specified and reported. Acceptable evidence of failure according to site is defined below. Any events not included in this section are considered unacceptable as evidence of recurrent disease. Failures include: local, regional, contralateral breast, and distant failures, second (non-breast) primaries, and deaths without recurrence. Histological confirmation of cytological evidence of recurrence is recommended in easily accessible lesions.

The date of failure event is the time of first appearance of a suspicious lesion, later proven to be a definitive recurrence or metastasis. All events described below should be recorded on the Follow-up Form (E).

6.2.1. Local failure

Local failure is defined as a tumor recurrence in any soft tissues of the ipsilateral, conserved breast or the chest wall, mastectomy scar, and/or skin.

Acceptable for recurrence in conserved breast: positive cytology or histology.

Acceptable for recurrence in chest wall, mastectomy scar, and/or skin: positive cytology or histology or evidence of new lesions (by CT or MRI) without any obvious benign etiology.

Suspicious: a visible or palpable lesion.

Appearance of DCIS or LCIS either in the ipsilateral or in the contralateral breast is not considered a BCFI event, but should be recorded on the Follow-up Form (E).

6.2.2. Regional Failure

Regional failure is defined as a tumor recurrence in the ipsilateral axillary lymph nodes, extranodal soft tissue of the ipsilateral axilla, ipsilateral internal mammary lymph nodes, and/or ipsilateral supraclavicular lymph nodes.

Acceptable: positive cytology or histology or evidence of new lesions by CT or MRI without a benign etiology.

Suspicious: a visible or palpable lesion.

6.2.3. Contralateral breast failure

Acceptable: positive cytology or histology.

Suspicious: a visible or palpable lesion, suspicious mammogram, ultrasound, or MRI.

Appearance of DCIS or LCIS in the contralateral breast is not considered an event for DFS.

6.2.4. Distant failure

Tumors in all areas other than those defined above are considered distant metastases. The following criteria apply:

6.2.4.1. Bone marrow

Acceptable: positive cytology, aspiration, or biopsy.

Suspicious: unexplained depression of peripheral blood counts and/or a leucoerythroblastic blood picture.

6.2.4.2. Lung

Acceptable: positive cytology or histology or a positive CT or MRI without obvious benign etiology or evidence of progressive disease. (Progressive disease is confirmed by two consecutive X-rays with the second showing worsening disease.)

Suspicious: new radiological lesion(s).

6.2.4.3. Pleura

Acceptable: positive cytology or histology.
Suspicious: new pleural effusion.

6.2.4.4. Bone

Acceptable: positive cytology or histology or a positive X-ray, MRI, or CT, one bone scan with new multiple lesions and no obvious benign etiology.

Suspicious: skeletal symptoms or positive scan showing only one new lesion (until confirmed by other imaging study).

6.2.4.5. Liver

Acceptable: positive cytology or histology, or positive CT or MRI without an obvious benign etiology, or evidence of progressive disease by ultrasound. (Progressive disease in this case is confirmed by two ultrasounds with the second showing worsening disease).

Suspicious: any two of the following: hepatomegaly on physical examination, equivocal ultrasound and abnormal liver function test.

6.2.4.6. Central nervous system

Acceptable: positive cytology or histology. Positive MRI or CT when the clinical picture is suspicious.

Suspicious: any other clinical findings suggestive of this diagnosis.

6.2.4.7. Distant lymph nodes

Acceptable: positive cytology or histology, or enlarged lymph nodes in CT or MRI, or progressive disease by physical exam without an obvious benign etiology.

Suspicious: evidence of enlarged lymph nodes by physical exam.

6.2.4.8. Other sites

Acceptable: positive cytology or histology or evidence of progressive disease if only indirect means of diagnosis were used (e.g., X-ray).

Suspicious: clinical and radiological evidence of a tumor.

6.3. Other Events

6.3.1. Second (non-breast) malignancy

Any positive diagnosis of a second (non-breast) malignancy other than basal cell or squamous cell carcinoma of the skin, cervical carcinoma *in situ,* or bladder cancer *in situ* is considered an DFS event and should be reported on the Follow-up Form (E) and on the Serious Adverse Event Forms (SAE-A and SAE-B).

6.3.2. Death without prior cancer event

Any death without a prior cancer event described in 6.2.1 through 6.2.4 above is considered a DFS event. The death, date, and the cause should be reported on the Follow-up Form (E) regardless of whether it occurs during or after trial treatment, and on the Serious Adverse Event Forms (SAE-A and SAE-B) if occurring during trial treatment.

6.3.3. Other noteworthy events

These events are NOT considered endpoints in this trial, but must be recorded on the Follow-up Form (E).

- Ipsilateral and contralateral breast cancer *in situ*
- Cervical carcinoma in situ, bladder cancer *in situ*
- Basal or squamous cell carcinoma of the skin

7. Study parameters

7.1. Table of study parameters

Visit	1 A	2	3	4	5	6	7	8	9	10	11	Yearly until death
Year	0	1	1	2	2	3	3	4	4	5	5	
Trial month	0	6	12	18	24	30	36	42	48	54	60	
Informed consent and pathology material consent	x											
Check of inclusion & exclusion criteria	x											
History	x											
Physical examination including weight	x	x	x	x	x	x	x	x	x	x	x	x
Estradiol, FSH, LH[B]	m											
Adverse Events (AE)[C]	x	x	x	x	x	x	x	x	x	x	x	
Late AEs[D]												x
Laboratory tests												
Hematology[E]	r	m	m	m	m	m	m	m	m	m	m	
Blood chemistry[F]	r	m	m	m	m	m	m	m	m	m	m	
Investigations												
Mammogram[G]	r		r		r		r		r		r	
Chest-X-ray[H] (PA and lateral views)	r	m	m	m	m	m	m	m	m	m	m	
Bone scan[I]	m	m	m	m	m	m	m	m	m	m	m	
Abdominal US, CT or liver scan[J]	r	m	m	m	m	m	m	m	m	m	m	
Gynecological exam[K]	m		m		m		m		m		m	
Bone mineral densitometry[L]	m		m		m		m		m		m	

x = mandatory
r = recommended
m = if medically indicated

Legend to Table 7.1

A. The day of randomization is considered Day 0 for the purpose of follow-up.

B. Biochemical evidence of definite postmenopausal status, defined as estradiol, FSH, and LH in the postmenopausal range, is required at study entry for some patients as defined in section 3.1.1.

C. Adverse events should be graded using the NCI CTCAE V.3 (Appendix II). The following targeted adverse events should be recorded on the CRF during the reporting period in which they occur:

- Hot flashes/flushes
- Osteoporosis

- Bone fracture
- Musculoskeletal symptoms (myalgia, arthralgia (joint pain), stiffness not including bone fractures)
- Mood alteration / depression
- Hypertension
- Cardiac ischemia/infarction
- Thrombosis / thrombus / embolism
- CNS cerebrovascular ischemia
- Hemorrhage, CNS
- Insomnia
- Fatigue
- Bone pain
- Other Grade 3 or higher adverse events

D. Late adverse events (adverse events occurring after trial treatment is completed) should be recorded on Follow-up Form (E).

E. Hematology is recommended within 2 months prior to randomization and should be done whenever medically indicated.

F. Blood chemistry (includes liver function tests with alkaline phosphatase) is recommended within 2 months prior to randomization and should be done whenever medically indicated.

Radiological assessments

G. A bilateral mammography is recommended within one year prior to randomization. A mammography of the conserved and contralateral breast is recommended at yearly intervals or should be done according to national standards or hospital specific requirements.

H. A chest X-ray is recommended prior to randomization. A chest X-ray should be performed any time it is medically indicated or according to specific local requirements. Both PA view and lateral view should be done.

I. A bone scan should be done at baseline if clinically indicated. A bone scan should be performed during treatment with trial drug if alkaline phosphatase is significantly elevated (e.g. > 3 x ULN) or if medically indicated otherwise (e.g. bone pain). If the bone scan showed areas suspicious for tumor then these areas should be confirmed by X-ray or CT or MRI.

J. Abdominal ultrasound or liver scan or abdominal CT is recommended prior to randomization or during treatment if liver function tests are significantly abnormal or if medically indicated or according to specific local requirements.

Other procedures

K. In the event of a pelvic complaint (i.e., abnormal vaginal bleeding) patients should have a gynecological examination.

L. Bone densitometry by DEXA should be done at baseline and then yearly for 5 years if medically indicated.

7.2. Adverse event reporting

The main criterion for tolerability is the occurrence of toxicities and adverse events. The severity and causality will be classified according to the NCI CTCAE Version 3.0. The CTCAE is available for downloading on the internet at (http://ctep.cancer.gov/reporting/ctc.html).

An adverse event is defined as any untoward medical occurrence that occurs from the first dose of study medication until 30 days after the final dose, regardless of whether it is considered related to a medication.

In addition, any known untoward event that occurs subsequent to the adverse event reporting period that the investigator assesses as possibly related to the protocol treatment should be considered an adverse event.

Symptoms of the targeted cancer (if applicable) should not be reported as adverse events.

The adverse event severity grade provides a qualitative assessment of the extent or intensity of an adverse event, as determined by the investigator or as reported by the subject. The severity grade does not reflect the clinical seriousness of the event, only the degree or extent of the affliction or occurrence (e.g. severe nausea, mild seizure), and does not reflect the relationship to study drug.

Severity grade for other adverse events, not covered in the toxicity grading scale:

1 = Grade 1	Mild
2 = Grade 2	Moderate
3 = Grade 3	Severe
4 = Grade 4	Life-threatening
5 = Grade 5	Fatal

7.3. Serious Adverse Event (SAE) reporting

7.3.1. Definition

An SAE is defined in general as any undesirable medical occurrence/adverse drug experience that occurs during or within 30 days after stopping study treatment that, at any dose, results in any of the following:

- is fatal (any cause)
- life-threatening,
- requires or prolongs inpatient hospitalization,
- results in persistent or significant disability/incapacity
- is an unexpected grade 4 toxicity
- is a congenital anomaly or birth defect
- is a secondary (non-breast) malignancy
- requires significant medical intervention

Second (non-breast) malignancies are always considered SAEs, no matter when they are diagnosed. These events should be reported on the Serious Adverse Event Forms (SAE-A and SAE-B) and on the Follow-Up Form (E).

Other significant/important medical events which may jeopardize the patient are also considered serious adverse events.

Serious also includes any other event that the investigator or the IBCSG Safety Office judges to be serious or which is defined as serious by the regulatory agency in the country in which the event occurred.

An unexpected adverse event is one that is not listed as a known toxicity of the investigational drug in the summary of product characteristics.

A related adverse event is one for which the investigator assesses that there is a reasonable possibility that the event is related to the investigational drug. All adverse events judged as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

7.3.2. Exceptions to the definition

Any death or serious adverse event that occurs more than 30 days after stopping study treatment but is considered to be at least possibly related to previous trial treatment is also considered an SAE. All serious adverse events must also be reported for the period in which the trial protocol interferes with the standard medical treatment given to the patient. Cases of second (non-breast) malignancies and congenital abnormalities are to be regarded as SAEs, regardless of whether they occur during or after study treatment.

Events not considered to be serious adverse events are hospitalizations occurring under the following circumstances:

- elective surgery;
- occur on an outpatient basis and do not result in admission (hospitalization < 24 h);
- are part of the normal treatment or monitoring of the studied treatment;
- progression of disease.

7.3.3. Reporting SAEs

Any serious adverse event occurring in a patient after providing informed consent must be reported. Information about all serious adverse events will be collected and recorded on the IBCSG Serious Adverse Event Report Forms (SAE–A and SAE-B).

To ensure patient safety, the IBCSG must learn of each SAE using the procedures described below:

- The investigator/MD responsible for the patient must FAX a signed Serious Adverse Event Form (SAE-A) in English within 24 hours to the DataFax data submission fax number for the Participating Center. A copy is automatically forwarded to the IBCSG Coordinating Center for medical review.
- Follow-up information should be completed on the Serious Adverse Event Form (SAE-B) within 15 days of the initial report and must be faxed to the DataFax data submission fax number for the Participating Center. A copy is automatically forwarded to the IBCSG Coordinating Center. If the event is not resolved within 15 days, submit an additional Serious Adverse Event Form (SAE-B) to report the final resolution.
- If a non-serious adverse event becomes serious, this and other relevant follow-up information must also be provided within 24 hours.

The original Serious Adverse Event Forms (SAE-A and SAE-B) and the fax confirmation sheet(s) must be kept with the CRFs at the Participating Center.

The IBCSG Coordinating Center will inform Novartis Corporation and other appropriate persons about all SAEs related to trial medication (per either investigator or IBCSG medical review) within 24 hours of receipt at the IBCSG Coordinating Center.

The IBCSG Coordinating Center will record the SAE and prepare a summary report of all SAEs received at the end of each month. Principal Investigators will receive the summary report on a monthly basis, and these reports can be found on the IBCSG web site (www.ibcsg.org).

8. Data submission

We will conduct the trial according to the ICH Good Clinical Practice (GCP) guidelines. Keeping accurate and consistent records is essential to a cooperative study. The following forms are to be submitted at the indicated times by the participating institutions for each patient:

8.1. Case report forms schedule

RANDOMIZATION FORMS		
Informed Consent Form	Consent to participation in clinical trial	Obtain before randomization and keep with patient records.
Pathology material consent	Consent to submission of pathology material	Obtain before randomization and keep with patient records.
Form PMC	Pathology Material Consent Form	DataFax after randomization with Form 35-A.
Form 35-A	Confirmation of Registration Form	Fill in before contacting your Randomization Center or entering the IBCSG Registration/Randomization system to randomize. DataFax completed form for all patients randomized.
BASELINE FORMS		
Form 35-H	History Form	DataFax within 1 month of randomization.
Pathology Report	Pathology Report of original diagnosis	DataFax within 1 month of randomization. See Section 10 for pathology material requirements.
Form 35-AE	Adverse Event Form	Complete prior to starting protocol treatment (letrozole) and DataFax within 1 month of randomization. This form is also required at follow-up (see instructions below).
Form 35-CCM	Concomitant Medications Form	Complete prior to starting protocol treatment (letrozole) and DataFax within 1 month of randomization. This form is also required at follow-up (see instructions below).
FOLLOW-UP FORMS		
Form 35-E	Follow-Up Form	DataFax every 6 months during Years 1-5, and yearly until death.
Form 35-L	Letrozole Form	DataFax at each follow-up period until the completion of letrozole.
Form 35-AE	Adverse Event Form	DataFax at each follow-up period during protocol therapy (letrozole). This form is also required at baseline.
Form 35-CCM	Concomitant Medications Form	DataFax at each follow-up period during protocol therapy (letrozole). This form is also required at baseline.
EVENT-DRIVEN FORMS		
Form 35-SAE-A	Serious Adverse Event Form (A)	DataFax within 24 hours when SAE occurs, see Section 7.3.
Form 35-SAE-B	Serious Adverse Event Form (B)	DataFax within 15 days of the initial report and/or at the definitive SAE outcome, see Section 7.3.

The Data Managers' Manual for this trial contains instructions for submitting forms using the DataFax system.

8.2. Signing and submitting forms

All forms should be signed by the Principal Investigator or designee. An authorization log (see Appendix IV) should be completed at each participating center.

CRFs should be faxed to an IBCSG DataFax number. SAE Forms should also be faxed to an IBCSG DataFax number for automatic transmission to the IBCSG Coordinating Center. Full instructions on submitting forms will be distributed to each Participating Center and are available on the IBCSG website (www.ibcsg.org). Also available on the website is a list of fax numbers that are available for faxing CRFs.

For Centers participating through a Group: Please consult your Participating Group Specific Logistical Information (Appendix VI) for special instructions about how to submit data.

8.3. Data management

Data collected in this trial will be sent to the IBCSG Data Management Center in Amherst, NY USA. The Data Management Center will process the data and will generate queries and forms requests. The IBCSG Coordinating Center in Bern, Switzerland will provide medical review and summary of SAEs. The IBCSG Statistical Center in Boston, MA USA will perform the data analysis.

8.4. Investigators' file

Each Participating Center should keep documentation about this trial in an investigators' file, which should include the following documents:

- Protocol and appendices
- Amendments
- Signed Protocol Signature Pages
- Sample CRFs including blank SAE Forms
- Data Managers' Manual
- Obvious Corrections Document
- Randomization Manual
- Patient information and Informed Consent templates approved by Ethics Committee
- Investigator's Brochure and updates
- Ethics Committee approval of protocol, Patient Information Sheet and IC, amendments
- Ethics Committee review of SAE, investigators' alert, and other documents
- Correspondence with Ethics Committee
- Malpractice insurance information
- Agreement with IBCSG
- Correspondence with IBCSG Coordinating Center, Data Management Center
- SAE reports from IBCSG Coordinating Center
- Accrual reports from IBCSG

- Normal laboratory values
- Laboratory Certifications
- CV of Principal Investigator and co-investigators
- Authorization log
- Patient identification log
- Drug accountability log (incl certificates of destruction if applicable)
- ICH GCP guidelines/Declaration of Helsinki and updates
- Audits/monitoring reports

8.5. Authorization log

The Principal Investigator (PI) should identify the other members of the Clinical Trial Team who are supervised by the PI and approved to provide information in CRFs, queries, etc. (See template in Appendix IV.)

