SWOG CANCER RESEARCH NETWORK

December 10, 2021

Adult CIRB - Late Phase Emphasis

RE: **S1007**, "A Phase III, Randomized Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients with 1-3 Positive Nodes, Hormone Receptor-Positive and HER2-Negative Breast Cancer with Recurrence Score (RS) of 25 or Less. RxPONDER: A Clinical Trial Rx for Positive Node, Endocrine Responsive Breast Cancer." Study Chairs: Drs. K. Kalinsky, J.R. Gralow, F. Meric-Bernstam, G.N. Hortobagyi, K.S. Albain and W. Barlow.

RESPONSE TO CTEP REVIEW COMMENTS (Protocol Version Date: 10/08/21)

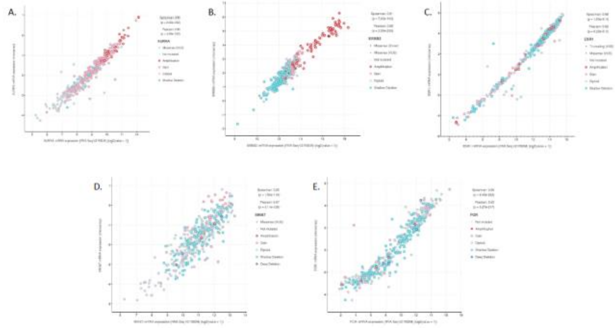
Dear CTEP,

Thank you for your consideration of the above-referenced SWOG protocol. The following concerns were expressed by CTEP in review of S1007 protocol Revision #18 (Version Date 10/08/21).

Please find the study team responses and associated modifications below.

I. Comments Requiring a Response – CTEP Stipulations:

#	Section	Comments
1.	18.2	Although GEM ExTrA is a reasonable platform for the investigators to use and using a single platform for genomic signature analyses will increase comparability across signatures, it is unclear how the investigators intend to calculate the scores from the signatures they lift. While the loci used to determine each score are known, the exact risk score calculations are proprietary information. If the investigators intend to "reinvent" the signatures using these data, their analysis plan should detail the procedures they intend to use (e.g., separating the data into training/validation sets, models, etc.) and address the limitations of this approach. Otherwise, the investigators should confirm that they have access to the information necessary to calculate the risk scores for the signatures of interest. **Study Team Response:** In the correlative proposal, we will be using GEM ExTrA platform to assess expression of multiple genes sets, proposed to be prognostic or predictive in breast cancer. We realize this approach has a few caveats: • Most of the gene sets were originally discovered by microarray technology. In preliminary studies we compared the concordance of RNA expression measurements based on Agilent custom gene expression arrays and Illumina RNA sequencing platforms that have been previously published(PMID: 26451490). We used breast cancer patients' samples spanning all breast cancer subtypes from the TCGA project (N=421). Only the samples with both microarray and RNA sequencing data were included in the analysis. We demonstrated the high correlation between log transformed RNAseq and microarray measurements suggesting with proper transformation both data platforms are provided may be adapted for risk predictions. The plots below are for the genes ERBB2, ESR1, MKI67, ERBB2 and PGR (Figure 1), demonstrating significant correlations between RNA expression by array and RNAseq approaches.



- There are currently several prognostic and predictive signatures proposed for use in breast cancer. Many of these gene sets differ in the genes included and it is not possible to test the contribution of other gene sets without using a central platform approach:

Overlap of published gene lists at the gene level

OVERLAP PAIRWISE							
	MP	PS	EP	GGI	BCI	SET	RS
MammaPrint®	64	3	0	8	1	0	1
Prosigna®		50	2	12	1	7	11
EndoPredict®			8	1	0	1	1
Genomic Grade Index				94	4	0	5
Breast Cancer Index®					7	0	0
Sensitivity Endocrine Treatment						162	0
Recurrence Score®							16
OVERLAP							
Genes in two or more lists	10	28	3	21	4	8	13

- Using a RNAseq based approach rather than using selected multigene panel commercial assays will be more tissue sparing, allowing us to test multiple gene sets and will also leave to potential for new signature discovery (as an exploratory endpoint). Importantly the RNAseq and WES will be made publicly available after the initial publication of our biomarker work and will provide an unparalleled resource for discovery to the scientific community to address additional questions about breast cancer.

- We appreciate the concern raised that derived signatures would not be able to calculate risk scores in the same way that other commercial assays would. With our approach we will be deriving **"pseudoscores"** using **principal component analysis**. In the revised protocol we expanded the analysis section to clarify the methodology we will be using with references to prior work. The new section is included below:

Discovery Analysis Methods

Genes considered will be all the constituent genes (except reference genes, where present) from the MammaPrint, PAM50, SET Index, Endopredict, Breast Cancer Index and Recurrence Score tests. For each set of genes examined, a score will be constructed using the first principal component of the gene set.

For assessment of chemotherapy effect prediction, a Cox proportional hazards regression model will be fit with endpoint iDFS and terms for the gene set score, treatment and the interaction of the gene set score with treatment. The log standardized hazard ratio for interaction (Crager 2020) and its variance will be computed for each gene set score.

For assessment of prognosis, a Cox model will be fit with a single term for the gene score using the patients who were randomized to endocrine therapy alone. For assessment of residual risk, the same procedure will be used for patients randomized to chemo-endocrine therapy.

False discovery rates (Storey 2002) and log standardized hazard ratios with correction for regression to the mean (Crager 2010, Crager 2012) will be calculated using model space sampling considering the universe of gene set scores selected from all genes under consideration and gene sets from 1 to 40 genes.

If prognostic gene sets are discovered at FDR 10%, their prognostic efficacy will be described using predictiveness curves (Huang, Pepe and Feng, 2007) corrected for regression to the mean.

If predictive gene sets are discovered at FDR 10%, their predictive efficacy will be described using treatment effect predictiveness curves, that is, predictiveness curves applied to the distribution of estimated treatment hazard ratio with correction for regression to the mean. Potential gene set score cut-points for identifying patients with substantial treatment benefit versus no substantial benefit will be assessed based on these curves.

These discovery analyses will be conducted separately for pre-menopausal patients and post-menopausal patients.

Pseudoscores were previously constructed using RNASeq of the SWOG 8814 study and the constituent genes of the MammaPrint®, Prosigna®, EndoPredict®, Genomic Grade Index, Breast Cancer Index® and Sensitivity Endocrine Treatment (SET) scores as well as the Oncotype DX Recurrence Score®. Each pseudoscore was constructed using the coefficients of the first principal component of the constituent genes. These pseudoscores will be evaluated and compared as continuous numeric biomarkers for prognosis of iDFS and prediction of the effect of chemotherapy using the RxPONDER data set and standardized hazard ratios. Categorical analyses for both prognosis and prediction will use equivalent cut-points using population quantiles. Since SWOG 8814 included only post-menopausal women, the pseudoscores will be re-derived separately using pre-menopausal and post-menopausal women in RxPONDER and the scores compared between pre- and post-menopausal women. If it is concluded that the premenopausal pseudoscores are sufficiently different from the postmenopausal pseudoscores, then it may require regeneration of the first principal component weights using five-fold cross-validation. Overall, it is recognized that the pseudoscores are not an exact match for the actual gene signatures, as they use different coefficients and analytical platforms, so that the performance of the actual signatures might be different. This analysis seeks to generally evaluate the information content of the constituent genes in each gene list.

Because of the restriction of the S1007 study population to patients with Recurrence Score result 0 – 25, it is recognized that the estimated prediction of effects of genes from the Recurrence Score and other genes substantially correlated with these genes will be biased downward.

References

Crager MR (2010). Gene identification using true discovery rate degree of association sets and estimates. *Statistics in Medicine* **29**:33-45.

Crager MR (2012). Generalizing the standardized hazard ratio to multivariate proportional hazards regression, with an application to clinical-genomic studies. *Journal of Applied Statistics* **39**:399-417.

Crager MR (2020). Extensions of the absolute standardized hazard ratio and connections with measures of explained variation and variable importance. *Lifetime Data Analysis* **26**:872-892.

		Huang Y, Pepe MS, Feng Z. Evaluating the predictiveness of a continuous marker. *Biometrics* **63**:1181-1188. Storey JD (2002). A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B.* **64**:479–498.
2.	18.2	No statistical plan is provided for the cfDNA analyses; additionally, it is unclear whether the bespoke ctDNA analysis intended by the investigators performs comparably between EDTA and Streck-preserved specimens, and the investigators state that they intend to use both types of specimens for this analysis. The investigators should consider amending the protocol to add these analyses at a later date, when they have a full statistical plan and have assay validation data confirming that the EDTA and Streck tubes have good concordance. **Study Team Response:** Thank you. The cfDNA analyses have been removed per CTEP request.
3.	18.2	Please indicate the funding secured for performing the described analyses. **Study Team Response:** Thank you. The contract with Exact Sciences is still in process. Exact Sciences Corporation has agreed to perform analytic assays (as described above) in kind and has agreed to perform sequencing on approximately 2,500-3,000 S1007 study specimens using GEM ExTra whole exome analysis of tumor and normal tissue, and RNAseq sequencing of tumor tissue. The cost of the hormone analyses will be offset by the Biobanking and Biomarker Validation Core (part of the Kansas Institute for Precision Medicine COBRE), with reagent costs offset via Hematology and Medical Oncology-associated funds provided by the Winship Cancer Institute of Emory University.

Please direct any inquiries to my attention at the SWOG Operations Office.

Sincerely,

Alicia Aranda
Protocol Project Manager
PC/aa

Enclosures: Protocol (Version Date: 12/10/2021)
Consent – Main (Version Date: 12/10/2021)

cc:
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Version Date: December 10, 2021

TO: ALL NATIONAL CLINICAL TRIALS NETWORK (NCTN) MEMBERS (U.S.)

