



**INTERNATIONAL BREAST CANCER
STUDY GROUP**

IBCSG 35-07

BIG 1-07



Study of Letrozole Extension

SOLE-EST

SOLE Estrogen Substudy

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

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Protocol Signature Page

IBCSG 35-07/ BIG 1-07

Study of Letrozole Extension – SOLE

SOLE Estrogen Substudy – SOLE-EST

Approved by:

[Redacted Signature]

(Signature on file)
Signature

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(Signature on file)
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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

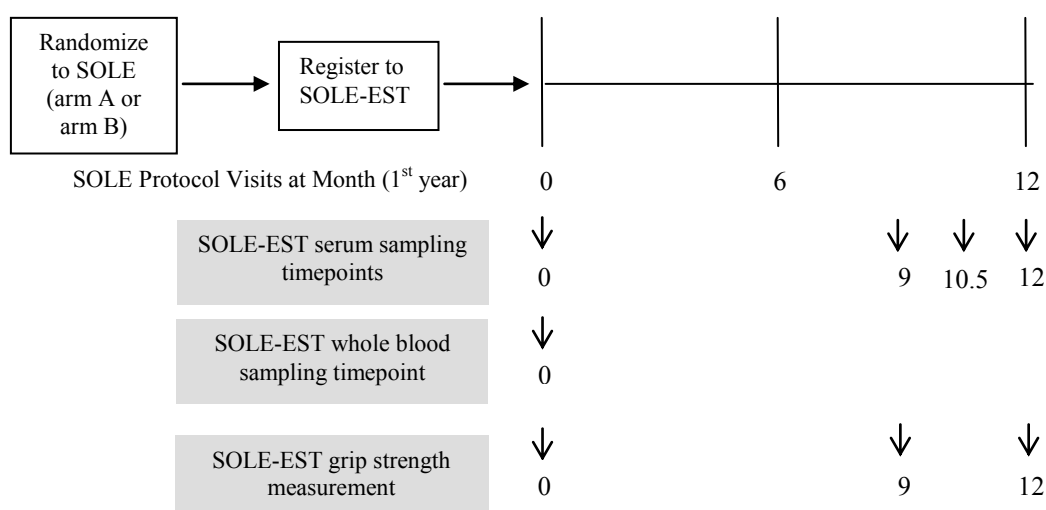


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1. Introduction and Rationale

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 hardware⁴. 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 study⁵ including 374 postmenopausal women, the mean E_2 level was 5.6 pg/ml ($\times 3.67=20.5$ pmol/L). In a second study⁹, 147 postmenopausal patients with early breast cancer were

randomized to receive placebo or exemestane. Mean baseline values for E₂, E₁ and E₁S 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 E₂, E₁ and E₁S were reduced by 83, 93 and 93%, respectively. No individual values or estrogen levels were reported at 12 months. In a third report¹⁰, 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 E₂ 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 (anastrozole 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 E₂ 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 E₂ is 2pg/ml (7.34 pmol/L) for E₁ is 3pg/ml (11.01 pmol/L) and for E₁S 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 regression¹⁶ 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 months¹⁷. In addition, in a clinical study of estrogen deprived breast cancer there was also some evidence that estradiol (E₂) may produce a treatment response¹⁷.

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 (E₂) and other estrogen levels.

1.4 Toxicity and Quality of Life

Toxicity from letrozole is well documented¹⁹ 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 sexuality²³. 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 sphygmomanometer¹⁹. 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 metabolism 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 genotypes²⁷. 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 study³⁰. 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 (E₂), Estrone (E₁) and Estrone Sulphate (E₁S) and Sex Hormone Binding Globulin (SHBG) during letrozole treatment.
- 2.1.2. To determine the degree of recovery of Estradiol (E₂), Estrone (E₁) and Estrone Sulphate (E₁S) 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 (E₂), Estrone (E₁) and Estrone Sulphate (E₁S) at 0, 9, 10.5 and 12 months from randomization to the core protocol.
- 2.3.2 % 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.

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 (E₂), estrone (E₁), and estrone sulphate (E₁S). 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 °C 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 (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.

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 Scales³¹ 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 $>12\text{pmol/L}$ (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 $\alpha=0.05$).

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 $\alpha=0.05$, 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.

9. References

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