8.6. Patient identification log

As per GCP, patients have the right to confidentiality. Therefore, no patients' names should be used in CRFs or any other documentation transmitted to IBCSG central offices. Items that are used to identify a patient include initials of patient's name, date of birth, randomization number. When no names are used, at least 2 of the above are usually required to identify the patients' records. It is therefore imperative that the local data manager keeps an identification log for all patients entered in this trial including:

- Patient's name
- Patient's initials
- Randomization number
- Date of birth

Other items that could be included are date of randomization and treatment arm.

9. Statistical considerations

9.1. Study design, objectives, and stratification

The SOLE trial is a multinational Phase III randomized clinical trial designed to compare continuous letrozole for 5 years with intermittent letrozole over a 5-year period among postmenopausal women who are disease-free following 4 to 6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive node-positive operable breast cancer. The hypothesis is that introducing 3 month treatment-free intervals during the course of five years of extended letrozole will improve disease-free survival.

Randomization will be stratified according to participating center and prior SERM/AI endocrine therapy use (SERM(s) alone, AI(s) alone, both SERM(s) and AI(s)).

9.2. Data analyses

The primary analysis will be undertaken with the intention-to-treat population of all randomized patients. The primary endpoint is disease-free survival (DFS: Section 6.1.1) and will be compared between treatment arms using a two-sided stratified logrank test with an overall experiment-wise alpha level equal to at most 0.05. Kaplan-Meier estimates of the DFS distributions will be calculated for each of the two treatment arms. Cox proportional hazards regression models will be used to investigate whether the treatment comparison is modified by adjustments for various covariates.

Other factors will be used to characterize the patients enrolled in the study and to provide descriptive statistics of outcomes according to subgroups of the population. These factors include age at randomization, body mass index, tumor size, tumor grade, number of positive lymph nodes, ER/PgR and HER2 status of the primary tumor, type of prior endocrine therapy, interval of time since the cessation of prior endocrine therapy. These analyses will be considered as secondary and descriptive.

The following secondary endpoints will be assessed: overall survival, distant disease-free survival, breast cancer-free interval, sites of first DFS failure, incidence of second (non-breast) malignancies, deaths without prior cancer event, incidence of targeted adverse events.

9.3. Sample size considerations

Postmenopausal women with hormone receptor positive early breast cancer continue to be at risk for disease recurrence following completion of 4 to 6 years of adjuvant endocrine therapy. The MA.17 trial provides an estimate for the baseline risk of an event defining DFS for patients enrolled in the continuous letrozole group. Overall, the 4-year DFS in the update of MA.17 was 94.4% [22]. Among the subgroup of patients with node-positive disease, the 4-year DFS was 91.8% [32]. DFS in the MA.17 trial considered only breast cancer recurrence and contralateral breast cancer as events; specifically it did not consider deaths prior to recurrence or second (non-breast) malignancies as events. Therefore, in the eligible population of patients with node-positive disease at initial diagnosis, the baseline risk following randomization used for sample size determination is assumed to be 90% at 4 years.

The sample size was determined to provide 80% power to detect a 20% reduction in the risk of an event defining DFS (Section 6) associated with intermittent letrozole compared with continuous letrozole (hazard ratio = 0.80; 25% increase in 4-year DFS from 90% to 91.917%) using a two-sided 0.05 level test of significance.

To achieve this goal requires 647 events defining DFS, assuming 4800 patients are accrued (1600 patients per year for 3 years), 5% non-assessability at 4 years, and approximately 5 years of additional follow-up [33]. One year of start-up time, as Participating Centers obtain Ethics Committee approval and complete regulatory processes, is anticipated.

9.4. Interim monitoring

A group sequential design with two interim analyses and one final analysis is used [33]. The target number of events for the final analysis is 647, and interim analyses are planned after 40% and 70% information (259 and 453 events observed respectively). At each interim analysis and at the final analysis, testing will be performed using O'Brien-Fleming boundaries [34].

9.5. Data and Safety Monitoring Committee (DSMC)

The study will be presented for review by the IBCSG Data and Safety Monitoring Committee (DSMC) at each of their semi-annual meetings. Accrual, safety, events, and deaths will be monitored. Analyses of efficacy according to randomization group will be presented only at the time points specified for formal interim analysis. The DSMC will also make recommendations concerning potential modifications to the design criteria for this study if the assumptions used in the design are found to be inaccurate. A formal review of the accrual rate will be performed two years after study activation to assess whether modifications are required.

10. Additional protocol-specific evaluations

10.1. Pathology and pathology material banking

10.1.1. Pathology requirements

The work of the pathologist is basic to the success of all studies. Each Participating Center should identify a pathologist responsible for study patients. The pathologist determines the diagnosis, classification, and grading of the primary tumor; and evaluates the non-tumor breast tissue and local or regional spread as found in the biopsy and/or mastectomy specimen, including precise documentation of tumor size, margins of the primary, the total number of lymph nodes examined, and the number of nodes involved. All lymph nodes must be examined from each patient. If the patient has received a sentinel node biopsy, each sentinel node must be evaluated. The central review pathologist will review the submitted specimens and complete the central pathology review.

The following items are required for all patients:

1. Pathology Reports (including steroid hormone receptor determination)
2. Tumor block for banking (Ideally the block should contain at least some invasive tumor taken from the periphery of the tumor.)
3. Normal tissue block for banking
4. Representative H & E sections of the above blocks

The tissue blocks may be returned to the Participating Center upon request after 4 1mm cores have been taken for preparing tissue micro-arrays (TMAs).

All reports, slides, and blocks must be marked with the randomization number. If materials are not properly marked, we cannot guarantee that the slides and blocks will be forwarded to the Central Pathology Review Office. Please ensure that the blocks and slides are carefully

packaged as otherwise they could easily get damaged during transport. The slides should be sent in customized slide boxes and should be wrapped with tissue paper to prevent any movement. The slides and blocks have not been packed securely enough if they move around when the box is shaken.

10.1.2. Pathology material banking

The IBCSG has established a central repository for tissue blocks and slides from every patient enrolled in IBCSG clinical trials. The required pathological material (described in the previous section) is submitted to, catalogued, and maintained in the IBCSG Coordinating Center Office in Bern (**IBCSG Coordinating Center, Pathology Coordinating Office, Effingerstrasse 40, CH- 3008 Bern**). The primary tumor H&E section and block are sent for central pathology review to the European Institute of Oncology in Milan, Italy, and then returned to the IBCSG Tissue Bank in Bern for storage. Central pathology review reports will be available to institution pathologists who wish to see them. Central pathology review will include histopathological parameters (tumor type and grade, occurrence of peritumoral vascular invasion), hormone receptors (estrogen and progesterone receptors), HER2 status and the tumor proliferative fractions (Ki-67 immunolabelling). Testing for genes which may be inherited is not a part of the central pathology review of this study. The blocks will be available for prospective and retrospective studies approved by the IBCSG Biological Protocols Working Group and by the IBCSG Ethics Committee.

The IBCSG requires a suitable tissue bank for application of the newer assays which are likely to become available in the very near future. In particular, IBCSG expects that novel predictive parameters will be identified by gene expression profiling. This will open at least one of the following possibilities:

1. The application of gene expression profiling to paraffin embedded material
2. The identification of specific mRNAs which could be detectable by molecular biology assays (RT-PCR, in situ hybridization, etc) in paraffin-embedded tissue
3. The identification of protein molecules detectable by immunohistochemistry.

These assays will most likely require a comparison between neoplastic and normal tissue, and this is why IBCSG is banking two sets of samples per patient. In many cases (but not all) normal tissue may be found around the invasive tumors. Separate blocks are necessary for extractive techniques in order to avoid neoplastic cells contaminating the normal sample.

10.2. Patient Reported Symptoms and Quality of Life Substudy

Some of the Participating Centers will participate in the ancillary study of Patient Reported Symptoms and Quality of Life. Details of the rationale, logistics, and statistical considerations are in Appendix III.

11. Regulatory approval procedures and Patient Informed Consent

11.1. Ethical Review Board/Ethics Committee

All protocols and the patient informed consent forms must have the approval of a properly constituted committee or committees responsible for approving clinical trials. The ERB/IRB written, signed approval letter/form must contain approval of the designated investigator, the protocol (identifying protocol title and version number), and of the patient informed consent. Documentation of Ethics Committee approval must be sent to the IBCSG Coordinating Center prior to enrollment of the first patient. The IBCSG Ethics Committee also approves the protocol and reviews it annually.

11.2. Regulatory approval procedures

If applicable, in addition to the approval of the Ethics Committee according to national legislation, the protocol, other protocol related documents including patient information and informed consent and other documents as required locally must be submitted to and be approved by the health authority. Documentation of health authority approval must be sent to the IBCSG Coordinating Center prior to Participating Center activation.

11.3. Protection of human subjects

The IBCSG has an Office for Human Research Protection (OHRP) Federal Wide Assurance (FWA00009439) and follows all of the policies and procedures that are part of that assurance. All potential subjects for this trial will receive a full explanation of the trial, its purpose, treatments, risks, benefits, and all of the other items listed in Section 11.4. Additional institution-specific sections should be added to Appendix I as described in Section 11.4.

The medical record must be available for review by the IBCSG audit team as described in Section 11.5.

Serious adverse event (SAE) reports are distributed monthly. In addition they are available on the IBCSG website (www.ibcsg.org) for IBCSG member institutions.

11.4. Informed consent

Informed consent for each patient will be obtained prior to initiating any trial procedures in accordance with the “IBCSG Patient Information and Informed Consent." (See Appendix I.) One signed and dated copy of the informed consent must be given to each patient and the original copy must be retained in the investigator's trial records. The informed consent form must be available in the case of data audits. Verification of signed informed consent and the date signed are required for randomization to this trial.

The "Declaration of Helsinki" (http://www.wma.net/e/policy/b3.htm) recommends that consent be obtained from each potential patient in biomedical research trials after the aims, methods, anticipated benefits, and potential hazards of the trial, and discomfort it may entail, are explained to the individual by the physician. The potential patient should also be informed of

her right to not participate or to withdraw from the trial at any time. The patient should be told that material from her tumor will be stored and potentially used for additional studies not described in this protocol.

If the patient is in a dependent relationship to the physician or gives consent under duress, the informed consent should be obtained by an independent physician. If the patient is legally incompetent (i.e., a minor, or mentally incompetent), informed consent must be obtained from the parent, legal guardian, or legal representative in accordance with the law of the country in which the trial is to take place. By signing this protocol, the investigator agrees to conduct the trial in accordance with Good Clinical Practice and the "Declaration of Helsinki."

The IBCSG recognizes that each institution has its own local, national, and international guidelines to follow with regard to informed consent. Therefore, we provide a template information sheet and informed consent form (Appendix I), which can be downloaded and edited to incorporate information specific to your institution (see www.ibcsg.org) for IBCSG members. The template Patient Information Sheet and Informed Consent has been written according to ICH guidelines which state the Informed Consent should adhere to GCP and to the ethical principles that have origin in the “Declaration of Helsinki”. The final version should receive the Institutional Review Board/ Local Ethics Committee approval in advance of its use.

11.5. Quality Assurance

The IBCSG conducts trials according to the ICH Good Clinical Practice (GCP) guidelines. The Study Data Manager reviews each CRF as it is received. In addition, the IBCSG Medical Reviewer reviews each case at specific timepoints. The IBCSG conducts periodic audit visits to ensure proper trial conduct, verify compliance with GCP, and perform source data verification.

12. Administrative Considerations

12.1. Insurance

The IBCSG will contract the appropriate liability insurance for this trial. Patients who suffer injuries due to the trial should report them immediately to their physician. The local group/institution should report all alleged claims immediately to the IBCSG Coordinating Center.

The IBCSG insurance does NOT cover patients from the United States of America or from Canada. Each group will be responsible for obtaining proper insurance coverage.

12.2. Steering Committee

A Steering Committee will be constituted for this trial. The primary responsibilities of the Steering Committee are twofold. First, the Steering Committee is responsible for maintaining the scientific integrity of the trial, for example, by recommending changes to the protocol in

light of emerging clinical or scientific data from other trials. Second, the Steering Committee is responsible for translation of recommendations of the IBCSG Data and Safety Monitoring Committee into decisions (see section 9.5.). Membership will include IBCSG officials, study chair and co-chairs, trial statisticians, representatives from some participating institutions and groups, and representatives from Novartis.

General partition of responsibilities:

The Steering Committee has the authority to make and implement any final decisions, such as substudies of the trial or amendments to the trial protocol, and may recommend the termination of the trial.

The IBCSG Executive Committee is responsible for the implementation of all final decisions taken by the Steering Committee.

The IBCSG Foundation Council decides on the termination of the trial.

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SOLE

Study of Letrozole Extension

Appendix III

Patient Reported Symptoms and Quality of Life Substudy

Table of Contents

1. Introduction

Information about concomitant and late symptoms of adjuvant endocrine therapies for breast cancer is crucial to prepare women for physical and psychological consequences of treatment and for making informed decisions. Several randomized trials in the adjuvant setting assessed self-reported symptoms related to endocrine therapy in postmenopausal women with breast cancer [1-4]. In the ATAC trial, patients of all three treatment arms reported a worsening of symptoms related to endocrine therapy (overall endocrine symptom score) during the first 3 months after study entry, which subsequently stabilized or slightly improved at 2- and 5-year follow-up. However, specific side-effects were significantly more frequent with anastrozole compared to tamoxifen (i.e., diarrhea, vaginal dryness, diminished libido, and dyspareunia), whereas dizziness and vaginal discharge were less frequent with anastrozole compared to tamoxifen [1, 2]. Postmenopausal women participating in the Intergroup Exemestane Study (IES) reported a high severity of vasomotor symptoms and sexual problems. The switch from tamoxifen to exemestane neither increased nor decreased endocrine symptoms after 2 to 3 years of tamoxifen [3]. In the MA.17 extended-adjuvant trial, only a minority of patients receiving either letrozole or placebo after five years of tamoxifen indicated symptoms to be bothersome [4]. Small but statistically significant differences of mean change scores from baseline to month 6, 12 and 24 showing a worsening for the letrozole group were seen for vasomotor symptoms (i.e., hot flushes/flashes and night sweats) and a number of quality of life (QL) domains including physical function, bodily pain, vitality and sexuality.

Although these studies revealed no major effect on overall QL by the different endocrine agents [1-4], differences in some QL domains were observed. An extension of treatment implies a continuation of symptoms, which may be a burden to the patient. Patient-reported symptoms (PRS) will therefore be evaluated in a subsample of patients participating in the IBCSG Trial 35-07 by comparing the occurrence and severity of symptoms between the two different administration schedules, and their relative impact on global QL indicators.

2. Objectives

The objective of this QL substudy is to compare differences in patient-reported symptoms and quality of life between continuous letrozole for 5 years and intermittent letrozole over a 5-year period among postmenopausal women who are disease-free following 4 to 6 years of prior adjuvant endocrine therapy for endocrine-responsive node-positive operable breast cancer. We hypothesize that introducing 3-month treatment-free intervals during the course of 5 years of extended letrozole will reduce both the number and the bother of symptoms associated with letrozole in this patient population.

Despite the potential decrease of symptoms in the intermittent arm, we hypothesize that starting and stopping treatment repeatedly over 5 years may be more burdensome on a global QL level than undergoing continuous treatment for 5 years.

2.1 Primary Hypothesis

Twelve months after randomization (i.e., before patients in the intermittent group start letrozole again) hot flushes/flashes will be worse in patients randomized to receive continuous letrozole compared with patients randomized to receive intermittent letrozole.

2.2 Secondary Hypotheses

Twelve months after randomization, both musculoskeletal and vaginal problems will be worse in patients randomized to receive continuous letrozole compared with patients randomized to receive intermittent letrozole.

Starting and stopping treatment repeatedly over the course of 5 years for patients randomized to receive intermittent letrozole is more burdensome on a global QL level compared with patients randomized to receive continuous letrozole for 5 years.