FROM: Cara Laubach, Lead Protocol Coordinator (E-mail: claubach@swog.org)

RE: **S1007**, "A Phase III, Randomized Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients with 1-3 Positive Nodes, Hormone Receptor-Positive and HER2-Negative Breast Cancer with Recurrence Score (RS) of 25 or Less. RxPONDER: A Clinical Trial Rx for Positive Node, Endocrine Responsive Breast Cancer." Study Chairs: Drs. K. Kalinsky, J.R. Gralow, F. Meric-Bernstam, G.N. Hortobagyi, K.S. Albain and W. Barlow.

REVISION #18

Study Chair: Kevin Kalinsky, M.D.
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IRB Review Requirements
(√) Expedited review allowed

Protocol changes
(√) Scientific / Statistical Consideration changes
(√) Editorial / Administrative changes

Sites using the CIRB as their IRB of record: The protocol and/or informed consent form changes have been approved by the CIRB and must be activated within 30 days of the CIRB posting of this notice.

Sites not using the NCI CIRB: Per CTMB Guidelines, the protocol updates and/or informed consent changes must be approved by local IRBs within 90 days of distribution of this notice.

REVISION #18

Revision 18 was prepared to incorporate update to integrated translational medicine objectives for planned integrated biomarker analyses to be completed on already banked samples and to add integrated hormone level analyses (on samples previously collected at baseline) (Sections 1.2, 2.3, 18.2, 18.3 and 18.9).

Protocol Changes:

The following specific changes were incorporated into the protocol:

1. General: Formatting, hyperlinks, and intra-document section references were updated and/or corrected throughout the protocol.

2. The Version Date and Table of Contents were updated.

3. Sections 1.2c and 1.2h were updated and Section 1.2l was inserted. These changes were also reflected in the secondary objectives included at the onset (sub section 1.4) of Section 18.3.

4. Section 2.3 was inserted to provide background on integrated translational medicine objectives to be completed on already banked samples.

5. Sections 5.3 and 15.3b were updated to better clarify that two separate collection kits must be ordered for the CBALR substudy.

6. Section 10.7 was inserted to provide the definition of Invasive Breast Cancer-Free Survival that will be utilized in association with translational medicine analyses. The subsequent section was renumbered.

7. Section 18.2: This section was updated in entirety to be reflective of current analytical technologies that will be utilized to accomplished planned integrated objectives. The updated section title was also reflected in Section 18.0.

8. Section 18.8e: Additional clarification was inserted regarding planned utilization of samples banked in association with the CBALR substudy for cfDNA analyses, pending the forthcoming statistical plan that will be included with a future revision.

9. Section 18.9: Distribution instructions (for samples that are already banked at the SWOG Biospecimen Repository) were inserted.

Model Consent Form Changes:

The **S1007** Model Consent Forms were updated as follows:

- The Version Date of the consent documents has been updated for congruence with the protocol. There were no substantive changes to the **S1007** Model Consent Forms for Step 1, Step 2, or Step 3 registration.

The updated protocol (Version Date: 12/10/2021) and Model Consent Forms (Version Date: 12/10/2021) can be accessed from the CTSU website (www.ctsu.org). Please discard any previous versions of the protocol and attach this memorandum to the front of your copy of **S1007.**

This study has been reviewed and approved by the NCI's Central Institutional Review Board (CIRB).

This memorandum serves to notify the NCI, CIRB, and SWOG Statistics and Data Management Center.

cc: PROTOCOL & INFORMATION OFFICE
Ruth Campo – GEICAM
Jerome Lemonnier - UNICANCER

PRIVILEGED COMMUNICATION
FOR INVESTIGATIONAL USE ONLY

Activation January 15, 2011

SWOG CANCER RESEARCH NETWORK

A PHASE III, RANDOMIZED CLINICAL TRIAL OF STANDARD ADJUVANT ENDOCRINE THERAPY +/- CHEMOTHERAPY IN PATIENTS WITH 1-3 POSITIVE NODES, HORMONE RECEPTOR-POSITIVE AND HER2-NEGATIVE BREAST CANCER WITH RECURRENCE SCORE (RS) OF 25 OR LESS. RXPONDER: A CLINICAL TRIAL RX FOR POSITIVE NODE, ENDOCRINE RESPONSIVE BREAST CANCER

NCT#01272037

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(CTEP IND-)

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NCIC-CTG/NCIC Clinical Trials Group
SWOG/SWOG Cancer Research Network
GEICAM/GEICAM (Spain)
UNICANCER/UNICANCER (France)

(see Section 18.5 for a list of approved UNICANCER and GEICAM sites and participating investigators)

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S1007 PROTOCOL CONTACT INFORMATION

Eligibility and Data Submission Questions:	SWOG Statistics and Data Management Center E-mail: breastquestion@crab.org 206/652-2267
Medical Queries (treatment or toxicity related questions):	Study Chairs: E-mail: **S1007**question@swog.org
Regulatory, Protocol, Informed Consent:	SWOG Operations Office: E-mail: protocols@swog.org Phone: 210/614-8808
Specimen Tracking System (STS) Amendments, Errors, Connectivity Issues and Technical issues with the SWOG CRA Workbench:	E-mail: technicalquestion@crab.org
Ordering Circulating Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) Substudy sample collection kits: Sample Collection Kit Ordering questions:	Streck cfDNA and CellSave® kits may be ordered by using the SWOG Biospecimen Bank Kit Management Application at: https://kits.bpc-apps.nchri.org E-mail: bpcbank@nationwidechildrens.org Phone: 614/722-2865
Circulating Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) Substudy Sample Shipping questions:	For questions pertaining to the **two Streck cfDNA tubes being shipped to Epic Sciences Lab**, contact: Lab #236: Epic Sciences via Email: partners@epicsciences.com / Attn: **S1007** For questions pertaining to the the **two Streck cfDNA tubes being shipped to the SWOG Biospecimen Bank,** contact: Lab #201: SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201 via E-mail: bpcbank@nationwidechildrens.org Attn: **S1007** For questions pertaining to the **Cellsave® tube collection or shipping** contact: Lab #122: Menarini Silicon Biosystems Labs via Email: msb-labservicesus@siliconbiosystems.com Attn: **S1007**
Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM):	CTEP-IAM account can be checked or new accounts can be created and updated: https://ctepcore.nci.nih.gov/iam/index.jsp
Serious Adverse Event Reporting questions:	See Protocol Section 16.1 Email: adr@swog.org
Patient Transfers:	patienttransfer@crab.org

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

To submit site registration documents:	For patient enrollments:	Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone: 1-866-651-CTSU Fax: 215-569-0206	Refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN related questions by phone or email : 1-888-823-5923, or ctsucontact@westat.com.	Online Data Submission: Institutions participating through the CTSU are required to submit and amend their data electronically via Online Data Submission. Access the SWOG Workbench using your CTSU user ID and password at the following url: https://crawb.crab.org/TXWB/ctsulogon.aspx. Exceptions: Data items that are not available for online submission (operative and pathology reports, scan reports, etc.) may be submitted by fax at 800-892-4007. Do not submit data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

The most current version of the **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.

CTSU sites should follow procedures outlined in the protocol for Site registration, Patient Enrollment, Adverse Event Reporting, Data Submission (including ancillary studies), and Drug Procurement.

For patient eligibility or data submission questions contact the SWOG Statistics and Data Management Center by phone at 206/652-2267 or by email at breastquestion@crab.org.

For clinical questions (treatment or toxicity related) contact the Study Chairs at S1007question@swog.org.

For non-clinical questions (unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or e-mail:
CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.

For detailed information on the regulatory and monitoring procedures for CTSU sites please review the CTSU Regulatory and Monitoring Procedures policy located on the CTSU members' website https://www.ctsu.org education and resources tab > CTSU Operations Information >CTSU Regulatory and Monitoring Policy.

The CTSU Web site is located at https://www.ctsu.org

SCHEMA

Node-positive (1-3 nodes) HR-positive and HER2-negative breast cancer

Patients consent to Recurrence Score (RS) testing, discussion of potential randomization on this trial.

Step 1 Registration and tumor tissue submission for RS testing

Recurrence Score

RS > 25

Discuss alternative trials for high risk patients

RS ≤ 25

Physician and patient discuss randomization knowing the RS

Refuse

Record chosen therapy

Accept

RS already available and ≤ 25. Physician and patient discuss randomization knowing the RS

Step 1 Registration

Step 2 Registration/ Randomization stratified by:

1. RS 0-13 vs. 14-25
2. Menopausal Status
3. Axillary node Dissection vs. Sentinel node biopsy

Chemotherapy; endocrine therapy

No Chemotherapy; endocrine therapy

If disease-free at time of IRB approval of Revision 16, offer participation in correlative CTC TM Assessment)

Patient Consent

Step 3 Registration

1.0 OBJECTIVES

1.1 Primary Objective

a. To determine the effect of chemotherapy in patients with node positive breast cancer who do not have high Recurrence Scores (RS) by Oncotype DX®. In patients with 1-3 positive nodes, and hormone receptor (HR)-positive, HER2-negative breast cancer with RS ≤ 25 treated with endocrine therapy we will test whether the difference in disease-free survival for patients treated with chemotherapy compared to no chemotherapy depends directly on the magnitude of RS. If benefit depends on the RS score, the trial will determine the optimal cutpoint for recommending chemotherapy or not.

