



International Breast Cancer Study Group Statistical Center

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

Statistical Analysis Plan

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

1 INTRODUCTION

1.1 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.

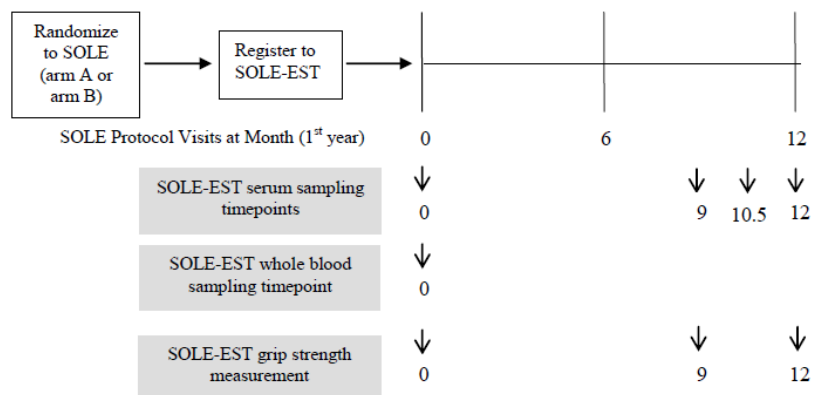
SOLE-EST was activated in IBCSG Participating Centers located in Belgium, Australia, and Milan on 19 November 2010 and the first patient enrolled on 31 January 2011. Enrollment was closed 12 July 2012, with a final accrual of 104 patients (25 from the continuous arm and 79 from the intermittent arm)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

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 $\alpha=0.05$).

Further, the sample size will allow exploration of the relation of other factors – specified patient characteristics, clinical measures and germline SNPs – with 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 $\alpha=0.05$, two-sample t-test). For a continuous factor a Pearson's correlation coefficient of 0.36 vs. null value of 0 would be detectable.

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)
 $\ln E2(9) = \ln E2(0) + \text{age}$ (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: $\ln E2(10.5, 12) = \ln E2(9) + \text{timepoint as covariate} + \text{age}$ (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