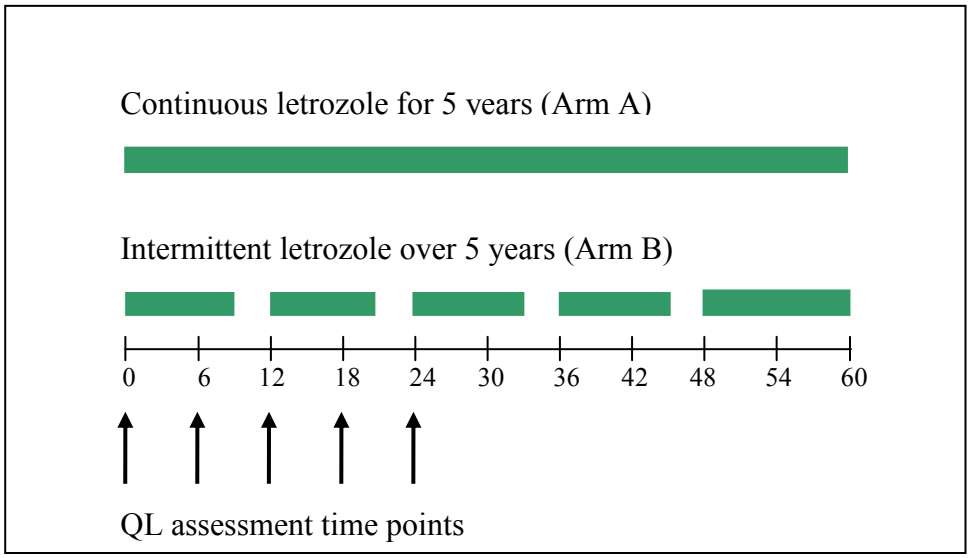
3. Patient selection

The QL assessment will be conducted in selected centers participating in the IBCSG Trial 35-07. It is strongly recommended that patients complete a baseline QL and PRS Form prior to randomization, and the baseline forms must be completed prior to start of treatment. If the patient is randomized beforehand, the patient should be informed of the randomization result only after completion of the baseline forms. The only exceptions are physical impairment that interferes with any assessment, or inability to read any of the languages available on the QL/PRS Forms.

4. Study design

In order to evaluate intermediate and long-term effects, a longitudinal design is used, including a baseline assessment and assessments at 6, 12, 18 and 24 months after randomization (Figure 1). To eliminate any differential anticipatory effects on baseline scores and to help insure compliance with the protocol requirements, the baseline QL/PRS Forms should be completed prior to randomization.

Figure 1: QL assessment time points



5. Measures

Patient-reported endocrine symptoms will be assessed by the Breast Cancer Prevention Trial (BCPT) Symptom Scales [5]. These scales represent a refinement of the 42-item BCPT symptom checklist reducing the original scale to meaningful symptom dimensions. Both exploratory and confirmatory factor analysis revealed eight scales corresponding to the physical symptom dimensions associated with cancer treatment, chemoprevention, menopause or normal aging: 1) hot flushes/flashes (2 items), 2) nausea (2 items), 3) bladder control (2 items), 4) vaginal problems (2 items), 5) musculoskeletal pain (3 items), 6) cognitive problems (3 items), 7) weight problems (2 items), 8) arm problems (2 items). Patients are asked to indicate how much they were bothered by each symptom during the past 4 weeks on a 5-point severity scale (0= not at all; 1= slightly; 2= moderately; 3= quite a bit; 4= extremely). In addition to the eight scale scores, a total score will be computed.
The psychometric properties of the scales are satisfying, but limited to data provided by patients who were previously diagnosed with breast cancer (i.e., breast cancer survivors) or women at risk for breast cancer, but not by patients currently undergoing chemotherapy or endocrine treatment [5].

Several global QL indicators will be assessed using selected items from the IBCSG QL Core Form, which was developed in 1986 and was subsequently revised for IBCSG Trials 10-93 through 14-93 (starting on May 1, 1993) [6, 7] and the IBCSG QL Module Form developed for IBCSG Trial 24-02. The IBCSG Trial 35-07 QL Form includes global linear analogue self-assessment (LASA) indicators for physical well-being, mood [8], and coping (PACIS [9]). The indicators for physical well-being, mood and coping were confirmed to be responsive to mental distress and psychosocial dysfunction in patients with early breast cancer [10]. They are suitable to describe patients' adaptation over time. Validation studies are summarized elsewhere [7]. In addition, one global indicator is included for overall treatment burden ("Overall, how much are you bothered by any treatment related difficulties?"). This indicator has been shown to be responsive to side-effects of anti-emetic and cytotoxic therapies [11] and to endocrine symptoms [12]. These global indicators are complemented with LASA indicators specific to symptoms which are not covered by the BCPT Checklist: Tiredness and sleep disturbance, as well as two indicators referring to sexuality (i.e., loss of sexual interest and difficulties becoming aroused). The latter question has to be answered only if a patient indicates that she has been sexually active during the past 6 months. As a reference measure we include the symptom specific LASA indicator for hot flushes/flashes. In IBCSG Trial VIII [13], scores of this measure were analyzed in relation to amenorrhea, providing further information for interpretation. In addition, the responsiveness of the LASA indicator for hot flushes/flashes will be compared with the corresponding item and subscale of the BCPT Symptom Scales.

5.1 Patient characteristics and Co-morbidity

Patient characteristics and co-morbidity are part of the standard study documentation. Thus, this information will not be collected as part of this QL study; however, the information will be used in the PRS/QL analyses.

6. Timing requirements, Data Collection and Local Data Management

6.1 Timing requirements

Assessment time points are determined by the interval from date of randomization, and coincide with the required clinical follow-up time points. The QL assessment time points are illustrated in Figure 1.

The schedule for submitting the QL/PRS Forms must be followed as closely as possible. If exact timing is not possible, assessment should be done as close as possible to the required date, but before restart of treatment at the 12 and 24 month time points for patients randomized to the intermittent letrozole arm. The QLC/PRS Forms must always be completed prior to any communication about the patient's medical information.

For methodological reasons, the required schedule has to be followed exactly, with neither more nor fewer assessments. Shortly after randomization, the IBCSG sends the local investigator a schedule of the dates of required QL/PRS Forms. This list should be put into each patient's chart to aid in the correct timing for completing the QL/PRS Forms.

6.2 Data Collection and Local Data Management

Within the first 2 years, every patient participating in the QL substudy is to complete the QL/PRS Forms at each scheduled assessment time point, as described in Figure 1.

If the patient does not complete the required QL/PRS Forms, then the reason why the assessment was not completed must be provided. This can be done by completing the Assessment Form (Form 35-AC)

The QL/PRS Forms are to be filled in at the clinic. If the patient is being followed elsewhere, arrangements are to be made with the clinic or physician to have the patient fill in the forms as required. If, for administrative reasons, the forms have not been presented to the patient, they may be filled in at home and mailed.

For the first assessment, the QL/PRS Forms have to be explained to the patient, with particular emphasis on making sure the patient understands both the categorical response format and the LASA format. For later assessments, the patient should be instructed to seek help only if she has problems in understanding any of the items in the form.

All questions on the QL/PRS Forms should be answered. The forms should be checked after completion and, if necessary, the patients should be asked to fill in missing answers. Patients may wish to leave some questions unanswered if they make them feel very uncomfortable. They should be encouraged to answer all items, however, especially those concerning symptoms, as they represent a primary objective of the ancillary study.

6.3 Central Data Management

Computerized data quality control measures will be used to monitor the submission rates of the QL forms and the timing of assessment as required by the study protocol. Institutions will receive feedback on their performance and specific problems on a regular basis.

7. Statistical Considerations

The QL substudy of this phase III randomized clinical trial is designed to compare differences in patient reported symptoms and quality of life between continuous letrozole for 5 years and intermittent letrozole over a 5-year period among postmenopausal women who are disease-free following 4 to 6 years of prior adjuvant endocrine therapy for endocrine-responsive node-positive operable breast cancer. We hypothesize that introducing 3-month treatment-free intervals during the course of 5 years of extended letrozole will reduce both the number of symptoms and the bother of symptoms associated with letrozole in this patient population. We will test this hypothesis by comparing the two treatment arms using patient reported symptoms at 5 time points over the first 24 months of treatment. In addition, despite the hypothesized decrease in symptoms associated with intermittent letrozole, we hypothesize that repeatedly starting and stopping treatment over 5 years may be more of a burden on patients' global QL than undergoing continuous treatment with letrozole for 5 years. We will test this secondary hypothesis by comparing the longitudinal pattern of patient reported overall treatment burden between the two treatment arms over the first 24 months of treatment.

7.1 Sample Size Determination

The hot flushes/flashes symptom scale captured on the BCPT Symptom Scales [5] is selected as the primary endpoint. This scale consists of 2 items of patient reported hot flushes/flashes and night sweats which have been shown to be a measurable side-effect of letrozole. Secondary endpoints include the musculoskeletal pain and vaginal problems scales, both anticipated symptoms induced by letrozole. Musculoskeletal pain consists of 3 items on the BCPT Symptom Scales, namely, general aches and pains, joint pains, and muscle stiffness while vaginal problems is captured by pain with intercourse and vaginal dryness.

The BCPT Symptom Scales asks the patient to indicate how much they were bothered by each symptom during the past 4 weeks on a 5-point severity scale (0= not at all; 1= slightly; 2= moderately; 3= quite a bit; 4= extremely). Scales are formed by averaging the scores on items forming each scale.

The sample size for this QL substudy will be based on the between-group comparison of the change from baseline to 12 months in the hot flushes/flashes scale. The sample size will be selected to achieve 90% statistical power to detect an effect size of 0.25 between the two groups using a two-sided 0.05 level t-test. Three hundred and thirty eight patients in each randomized treatment arm (676 total) will be sufficient to achieve the stated objective. To allow for a 10% non-compliance rate, the sample size is inflated to 744 patients.

The between-group comparison of the change from baseline to 12 months for the two secondary endpoints, musculoskeletal pain and vaginal problems scales, will also be performed. In order to control the inflated Type I error rate, evaluation of each of these two other important comparisons will be conducted using a two-sided alpha level of 0.025. The power to detect an effect size of 0.25 for each secondary endpoint is 84%.

The QL substudy will be conducted in selected centers and the centers will be compensated for participating. This approach is intended to result in at least a 90% completion rate for each patient at each of the 5 time points (baseline, and at months 6, 12, 18, and 24 post-randomization).

7.2 Statistical Analysis

The statistical analysis plan will involve five separate steps aimed at minimizing bias and emphasizing the clinical meaningfulness of the data: a comparison of the completion rates for the BCPT Symptom Scales and QL measures between groups; a comparison of baseline scores between groups; a comparison of mean change of the symptom scales and QL measures from baseline; a comparison between groups of the longitudinal pattern of symptoms and QL measures during the first 24 months; and a comparison of the proportion of patients with improved, stable, or worsened symptoms between groups. The latter is called a response analysis and identifies the proportion of patients demonstrating a minimally important improvement or worsening of symptoms at any time during follow-up.

The primary analyses will be based on treatment differences (continuous letrozole for 5 years vs. intermittent letrozole over a 5-year period) of the change in each symptom scale from baseline to 12 months post-randomization. In addition, we will analyze treatment differences of the change from baseline to month 24 as well as at each assessment time point for both the BCPT Symptom Scales and QL measures. The two-sample t-test will be the basis of comparisons between the groups.

Demographic factors will be collected from each patient and incorporated in the data analysis. We will use linear regression to analyze treatment differences at each time point and of the change in the bother of symptoms and QL measures from baseline adjusting for patient's culture, type of prior adjuvant endocrine therapy, and other demographic factors as necessary. In particular, in the event that we detect any baseline imbalances we will adjust for these factors. A linear mixed-effects model will also be used as a longitudinal analysis to estimate and describe treatment effects on symptoms and QL measures, in particular overall treatment burden, over time and at specific time points. This model uses all available PRS/QL information obtained on the patient while allowing for the repeated measurements to exhibit within-subject correlation of observations over time. A compound symmetry covariance matrix will be assumed and used to estimate and describe treatment effects, but other covariance structures will be explored.

The response analysis will be based on the proportion of patients observed to have an improved (important reduction in the bother of the symptom from baseline), stable (did not experience an important change in the bother of the symptom from baseline), or worsened score (important increase in the bother of the symptom from baseline) during the course of the study. A minimally important change in the bother of the symptom will be calculated by using a half standard deviation of the scale [14]. As additional analyses, we will perform a logistic regression to evaluate baseline characteristics that predicts a minimally important worsening in symptoms at any time point.

To gain a better understanding of what specific symptoms might be affected by letrozole, we will also look at each individual item on the BCPT Symptom Scales. We will compare between groups the proportion of patients after random assignment who report being very bothered by a symptom with a score of 3 or 4 on a scale ranging from 0 (not at all bothered) to 4 (extremely bothered) and for each item. The $\chi 2$ test will be used to test for statistical significance between the groups.

Further exploratory analyses will address the relationships between patient reported symptoms captured on the BCPT Symptom Scales and the corresponding LASA indicator (i.e. hot

flushes/flashes) as well as the global QL indicators (physical well-being, mood, coping, overall treatment burden).

We will be collecting information on reasons why the patient did not fill out the QL/PRS Forms form. As an additional analysis, this information will be used to impute patients' missing QL scores. A sensitivity analysis will be done in parallel with various imputation techniques to validate the effect that imputing values has on the parameter estimates.

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INTERNATIONAL BREAST CANCER STUDY GROUP
IBCSG 35-07
BIG 1–07

SOLE
Study of Letrozole Extension

SOLE-EST
SOLE Estrogen Substudy

Investigating changes in estrogen levels and grip strength for patients participating in the SOLE Trial

Coordinating Group: International Breast Cancer Study Group (IBCSG)

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GROUP SPECIFIC CONTACT INFORMATION

Please refer to Section 1 of Appendix V of the SOLE protocol for group-specific contact information to direct your inquiries about participation/eligibility/treatment for the SOLE parent trial.

Protocol Signature Page

IBCSG 35-07/ BIG 1-07

Study of Letrozole Extension – SOLE

SOLE Estrogen Substudy – SOLE-EST

Approved by:
Anita Hiltbrunner
Director, International Breast Cancer Study Group

(*Signature on file)*	*26Oct10*
Signature	Date

Approved by:
Dr. Meredith M. Regan
Group Statistician, International Breast Cancer Study Group

(*Signature on file)*	*26Oct10*
Signature	Date

Principal Investigator Protocol Signature Page

SOLE Estrogen Substudy (SOLE-EST) of IBCSG 35-07 / BIG 1-07 / SOLE
Version 1.0 26Oct10

I have read the protocol and agree that it contains all necessary details for conducting this substudy. I will conduct the substudy as outlined in the following protocol and in compliance with GCP. I will provide copies of the protocol **to all physicians responsible to me who participate in this substudy. I will discuss this material with them to assure that they are fully informed** regarding the conduct of the substudy. I agree to keep records on all patient information (Case Report Forms and patient's Informed Consent statement), and all other information collected during the substudy for a minimum period of 15 years.

Name of Principal Investigator:_____

_____

Signature Date

Substudy Summary and Schema

SOLE Estrogen Substudy (SOLE-EST)

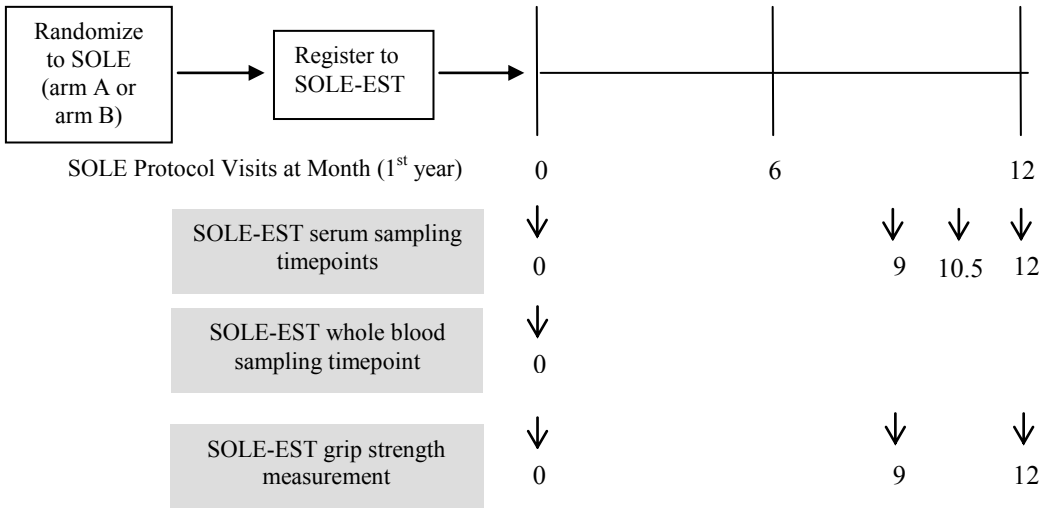
A substudy of the SOLE Trial to investigate estrogen levels for patients participating in the SOLE Trial

Patient Population: Postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with selective estrogen receptor modulator(s) (SERM) and/or aromatase inhibitor(s) (AI) for endocrine-responsive, node-positive operable breast cancer, who have been randomized into the SOLE Trial and participate in the Quality of Life substudy.