1.2 Secondary Objectives

a. To compare overall survival (OS), distant disease-free survival (DDFS) and local disease-free interval (LDFI) by receipt of chemotherapy or not and its interaction with RS.

b. To compare the toxicity across the treatment arms.

c. To perform other molecular assays or test other signatures that measure prognosis and potential benefit of chemotherapy and compare them to Oncotype DX®.

d. To determine the impact of management with Oncotype DX® on patient-reported anxiety (co-primary Health-Related Quality of Life [HRQL] outcome) prior to screening, after disclosure of test results, and during the randomized trial.

e. To determine the impact of Oncotype DX® on the initial management cost of node-positive, HR-positive, HER2-negative breast cancer.

f. To compare patient-reported utilities (e.g., QOL) for those randomized to chemotherapy versus no chemotherapy.

g. Using modeling and DFS information from the trial, to estimate the cost-effectiveness of management with Oncotype DX® vs. usual care.

h. To determine the role of other assays as predictors of DFS, DDFS and LDFI for patients randomized to chemotherapy versus no chemotherapy.

i. To determine the impact of treatment with chemotherapy versus no chemotherapy on patient-reported fatigue and cognitive concerns (secondary HRQL outcomes).

j. To determine the impact of management with Oncotype DX® on patient-reported decision conflict, perceptions regarding Oncotype DX® testing, and survivor concerns prior to screening, after disclosure of test results, and during the randomized trial (secondary HRQL outcomes).

k. The presence of circulating tumor cells (CTC+) using two CTC platforms will be assessed at up to two time points to assess late recurrence in those still at risk for the primary outcome. Invasive disease-free survival (IDFS) will be compared between CTC+ versus CTC-, incorporating use of endocrine therapy.

l. To compare clinically reported menopausal status with status categorized by serum hormone levels determined from baseline serum in women under age 55 years and to assess subsequent association with outcomes.

1.3 Banking Objective

To bank specimens for future correlative studies.

2.0 BACKGROUND

2.1 Background / Rationale

Prospective randomized trials indicate that patients with Hormone Receptor (HR)-positive primary breast cancer benefit from the addition of chemotherapy to adjuvant endocrine treatment overall. *(1)* The meta-analysis of randomized trials demonstrates that the addition of taxanes to anthracycline-base therapy improves Disease Free Survival (DFS) and Overall Survival (OS) in all patients with node-positive breast cancer. *(2)* However, several retrospective analyses of prospective clinical trials indicate that some patients may not benefit from chemotherapy; specifically, patients with well-differentiated tumors, low grade, those with high expression of HR, or those with low or intermediate Recurrence Score (RS) as defined by the Oncotype DX® assay. *(3,4,5,6)* Other retrospective analyses indicate that not all breast cancer groups may benefit equally from the addition of taxanes, i.e. patients with Estrogen Receptor (ER)-positive disease may benefit the least. *(7)* At the same time, these patients still have a substantial risk of death or recurrence despite effective endocrine therapy; such risk reaches 40% at 10 years for node-positive, low RS tumors. *(8)* Therefore, there is a need to develop additional, effective treatments for this population. Therefore, although current state-of-the-art chemotherapy appears to be more effective than the chemotherapy used in **SWOG-8814**, evidence is not strong that it is efficacious in all subsets of breast cancer. *(9)* Nevertheless, and in order to derive optimal benefit from chemotherapy, this trial will use the regimens that have proven to be of greater benefit than earlier chemotherapy regimens in modern randomized trials. The study will use a simple parallel two-group randomization to chemotherapy followed by endocrine therapy or endocrine therapy alone in a lower risk, 1-3 node-positive population (identified by intermediate or low Recurrence Score) to determine if modern chemotherapy is efficacious and to identify those patients who will benefit from the addition of chemotherapy.

Multi-gene tumor assays have provided clinically useful prognostic information for patients with node-negative breast cancer. The 21-gene RS has been shown to be both prognostic for patients with ER-positive disease if treated with tamoxifen alone, as well as predictive of benefit from adding chemotherapy. In retrospective analyses, patients with high RS appeared to benefit greatly from the addition of standard chemotherapy to tamoxifen, whereas those with low RS did not. *(10,11)* This assay now helps guide patient and physician decision-making for determining therapy for patients with node-negative, HR-positive disease. Analytical performance data on the RS assay were published by Cronin and colleagues in 2007. *(12)* Furthermore, retrospective and prospective studies indicate that RS results change the adjuvant recommendation in an average of 30% of the time in clinical practice. *(13,14)* However, to generate additional data about the clinical utility of the Oncotype DX® assay, a large, multicenter, prospective randomized trial will recruit > 11,000 patients with lymph node-negative primary breast cancer and randomize those with mid-range RS to endocrine therapy alone or the sequential administration of chemotherapy and endocrine therapy. Preliminary evidence now exists that RS may also allow determination of chemotherapy benefit in node-positive, HR-positive disease. The evidence is stronger for low RS while debatable for intermediate RS of 18-30. *(15)* This trial will to use the same upper limit of 25 as used by the ECOG **PACCT-1** (TAILORx) trial to both avoid confusion and to avoid the potential risk to patients with RS > 25. While **SWOG-8814** suggests no statistically significant benefit overall in RS 14-25, this trial will restrict the analysis to nodes 1-3 where an efficacious effect of chemotherapy has not been demonstrated. The retrospective analysis suggests that RS 19 may be the point of equivalence and that some benefit of chemotherapy could emerge in RS values greater than 19. It is necessary emphasize that this retrospective analysis was at best hypothesis-generating and should not be used for clinical decision-making until prospectively

validated. Modern chemotherapy may indeed show a benefit for higher RS starting somewhere in the 20 to 30 range. If this trial can demonstrate that there is little benefit to chemotherapy for women with lower RS (e.g. RS ≤ 25), it will be possible to eliminate the morbidity and costs of chemotherapy in approximately 67% of patients with HR-positive breast cancers and 1-3 node-positive lymph nodes, but continue to give effective chemotherapy to the 33% of women with RS > 25. However, the RS cutpoint of 25 is used here only for illustration purposes as the point of equivalence and the actual value for decision making will be estimated from the trial data.

Since the development of the Oncotype DX® assay, other prognostic and predictive molecular indices have been developed. Some of these indices have been tested in multiple retrospective sets, but lack the validation of a prospective trial. *(16)* One of the objectives of this protocol is to validate these early findings with some of these additional molecular predictive tests/assays. Further, since tissues and serum will be banked, it will be possible to test other molecular profiles that are developed when the study is on-going or subsequent to study completion.

Overall SWOG-8814 Results *(17)*

Until recently, there was no information on the potential value of the RS assay in patients with positive axillary nodes and HR-positive disease from a study that contains a similar tamoxifen-alone control arm since today these patients are routinely treated with adjuvant chemotherapy as well as adjuvant endocrine therapy. **SWOG-8814** was a practice-changing Phase III trial for postmenopausal women with node-positive, ER-positive breast cancer which demonstrated that cyclophosphamide, doxorubicin (Adriamycin) and 5-fluorouracil (CAF) chemotherapy added significant survival benefit to tamoxifen (T), especially in the sequential setting, with CAF preceding the initiation of tamoxifen therapy. The study had optional specimen banking that yielded tumor specimens for RS determination by the Oncotype DX® gene assay. When comparing the tamoxifen and the sequential CAF-T arms in tissues from 367 patients, the RS was prognostic for DFS in the tamoxifen-alone arm (p=0.006). In this study SWOG used the RS groupings defined by Paik et al. *(18)* There was no apparent CAF benefit in the low RS (0-17) group (p=0.97) or the intermediate RS (18-30) group (p=0.48), but a significant DFS improvement was detected for the high RS (31-100) subset (p=.03). Due to failure of the proportional hazards assumption, separate analyses were performed for the first five years of follow-up and the period beyond five years. The RS-by-treatment interaction was significant in the first 5 years for DFS (p=0.029), with no additional prediction of CAF benefit beyond 5 years (p=0.58) but no loss of initial effect at 10 years. No impact of CAF was observed in the lowest RS group, regardless of nodal status. Results were similar for OS. *(19)*

In this trial, only women with RS ≤ 25 and 1-3 positive nodes will be included. SWOG investigators reexamined the **SWOG-8814** data using this cutoff and 10 years of survival using a standard Cox model. The Kaplan-Meier graph below shows little difference between the two groups (Figure 1) even if we restrict attention to those with RS ≥ 14 (Figure 2). However, the Kaplan-Meier graphs may obscure a possible difference since they do not use the continuous RS. If one fits a more complex model using continuous RS and its interaction with chemotherapy, then a pattern emerges even though there is still no significant interaction of RS and treatment in this patient subset with RS ≤ 25. The Cox model gives an estimate of the log hazard ratio (relative failure rates) by RS with high hazard ratios indicating worse DFS. The hazard ratios apply at any time point (e.g. 5 or 10 years) which is why we prefer to illustrate them here. In the simplest case we use a Cox model and allow for a linear interaction of RS and treatment (Figure 3). The hazard ratios cross, indicating there may be a point of equivalence where a chemotherapy benefit may start to emerge, but of course the difference would have to be large enough to be both clinically and statistically significant. For nodes 1-3 this point of equivalence was about RS=19 in our retrospective analysis. Based on this model, the estimated hazard ratio at RS=22 would be 0.84 for chemotherapy versus no chemotherapy, but the 95% CI of this

estimate is 0.28-2.49 due to the small sample size. Note that the effect in very low RS scores may be exaggerated due to the sparseness of data. This model is based on sparse data and is perhaps too simple, but does indicate that using continuous RS may provide more insight and power than simple categorization. However, interaction alone is also not sufficient and needs to be supplemented by a clinically useful cutoff.

Figure 1
All patients with RS ≤ 25

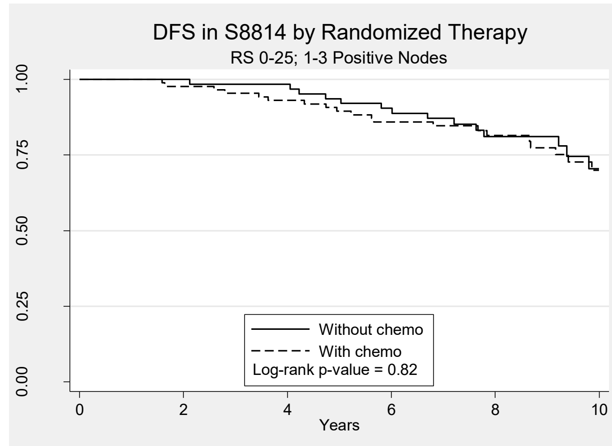


Figure 2
Patients with 14-25

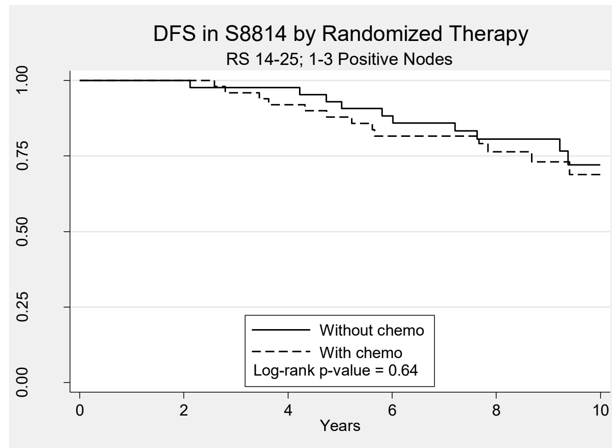
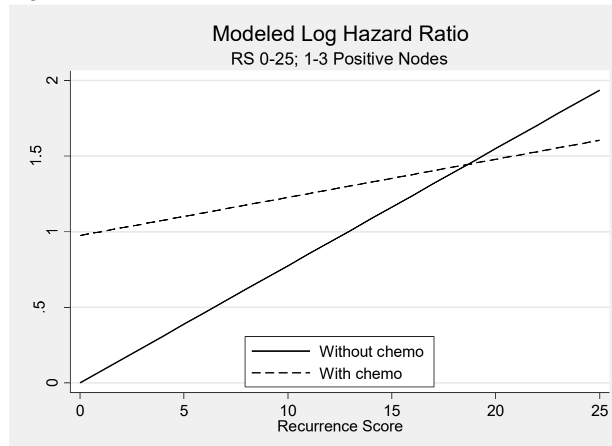


Figure 3



Based on **SWOG-8814** data alone it is difficult to conclude that there is benefit of chemotherapy in patients with RS ≤ 25. The sample size is small, and there are some trends in the data that support chemotherapy at the higher ends of the RS range. Furthermore, more effective chemotherapy regimens have been adopted since **SWOG-8814** was conducted. There have also been improvements in hormonal therapy, surgery, and radiation therapy, though these would apply equally to the two randomized groups. How representative of current outcomes are the **SWOG-8814** data given that patients were randomized in the early 1990s? SEER does not provide DFS, but does provide some information about overall survival (OS). Using SEER, one can examine some of this improvement by tumor stage. From SEER one can derive the estimated overall survival of 52,592 women aged 55-74 diagnosed with hormone receptor-positive, node-positive breast cancer in the years 1996-2003. In this group, overall survival was 83% at 5 years and 64% at 10 years. Note that **SWOG-8814** had an overall survival of 82% at five years and 64% at ten years, so the outcomes are almost identical to current population results. Nonetheless, randomized trials have clearly established that better chemotherapies are available today, including taxanes and the dose-dense concept. This would strengthen the importance of this trial since we would have a randomized comparison with modern chemotherapy, rather than observational data based on outdated chemotherapy.

The high failure rate in **SWOG-8814** has been criticized but, as outlined above, the overall survival is exactly what one would expect in a comparable population. The recurrence results for **SWOG-8814** have also been compared with those of TRANS-ATAC at 5 years. Their primary outcome is disease-free interval (DFI), which censors deaths that are not associated with a recurrence. Ten deaths in **SWOG-8814** occurred within 5 years and had no evidence of recurrence so are treated as censored in this analysis, but would be considered as failures for DFS. The TRANS-ATAC investigators kindly provided comparison data, but collapsed over treatment group. Recurrence rates (events/person years) suggest little difference between ATAC and **SWOG-8814**. Adjusting for number of nodes and RS risk group (< 18, 18-30, > 30) showed no statistically significant difference in DFI between ATAC and **SWOG-8814**. There are not appreciable differences in the TRANS-ATAC and **SWOG-8814** outcomes when RS groupings and number of nodes are considered. Thus, the **SWOG-8814** data still provide an excellent reference point for the proposed trial.

The percentage of patients in **SWOG-8814** with > 3 positive nodes was 43%. This proposed trial will restrict eligibility to patients with 1-3 positive nodes. In women with 1-3 positive nodes, RS was ≤ 25 in 67% of patients in the **SWOG-8814** study. Since the Oncotype DX® test became available for clinical practice for node positive patients in 2008, it has been observed that the proportion of tested patients with RS ≤ 25 has been greater than 75%, consistent with the use of test more often in patients who appear lower risk by other prognostic factors such as patient age, tumor size, tumor grade, and/or extent of hormone receptor staining by IHC. Since the point of equivalence is unknown, it is necessary to have a wider range of RS scores to distinguish those who may need chemotherapy from those who do not.

To summarize, **SWOG-8814** provides general support for chemotherapy in patients with receptor-positive, node-positive disease, but no strong evidence of benefit in the subset with RS ≤ 25. Looking more carefully using the actual value of RS, does reveal that treatment effects may start to diverge at higher values of RS, but this would need to be supported by data using modern endocrine and chemotherapy and many more patients. This study predicts that continuous RS will be directly associated with the degree of benefit. It also predicts there may be an equivalence point between RS 0 to 25 after which a benefit to chemotherapy may emerge. When categorized, we expect no benefit below that cutpoint, but a clinically significant benefit above the cutpoint.

Translational Medicine

Comparative Effectiveness Evaluation of Oncotype DX® for Women with Node-positive, Hormone-responsive and HER2-negative Breast Cancer

While it is recognized that the majority of the acute effects of chemotherapy (e.g. nausea, vomiting, diarrhea, stomatitis, alopecia and neutropenia) will resolve, sub-acute effects and long-term sequelae may have a lasting impact on the quality of life of survivors. Adjuvant chemotherapy is associated with premature menopause in some women who are less than 40 years of age (estimates from 13%-38%) and in the vast majority of women over the age of 40 (estimates from 57%-96%). Weight gain has been reported in 50% or more of women receiving adjuvant chemotherapy. Weight gain is likely to have a profound influence on a woman's physical and psychological health. The long-term effects of chemotherapy associated with thrombosis, myelodysplastic syndromes, acute leukemia and cardiac disease may also contribute to the decreased quality of life of women. *(20,21,22,23)* With the increasing use of taxane based and dose-intensive regimens, previously unobserved sequelae such as neuropathy and myalgias are more common. Many of these symptoms are slow to resolve and may have long-term consequences for women. While the acute effects of chemotherapy on fatigue has long been recognized, a number of studies have reported problems with fatigue for lasting months or even years after adjuvant chemotherapy. Such fatigue was associated with a decrease in daily functioning. *(24)*

In addition, there has been increased awareness of cognitive dysfunction associated with adjuvant chemotherapy in women with breast cancer. *(25)* Schagen, et al, evaluated 39 women at approximately 2 years following 6 cycles of chemotherapy compared to 34 women who had received local therapy only; 28% of the patients treated with CMF, compared to 12% of control groups, showed evidence of cognitive dysfunction characterized by difficulty with concentration, memory, and word finding. *(26)* van Dam, et al, suggested that such symptoms were worse in patients treated with high-dose chemotherapy. *(27)* While these studies suffer from lack of good controls and poor association between reports of cognitive dysfunction and scores on formal testing, they remain concerning. Anecdotally many breast cancer patients complain of forgetfulness, difficulty concentrating or "chemo brain". These effects are likely to have significant long-term consequences for women's quality of life.

In a single arm observational study, Ganz, et al, demonstrated the potential negative effects of chemotherapy on the long-term health-related quality of life (HRQOL) of breast cancer survivors, including decreases in sexual activity and overall well being. *(28,29)* While earlier studies have shown some of the negative effects of adjuvant chemotherapy on HRQOL, changes in both regimens and supportive care practices mean that these data are much less relevant to modern practice. The impact that chemotherapy has on health-related quality of life is key and central for patients and physicians deciding whether or not to receive adjuvant chemotherapy in order to prevent further recurrence. This study provides a unique opportunity to prospectively and quantitatively evaluate the impact of modern chemotherapy on the quality of life of women with early breast cancer. It is critical now to take the opportunity to integrate quality of life assessment into this unique randomized trial. While it is hypothesized that chemotherapy is unlikely to have an effect on cancer outcomes in those with an RS < 25, it is possible that a subset of patients with RS between 11 and 25 may achieve small benefits in reduction in recurrence. If so, the impact of chemotherapy on quality of life will be particularly relevant for this group of patients. Such information is also likely to be useful to all women with breast cancer and their physicians who are considering adjuvant chemotherapy. This study will also provide an ideal opportunity to evaluate, in the context of a randomized trial, the impact of chemotherapy on long-term sequelae such as premature menopause and weight gain, and the quality of life associated with these events.