Entry: Patients should be enrolled to SOLE-EST at the time of entry into the main study and the Quality of Life substudy. The first serum sample must be obtained after randomization but prior to starting letrozole on study according to the guidelines in the SOLE-EST Manual for Blood Sample Logistics and Vigorimeter Use.

Sample size: 100 patients, 25 from arm A (continuous letrozole) and 75 from arm B (intermittent letrozole).

SOLE-EST schema and blood sampling timepoints



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1. Introduction and Rationale

Third generation aromatase inhibitors such as letrozole profoundly suppress estrogen levels in postmenopausal women, which is the mechanism of their action against hormone receptor positive breast cancer. The hypothesis under investigation in the SOLE Trial is that removing this suppression for a period of time will allow re-sensitization of breast cancer cells to estrogen deprivation resulting in a delay or prevention of the development of resistance to the anti-cancer effect of letrozole.

The estrogen substudy will investigate the changes in estrogen levels that occur on letrozole and during the three month gap phase without letrozole. Estrogen suppression and recovery may also be linked with clinical outcomes including toxicity and disease free survival. In addition, the substudy will address the relationships between changes in patient reported musculoskeletal symptoms, grip strength, and QoL and related changes on letrozole and during the gap phase. As genes important for the metabolism and activity of letrozole may influence estrogen levels, single nucleotide polymorphisms **(**SNPs) related to these will be assessed and analyzed.

1.1. Estrogen levels

There are three biologically important circulating estrogens present in postmenopausal women:

- 17 β–Estradiol (E_2) is a product of both estrone and testosterone and the most abundant circulating estrogen in premenopausal women
- Estrone (E_1) is the major product of aromatase enzyme activity in postmenopausal women
- Estrone sulphate (E_1S) is the most stable estrogen fraction measured and provides a robust surrogate-parameter for suppression of estrogen biosynthesis in vivo.

Mean plasma levels of estradiol (E_2), estrone (E_1) and estrone sulphate (E_1S) in postmenopausal women are reported to be 20, 80 and 4-500 pmol/L[1,2,3] respectively. Suppression of estrogen production by letrozole reduces levels close to or below the level of detection in many assays.

1.2. Measuring estrogen levels

Gas chromatography/tandem mass spectrometry (GC-MS/MS) is considered the reference standard for measuring both male and female sex steroids. However, GC-MS/MS is not presently practical for the analysis of large sample sets and as with all assays, many variables factor into the accuracy of mass spectrometry methods including standardizing reagents, procedures, and hardware[4]. In postmenopausal women with low E_2, indirect E_2 assays correlate best with GC-MS/MS[1,5], the extraction step in indirect assays removing potentially interfering substances, particularly cross-reacting water-soluble steroid conjugates. It is critical to note that direct assay measurements systematically overestimate E_2 levels and as E_2 levels increase, the overestimation increases[5,6]. It is also important to measure sex hormone binding globulin (SHBG) as it is the major determinant of bioavailability of E_1 and E_2 in serum. Serum estrogen levels in postmenopausal women will also vary substantially by years since menopause, body mass index (BMI) and level of physical activity[6,7].

Data regarding estrogen levels using GC-MS/MS come from three main studies. In a large study[5] including 374 postmenopausal women, the mean E_2 level was 5.6 pg/ml (x 3.67=20.5 pmol/L). In a second study[9], 147 postmenopausal patients with early breast cancer were

randomized to receive placebo or exemestane. Mean baseline values for E_2, E_1 and E_1S were 4.0 and 4.8 pg/ml, 21 and 24.9 pg/ml and 179 and 212 pg/ml in placebo and exemestane groups, respectively. After 12 months of treatment with exemestane, mean values for E_2, E_1 and E_1S were reduced by 83, 93 and 93%, respectively. No individual values or estrogen levels were reported at 12 months. In a third report[10], 55 postmenopausal women were treated either with atamestane/toremifene or letrozole/placebo. After a maximum of 12 weeks of aromatase inhibitor treatment estradiol levels fell from a mean value of 5.9 to <0.63 pg/ml.

In the NSABP P-1 study, the median plasma E_2 concentration using a validated indirect assay among untreated case subjects was 21 pmol/L (25th–75th percentile, 12 to 33 pmol/L) and the median SHBG plasma concentration among case subjects was 33 nmol/L (25th–75th percentile, 25 to 49 nmol/L)[11]. In a study of 54 patients receiving adjuvant AIs (anastrazole and letrozole), mean estrone sulphate levels were in the order of 1,054 pmol/L untreated (95% CI ,854 to 1,302) and with letrozole therapy fell to a mean of 20.7 pmol/L (95% CI,16.5 to 25.9)[12] In this study, baseline E_2 levels ranged from 3 pmol/L to 91 pmol/L with a mean of 25.7pmol/L and with letrozole were suppressed to a mean of 1.560 (95%CI,1.37 to 1.780). Other studies have shown similar suppression of estrogen levels by letrozole.[1]

In conclusion, estrogen levels on letrozole are very low[1,2,3,10,11,12,13]. Measurement by extraction-based indirect assay appears to correlate well with the gold standard GC-MS/MS and is the most practical for this study. The laboratory of Frank Stanczyk will perform these assays; the lab sensitivity for E_2 is 2pg/ml (7.34 pmol/L) for E_1 is 3pg/ml (11.01 pmol/L) and for E_1S RIA is 9 pg/ml (24.57 pmol/L).

1.3. Rationale for present study (SOLE-EST)

In the SOLE Trial, regular repeated episodes of low level estrogen exposure will occur during the break from letrozole due to renewed peripheral and potentially intra-tumoral aromatization of androgens. It is hypothesized that this may help to prevent development of resistance thereby increasing the efficacy of the extended adjuvant letrozole when given intermittently rather than continuously. There are a number of preclinical and clinical observations that support this hypothesis.

After a time of very low estrogen levels breast cancer cells may develop estrogen independent growth and progress despite ongoing estrogen deprivation [14,15]. It has also been shown that in cancer cell lines that are resistant to estrogen deprivation, estrogen can produce apoptosis and tumor regression [16] and there is also evidence that re-exposure to estrogen after a period of estrogen deprivation can re-sensitize breast cancer cells to estrogen deprivation, thereby overcoming resistance. Further animal studies (Long Term Letrozole Treated MCF-7Ca xenografts) have also demonstrated that stopping treatment with letrozole can reverse acquired resistance – in this study the break from letrozole was 4 months[17]. In addition, in a clinical study of estrogen deprived breast cancer there was also some evidence that estradiol (E_2) may produce a treatment response[17].

The above hypothesis is however dependent on the renewed estrogen production following cessation of letrozole. The size and timing of this renewed estrogen production may vary between patients and may therefore potentially influence the value of this intervention. There is no clinical data on the effect of letrozole cessation on estradiol (E_2) and other estrogen levels.

1.4 Toxicity and Quality of Life

Toxicity from letrozole is well documented[19] and includes hot flushes, arthralgia, anxiety and depression, diarrhea and fatigue. A reduction in bone density and increased risk of fracture is also associated with its use. Several randomized trials in the adjuvant setting assessed self-reported symptoms related to endocrine therapy in postmenopausal women with breast cancer[20,21,22,23]. Although these studies revealed no major effect on overall quality of life (QoL) by the different endocrine agents, difference in some QoL domains were observed. In the MA 17 extended-adjuvant trial comparing letrozole to placebo after 4.5-6 years of adjuvant tamoxifen, small but statistically significant differences of mean change scores from baseline to month 6, 12 and 24 showing a worsening for the letrozole group were seen for vasomotor symptoms (i.e., hot flushes and night sweats) and a number of QoL domains including physical function, bodily pain, vitality and sexuality[23]. Regarding the proportion of patients who demonstrated an important change in QoL, a significant difference was seen between groups with a higher percentage of patients in the letrozole group reporting a worsening in bodily pain and the vasomotor domains. Musculoskeletal side effects are amongst the most common and most troublesome side effects associated with aromatase inhibitors[24,25,26]and have been assessed in a number of ways. A simple and validated measure is grip strength using a modified sphygmomanometer[19]. The side effects and their impact on QoL due to treatment with an aromatase inhibitor for a duration longer than 5 years is as yet unknown. In addition, the effect of a three month break is not known. It is hypothesized that the increase in estrogen levels during the three month gap will reduce side effects, especially musculoskeletal side effects and patient-reported symptoms and that this will correlate with the absolute estrogen levels. It is also hypothesized that the increase in estrogen levels will improve QoL overall.

The SOLE-EST Substudy will test these hypotheses by using the toxicity data collected in the main study and the QoL data collected in the QoL substudy and by measuring the grip strength of the patients.

1.5 Single Nucleotide Polymorphisms (SNPs) and relationship to estrogen levels

Changes in the metabolisation of letrozole may translate into altered estrogen levels, and may therefore affect efficacy.

Letrozole is metabolized by cytochrome P450 (CYP) isoenzymes including CYP2A6 and CYP3A4. Genetic variations in the genes encoding for those enzymes can alter the metabolism and therefore plasma levels of letrozole. This can have an impact on the efficacy of letrozole. Desta *et al* found significant differences in letrozole concentrations between slow, intermediate and normal CYP2A6 genotypes[27]. Therefore, SNPs on alleles CYP2A6*2, CYP2A6*9, CYP2A6*12 and CYP2A6*35 will be analyzed. In the CYP3A4 gene, only the CYP3A4*1B and CYP3A4*7 SNP have a known effect on the enzymatic activity and a frequency of more than 1% in the Caucasian population. Therefore, for the CYP3A4 gene, only those two SNPs will be genotyped.

The gene encoding CYP19 (the aromatase enzyme that produces estrogen from testosterone) is a highly polymorphic gene that may influence the efficacy of letrozole through an altered (increased or decreased) activity. For this reason, the SNPs with a frequency of >10% in the Caucasian population and with a known effect on the enzymatic activity will be analyzed, namely rs4646, rs10046, rs727479, rs10459592, rs4775936, rs6493497 and rs7176005[28,29,32].

Rs4646, rs10459592 and rs4775936 have already been associated with an improved efficacy of letrozole[28,29].

There is also some evidence that genetic variation may explain the variation of the degree of side effects amongst women on letrozole. Ingle *et al* found 4 SNPs (rs7158782, rs7159713, rs2369049 and rs6637820) that were associated with the degree of musculoskeletal symptoms using a genome-wide association case-control study[30]. These SNPs will also be analyzed. The SNPs will be analyzed using the Sequenom MassARRAY® technology and the presence of the SNPs will be correlated with estrogen levels.

In addition, further SNPs will be analyzed based on scientific evidence available at the time of evaluation.

2. Objectives and endpoints

2.1. Primary objective

2.1.1. To determine the serum level of estrogens Estradiol (E_2), Estrone (E_1) and Estrone Sulphate (E_1S) and Sex Hormone Binding Globulin (SHBG) during letrozole treatment.

2.1.2. To determine the degree of recovery of Estradiol (E_2), Estrone (E_1) and Estrone Sulphate (E_1S) during the 3 month gap.

2.2. Secondary objectives:

2.2.1. To link the estrogen level changes with the clinical outcomes of toxicity and QoL.

2.2.2. To determine the effect of the following factors on estrogen levels:

- prior adjuvant endocrine therapy
- age
- BMI
- type of menopause

2.2.3. To explore variability of estrogen level changes and link these with germline SNPs.

2.3. Primary endpoints

2.3.1 Levels of Estradiol (E_2), Estrone (E_1) and Estrone Sulphate (E_1S) at 0, 9, 10.5 and 12 months from randomization to the core protocol.

2.3.2 % change (suppression or recovery) of E_2, E_1 and E_1S from baseline at 9 months, 10.5 months and at 12 months from randomization to the core protocol.

2.4. Secondary endpoints

2.4.1. Toxicity grade changes (for arthralgia, hot flushes and insomnia) between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.

2.4.2. Quality of life score change between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.

2.4.3. Changes in grip strength score at 9 months and 12 months.

3. Study design

Collection of blood for SNPs will be at baseline.

Time points of serum collection for measurement of estrogen levels are 0 (pre-treatment), 9, 10.5 and 12 months.

Time points for measurement of grip strength are 0 (pre-treatment), 9 and 12 months.

The substudy will include 75 patients from the intermittent arm and 25 from the continuous arm of the SOLE parent trial. The first 50 patients will be included from both arms (25 patients from each arm), and the last 50 patients will be included from arm B (intermittent arm) alone.

4. Patient Selection

4.1. SOLE-EST Participating Centers

SOLE-EST will be conducted in selected countries and Centers. These Centers will also be participating in the QoL substudy. All eligible patients randomized to SOLE from these Centers should be offered participation in SOLE-EST, but inclusion is not mandatory.

4.2. Inclusion criteria

4.2.1. Patient must be randomized to the parent SOLE Trial.

4.2.2. Patient must participate in QoL substudy.

4.2.3. Written informed consent for the SOLE-EST Substudy must be obtained.

5. Patient Registration

Registration to SOLE-EST should immediately follow randomization to the parent SOLE Trial, using the IBCSG Registration/Randomization System. The Confirmation of Registration Form (35-SE-A) should be completed prior to registration to confirm eligibility. Patients must be entered in the substudy immediately after their randomized allocation in the main SOLE Trial is known and prior to starting allocated treatment in order to obtain pretreatment baseline estrogen levels. A separate consent form applies to the substudy.

During the recruitment period of the substudy, participating Centers are encouraged to approach all patients who enroll in SOLE for SOLE-EST participation. It is recommended that informed consent for SOLE-EST be obtained at the same time as for the parent trial. The baseline sample must be obtained prior to receiving protocol treatment.

6. Study Parameters and Data Submission

6.1. Assessments

A complete "estrogen profile" will be performed centrally: estradiol (E_2), estrone (E_1), and estrone sulphate (E_1S). SHBG will also be performed centrally. The results of these assessments will not be available to make treatment decisions during the protocol treatment period. Blood will also be drawn at baseline for SNP analysis.

6.2. Timing of assessments

Assessment timepoints are determined by the interval from date of randomization to the core protocol and some do not coincide with the required clinical follow-up timepoints of the SOLE Trial.

Substudy Parameters					
Regular SOLE visit number	1	2			3
Year	1	1	1	1	2
Trial Month	0	6	9	10.5	12
Visit only for SOLE-EST Substudy			x	x	
Whole blood sample	x				
Serum sample	x		x	x	x
Grip strength	x		x		x
Form 35-SE-SC	x		x	x	x

x = mandatory
Note: Serum and Form 35-SE-SC must also be submitted at discontinuation (see below)

Blood samples can be taken at any time of day and do not need to be fasting.

6.3. Required Samples

Baseline sample (Month 0): After randomization to the SOLE Trial but prior to commencement of SOLE protocol therapy, a whole blood sample and a serum sample have to be drawn.

Months 9 and 10.5 samples: Special visits only for the substudy. Only serum sample is needed.

Month 12 sample: At the required clinical follow-up visit. For patients in arm B, it is mandatory that the blood is drawn prior to restart of letrozole. Only serum sample is needed.

The Sample Collection Form (35-SE-SC) should be completed and submitted via DataFax at each collection timepoint listed above. This Case Report Form (CRF) confirms that the required samples were obtained, and can be submitted with the other CRFs for the SOLE Trial. Grip strength will also be reported on this form.

For patients who come off study drug in Year 1 of trial treatment, a final blood sample will be collected. If a patient discontinues the SOLE-EST Substudy prior to the 12-month sample, for any reason (e.g., relapse or drug intolerance), then an **Early Discontinuation (35-SE-ED) Form** should be completed and submitted via DataFax.

6.4. Forms submission

The Data Management Manual for this substudy contains instructions for submitting forms using the DataFax system.

IC Form	Informed Consent Form	Obtain before registration for SOLE-EST and keep with patient records.
Form 35-SE-A	Confirmation of Registration Form	Complete before contacting your Randomization Center or entering the IBCSG Registration/Randomization system to register. DataFax the completed form for all patients registered or enter it into iDataFax.
Form 35-SE-BMC	Biological Material Consent Form	DataFax after registration with Form 35-SE-A or enter into iDataFax.
Form 35-SE-SC	SOLE-EST Sample (and grip strength) Collection Form	DataFax (via fax or iDataFax) at baseline (after randomization to SOLE but prior to commencement of protocol therapy), Months 9, 10.5 and 12 (grip strength not tested at Month 10.5)
Form 35-SE-ED	SOLE-EST Early Discontinuation Form	Submit to DataFax (via fax or iDataFax) once upon early discontinuation of the substudy.