Another central question is whether women will accept a recommendation not to receive chemotherapy based on the results of Oncotype DX®, given the findings of benefit for adjuvant therapy in this population. Recent studies in doctor/patient decision making suggest that women are likely to accept adjuvant chemotherapy for little or minimal benefits. *(30)* Thus, it is possible that a woman will experience anxiety about the idea of forgoing chemotherapy, even if the RS predicts that chemotherapy is unlikely to provide benefit. Such anxiety might be debilitating or lead to a decision to proceed to chemotherapy despite a low RS for those randomized to endocrine therapy alone. If this occurs in this trial, it will be important to understand the reason for this choice.

Background and Significance: Cost

Direct expenditures on breast cancer were estimated to be about $6 billion in 1996 (the last year such estimates were made), and are surely higher today. Gene expression profile (GEP) tests are expensive, costing approximately $4,000 per patient, yet adjuvant chemotherapy is much more expensive, costing $20,000 – $26,000 (upper ranges are closer to $50,000) (2003 dollars). *(31)* The immediate impact of GEP on breast cancer expenditures will depend on the degree to which the test spares women from undertaking costly chemotherapy. Based on current evidence regarding test outcomes, GEP could reduce initial breast cancer treatment costs by hundreds of millions of dollars. The long-term budget impact, however, will depend on the ability of the test to distinguish those who ultimately would experience breast cancer recurrence from those who would not. If GEP is a poor predictor of recurrence, the testing strategy could be more expensive than current practice while at the same time producing poorer outcomes. On the other hand, if GEP can better target women who will recur, risk profiling will substantially improve the cost-effectiveness of adjuvant therapy. The successful use of adjuvant chemotherapy in a highly targeted population thus represents a paradigm shift, both clinically and from an economic value standpoint. An accurate understanding of the changing economic value of adjuvant chemotherapy will be essential to ensure appropriate reimbursement policies.

Given the proliferation of GEP tests, their potential role in clinical practice, and the national clinical and economic burden of breast cancer, quantitative evaluations of the economic outcomes associated with GEP are warranted. There have been two published cost-effectiveness evaluations of GEP for women with localized breast cancer. Both used simulation modeling and available data, but came to very different conclusions. One analysis, basing its estimates on the performance characteristics of Oncotype DX®, found that risk stratification using GEP would reduce cancer care costs and increase quality-adjusted survival. (*32*) The other based its analysis on the MammaPrint assay, and found that GEP increased costs and reduced quality-adjusted survival. *(33)* While some might conclude that these analyses support the superiority of Oncotype DX® vs. MammaPrint, differences in the model structures and other input parameters in these studies make such conclusions tentative at best. Moreover, no study has evaluated costs and outcomes of GEP tests in the management of node-positive breast cancer.

Clinical trials evaluating medicines, medical devices and procedures now commonly assess the economic value of these interventions. "Piggybacking" cost-effectiveness analyses alongside clinical trials offers many advantages over 'pure' modeling approaches: combining studies is efficient; the internal validity of both studies is maximized, and economic data is made available alongside clinical data in a timely fashion. *(34)* A trial-based economic analysis will provide the most accurate estimates of the cost-effectiveness of GEP. Decision makers in many countries now consider clinical and economic evidence together for formulary and insurance coverage policies, as will surely be the case for GEP.

2.2 Inclusion of Women and Minorities

This study was designed to include women and minorities, but was not designed to measure differences of intervention effects.

Ethnic Category			
	Females	**Males**	**Total**
Hispanic or Latino	178	0	178
Not Hispanic or Latino	3,822	0	3,822
Total Ethnic	4,000	0	4,000
Racial Category			
American Indian or Alaskan Native	31	0	31
Asian	154	0	154
Black or African American	433	0	433
Native Hawaiian or other Pacific Islander	12	0	12
White	3,370	0	3,370
Racial Category: Total of all Subjects	4,000	0	4,000

2.3 Rationale for Additional Biomarker Analysis

Gene Expression Based Prognostic and Predictive Markers in Hormone Receptor Positive Breast Cancer

Although in **S1007** the 21-gene RS was used for patient stratification, several other gene expression signatures are commonly used as prognostic/predictive markers including 70-gene MammaPrint signature and PAM50 gene intrinsic subtype, SET index, Endopredict and Breast cancer index. Some of these expression profiles are described below.

While comparison amongst genomic assays was performed in the TransATAC trial, generalizability concerns of these analyses include the study was limited to postmenopausal patients and patients did not receive chemotherapy *(35, 36)*. There remains an unmet need to identify predictors of chemotherapy benefit within premenopausal as well as postmenopausal patients. Molecular analysis will allow for testing of prognostic and predictive biomarkers in patients who received chemotherapy on the same platform across the entire patient cohort, and will determine the underlying biological differences between premenopausal and postmenopausal patients.

Brief description of additional assays and signatures

70-gene MammaPrint signature: MammaPrint is a 70-gene signature which classifies tumors into groups that are associated with a good or poor prognosis on the basis of distant metastasis-free survival (DMFS) at 5 years and at 10 years. *(37)* Among the 658 women with HR+/HER2-, N1 breast cancers in the MINDACT (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy) trial who had clinical high but genomic low risk as determined by the 70-gene MammaPrint assay (Agendia) there

was a 2.6% improvement in 8-year DMFS with chemotherapy. *(38, 39)* An exploratory subgroup analysis demonstrated an age-dependent effect of chemotherapy, in which the magnitude of chemotherapy benefit reached 5% in women age $\leq$50 and <1% benefit if age >50.

PAM50 signature: Gene expression profiling classifies breast cancer into "intrinsic subtypes" based on the biology of the underlying disease pathways. *(40)* This has been developed as Prediction Analysis of Microarray 50 (PAM50) Risk of Recurrence (ROR) score (Veracyte Technologies, previously Prosigna). The ROR Score was validated to determine the risk of recurrence of disease in HR+ breast cancer after surgery and treatment with 5 years of endocrine therapy. The ROR score depends on the intrinsic subtype, proliferation score of the tumor, and the tumor size. *(41, 42, 43)*

SET ER/PR and SET2,3 index: Sensitivity of endocrine therapy (SET) ER/PR index was developed to measure gene expression microarray probe sets that associate with hormone receptors (ESR1 and PGR). Higher SET ER/PR index in MBC samples predicted improved PFS and OS when patients received endocrine therapy as next treatment, even after adjustment for clinical-pathologic risk factors (PFS: HR 0.534, 95% CI 0.299 to 0.955, p = 0.035; OS: HR 0.315, 95% CI 0.157 to 0.631, p = 0.001). *(44)* SET2,3 index was proposed as a test for sensitivity to adjuvant endocrine therapy for patients with stage II-III breast cancer by measuring transcription related to estrogen and progesterone receptors (SET ER/PR index) adjusted for a baseline prognostic index combining clinical tumor and nodal stage with molecular subtype by RNA4 (ESR1, PGR, ERBB2, and AURKA). In HR+ patients who underwent neoadjuvant therapy, SET2,3 index was found to add independent prognostic information in addition to residual cancer burden in two separate cohorts. *(45)*

EndoPredict (EP; Myriad Genetics, Cologne, Germany): EndoPredict (EP) is an RNA based multigene test that predicts the likelihood of distant recurrence in patients with HR+ breast cancer being treated with adjuvant endocrine therapy. In the GEICAM 9906 trial, EP was an independent prognostic parameter in node-positive, HR+ breast cancer patients treated with adjuvant chemotherapy followed by hormone therapy. *(46, 47)* The EP assay is based on the quantification of eight cancer-related genes of interest and three reference genes.

Breast Cancer Index (BCI; Biotheranostics,San Diego, CA): The Breast Cancer Index test analyzes the activity of seven genes to help prognosticate the risk of recurrence in patients with HR+ breast cancer 5 to 10 years after diagnosis. BCI can be used for prediction with the benefit of extended adjuvant endocrine therapy. *(48, 49)*

Genomic Alteration in Breast Cancer as Prognostic Markers

Genomic characterization of breast cancer has become standard of care for metastatic breast cancer (MBC) patients with HR+ cancer. There is already one therapy FDA-approved linked to a genomic biomarker for MBC: PI3K inhibitor alpelisib in combination with endocrine therapy for *PIK3CA* mutant HR+ breast cancer. There are several other genomically matched therapies under investigation in MBC, with expected increase in clinical utility of genomic testing in MBC.

Although genomic testing is not standard of care in non-metastatic breast cancer, we and others have already demonstrated that several key genomic alterations are associated with an increased risk of relapse and/or endocrine resistance in HR+ breast cancer including *TP53* mutations, *(50)* and alterations in MAPK pathway such as *NF1* loss. *(51, 52, 53)* Notably, *ESR1* mutations have also been associated with endocrine resistance but this has been primarily found in metastatic tumors, as a mechanism of acquired resistance. *(54)*

Taken together, there are several different prognostic signatures already developed for HR+ breast cancer and many genomic features associated with recurrence. We *hypothesize* that three established prognostic signatures (21-gene signature, breast cancer intrinsic subtype and 70-gene signature) based on RNAseq are associated with prognosis in premenopausal and postmenopausal patients with 1-3 LN+. Prognostic endpoints include IDFS, DDFS, LDFS, and OS. We also hypothesize that these prognostic signatures alone or integrated together will predict chemotherapy benefit in premenopausal patients with 1-3 LN+. The prognostic and predictive value may be further enhanced with integration of additional gene expression sets (*e.g., SET2,3*, RNA4 index, *MKI67* gene expression) and breast cancer genomics and proteomics.

Baseline serum hormone levels

In addition to clinical characteristics, serum hormone levels may be able to further discriminate menopausal status. Beyond self-reporting of menopausal status, serum levels can offer an objective measure. The mean age at onset of menopause is 51 years in Western countries, and by age 55 approximately 85% of women have undergone menopause, whereas less than 10% of women experience menopause at or before age 45. *(55, 56)* In clinical practice, estradiol, luteinizing hormone, and follicle-stimulating hormone are often evaluated for determination of whether a patient is pre- or post-menopausal. Anti-Müllerian Hormone (AMH), also called Müllerian inhibiting factor (MIF) is an additional indicator available as to whether a woman is approaching or is likely to have reached her final menstrual period. Given that there is a significant interaction between menopausal status, as determined by clinical characteristics, and IDFS and DDFS in RxPONDER, we propose evaluating hormone levels in pre-treatment baseline samples to assess whether menopausal status is further refined and whether an interaction term remains statistically significant based upon menopausal status per serum hormone levels.

2.4 Rationale for Circulating Biomarker Assessment for Late Relapse Translational Medicine Substudy

S1007 is a trial of patients with HR+/Her2- breast cancer, with 1-3 lymph nodes involved. Patients with an Oncotype Recurrence Score ≤ 25 were randomized to hormone therapy alone vs. hormone therapy plus chemotherapy. **S1007** was initiated in 2011 and completed accrual in October 2015 at NTCN sites. UNICANCER independently accrued ~1,000 patients, which completed accrual October 2017. Ultimately, 5,083 patients were randomized to **S1007**.

S1007 is an ideal trial to collect blood samples in patients who have not developed a recurrence for evaluation of blood-based biomarkers. We have baseline clinical and pathologic data on all randomized patients and will be following patients for several more years to determine recurrence rate. Identifying potential markers for late recurrence in patients who have taken endocrine therapy remains an important unmet need and, as these patients are high-risk, given their nodal involvement, this study includes the population for which this question should be addressed. Also, **S1007** exclusively enrolled patients with HR+/HER2- breast cancer, a subtype with a risk of late recurrence. **S1007** initially included an optional baseline tumor and nodal tissue collection, as well as blood samples collection, allowing for comparison of changes over time, depending on tissue availability. In addition, this study and other previously completed SWOG trials collected samples from patients prior to initiating adjuvant therapy.

The circulating biomarker assessment for late relapse will involve Streck, Cellsave®, and STS blood and serum sample collection (with patient consent to the substudy) for patients who are between 5 and 8 years following start of endocrine therapy. Previously, EDTA tubes were collected (with patient consent) for banking at baseline registration to **S1007**.

This will provide a unique opportunity to compare the additional substudy samples to originally banked **S1007** pre-treatment samples, which may, in turn, allow for identification of three groups of patients: 1) those who have not escaped dormancy (and may not need continued hormonal or other therapy), 2) those who are escaping dormancy and will relapse in the near future (and may need to modify treatment immediately, identifying the highest risk group for future clinical trial approach considerations), and 3) those who still are in dormancy and may experience a later relapse (and may need to switch hormonal therapy and/or add a new targeted treatment: again, a population for future trial selection).

In summary, these findings support the design of utilizing plasma repositories from large adjuvant trials of endocrine therapy. A number of critical questions remain in early-stage breast cancer. For instance, if CTCs are identified in a patient who is still on adjuvant therapy without a recurrence, should we consider switching the systemic therapy to decrease the risk for late recurrence? Is there a role for detection of ESR1 mutations, or other frequent mutations such as PIK3CA mutations, during adjuvant AI therapy? Will the ESR1 mutation status results observed in the metastatic Bolero-2 trial (endocrine therapy +/- everolimus) be recapitulated in the operable setting (endocrine +/- everolimus)? If ESR1 mutations are identified, should these patients be switched to an alternative adjuvant therapy, such as a selective estrogen receptor downregulator (SERD)? Perhaps switching strategies or combination of hormone therapy with a SERD should be utilized to overcome endocrine resistance in operable breast cancer? Establishing a biorepository in this node positive population after 5 years of endocrine therapy offers a unique opportunity to evaluate whether we can identify blood-based predictors of late relapse.

While there are commercially available tumor-tissue based genomic tests looking at rate of distant relapse, including breast cancer index, these are looking at baseline, pre-treatment samples. This substudy will be looking at real-time, on-treatment predictors, which may be more reflective of current tumor biology due to selective treatment pressure, dormancy escape, etc. In addition, other previously described blood-based markers, such as serum tumor markers, in patients with breast cancer can be unreliable, including in patients with metastatic breast cancer. CTC enumeration and the association with risk of late relapse was selected as the primary outcome, given that preliminary data with CELLSEARCH in the adjuvant setting has been previously described, allowing for appropriately powered calculations in this concept. It is critical to further understand the differences in the technologies and whether a) the rate of CTC detection vary with a different platform in the same population and b) there are differences in the dynamic CTC changes with a different platform. We hypothesize that invasive disease-free survival will be poorer in CTC-positive patients compared to those patients who are CTC-negative. The two platforms for CTC's (Menarini and Epic Sciences) will be compared to assess positivity rates between these two systems at each timepoint as well as whether changes between timepoints are also consistent. Ultimately, the goal of this project is to identify early predictors of dormancy escape and late recurrence in patients with operable breast cancer, which can serve as the basis for future randomized, interventional trials.

2.5 Rationale for Utilization of Remote Consent Procedures for Patient Consent to the Circulating Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) Substudy

The patient population being considered for inclusion in the CBALR TM substudy (Registration Step 3) is currently in annual follow-up. There is strong rationale for the allowance for participating site implementation of remote consent procedures (especially with consideration for the ongoing effects of the COVID-19 pandemic on the healthcare environment) to consent prospective participants (who meet the eligibility criteria defined in Section 5.3) via telehealth methods prior to the patient's next scheduled annual follow-

up visit. Herein, an allowance for utilization of remote consent procedures has been requested from the NCI CIRB for U.S. sites utilizing the NCI CIRB. Please see Section 16.0 for **S1007** Remote Consent Procedures for the CBALR TM substudy.

3.0 DRUG INFORMATION

(This study is being conducted under CTEP IND #)

Please refer to the package insert for the drugs that the individual patient will receive for approved language related to information on dosing, toxicities, preparation and administration of these agents.

4.0 STAGING CRITERIA

N1 Metastasis to movable ipsilateral axillary lymph node(s)
pN1 Micrometastasis or Metastasis in 1 to 3 axillary lymph nodes, and/or in internal mammary nodes with metastasis by sentinel lymph node biopsy but not clinically detected
pN1mi Micrometastases (greater than 0.2 mm and/or more than 200 cells, but none greater than 2.0 mm)
pN1a Metastases in 1 to 3 axillary lymph nodes, at least one metastasis greater than 2.0 mm
pN1b Metastases in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node dissection but not clinically detected
pN1c Metastases in 1 to 3 axillary lymph nodes and in internal mammary nodes with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected

5.0 ELIGIBILITY CRITERIA

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. Use the spaces provided to confirm a patient's eligibility. For each criterion requiring test results and dates, please record this information on the **S1007** Prestudy Form and submit to the SWOG Statistics and Data Management Center in Seattle (see Section 14.0). Any potential eligibility issues should be addressed to the SWOG Statistics and Data Management Center in Seattle at 206/652-2267 prior to registration.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday 4 weeks later would be considered Day 28. This allows for efficient patient scheduling without exceeding the guidelines. **If Day 14, 28, 42, 56 or 84 falls on a weekend or holiday, the limit may be extended to the next working day.**

5.1 STEP 1 REGISTRATION (Oncotype DX® Screening)

If patient has previously been tested by Oncotype DX®, then she still must satisfy these eligibility criteria to be considered for randomization

a. Patients must have a histologically confirmed diagnosis of node positive (1-3 nodes) invasive breast carcinoma with positive estrogen and/or progesterone receptor status, and negative HER-2 status. Estrogen and progesterone receptor positivity must be assessed according to ASCO/CAP guidelines as either ER or PR ≥ 1% positive nuclear staining. HER-2 test result negativity must be assessed as per ASCO/CAP 2013 guidelines using IHC, ISH or both. HER-2 is negative if a single test (or all tests) performed in a tumor specimen show: a) IHC negative (0 or 1+) or b) ISH negative using single probe or dual probe (average HER-2 copy number < 4.0 signals per cell by single probe or HER-2/CEP ration < 2.0 with an

average copy number < 4.0 signals per cell by dual probe). If HER-2 IHC is 2+, evaluation for gene amplification (ISH) must be performed and the ISH must be negative; ISH is not required if IHC is 0 or 1+. HER-2 equivocal is not eligible.

b. Patients with multifocal, multicentric and synchronous bilateral breast cancers are allowed.

- Multifocal disease is defined as more than one invasive cancer < 2 cm from the largest lesion within the same breast quadrant. (NOTE: The Oncotype DX® testing must be completed on the largest lesion.)

- Multicentric disease is defined as more than one invasive cancer ≥ 2 cm from the largest lesion within the same breast quadrant or more than one lesion in different quadrants. (NOTE: Oncotype DX® testing should be completed on all tumors and the determination for eligibility should be made on the highest recurrence score.)

- Synchronous bilateral disease is defined as invasive breast cancer with positive lymph nodes (axillary or intramammary) in at least one breast, diagnosed within 30 days of each other. (NOTE: The Oncotype DX® testing should be completed on both tumors and the tumor with the highest recurrence score should be used.)

c. Patients will have undergone axillary staging by sentinel node biopsy or axillary lymph nodes dissection (ALND). Patients must have at least one, but no more than three known positive lymph nodes (pN1a, pN1b or pN1c), see Section 4.0 for definitions. Patients with micrometastases as the only nodal involvement (pN1mi) are not eligible. Patients with positive sentinel node are not required to undergo full axillary lymph node dissection. This is at the discretion of the treating physician.