6.5. Sample collection logistics

Blood collection kits containing cryovials, labels and cryoboxes will be supplied to the participating Centers. These kits must be used for collection, storage and shipment of serum samples. At baseline a volume of 10 mL of blood has to be drawn in an EDTA treated tube and be stored at -20°C until shipment for SNP analysis to Vesalius Research Center, K.U. Leuven and VIB , Leuven, Belgium. At Months 0, 9, 10.5 and 12, a volume of 7.5-10 mL of blood should be drawn and processed to obtain two aliquots of serum, according to the SOLE-EST Manual for Blood Sample Logistics and Vigorimeter Use. Every aliquot should be labeled as instructed in the SMM and stored at -20 ºC until delivery to the central laboratory, Reproductive Endocrine Research Lab Los Angeles, USA, for estrogen assays. All samples must be labeled with pre-printed self-adhesive labels, contained in the blood collection kits. The Sample Collection Form (Form 35-SE-SC) should be faxed into DataFax or entered into iDataFax at the time of the blood draw to inform IBCSG that the collection has been made. Samples have to be stored and shipped at -20°C. All assay results will be assessed centrally and results submitted to the IBCSG Data Management Center. The SOLE-EST Manual for Blood Sample Logistics and Vigorimeter Use contains the details for sample collection, storage, and shipping, as well as measuring grip strength.

Samples will be anonymized and confidentiality will be maintained during the entire process. The costs of blood collection will be covered by the additional patient fee and shipping will be free of charge.

6.6. Grip strength measurement

Grip strength will be measured using the Martin Vigorimeter and recorded on Form 35-SE-SC. The Vigorimeter will be provided or reimbursed by IBCSG. Grip Strength will be measured at Baseline and Months 9 and 12.

6.7. Blood banking

Surplus DNA isolated from the whole blood as well as surplus serum will be transferred to the IBCSG Tissue Bank located at the IBCSG Central Pathology Office, Milan, Italy, for use in not yet specified future research. As part of the informed consent process, patients are asked

to indicate whether they agree that their sample is used for such research. The use of the DNA and serum for unspecified future research will be under the auspices of the IBCSG Biological Protocols Working Group and any project has to be approved by the IBCSG Ethics Committee.

6.8. Determination of estrogen levels and SHBG

Estrogen levels must be measured using a highly sensitive assay. Direct radioimmunoassay (RIA) does not provide adequate sensitivity at low levels. RIA specificity can be increased by an organic solvent extraction step, which if combined with chromatographic separation of estrogen from interfering steroids, will enable reliable measurement of the steroid.

A central laboratory (Reproductive Endocrine Research Lab, Los Angeles, USA) will do the assays, and the sensitivity they quote for their RIA using a 1.0 ml aliquot of serum is as follows:

- for estradiol (E_2) is 2 pg/ml (7.34pmol/L)
- for estrone(E_1) is 3 pg/ml (11.01pmol/L)
- for estrone sulphate (E_1S) is 9 pg/ml (33.03pmol/L)

It is considered that a 50% increase in estradiol (E_2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay) after cessation of letrozole would be clinically significant.

6.9. SNP analysis

The Sequenom MassARRAY® technology, which is an innovative technology platform for high-throughput genotyping of gene polymorphisms, will be used for the SNP analysis. It efficiently multiplexes up to 40 different genotypes into one reaction, and thereby provides very flexible, effective, rapid and accurate genotypes at an affordable price. The Sequenom platform is fully operational at the Vesalius Research Center, K.U. Leuven and VIB, Belgium.

7. Measurement of toxicity and quality of life

Collection of toxicity information:

Adverse event information will be collected at baseline and at 6 and 12 months as per the main study on Form 35-AE.

Measurement of musculoskeletal toxicity:
An additional assessment of musculoskeletal toxicity will be undertaken with the use of a grip strength measure at baseline, 9 and 12 months using a modified sphygmomanometer (Martin Vigorimeter). To perform the hand grip test, the patient is asked to squeeze the balloon of a modified sphygmomanometer three times with maximal force and the maximal value of three trials of each hand will be used for evaluation. This assessment has high inter-rater reliability and takes a minimal amount of time.

Quality of life assessment:
Patient-reported symptoms will be assessed by the Breast Cancer Prevention Trial (BCPT) Symptom Scales[31] on Form 35-PRS. In addition, several global QoL indicators will be assessed using the IBCSG Trial 35-07 QL Form. For detailed descriptions of the measures

please refer to Appendix III of the IBCSG 35-07/BIG 1-07 Protocol: Patient Reported Symptoms and Quality of Life Substudy.

8. Statistical Considerations

8.1. Study design and objectives

The study will use a longitudinal design with samples drawn over a 12-month period (0, 9, 10.5 and 12 months after randomization to the core protocol). Only patients enrolled in the parent trial are eligible. The target accrual to the substudy is 100 patients. The main objectives are:

8.1.1. To describe estrogen levels (E_2, E_1 and E_1S) at different timepoints during the first 12 months of protocol treatment, including:

- The degree of recovery of E_2, E_1 and E_1S during the 3 month break from letrozole.
- Levels and degree of suppression from baseline of E_2, E_1 and E_1S during 12 months of treatment with letrozole taken either continuously or intermittently.
- The within-patient variability of E_2, E_1 and E_1S across time among patients continuously taking letrozole.
- Whether levels or degree of suppression of estrogens vary according to:
  - prior adjuvant endocrine therapy (type, duration, duration without therapy prior to study entry)
  - current age
  - type of menopause
  - BMI

8.1.2. To assess the correlation of estrogen levels and of changes in estrogen levels from baseline with clinical outcomes of toxicity (musculoskeletal toxicity [as assessed by grip strength], arthralgia, hot flushes and insomnia) and quality of life measures and with germline SNPs.

8.2. Sample size considerations

The target accrual to the substudy is 100 patients, 25 in arm A (continuous letrozole) and 75 in arm B (intermittent letrozole). Taking into account the actual accrual of participating Centers, enrollment is expected to be completed at the time the parent trial closes to accrual.

Sample size was determined based on a conservative approach to the primary analysis in which patients are classified as to whether or not the change in E_2 between 9 and 12 months exceeds a certain threshold indicative of clinically significant recovery of E_2. Clinically significant recovery is defined as a 50% increase in estradiol (E_2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay). With a sample size of 100 patients, 25 in arm A (continuous letrozole) and 75 in arm B (intermittent letrozole), and assuming conservatively that only 80% of patients have paired samples at 9 and 12 months, then there is over 90% power to detect a difference between 10% vs. 50% of patients having recovery of E_2 during the 3-month interval (Fisher's exact test; two-sided α=0.05).

Further, the sample size will allow exploration of the relation of other factors – specified patient characteristics, clinical measures and germline SNPs – with E_2 changes measured on the continuum. Again focusing on the change in E_2 between 9 and 12 months among patients in arm B, and considering a range of the frequency of a binary factor in the population from

50-50% to 10-90% , the detectable effect size between two groups ranges from about 0.75 to 1.25 SD (80% power, two-sided α=0.05, two-sample t-test). For a continuous factor a Pearson's correlation coefficient of 0.36 vs. null value of 0 would be detectable.

8.3. Data analyses

Estrogen levels (E_2, E_1, E_1S) will be summarized over time among all patients and by treatment arm using descriptive statistics (mean, SD, median, quartiles) and graphically. It is anticipated based on the literature that log-transformation of estrogen levels may be required, in which case the geometric mean would be reported. Values will also be summarized as percent change from baseline levels.

Using linear mixed modeling of all available data over the 4 timepoints (or of 3 timepoints relative to baseline), each estrogen level will be modeled as a function of time and treatment arm and the interaction of the two factors to investigate the time pattern of estrogen levels and differences in levels between treatment arms. Of primary interest is whether the change from 9 to 10.5 and 12 months differs among patients assigned to intermittent vs. continuous letrozole. It may be the case that estrogen levels while on treatment are below the assay lower limit of detection, in which case the analysis method would be changed to account for this sort of "floor effect" or "left censoring" of data. Relationships of specified patient characteristics (prior endocrine therapy, age at and/or type of menopause, BMI) with the estrogen levels and changes in levels will also be investigated, as well as specific toxicities and quality of life measures and germline SNPs.

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International Breast Cancer Study Group Statistical Center

SOLE (Trial 35-07/BIG 1-07)

Statistical Analysis Plan

Version	Author	Date	Status
1	Weixiu Luo	Jan 2017	For primary trial report
2	Meredith Regan, ScD	May 2019	For final, updated report [updates/changes to SAP from 2017 report tracked, marked as edited or marked as new]

1 INTRODUCTION

1.1 BACKGROUND

In 2006, the standard duration of adjuvant endocrine therapy for breast cancer (either SERMs or AIs) is five years. Patients who receive extended adjuvant letrozole for five years following approximately five years of tamoxifen obtain further benefit compared with the five years of tamoxifen alone. Similarly, benefit has been demonstrated for switching from tamoxifen to an AI after 2 to 3 years of tamoxifen to complete five years of endocrine therapy, as well as initiating therapy with AI following surgery and administering the AI for five years.

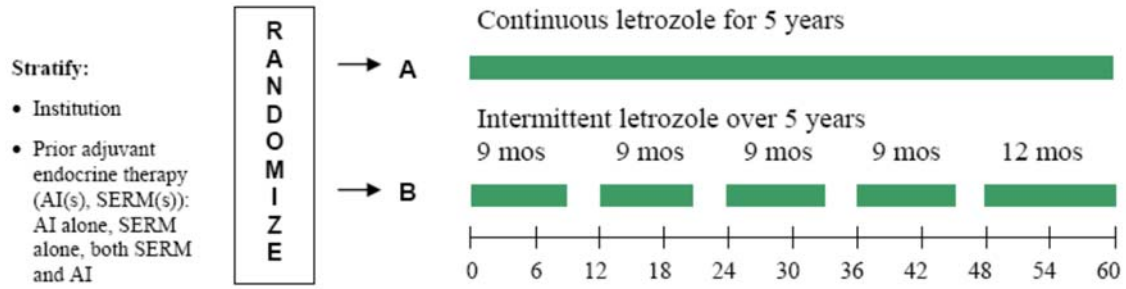
Questions remain about the optimal duration and best schedule of AIs in the extended adjuvant setting. This trial tests the hypothesis that introducing 3-month treatment-free intervals during the course of five years of extended adjuvant letrozole will improve disease-free survival. This hypothesis is based on the theoretical principle that letrozole withdrawal for 3 months will permit some estrogenic stimulation which makes residual resistant disease susceptible to letrozole reintroduction.

1.2 TRIAL DESIGN

SOLE (IBCSG 35-07 / BIG 1-07) OVERVIEW

Title:	**Study Of Letrozole Extension (SOLE):** A phase III trial evaluating the role of continuous letrozole versus intermittent letrozole following 4 to 6 years of prior adjuvant endocrine therapy for postmenopausal women with hormone receptor positive, node-positive early stage breast cancer.
Patient Population:	Postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with selective estrogen receptor modulator(s) (SERM) and/or aromatase inhibitor(s) (AI) for endocrine-responsive, node-positive early breast cancer.
Entry:	Patients must have stopped prior endocrine SERM/AI therapy, and must be randomized within 12 months (1 year) of the last dose of prior endocrine SERM/AI therapy.
Activation Date:	8 November 2007 (First patient randomized on 5 December 2007)
Target Accrual:	4800 patients
Closure Date:	12 July 2012 (Last patient randomized on 8 October 2012)
Final Accrual:	4884 patients
Last Visit:	31 December 2018

Trial Schema:



The SOLE trial is a multinational phase III randomized clinical trial designed to compare continuous letrozole for 5 years with intermittent letrozole over a 5-year period among postmenopausal women who are disease-free following 4 to 6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive node-positive operable breast cancer. The hypothesis is that introducing 3 month treatment-free intervals during the course of 5 years of extended letrozole will improve disease-free survival.

The randomization was stratified according to participating center and prior SERM/AI endocrine therapy (SERM(s) alone, AI(s) alone, both SERM(s) and AI(s)). Patients were randomized 1:1 to one of two extended adjuvant endocrine therapy groups:
A: Continuous letrozole 2.5 mg daily for 5 years
B: Intermittent letrozole 2.5 mg daily for the first 9 months of years 1 through 4, with treatment-free intervals for the last 3 months of years 1 through 4, followed by 12 months of letrozole 2.5 mg daily in year 5
Patients should start trial treatment as soon as possible after randomization. Trial treatment should begin no later than 6 weeks from the date of randomization. All patients will be followed every 6 months for years 1 to 5, and yearly thereafter for assessment of disease status and for survival data collection.

1.3 SAMPLE SIZE CONSIDERATIONS

The sample size was determined to provide 80% power to detect a 20% reduction in the risk of an event defining DFS associated with intermittent letrozole compared with continuous letrozole (hazard ratio = 0.80; 25% increase in 4-year DFS from 90% to 91.917%) using a two-sided 0.05 level test of significance.

To detect the treatment difference 647 DFS events are required, assuming 4800 patients are accrued (1600 patients per year for 3 years), 5% non-assessability at 4 years, and approximately

5 years of additional follow-up. One year of start-up time, as Participating Centers obtain Ethics Committee approval and complete regulatory processes, is anticipated.

1.4 Interim Monitoring Plan (from Protocol)

A group sequential design with two interim analyses and one final analysis is used. The target number of events for the final analysis is 647, and interim analyses are planned after 40% and 70% information (259 and 453 events observed respectively). At each interim analysis and at the final analysis, testing will be performed using O'Brien-Fleming boundaries.

1.5 Data and Safety Monitoring Committee Reviews

The Data and Safety Monitoring Committee (DSMC) reviews the safety information, including adverse events, second (non-breast) malignancies, and deaths without prior cancer event, every six months and has recommended the trial continue as planned. The first interim efficacy analysis was performed for spring 2014 DSMC report when 324 DFS events (50% information) were observed. At that time the O'Brien-Fleming boundary was not crossed and there was not enough evidence to support early stopping of the trial due to efficacy. The DSMC recommended that SOLE treatment administration and follow-up continue as planned. The same results held for the second interim efficacy analysis which was performed for spring 2015 DSMC report when 454 DFS events (70% information) were observed. The final DSMC review was undertaken on 13 November 2017 after all patients had completed the 5-year protocol therapy period, and determined that follow-up should continue as planned.

1.6 Primary Results

After a median follow-up of 5 years (data cut-off, 31 October 2016), DFS events were reported for 665 of 4851 patients in the intention-to-treat population. Patients assigned intermittent letrozole did not have a significantly reduced hazard of a DFS event vs. patients assigned continuous letrozole (HR=1.08; 95% CI, 0.93 to 1.26; P=0.31). The estimated 5-year DFS was 85.8% among patients assigned intermittent letrozole versus 87.5% for patients assigned continuous letrozole. Similar results were observed for secondary endpoints of breast cancer-free interval (431 events, HR=0.98; 95% CI, 0.81 to 1.18; P=0.84), distant recurrence-free interval (338 events, HR=0.88; 95% CI, 0.71 to 1.09; P=0.25), and overall survival (316 deaths, HR=0.85; 95% CI, 0.68 to 1.06; P=0.16). At 60 months median follow-up, 27% of patients continued on protocol treatment. 36.2% and 34.5% of patients assigned to intermittent versus continuous letrozole had grade 3-5 targeted AEs reported, of which grade 3-4 hypertension was most frequently reported.

The results were presented at the 2017 ASCO Annul Meeting in June 2017 [1] and subsequently published in *The Lancet Oncology* [2]. In postmenopausal women with hormone receptor-positive breast cancer, extended use of intermittent letrozole did not improve disease-free survival compared with continuous use of letrozole.

1.7 FINAL, UPDATED ANALYSIS

A decision was made following publication of the primary results to cease follow-up as of 31 December 2018 **(last patient, last FU visit)**, at which point the median follow-up would be extended from 5 to approximately 7 years (range 6 to 11 years). A SOLE Flash newsletter was distributed to investigators, providing information regarding closure of data collection at the end of 2018.

In addition to an update of the primary and secondary endpoints, adverse events and final treatment status (the 5 years of protocol treatment was reached as of October 2017) , it was decided that this final analysis would include also the SOLE-EST substudy and further investigate whether there is heterogeneity of treatment efficacy according to type and duration of prior endocrine therapies, .