   Axillary node evaluation is to be performed per the standard of care at each insititution.

d. Patients must not have inflammatory breast cancer and must not have metastatic disease.

e. Patients with a prior diagnosis of contralateral DCIS are eligible if they underwent a mastectomy or lumpectomy with whole breast radiation. Prior partial breast irradiation, including brachytherapy, is not allowed. Patients with a prior diagnosis of ipsilateral DCIS or invasive breast cancer who received radiation to that breast are not eligible.

f. Patients must have had either breast-conserving surgery with planned radiation therapy or total mastectomy (with or without planned postmastectomy radiation). Patients must have clear margins from both invasive breast cancer and DCIS (as per local institutional guidelines). LCIS at the margins is allowed.

g. Registration of patients who have not yet undergone Oncotype DX® screening must occur no later than 56 days after definitive surgery. (For all patients, Step 2 Registration must occur within 84 days after definitive surgery.) If the Oncotype DX® Breast Cancer Assay has not been performed, patients must be willing to submit tissue samples for testing to determine the Recurrence Score value. A representative block or unstained sections from the representative block are sent directly to Genomic Health for Oncotype DX® Breast Cancer Assay which will be performed according to the standard commercial process (see Section 15.1).

   If the Oncotype DX® Recurrence Score is already known and is 25 or less, the patient must be registered to Step 2 immediately following Step 1 registration. If

the Oncotype DX® Recurrence Score is already known and is greater than 25, the patient is ineligible.

h. Patients must be females ≥ 18 years of age. As the Oncotype DX® Recurrence Score has not been validated in men with breast cancer, men are not eligible for this study.

i. Patients must have a complete history and physical examination within 28 days prior to registration.

j. Patients must have a performance status of 0-2 by Zubrod criteria (see Section 10.7).

k. Patients must be able to receive taxane and/or anthracycline based chemotherapy.

l. Patients must not have begun chemotherapy or endocrine therapy for their breast cancer prior to registration.

m. Patients must not require chronic treatment with systemic steroids (inhaled steroids are allowed) or other immunosuppressive agents.

n. Patients must not have received an aromatase inhibitor (AI) or a selective estrogen receptor modulator (SERM) such as tamoxifen or raloxifene within 5 years prior to registration.

o. Patients must not be pregnant or nursing due to the possibility of harm to a fetus or nursing infant from this treatment regimen. Women of reproductive potential must have agreed to use an effective contraceptive method. A woman is considered to be of "reproductive potential" if she has had menses at any time in the preceding 12 consecutive months. In addition to routine contraceptive methods, "effective contraception" also includes heterosexual celibacy and surgery intended to prevent pregnancy (or with a side-effect of pregnancy prevention) defined as a hysterectomy, bilateral oophorectomy or bilateral tubal ligation. However, if at any point a previously celibate patient chooses to become heterosexually active during the time period for use of contraceptive measures outlined in the protocol, he/she is responsible for beginning contraceptive measures.

p. No other prior malignancy is allowed except for adequately treated basal cell (or squamous cell) skin cancer, in situ cervical cancer, or other cancer for which the patient has been disease-free for 5 years.

q. **The Quality of Life and Economic Substudy is permanently closed to accrual effective 12/1/12. Patients who consented to QOL prior to 12/1/12 should continue to complete QOL forms per their expectation report.** Patients who are able to complete a questionnaire in English must be offered the opportunity to participate in the Quality of Life and Economic Substudy. (The Quality of Life and Economic Substudy is available to U.S. INSTITUTIONS ONLY.) Patients who are not able to complete a questionnaire in English are registered to **S1007** without participating in the Quality of Life and Economic Substudy.

- Patients who consent to participate in the Quality of Life and Economic Substudy and who do not yet know the results of their Oncotype DX® screening must agree to complete the **S1007** Health-Related Quality of Life Questionnaire: Enrollment between 14 days prior to and 7 days after Step 1 Registration.

- Patients who consent to participate in the Quality of Life and Economic Substudy and who do already know their Oncotype DX® Recurrence Score (and it is 25 or less) will proceed to Step 2 Registration without completing the **S1007** Health-Related Quality of Life Questionnaire Enrollment Form (but will complete the **S1007** Health-Related Quality of Life Questionnaire: Randomized Study Form) as outlined in Section 5.2d.

r. Patients or their legally authorized representative must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines. For Step 1 registration of patients who have not yet submitted specimens for the Oncotype DX® Breast Cancer Assay, the appropriate consent form is the Step 1 Consent Form. For both Step 1 and Step 2 registration of patients whose Recurrence Score is already known and is 25 or less, the appropriate consent form is the Step 2 Consent Form.

s. As a part of the OPEN registration process (see Section 13.4 for OPEN access instructions) the treating institution's identity is provided in order to ensure that the current (within 365 days) date of institutional review board approval for this study has been entered in the system.

5.2 STEP 2 REGISTRATION (Randomization)

The following additional criteria must be met in order for a patient to be considered eligible for registration to the randomized trial. For each criterion requiring test results and dates, please record this information on the **S1007** Prestudy Form-Randomized Study and submit to the SWOG Statistics and Data Management Center in Seattle (see Section 14.0). Any potential eligibility issues should be addressed to the SWOG Statistics and Data Management Center in Seattle at 206/652-2267 prior to registration.

a. Recurrence score (RS) by Oncotype DX® must be ≤ 25.

b. Step 2 Registration must take place within 84 days after definitive surgery. Patients must not have begun chemotherapy or endocrine therapy for their breast cancer prior to randomization.

c. Patients randomized to either arm may also co-enroll in Phase III trials that compare local therapies, or compare systemic therapies (such as chemotherapy, if randomized to Arm 1 of **S1007)**.

d. **The Quality of Life and Economic Substudy is permanently closed to accrual effective 12/1/12.** Patients at U.S. INSTITUTIONS who consent to participate in the Quality of Life and Economic Substudy must agree to complete the **S1007** Health-Related Quality of Life Questionnaire: Randomized Study Form after Recurrence Score results and randomized treatment status are known but before treatment has been initiated.

e. **Patients** or their legally authorized representative must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines. For all patients the appropriate consent form for this registration is the Step 2 Consent Form.

5.3 **STEP 3 REGISTRATION (U.S. Sites Only) – CBALR TM Substudy**

U.S. Patients who meet the following criteria at time of participating site activation of Revision 16 (Version Date 03/24/21) must be offered participation in sample collection and banking, as indicated in Section 15.3, for the Circulating Biomarker Assessment for Late Relapse Translational Medicine Substudy:

a. Patients must be disease-free, with no prior invasive recurrence at time of registration to Step 3.

b. Patients must be registered to Step 3 no more than 8 years after randomization (Step 2 Registration) and must agree to have samples drawn within 28 days after registration to Step 3.

c. Patients must agree to have blood samples collected at up to 3 timepoints: 1) within 28 days after registration to Step 3, 2) 2-3 years after time of registration to Step 3, and 3) At time of invasive recurrence (if applicable). Patients must also agree to have tissue submitted at time of invasive recurrence (if applicable) from the invasive recurrence biopsy (where tissue is available).

**** NOTE: Two separate specimen collection kits must be ordered IMMEDIATELY after patient registration to Step 3. See Section 15.3b for kit ordering instructions.**

6.0 STRATIFICATION FACTORS

6.1 Patients will be randomized between Arm 1 (chemotherapy and endocrine therapy) and Arm 2 (endocrine therapy alone) using a dynamic balancing algorithm. *(57)* Treatment arms will be balanced with respect to the following stratification factors:

a. Recurrence score: 0-13 versus 14-25

b. Menopausal status: pre-menopausal versus postmenopausal as defined in the **S1007** Prestudy Form.

c. Type of nodal dissection: axillary lymph node dissection (with or without sentinel node mapping) versus sentinel node biopsy without axillary lymph node dissection.

7.0 TREATMENT PLAN

For questions regarding treatment regimen selection, please contact Dr. Kevin Kalinsky or Dr. Julie R. Gralow at **S1007**question@swog.org. For dosing principles or questions, please consult the SWOG Policy #38 "Dosing Principles for Patients on Clinical Trials" at https://www.swog.org/sites/default/files/docs/2017-11/Policy38.pdf. However, individual decisions about patient dosing and dose modification are at the discretion of the treating investigator (see Section 8.3).

7.1 General Consideration/Concomitant Therapies

a. It is recommended for patients to have adequate bone marrow function, as defined by peripheral granulocyte count of ≥ 1,500/mm^3, and platelet count ≥ 100,000/mm^3.

b. It is recommended for patients to have adequate renal function with creatinine levels ≤ the institutional upper limit of normal.

c. It is recommended for patients to have adequate liver function with a bilirubin ≤ the institutional upper limit of normal. Alkaline phosphatase and transaminases (ALT and AST) may be up to 1.5 x the institutional upper limit of normal (ULN).

d. If patient is clinical Stage IIIA (T3, N1, M0) a baseline chest x-ray, CT scan of the abdomen and pelvis, PET/CT, bone scan, or MRI is recommended.

e. Diagnostic bilateral mammogram to obtain a baseline is recommended prior to surgery.

f. MUGA or ECHO is recommended for patients who are randomized to receive chemotherapy and who choose to include anthracycline-based treatment in the regimen. It is recommended that these patients have a normal left ventricular ejection fraction of ≥ 50%.

g. In general, the use of any concomitant medication/therapy deemed necessary for the care of the patient is allowed, including drugs given prophylactically (e.g. antiemetics +/- steroids, granulocyte colony stimulating factors).

h. Clinical Trial Conduct during COVID-19 Pandemic (and other extenuating circumstances)

In order to provide participating sites flexibility in ongoing patient treatment in the current COVID-19 pandemic healthcare environment, utilization of offsite / local healthcare resources for conduct of participant's annual history and physical exam is allowable with appropriate oversight by the Responsible Investigator. This utilization of local healthcare providers does not need to be documented as a deviation. In addition, the following extended window for the follow-up assessments is allowable per protocol, where the Responsible Investigator determines the delayed assessment is in the best interest of the patient as indicated below.