2 EFFICACY ANALYSIS PLANS

2.1 OBJECTIVES

The primary objective is to compare continuous letrozole for five years with intermittent letrozole over a five year period for postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive, node-positive, operable breast cancer.

2.2 ANALYSIS POPULATIONS

The primary analysis will use an intention-to-treat (ITT) approach. The ITT population will include all randomized patients, regardless of eligibility status; the possible exceptions are patients who immediately withdrew consent prior to treatment initiation and declined all participation, patients determined (e.g., via audit) to be without documented informed consent, and/or patients at a participating center determined not to be compliant with protocol procedures. Any exclusion from the ITT population will be determined prior to the analysis and will be summarized in listing and CONSORT in the trial report. The final, updated analysis will continue to use an ITT approach and the ITT population as originally reported will be used, even if information has changed since the primary report; the only exception would be for a case in inadequately documented informed consent or withdrawal of consent to use any data.

2.3 ENDPOINT DEFINITIONS

2.3.1 Primary Endpoint

- Disease-free survival (DFS) is defined as the duration of time from randomization to the first indication of the following events: invasive recurrence at local (including recurrence restricted to the breast after breast conserving treatment), regional or distant sites; a new invasive cancer in the contralateral breast; any secondary (non-breast) malignancy; or a death without

prior cancer event. Appearance of DCIS or LCIS either in the ipsilateral or in the contralateral breast will not be considered as an event for DFS. In the absence of an event, DFS is censored at the date of last follow-up.

2.3.2 Secondary Endpoints

- Overall survival (OS) is defined as the duration of time from randomization to death from any cause, or is censored at the date last known alive. (Note, for patients who withdrew consent or were lost to follow-up but follow-up for survival was possible through hospital or registry records, OS is censored at the date last known alive rather than date of last follow-up/withdrawn consent).

- Distant disease-free survival (DDFS) was a secondary endpoint in protocol. It will be replaced by a more modern definition -- distant recurrence-free interval (DRFI) defined as the duration of time from randomization to the first indication of invasive breast recurrence at a distant site. In the absence of an event, DRFI is censored at the date of last follow-up or date of death without distant recurrence.

- Breast cancer-free interval (BCFI) is defined as the duration of time from randomization to the first indication of the following events: invasive breast recurrence at local, regional or distant sites; a new invasive cancer in the contralateral breast. In the absence of an event, BCFI is censored at the date of last follow-up or date of death without prior breast cancer event. (Second non-breast malignancies are ignored)

- Site of First Failure (i.e., DFS events): Hierarchy of failures from least to worst, following standard IBCSG definition:
  - Local
  - Contralateral Breast ± above
  - Regional ± above
  - Soft tissues/distant nodes ± above
  - Bone ± above
  - Viscera (including 'other') ± above

  as well as,
  - second (non-breast) malignancy,
  - death without prior cancer event (aka death without subsequent cancer event)
  - death with breast cancer recurrence suspected
  - death with no information about recurrence.

For "site of first failure," if there are 2 or more failure sites within 2 months of each other, then the "worst" site is considered as the site of first failure. For example if there is a regional recurrence and a bone recurrence within 2 months of each other, then the bone would be

considered the "worst" site of failure and considered as "site of first failure." If second malignancy is coincident with breast cancer recurrence then site of first failure is reported as the breast cancer recurrence.

Per IBCSG standard, the date of DFS event is the date a proven recurrence was first suspected. In rare cases that the date suspected is prior to randomization, date of randomization is considered as date of event. Since the first report, the 35-RC form SOP was clarified that in cases with multiple proven sites of first recurrence (within 2 months of each other), that the date first suspected should be the earliest of suspected dates from among those proven sites (which may not necessarily correspond to the site with the earlies proven date).

2.4 FOLLOW-UP

Number of DFS events per person-year of follow-up is calculated at each DSMC review time to guide the timing for the database lock. The data cut-off is planned for prior to November 1, 2016 when we expect the number of DFS events will reach the target of 647 for the analysis with database lock in Q1 2017. Data as of the database cut-off will be used for analysis.

Median follow-up is calculated from the Kaplan-Meier estimate of overall survival, with the event/censoring indicator inverted (i.e. alive as event and dead as censored).

The final, ~~U~~updated results will be presented ~~every~~ approximately two to three years ~~there~~after the primary report.

2.5 TESTS AND ESTIMATES

The primary endpoint, DFS, will be compared between treatment arms using a two-sided stratified logrank test with an overall experiment-wise alpha-level equal to at most 0.05. The test statistic and p-value will be taken from the stratified Cox PH model score test. Hazard ratios will be estimated from a stratified Cox PH model, with 95% CIs. Kaplan-Meier estimates of the DFS distributions will be calculated for each of the treatment arms, with reporting of the 5yr DFS. We will check the proportional hazards assumption by visually assessing the plot of log(-log(survival)) versus log of survival time for parallelism. This will be done overall, and according to strata.

2.5.1 Stratification

The stratification factor during randomization is prior SERM/AI endocrine therapy (SERM(s) alone, AI(s) alone, both SERM(s) and AI(s)) as reported on randomization (RA) form. Institutions were balanced using dynamic balancing. Logrank test and Cox PH model will be stratified by prior SERM/AI endocrine therapy.

Note about values of strata as provided at randomization vs. actual values: The randomization form (RA form) collects the values entered into the IBCSG randomization system and used for stratification of the randomization assignment. If these values are incorrect then they are amended on the Registration form (A-form). ~~We will cross-tabulate the stratification variables between RA and A forms to compare the information obtained at randomization versus that on the A forms.~~ [done, no longer needed] For primary and secondary overall analyses we will use the stratification factor entered on randomization form. For subgroup analyses, we will use the actual values entered in the A form when available.

2.6 ANALYSIS COMPONENTS [EDITED]

2.6.1 Enrollment, Follow-up Compliance

2.6.1.1 Overview of enrollment

Figures:

- Enrollment over time; x-axis time in 6-montly intervals; y-axis number enrolled

2.6.1.2 Follow-up submission

Tables:

- Institutional follow-up compliance by group/country (rows)

2.6.1.3 CONSORT

In the manner of the CONSORT diagram, the following will be summarized, with changes from the primary report highlighted.

Tables:

- CONSORT diagram content numbers by treatment assignment
    - Number of patients randomized
    - Number of patients included vs excluded from analysis population, with reasons [not expected to change since primary analysis]
    - Number in analysis population who never started protocol treatment [note any changes since primary analysis]
    - Number in analysis population who WC/LFU [update]
    - Number of patients analyzed in analysis population [same as number included; not expected to change since primary analysis]

2.6.1.3 Stratification

Tables

- Distribution of stratification factors, overall and according to treatment assignment
- Listing of any new information about actual (from A-form) values of stratification factors

2.6.1.4 Withdrawn consent/loss to follow-up status

Summary should mention any centers that were lost to follow-up since the primary report.

Tables:

- Withdrawn consent and lost to follow-up status, overall and according to treatment assignment

2.6.2 Patient, Disease and Prior Treatment Characteristics

Characteristics of the analysis population will be summarized overall and by treatment group. Continuous variables are summarized as mean, SD, min/max, and quartiles. Categorical variables are summarized as N(%); for variables with unavailable (missing, unknown, not done) values, the default approach is to include an unknown category that is included in the denominator for percentages (rather than just listing the number of unknowns as a category). The same tables will be re-run, and may differ slightly from the primary report as the data will reflect any updates to the database since the primary database lock.

Tables (overall and by treatment group, unless otherwise specified):

- Patient:
  - Age at randomization (continuous; categorized in 5-year intervals)
  - Race/ethnicity
  - ~~Performance status at randomization~~ [this was an error; not collected]
  - BMI at randomization
  - Menopausal status at primary cancer diagnosis
  - Symptoms (from baseline AE form) [n.b., slight redefinition]
- Treatment:
  - Local therapy (combining surgery [Mx/BCS] and radiotherapy [yes/no]
  - Chemotherapy (whether used; ~~regimen [anthracycline-based; taxane-based; both; other]~~
  - ~~Biologic therapy~~ [not reported; not useful]
  - Prior endocrine therapy [stratification factor; actual] and years of prior endocrine therapy [<4.5; 4.5-5.5; >5.5]; more detail will be added, see section 2.6.5 below
  - Duration from end of prior adjuvant therapy to randomization [≤1 vs >1 month]
- Disease:
  - ER/PgR and HER2 status (H form)
  - Nodal involvement (number of positive lymph nodes 0, 1-3, 4-9, 10+, unknown)
  - Tumor size (<1, 1-2, >2-5, >5 cm) and grade (1, 2, 3), primary histology (ductal vs lobular vs other; ~~and other details from~~ H form)
  - ~~Disease laterality and location~~

2.6.3 Primary Efficacy Analysis

The primary efficacy analysis will proceed as summarized in Section 2.5 above. The data cut-off and database lock dates used for the analyses and the median follow-up duration (and IQR and range; overall and by treatment assignment) will be reported. Numbers of outcome events at primary analysis and at final, updated analysis will be tabulated, for ease of reference.

2.6.3.1 Subgroup Analyses

The protocol pre-specified factors that will be used to characterize the patients enrolled in the study and to provide descriptive statistics of outcomes according to subgroups of the population. These factors include: age at randomization, body mass index, tumor size, tumor grade, number of positive lymph nodes, ER/PgR, HER2 status, type of prior endocrine therapy, duration of prior endocrine therapy by type, interval of time since the cessation of prior endocrine therapy till randomization. These analyses will be considered as secondary and descriptive.

The plans for these variables are summarized below:

- Age (5-year age groups (<55, 55-59, 60-64, 65-69, ≥70))
- BMI (obese (≥30), overweight (25 - <30), normal (<25), unknown)
- Tumor size (≤2 vs >2 cm; unknown)
- Tumor Grade (1,2,3,unknown)
- ~~ER/PgR subgroup (+/+; +/-; -/+; unknown and other)~~ [too few not +/+ to be meaningful]
- ~~HER2 status (positive, negative, unknown)~~ [too few positive to be meaningful]
- No. of positive lymph nodes (0, 1-3, 4+, unknown)
- Type of prior endocrine therapy (AI, SERM, AI and SERM) & more details?
- Duration of prior endocrine therapy (<4.5, 4.5-5.5, >5.5 years) & more details?
- Interval of time since the cessation of prior endocrine therapy till randomization (≤1, >1 mo)

2.6.3.2 Models

Stratified Cox PH regression models will be used to: estimate HRs (95% CI) for treatment effect, unadjusted and adjusted for these covariates in Section 2.6.3.1; and estimate HRs (95% CI) for treatment effect within subgroups by including treatment-by-covariate interaction in the model (but not other covariates) and using contrasts.

2.6.3.3 Tables and Figures

Tables:

- Primary treatment comparison: N events and patients, HR, 95% CI, log-rank test statistic and p-value, 5yr and 7yr DFS, SE and 95% CI
- Treatment effects within subgroups: N events and patients and 7yr rates within each subgroup, treatment HR, 95% CI, p-value for test of treatment-by-variable interaction

Figures:

- KM plot of DFS, by treatment group, for entire analysis population *(y-axis: Percent Alive and Disease-Free; x-axis: Time since Randomization (1-year intervals); x-axis limited to ~~6~~ 8 years (median follow-up plus 1 year) and numbers at risk at each yearly interval)*
- Forest plot of DFS, overall and for subgroups

2.6.4 Secondary Efficacy Endpoints

Breast cancer-free interval, distant recurrence-free interval and overall survival will be summarized as described for DFS.

Sites of first treatment failure will be summarized overall and by treatment group as N (%). Second (non-breast) malignancies as site of first failure, and deaths without prior cancer event will be summarized overall and by treatment assignment.

2.6.4.1 Tables and Figures

Tables:

- Primary treatment comparison for each of the 3 secondary endpoints: N events and patients, HR, 95% CI, log-rank test statistic and p-value, 5yr & 7yr DFS, SE and 95% CI
- Sites of first failure, overall and by treatment assignment
- Types of second non-breast malignancies, as site of first failure, overall and by treatment assignment.
- Second non-breast malignancies and Death without prior cancer event (list of patients with type of malignancy or cause of death, respectively)

Figures:

- KM plots of BCFI, by treatment group, for entire analysis population *(y-axis: Percent Free from Breast Cancer; x-axis: Time since Randomization (1-yr intervals); x-axis limited to ~~6~~8 years (median survival plus 1 year) and numbers at risk at each yearly interval)*
- KM plots of DRFI, by treatment group, for entire analysis population *(y-axis: Percent Free from Distant Recurrence; x-axis: Time since Randomization (1-yr intervals); x-axis limited to ~~6~~ 8 years (median survival plus 1 year) and numbers at risk at each yearly interval)*
- KM plot of OS, by treatment group, for entire analysis population *(y-axis: Percent Alive; x-axis: Time since Randomization (1-yr intervals); x-axis limited to ~~6~~ 8 years (median survival plus 1 year) and numbers at risk at each yearly interval)*

2.6.5 Treatment Efficacy in Relation to Prior Endocrine Therapy Type & Duration (new)

Motivation: Investigate whether duration of prior AI is associated with treatment responsiveness; hypothesize that different duration of prior AI may be differentially sensitive to AI interruption

on basis of heterogeneity of extended AI trial results depending on prior therapy of enrolled population.

Endpoints: BCFI, DRFI, DFS; OS to be analyzed only if other results of interest, to ensure consistency of message.

1) Define prior ET type and duration in more detail:

- number of months of prior AI, number of months of prior SERM;
- first drug taken, most recent drug taken;
- number of weeks of prior AI within the 1 yr before randomization.

Re-tabulate categories of AI duration, in particular looking more closely at those with both prior AI and SERM exposure to better differentiate those with sequential tam>AI (approx. 2>3 yrs) as separate from those that had a short duration of AI and mostly tamoxifen (i.e., more like SERM-only), or had at least 4 yrs of AI (i.e., more like AI-only). [0-<6m; 6m -<2y; 2-<3y; 3-<4y; ≥4y]

2) Assess relation of prior AI use with other covariates, especially age and menopausal status at diagnosis, and disease characteristics.

- Tabulate patient, disease and treatment characteristics reported at randomization, overall and according to AI duration categories above (without regard to treatment assignment).

3) Assess relation of prior AI use (AI duration categories above) with time to protocol therapy cessation (with DFS event as competing risk); with and without regard to treatment assignment.

- There is particular interest in a group of patients who had very short AI exposure (>0 but <6m to 1yr) early in adjuvant treatment and presumably stopped because of tolerability (i.e., do not include pts whose short AI exposure was just prior to randomization); the clinical question would be whether such patients should re-try AI for extended adjuvant or also very likely to cease early.

4) Assess relation prior AI duration with treatment comparison;

- STEPP analysis, on HR and 7-yr event rate, with continuous prior AI duration as x-axis; note this doesn't adjust for covariates.
- Using unstratified Cox model, test prior AI categories (as above) vs treatment assignment interaction, adjusted for selected patient and disease characteristics [age or menopausal status, BMI; nodes, tumor size, tumor grade, HER2 status; prior chemo? prior local-therapy?] (n.b., 3-category prior ET already done in forest plot, unadjusted; may want to repeat this in adjusted Cox model)
- If useful as part of unadjusted analyses, KM plots of subgroups defined by prior AI duration categories (as above) and treatment assignment, with 7-yr estimates.

2.6.6 Adverse Events / Safety

2.6.6.1 Population

The toxicity population is the subset of patients in the ITT analysis population who started protocol treatment. Any patients without at least 1 post-baseline AE form submitted will not be able to contribute; note any such patients in trial report (noting any difference from primary report).

2.6.6.2 Analysis

Targeted AEs, and other grade 3-5 AEs, are collected on CRFs. The grade and causality attribution are recorded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. The targeted AEs will be summarized by AE type and maximum grade over time, regardless of causality attribution. The maximum grade consolidates the reports of a given type of AE for a patient over time since randomization (i.e., baseline reports are excluded) by taking the maximum across time (i.e., a patient appears only once for a given type of AE). Patients with reports of multiple AEs of different types are reported multiple times under the relevant AE categories. Maximum grade 0 indicates that the AE type has not been reported. 95% exact CIs will be calculated for each targeted AE, according to treatment group.

Other grade 3 or higher AEs are also requested on CRFs, by write-in text. All will be tabulated, similarly according to max grade, but the intention is to focus on those deemed possibly, probably or definitely related to study treatment(s), which will also be tabulated.

Late adverse events (LAEs: cardiac ischemia/infarction, thrombosis/thrombus/embolism, CNS cerebrovascular ischemia, and fractures) were requested on CRFs, but very little was reported, thus we will not automatically summarize these data, as we're concerned that this would underrepresent the prevalence of such events.

2.6.6.3 Tables and Figures

Tables:

- Numbers of patients in the safety population, overall and by stratum and treatment assignment; listing of any changes since primary analysis and listing of any patients who started treatment but do not have AE CRFs submitted
- Targeted AEs by grade, reported according to treatment assignment; N, %
- Targeted AEs, % grade 1-5 AE with 95% CI; % grade 3-5 AE with 95% CI, reported according to treatment assignment
- Targeted AEs by grade, reported according to prior endocrine therapy and treatment assignment; N, frequency
- Other non-targeted grade 3-5 AEs, deemed possibly, probably or definitely related to study treatment(s) according to treatment assignment, N

2.6.7 Treatment

Final Protocol treatment status ~~as of the clinical data cut-off~~ will be summarized. In this report, status will be summarized by treatment assignment. Time from randomization to permanent treatment discontinuation will be summarized using KM curves according to treatment assignment; this will be done without regard to reason to summarize the exposure period, and secondarily with disease events as competing risks as a measure of adherence with 5-yr treatment period.

Details of scheduled (and unscheduled) interruptions were analyzed and reported in the primary Clinical Trial Report. These analyses below will not be repeated:

Per SOLE Data Manager's Hand Book v3.0 (dated 21Mar13), for Arm B patients, interruptions should be every 9 months and should last for 3 months in years 1-4. Any interruption up to 4 months is considered a "protocol-specified interruption" and the reason for interruption is defined as "protocol-defined interval" on L forms; any interruption of 4 months +1 day (121 days or more) is considered a "non-protocol interruption".

The Idealized Scheduler contains a link to an Excel file on the IBCSG web site. This file calculates the letrozole dispensation and interruptions (Arm B) based off of the Randomization Date.

Treatment variation scenarios:

- Patient on Arm B stops letrozole early (e.g., at Month 6 instead of Month 9). The patient should stop for three months. Patient should re-start letrozole and continue until the pre-defined date of the next interruption. This will get the patient back on schedule.
- Patient on Arm B took medication during the protocol-specified interruption. Patient should continue until Month 9 of the next year and then start the interruption. This will get the patient back on schedule.
- Patient forgot to take some pills and therefore has some left at the date the interruption should start. The patient should not finish the medication. The patient should interrupt on the pre-defined date to stay on schedule.

For Arm A patients, not-per-protocol interruptions are those of over a month in duration.

Reasons for treatment interruptions will be summarized by treatment assignment. Note that there could be more than one reason for an interruption spanning more than one L Form: for example, a patient start interruption per protocol on one L form, but due to adverse event, the interruption continues to the next L form without restarting after 4 months. The first reason would be "protocol-defined interval" and then the second reason would be "Adverse Event".

Based on information on Data Manager's Handbook, to describe adherence with protocol-assigned treatment, the following tables and figures are proposed.

Tables

- Final status of protocol-assigned treatment, overall and according to treatment assignment (same as CTR table 8.1.1)
- ~~Number (%) patients with per-protocol interruptions and resumptions for patients assigned intermittent letrozole (CTR table 8.3.1)~~
- ~~Number (%) patients who took not per-protocol interruptions over the 5 years, according to treatment assignment (CTR table 8.3.2)~~
- ~~Number (%) patients on treatment by month, according to treatment assignment (denominator is pts who have not perm. d/c at beginning of month; numerator is pts on treatment at beginning of month; %)~~

Figures

- Time from randomization to permanent treatment discontinuation, according to treatment assignment, with yearly estimates: all reasons (CTR Fig 8.2.1), and then with disease event as competing risk (x-axis: months 0-72 since randomization; y-axis: percent on treatment 0-100)
- ~~Percentage of patients on treatment by month, according to treatment assignment (x-axis: month 1 to 60, y-axis: percentage; CTR Fig 8.3.1)~~
- ~~Percentage of patients on treatment by 3-month intervals, according to treatment group (x-axis: interval 1 to 20 quarters, y-axis: percentage; CTR Fig 8.3.2)~~
- ~~Mean percentage of days on treatment during 3-month intervals, according to treatment group (x-axis: interval 1 to 20 quarters, y-axis: mean percentage; CTR Fig 8.3.3)~~

3 SOLE-EST SUBSTUDY

3.1 SUMMARY

Third generation aromatase inhibitors such as letrozole profoundly suppress estrogen levels in postmenopausal women, which is the mechanism of their action against hormone receptor positive breast cancer. The hypothesis under investigation in the SOLE trial is that removing this suppression for a period of time will allow re-sensitization of breast cancer cells to estrogen deprivation resulting in a delay or prevention of the development of resistance to the anti-cancer effect of letrozole.

The estrogen suppression substudy enrolled patients at randomization and aims to describe the changes in serum levels of estrogen levels that occur on letrozole and during the three-month treatment gap without letrozole. The blood sampling time points were over the first year since randomization (see schema). Estrogen suppression and recovery may also be linked with clinical outcomes including toxicity and disease free survival. SOLE-EST will also examine the relationship between estrogen level changes and changes in toxicity, patient-reported musculoskeletal symptoms and QL, and grip strength. As genes important for the metabolism and activity of letrozole may influence estrogen levels, single nucleotide polymorphisms (SNPs) related to these will be assessed and analyzed in relation to the variability of estrogen level changes.

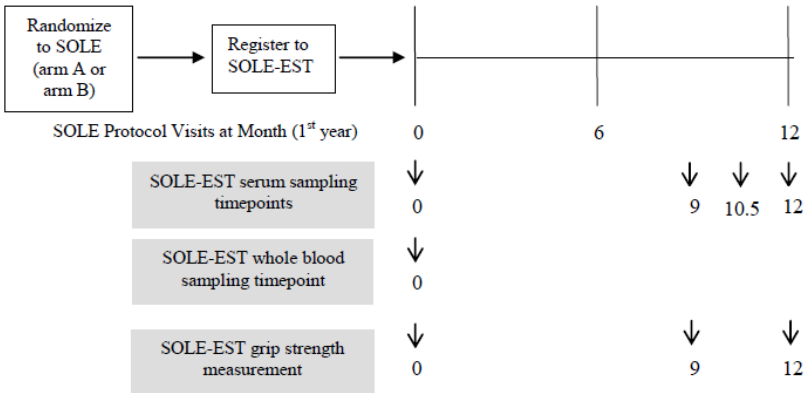
SOLE-EST was activated in IBCSG Participating Centers located in Belgium, Australia, and Milan on 19 November 2010 and the first patient enrolled on 31 January 2011. Enrollment was closed 12 July 2012, with a final accrual of 104 patients (25 from the continuous arm and 79 from the intermittent arm) enrolled at 14 Participating Centers.

All eligible patients randomized to SOLE from these Centers were to be offered participation in SOLE-EST, but inclusion was not mandatory.

SOLE-EST OVERVIEW

Title:	**SOLE-EST: SOLE Estrogen Substudy:** Investigating changes in estrogen levels and grip strength for patients participating in the SOLE Trial.
Patient Population:	Postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with selective estrogen receptor modulator(s) (SERM) and/or aromatase inhibitor(s) (AI) for endocrine-responsive, node-positive early breast cancer, who: have been randomized into the SOLE trial; participate in the Quality of Life substudy; provide written informed consent.
Entry:	Patients should be enrolled to SOLE-EST at the time of entry into the main study and the Quality of Life substudy. The first serum sample must be obtained after randomization but prior to starting letrozole on study according to the guidelines in the SOLE-EST Manual for Blood Sample Logistics and Vigorimeter Use.
Activation Date:	19 November 2010 (First patient enrolled on 31 January 2011)
Target Accrual:	100 patients: 25 from the continuous arm and 75 from the intermittent arm.
Closure Date:	12 July 2012 (Arm A closed 29 Sept 2011)
Final Accrual:	104 patients

SOLE-EST schema and blood sampling timepoints



3.2 BACKGROUND

There are three biologically important circulating estrogens present in postmenopausal women: 17 β-Estradiol (E2) is a product of both estrone and testosterone and the most abundant circulating estrogen in premenopausal women; estrone (E1) is the major product of aromatase enzyme activity in postmenopausal women; estrone sulphate (E1S) is the most stable estrogen fraction measured and provides a robust surrogate-parameter for suppression of estrogen biosynthesis in vivo. Mean plasma levels of E2, E1 and E1S in postmenopausal women are reported to be 20, 80 and 4-500 pmol/L respectively. Suppression of estrogen production by letrozole reduces levels close to or below the level of detection in many assays.

Musculoskeletal side effects are amongst the most common and most troublesome side effects associated with aromatase inhibitors and have been assessed in a number of ways. A simple and validated measure is grip strength using a modified sphygmomanometer. The effect of a three month break on QL was reported for the overall trial population. It was hypothesized that the increase in estrogen levels during the three month gap would reduce side effects, especially musculoskeletal side effects and patient-reported symptoms; and it is hypothesized that this will correlate with the absolute estrogen levels. It is also hypothesized that the increase in estrogen levels will improve QoL overall. The SOLE-EST substudy will test these hypotheses by using the toxicity data collected in the main study and the QoL data collected in the QoL substudy and by measuring the grip strength of the patients.

Letrozole is metabolized by cytochrome P450 (CYP) isoenzymes including CYP2A6 and CYP3A4. Genetic variations in the genes encoding for those enzymes can alter the metabolism and therefore plasma levels of letrozole. The gene encoding CYP19 (the aromatase enzyme that produces estrogen from testosterone) is a highly polymorphic gene that may influence the efficacy of letrozole through an altered (increased or decreased) activity. There is also some evidence that genetic variation may explain the variation of the degree of side effects amongst women on letrozole. Ingle *et al.* found 4 SNPs that were associated with the degree of musculoskeletal symptoms using a genome-wide association case-control study.

3.3 OBJECTIVES AND ENDPOINTS

Primary objective:

- To determine the serum level of estrogens E2, E1 and E1S and Sex Hormone Binding Globulin (SHBG) during letrozole treatment
- To determine the degree of recovery of E2, E1 and E1S during the 3 month gap.

Also noted, describe the within-patient variability of E2, E1 and E1S across time among patients continuously taking letrozole.

Secondary objectives:

- To link the estrogen level changes with the clinical outcomes of toxicity (musculoskeletal toxicity [as assessed by grip strength], arthralgia, hot flushes and insomnia) and QoL.

- To determine the effect of the following factors on estrogen levels: prior adjuvant endocrine therapy (type, duration, duration without ET prior to study entry); age; BMI; type of menopause.
- To explore variability of estrogen level changes and link these with germline SNPs.

Primary endpoints

- Levels of E2, E1 and E1S at 0, 9, 10.5 and 12 months from randomization to the core protocol.
- % change (suppression or recovery) of E2, E1 and E1S from baseline at 9 months, 10.5 months and at 12 months from randomization to the core protocol.

Secondary endpoints

- Toxicity grade changes (for arthralgia, hot flushes and insomnia) between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.
- Quality of life score change between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.
- Changes in grip strength score at 9 months and 12 months.

3.4 SUBSTUDY DESIGN AND PROCEDURES

3.4.1 Blood Samples and Assays

A whole blood sample was obtained at baseline. Serum samples were obtained at 4 time points (0, 9, 10.5 and 12 months); they were taken at any time of day and were not fasting. Samples were taken using provided blood collection kits and stored locally at -20°C. Assays were performed centrally.

To be checked. Estrogen levels must be measured using a highly sensitive assay. A central laboratory (Reproductive Endocrine Research Lab of Frank Stanczyk, Los Angeles, USA) will do the assays, and the sensitivity they quote for their RIA using a 1.0 mL aliquot of serum is as follows:

- for estradiol (E2) is 2 pg/ml (7.34pmol/L)
- for estrone(E1) is 3 pg/ml (11.01pmol/L)
- for estrone sulphate (E1S) is 9 pg/ml (33.03pmol/L)
- for SHBG .

It is considered that a 50% increase in estradiol (E2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay) after cessation of letrozole would be clinically significant.

The protocol stated the SNPs were to be analyzed using the Sequenom MassARRAY® technology in the laboratory of Vesalius Research Center, K.U. Leuven and VIB, Belgium:

- SNPs on alleles CYP2A6*2, CYP2A6*9, CYP2A6*12 and CYP2A6*35
- In the CYP3A4 gene, only the CYP3A4*1B and CYP3A4*7 SNP have a known effect on the enzymatic activity and a frequency of more than 1% in the Caucasian population. Therefore, for the CYP3A4 gene, only those two SNPs will be genotyped.
- The CYP19 SNPs with a frequency of >10% in the Caucasian population and with a known effect on the enzymatic activity will be analyzed, namely rs4646, rs10046, rs727479, rs10459592, rs4775936, rs6493497 and rs7176005.
- SNPs previously associated with musculoskeletal symptoms: rs7158782, rs7159713, rs2369049 and rs6637820.

3.4.2 Measurement of toxicity and quality of life

Adverse event information will be collected at baseline and at 6 and 12 months as per the main study. Primary AEs are arthralgia, hot flushes and insomnia.

Patient-reported symptoms will be assessed by the Breast Cancer Prevention Trial (BCPT) Symptom Scales on Form 35-PRS. In addition, several global QoL indicators will be assessed using the IBCSG Trial 35-07 QL Form.

Grip strength will be measured using the Martin Vigorimeter (a modified sphygmomanometer , provided or reimbursed by IBCSG) at 0, 9, 12 months. To perform the hand grip test, the patient is asked to squeeze the balloon of a modified sphygmomanometer three times with maximal force and the maximal value of three trials of each hand will be used for evaluation. This assessment has high inter-rater reliability and takes a minimal amount of time.

3.4.3 Sample size considerations

Sample size was determined based on a conservative approach to the primary analysis in which patients are classified as to whether or not the change in E2 between 9 and 12 months exceeds a certain threshold indicative of clinically significant recovery of E2. Clinically significant recovery is defined as a 50% increase in estradiol (E2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay). With a sample size of 100 patients, 25 in arm A (continuous letrozole) and 75 in arm B (intermittent letrozole), and assuming conservatively that only 80% of patients have paired samples at 9 and 12 months, then there is over 90% power to detect a difference between 10% vs. 50% of patients having recovery of E2 during the 3-month interval (Fisher's exact test; two-sided α=0.05).

Further, the sample size will allow exploration of the relation of other factors – specified patient characteristics, clinical measures and germline SNPs – with E2 changes measured on the continuum. Again focusing on the change in E2 between 9 and 12 months among patients in arm

B, and considering a range of the frequency of a binary factor in the population from 50-50% to 10-90% , the detectable effect size between two groups ranges from about 0.75 to 1.25 SD (80% power, two-sided α=0.05, two-sample t-test). For a continuous factor a Pearson's correlation coefficient of 0.36 vs. null value of 0 would be detectable.

3.4.4 Analysis plan (from protocol)

Estrogen levels (E2, E1, E1S) will be summarized over time among all patients and by treatment arm using descriptive statistics (mean, SD, median, quartiles) and graphically. It is anticipated based on the literature that log-transformation of estrogen levels may be required, in which case the geometric mean would be reported. Values will also be summarized as percent change from baseline levels.

Using linear mixed modeling of all available data over the 4 timepoints (or of 3 timepoints relative to baseline), each estrogen level will be modeled as a function of time and treatment arm and the interaction of the two factors to investigate the time pattern of estrogen levels and differences in levels between treatment arms. Of primary interest is whether the change from 9 to 10.5 and 12 months differs among patients assigned to intermittent vs. continuous letrozole. It may be the case that estrogen levels while on treatment are below the assay lower limit of detection, in which case the analysis method would be changed to account for this sort of "floor effect" or "left censoring" of data. Relationships of specified patient characteristics (prior endocrine therapy, age at and/or type of menopause, BMI) with the estrogen levels and changes in levels will also be investigated, as well as specific toxicities and quality of life measures and germline SNPs.

3.4.5 Analysis plan for trial reporting

We plan as part of the report of the parent trial to include the analysis of the SOLE-EST primary objective. The secondary objectives will be analyzed separately, with separate SAP.

3.4.5.1 Population and Available Samples

Accrual:
Tabulate by country, site: numbers of patients, first/last enrollment dates

Accounting:
* Patients: Pt participation over the 12 months, incl. protocol treatment status at 9 (arms A & B) and 12 (arm A) months
* Samples: Sample assay results availability, by timepoint; timing relative to planned collection timepoints
* Flow diagram

Characteristics, same tabs as parent trial:
* tabulate SE pts vs all ITT patients
* tabulate SE pts vs all ITT patients at the participating sites
* tabulate SE pts vs all ITT patients at the participating sites during SE enroll period

Additional Characteristics:
In addition to characteristics from the main trial, collected for SOLE-EST are:

- Dominant hand
- Type of menopausal will be derived from additional data collected on SE-A form:
    - menopause information (bilateral oophorectomy, RT ovarian ablation, chemotherapy-induced amenorrhea, natural menopause before BC diagnosis, natural menopause since BC diagnosis)
    - potential for residual ovarian function (whether age<55, ovarian function recovered following chemotherapy, receipt of GnRH/LHRH in past 2 yrs; last menstruation within past 2 yrs, other potential for residual ovarian function specify)

Hormone levels at baseline:
* descriptive statistics in relation to: prior ET; most recent prior ET; duration from end of prior ET to randomization; duration prior AI category, age, type menopause

Hormone levels over time:
** note, may need to look at variability in planned vs actual timing of samples and whether that influences levels;*
* descriptive statistics, boxplots, spaghetti plots according to timepoint and treatment assignment [absolute levels]
* descriptive statistics of percent change and fold-change from baseline, according to timepoint and treatment assignment
* descriptive statistics, graphical summaries of percent change and fold-change from 9m to 10.5 and 12m, according to timepoint and treatment assignment
    * according to other characteristics?

International Breast Cancer Study Group Statistical Center

SOLE-EST (Trial 35-07/BIG 1-07)

Statistical Analysis Plan

Version	Author	Date	Status
1	Subrina Farah	March 2020	Analysis of primary and secondary objectives

1 INTRODUCTION

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The estrogen suppression substudy enrolled patients at randomization and aims to describe the changes in serum levels of estrogen levels that occur on letrozole and during the three-month treatment gap without letrozole. The blood sampling time points were over the first year since randomization (see schema). Estrogen suppression and recovery may also be linked with clinical outcomes including toxicity and disease-free survival. SOLE-EST will also examine the relationship between estrogen level changes and changes in toxicity, patient-reported musculoskeletal symptoms and QL, and grip strength. As genes important for the metabolism and activity of letrozole may influence estrogen levels, single nucleotide polymorphisms **(**SNPs) related to these will be assessed and analyzed in relation to the variability of estrogen level changes.

SOLE-EST was activated in IBCSG Participating Centers located in Belgium, Australia, and Milan on 19 November 2010 and the first patient enrolled on 31 January 2011. Enrollment was closed 12 July 2012, with a final accrual of 104 patients (25 from the continuous arm and 79 from the intermittent arm) enrolled at 14 Participating Centers.

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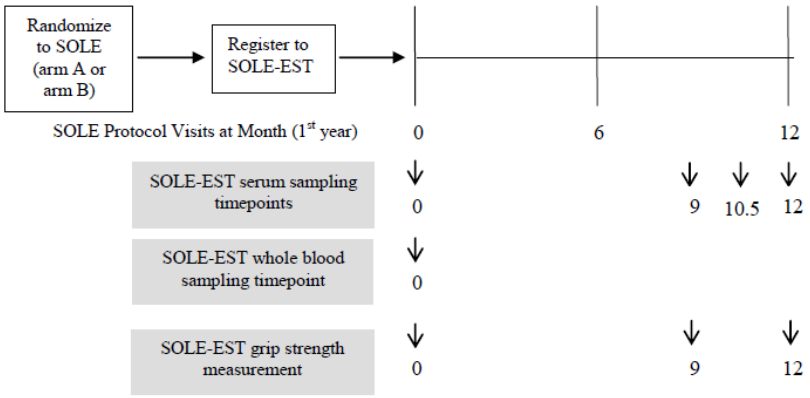
SOLE-EST schema and blood sampling timepoints



1.2 BACKGROUND

There are three biologically important circulating estrogens present in postmenopausal women: 17 β-Estradiol (E2) is a product of both estrone and testosterone and the most abundant circulating estrogen in premenopausal women; estrone (E1) is the major product of aromatase enzyme activity in postmenopausal women; estrone sulphate (E1S) is the most stable estrogen fraction measured and provides a robust surrogate-parameter for suppression of estrogen biosynthesis in vivo. Mean plasma levels of E2, E1 and E1S in postmenopausal women are reported to be 20, 80 and 4-500 pmol/L respectively. Suppression of estrogen production by letrozole reduces levels close to or below the level of detection in many assays.

Musculoskeletal side effects are amongst the most common and most troublesome side effects associated with aromatase inhibitors and have been assessed in a number of ways. A simple and validated measure is grip strength using a modified sphygmomanometer. The effect of a three-month break on QL was reported for the overall trial population. It was hypothesized that the increase in estrogen levels during the three-month gap would reduce side effects, especially musculoskeletal side effects and patient-reported symptoms; and it is hypothesized that this will correlate with the absolute estrogen levels. It is also hypothesized that the increase in estrogen levels will improve QoL overall. The SOLE-EST substudy will test these hypotheses by using the toxicity data collected in the main study and the QoL data collected in the QoL substudy and by measuring the grip strength of the patients.

Letrozole is metabolized by cytochrome P450 (CYP) isoenzymes including CYP2A6 and CYP3A4. Genetic variations in the genes encoding for those enzymes can alter the metabolism and therefore plasma levels of letrozole. The gene encoding CYP19 (the aromatase enzyme that produces estrogen from testosterone) is a highly polymorphic gene that may influence the efficacy of letrozole through an altered (increased or decreased) activity. There is also some evidence that genetic variation may explain the variation of the degree of side effects amongst women on letrozole. Ingle *et al.* found 4 SNPs that were associated with the degree of musculoskeletal symptoms using a genome-wide association case-control study.

2 OBJECTIVES AND ENDPOINTS

Primary objective:

- To determine the serum level of estrogens E2, E1 and E1S and Sex Hormone Binding Globulin (SHBG) during letrozole treatment
- To determine the degree of recovery of E2, E1 and E1S during the 3-month gap.

Also noted, describe the within-patient variability of E2, E1 and E1S across time among patients continuously taking letrozole.

Secondary objectives:

- To link the estrogen level changes with the clinical outcomes of toxicity (musculoskeletal toxicity [as assessed by grip strength], arthralgia, hot flushes and insomnia) and QoL.
- To determine the effect of the following factors on estrogen levels: prior adjuvant endocrine therapy (type, duration, duration without ET prior to study entry); age; BMI; type of menopause.
- To explore variability of estrogen level changes and link these with germline SNPs.

Primary endpoints

- Levels of E2, E1 and E1S at 0, 9, 10.5 and 12 months from randomization to the core protocol.
- % change (suppression or recovery) of E2, E1 and E1S from baseline at 9 months, 10.5 months and at 12 months from randomization to the core protocol.

  - Calculated as timepoint minus baseline, so that positive are increases from baseline and negative are decreases from baseline
- Addition: % change (suppression or recovery) of E2, E1 and E1S from 9 months at 10.5 months and at 12 months
  - Calculated as timepoint minus 9mo, so that positive are increases from 9mo and negative are decreases from 9mo

Secondary endpoints

- Toxicity grade changes (for arthralgia, hot flushes and insomnia) between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.
  - Calculated as 12mo minus 6mo, so that positive is increase in grade and negative is decrease in grade
- Quality of life score change between 6 months (on letrozole) and 12 months (off letrozole for 3 months) and correlation with % recovery of estrogen levels.
  - LASA scores higher value is better condition, thus change is calculated as 12mo minus 6 mo so that positive is improved condition and negative is declined condition.
- Changes in grip strength score [from baseline] at 9 months and 12 months.
  - Higher value is greater strength, thus change is calculated as 12mo minus 9 mos so that positive is improved condition and negative is declined condition.

3 SUBSTUDY DESIGN AND PROCEDURES

3.1.1 Blood Samples and Assays

A whole blood sample was obtained at baseline. Serum samples were obtained at 4 time points (0, 9, 10.5 and 12 months); they were taken at any time of day and were not fasting. Samples were taken using provided blood collection kits and stored locally at -20°C. Assays were performed centrally.

Estrogen levels must be measured using a highly sensitive assay. A central laboratory (Reproductive Endocrine Research Lab of Frank Stanczyk, Los Angeles, USA) will do the assays, and the sensitivity they quote for their RIA using a 1.0 mL aliquot of serum is as follows [to be confirmed with lab after assays]:

- for estradiol (E2) is 2 pg/ml (7.34pmol/L)
- for estrone(E1) is 3 pg/ml (11.01pmol/L)
- for estrone sulphate (E1S) is 9 pg/ml (33.03pmol/L).

It is considered that a 50% increase in estradiol (E2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay) after cessation of letrozole would be clinically significant.

The SNPs were to be analyzed using the Sequenom MassARRAY® technology in the laboratory of Vesalius Research Center, K.U. Leuven and VIB, Belgium [methodology to be re-confirmed]. The protocol noted the following.

- SNPs on alleles CYP2A6*2, CYP2A6*9, CYP2A6*12 and CYP2A6*35
- In the CYP3A4 gene, only the CYP3A4*1B and CYP3A4*7 SNP have a known effect on the enzymatic activity and a frequency of more than 1% in the Caucasian population. Therefore, for the CYP3A4 gene, only those two SNPs will be genotyped.
- The CYP19 SNPs with a frequency of >10% in the Caucasian population and with a known effect on the enzymatic activity will be analyzed, namely, rs10046, rs727479, rs10459592, rs4775936, rs6493497 and rs7176005.
- SNPs previously associated with musculoskeletal symptoms: rs7158782, rs7159713, rs2369049 and rs6637820.

The listing at the end of this document includes the notes from 2013 when SNP selection was planned; SNPs run are highlighted, and the few additional SNPs run (3 CYP19, 4 CYP17A1, 1 SLCO1B1) are added.

3.1.2 Measurement of toxicity and quality of life

Adverse event information will be collected at baseline and at 6 and 12 months as per the main study. Primary AEs are arthralgia, hot flushes and insomnia.

Patient-reported symptoms will be assessed by the Breast Cancer Prevention Trial (BCPT) Symptom Scales on Form 35-PRS. In addition, several global QoL indicators will be assessed using the IBCSG Trial 35-07 QL Form. QL measures were taken every 6 months until 24 months.

Grip strength will be measured using the Martin Vigorimeter (a modified sphygmomanometer , provided or reimbursed by IBCSG) at 0, 9, 12 months. To perform the hand grip test, the patient is asked to squeeze the balloon of a modified sphygmomanometer three times with maximal force and the maximal value of three trials of each hand will be used for evaluation. This assessment has high inter-rater reliability and takes a minimal amount of time.

3.1.3 Additional Characteristics

In addition to characteristics from the main trial, collected for SOLE-EST are:

- Dominant hand
- Type of menopausal will be derived from additional data collected on SE-A form:
    - menopause information (bilateral oophorectomy, RT ovarian ablation, chemotherapy-induced amenorrhea, natural menopause before BC diagnosis, natural menopause since BC diagnosis)

- potential for residual ovarian function (whether age<55, ovarian function recovered following chemotherapy, receipt of GnRH/LHRH in past 2 yrs; last menstruation within past 2 yrs, other potential for residual ovarian function specify)

3.1.4 Sample size considerations (from protocol)

Sample size was determined based on a conservative approach to the primary analysis in which patients are classified as to whether or not the change in E2 between 9 and 12 months exceeds a certain threshold indicative of clinically significant recovery of E2. Clinically significant recovery is defined as a 50% increase in estradiol (E2) levels or a level of >12pmol/L (for those whose levels were below the lower limit of sensitivity for the assay). With a sample size of 100 patients, 25 in arm A (continuous letrozole) and 75 in arm B (intermittent letrozole), and assuming conservatively that only 80% of patients have paired samples at 9 and 12 months, then there is over 90% power to detect a difference between 10% vs. 50% of patients having recovery of E2 during the 3-month interval (Fisher's exact test; two-sided α=0.05).

Further, the sample size will allow exploration of the relation of other factors – specified patient characteristics, clinical measures and germline SNPs – with E2 changes measured on the continuum. Again focusing on the change in E2 between 9 and 12 months among patients in arm B, and considering a range of the frequency of a binary factor in the population from 50-50% to 10-90% , the detectable effect size between two groups ranges from about 0.75 to 1.25 SD (80% power, two-sided α=0.05, two-sample t-test). For a continuous factor a Pearson's correlation coefficient of 0.36 vs. null value of 0 would be detectable.

3.1.5 Analysis plan (from protocol)

Estrogen levels (E2, E1, E1S) will be summarized over time among all patients and by treatment arm using descriptive statistics (mean, SD, median, quartiles) and graphically. It is anticipated based on the literature that log-transformation of estrogen levels may be required, in which case the geometric mean would be reported. Values will also be summarized as percent change from baseline levels.

Using linear mixed modeling of all available data over the 4 timepoints (or of 3 timepoints relative to baseline), each estrogen level will be modeled as a function of time and treatment arm and the interaction of the two factors to investigate the time pattern of estrogen levels and differences in levels between treatment arms. Of primary interest is whether the change from 9 to 10.5 and 12 months differs among patients assigned to intermittent vs. continuous letrozole. It may be the case that estrogen levels while on treatment are below the assay lower limit of detection, in which case the analysis method would be changed to account for this sort of "floor effect" or "left censoring" of data. Relationships of specified patient characteristics (prior endocrine therapy, age at and/or type of menopause, BMI) with the estrogen levels and changes in levels will also be investigated, as well as specific toxicities and quality of life measures and germline SNPs.

4 ANALYSIS PLAN

4.1.1 Primary objectives: To determine the serum levels during letrozole treatment:

- Descriptive statistics tabulation of E2, E1, E1s and SHBG values with respect to most recent ET taken prior to randomization.
- Descriptive statistics tabulation of E2, E1, E1s and SHBG values over time by treatment arms.
- Descriptive statistics tabulation of the percent change in values of E2, E1, E1s and SHBG from baseline to timepoint by treatment arms.
- Descriptive statistics tabulation of the fold change in values of E2, E1, E1s and SHBG from baseline to timepoint by treatment arms.
- Scatter box plots to depict level of E2, E1, E1s and SHBG over time by treatment arms.
- Spaghetti plots of E2 levels over time for each treatment arm separately.

4.1.2 Primary objectives: To determine the degree of recovery during the 3-month gap:

- Descriptive statistics tabulation of the percent change in values of E2, E1, E1s from month 9 to timepoint by treatment arms.
- Descriptive statistics tabulation of the fold change in values of E2, E1, E1s from month 9 to timepoint by treatment arms.
- Statistical testing of E2 recovery for intermittent group from 9 to 12 months and secondarily from 9 to 10.5 months (i.e., is recovery already evident by 10.5 mos). (Wilcoxon Signed Rank Test)
- E2 recovery comparison test for intermittent vs. continuous group from 9 to 12 months and 9 to 10.5 months. (Wilcoxon Rank sum Test), to confirm that recovery is greater than variability of E2 while continuing treatment.

4.1.3 Secondary Objective: To link the estrogen level changes with the clinical outcomes of toxicity, QoL and grip strength

- Scatter plot E2 % change 9-12 mos. vs change of QoL LASA measures 6-12 mos. (except coping effort and treatment burden) and calculate corresponding Spearman correlation coefficients.
- Scatter plot E2 % change 9-12 mos. vs change 9-12 mos of grip strength dominant and non-dominant hand and Spearman correlation coefficients.
- Scatter and box plots of AE toxicity grade changes (no grade, positive and negative) 6-12 mos. with respect to E2 % change 9-12 mos. by treatment assignment.

4.1.4 Secondary Objective: Effect of factors on estrogen levels

- Tabulation of baseline E2, E1, E1s and SHBG by characteristics (categorized where needed): most recent ET, duration of prior AI, duration of prior ET to rando, age, BMI, types of menopause; and scatterplots baseline E2 with continuous characteristics. Transpose table, Spearman correlation coeff matrix with all cont. variables by treatment arms.
- Tabulation of E2 % change (9-12 mos and 9-10.5m) by characteristics: most recent ET, duration of prior ET, duration of prior AI, duration of prior ET to rando, age, BMI, types of menopause; transpose table and scatterplot E2 % change 9-12 mos with continuous characteristics. One correlation coeff matrix.
- Statistical modeling of natural log transformation of fold change from 9-12 months (linear regression multivariate analysis) with respect to characteristics.
    - Investigate relations between the characteristics, tabulations and scatterplots and correlation coefficient matrix depending on what's helpful
    - Effect on E2 over 9m of AI (all patients without regard to treatment assignment) lnE2(9)=lnE2(0)+age (without treatment assignment). (series of mixed model with one covariate at a time and final model with all covariates)
    - Effect on E2 recovery (intermittent only) from 9 to 10.5 and 12 months Mixed modelling: lnE2(10.5,12)=ln E2(9) + timepoint as covariate + age (series of mixed model with one covariate at a time and final model with all covariates)

4.1.5 Secondary Objective: Link of estrogen level changes with germline SNPs

To be added in the future