Extended Window for Follow-up Disease Assessment and Mammograms:

The allowable best practices window for the **S1007** disease assessments, occurring every 6 months until 5 years after time of randomization and then annually thereafter until 15 years after randomization, and annual mammograms is being extended to +/- 30 days, where the Responsible Investigator determines that the delayed assessments help to assure the safety of the patient, with consideration for the COVID-19 pandemic and related extenuating circumstances.

The Responsible Investigator rationale for utilization of the extended window outlined above must be carefully documented in the patient chart as resultant from the COVID-19 pandemic and extenuating circumstance. Please note the allowable best practices windows for the disease assessments and mammograms, indicated in Section 9.0, otherwise remain applicable for all patients, where there is not a COVID-19 pandemic-related extenuating circumstance.

7.2 Oncotype DX® Assay

If not already done prior to Step 1 Registration, tumor sample will be submitted to Oncotype DX® Assay Genomic Health for the Oncotype DX® assay, and will be evaluated for recurrence score (RS). Patients with RS ≤ 25 will undergo discussion of this trial in consultation with their oncologist considering known RS value and number of positive nodes. Patients will then be randomized to one of two arms (Step 2).

Radiation is recommended per institutional and National Comprehensive Cancer Network (NCCN) guidelines (http://www.nccn.org) and may be given after chemotherapy and during endocrine therapy.

7.3 Arm 1 (chemotherapy and endocrine therapy)

All Arm 1 patients will receive a protocol-approved chemotherapy regimen (see below), followed by a protocol-approved endocrine therapy (see below). The approved chemotherapy and endocrine therapy regimens are listed below, though these may be expanded or contracted during the course of the trial as standard of care changes occur.

a. Chemotherapy: All patients will receive a second or third generation chemotherapy. Choice of chemotherapy will depend on patient/physician preference.

1. Second Generation Regimens:

Agents	**Schedule**	**Cycle length**
Docetaxel and cyclophosphamide	4-6 cycles	
5-FU, doxorubicin (or epirubicin), and cyclophosphamide*	6 cycles	
Doxorubicin (or epirubicin) and cyclophosphamide (AC/EC) followed by paclitaxel	4 cycles each	q 3 weeks
5-FU, doxorubicin (or epirubicin) and cyclophosphamide followed by docetaxel	3 cycles each	q 3 weeks

* includes CAF/CEF with oral cyclophosphamide and FAC/FE(100)C with intravenous cyclophosphamide

2. Third Generation Regimens:

Agents	**Schedule**	**Cycle length**
Doxorubicin (or epirubicin) and cyclophosphamide (AC/EC) followed by paclitaxel	4 cycles for AC/EC;12 cycles for paclitaxel	q 2 weeks or q 3 weeks for AC; weekly for paclitaxel
5-FU, doxorubicin (or epirubicin), and cyclophosphamide followed by docetaxel	3 cycles each	All cycles q 3 weeks
5-FU, doxorubicin (or epirubicin), and cyclophosphamide followed by docetaxel	3 cycles each	
Dose dense doxorubicin and cyclophosphamide followed by dose dense paclitaxel	4 cycles each	All cycles q 2 weeks
Docetaxel, doxorubicin, and cyclophosphamide	6 cycles	
5-FU, doxorubicin (or epirubicin) and cyclophosphamide (FAC) followed by paclitaxel	4 cycles for FAC;12 cycles for paclitaxel	Weekly for paclitaxel
Paclitaxel followed by 5-FU, doxorubicin (or epirubicin) and cyclophosphamide (FAC)	12 cycles for paclitaxel; 4 cycles for FAC	Weekly for paclitaxel

b. Endocrine therapy: All patients will receive endocrine therapy. Choice of therapy will depend on menopausal status (see below) and patient/physician preference. Anyone not defined as postmenopausal per institutional standards should be treated as premenopausal. Treatment should be at least 5 years but can be extended. Switching from one therapy to another is allowed.

1. Approved Endocrine Therapy Regimens for **Premenopausal** women:

Treatment	**Dose**	**Treatment duration**
Tamoxifen	20 mg daily	5 years
Ovarian suppression or ablation		5 years
Tamoxifen combined with ovarian suppression or ablation	20 mg daily	5 years
Aromatase inhibitor (AI) combined with ovarian suppression or ablation*	Approved dose for AI	5 years
Tamoxifen followed by an aromatase inhibitor (AI)**	20 mg daily for tamoxifen; approved dose for AI	2-3 years each
Tamoxifen followed by an aromatase inhibitor (AI)**	20 mg daily for tamoxifen; approved dose for AI	5 years each

* if the patient cannot tolerate tamoxifen or tamoxifen is contraindicated
** If the patient becomes postmenopausal

2. Approved Endocrine Therapy Regimens for **Postmenopausal** women:

Treatment	**Dose**	**Treatment duration**
An aromatase inhibitor	Approved dose	5 years
Tamoxifen*	20 mg daily	5 years
Tamoxifen followed by an aromatase inhibitor	20 mg daily; approved dose	2-3 years each
An aromatase inhibitor followed by tamoxifen	approved dose; 20 mg daily	2-3 years each
Tamoxifen followed by an aromatase inhibitor	20 mg daily; approved dose	5 years each

* if the patient is unsuitable for, cannot tolerate, or refuses an aromatase inhibitor

NOTE: All postmenopausal patients are encouraged to receive an aromatase inhibitor sometime during their course of adjuvant endocrine therapy.

The approved regimens may be expanded or contracted if there is a shift in standard of care during the course of the trial.

7.4 Arm 2 (endocrine therapy alone)

Patients will receive a protocol-approved endocrine therapy.

Endocrine therapy: All patients will receive endocrine therapy. Choice of therapy will depend on menopausal status (see above) and patient/physician preference. Anyone not defined as postmenopausal per institutional standards should be treated as premenopausal. Treatment should be at least 5 years but can be extended. Switching from one therapy to another is allowed.

The approved endocrine therapy regimens are listed above under Arm 1, although these may be expanded or contracted during the course of the trial as standard of care changes occur.

Radiation is recommended per institutional guidelines and may be given before or during endocrine therapy (the start date of treatment is the start date of endocrine therapy)

7.5 Criteria for Removal from Protocol Treatment (Step 2)

a. Invasive recurrence/progression/relapse of disease (as defined in Section 10.1).

b. Completion of 5 years from randomization. (NOTE: Refusal of randomized treatment arm assignment, completion of chemotherapy, and/or early discontinuation of endocrine therapy are **not** considered criteria for removal from protocol treatment for this study.)

c. The patient may withdraw from the study at any time for any reason. However, refusal of the randomized treatment arm assignment *alone* is **not** a reason to take the patient off study. Randomized patients should be continued on study for 5 years from initial registration regardless of actual treatment received.

NOTE: Patients who develop a second primary malignancy are not required to be removed from protocol treatment, and they are encouraged to remain on study unless it is not appropriate for their continued care in the opinion of the treating physician.

7.6 Discontinuation of Treatment

All reasons for discontinuation of treatment must be documented in the Off Treatment Notice.

7.7 Follow-Up Period

All patients will be followed until death or **15** years after randomization, whichever occurs first.

8.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

8.1 NCI Common Terminology Criteria for Adverse Events

Two different versions of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be used on this study.

a. Serious Adverse Event (SAE) reporting

The CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 5.0 will be utilized **for SAE reporting only**. The CTCAE Version 5.0 can be downloaded from the CTEP home page (https://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 5.0.

b. Routine toxicity reporting

This study will utilize the CTCAE Version 4.0 for routine toxicity reporting. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP home page (https://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE Version 4.0.

8.2 General Considerations

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed (e.g., bisphosphonates for osteoporosis), including drugs given prophylactically (e.g. antiemetics +/- steroids, granulocyte colony stimulating factors),

8.3 Dose Modifications

Treatment and dose modifications are at the discretion of the treating investigator. Use of granulocyte growth factor support is recommended to maintain planned doses of chemotherapy.

8.4 Adverse Event Reporting

Toxicities (including suspected reactions) that meet the expedited reporting criteria as outlined in Section 16.0 of the protocol must be reported to the Operations Office, Study Chair and NCI via CTEP-AERS, and to the IRB per local IRB requirements.

9.0 STUDY CALENDAR

REQUIRED STUDIES	PRE STUDY	PRE Reg #2	During Chemotherapy Treatment *	During Endocrine Therapy Treatment Ω	Clinic Visits Prior to Invasive Recurrence/ Progression/ Relapse Ω	At time of Invasive Recurrence	Follow-Up Post-Invasive Recurrence/ Progression/ Relapse ^
PHYSICAL*							
History & physical √	X		X	X	X	X	X
Wt. & Performance Status	X		X	X	X	X	X
Disease Assessment	X		X	X	X	X	X
Toxicity Notation			X	X			
HRQL ¿	X	X			X	X	X
LABORATORY*							
AST/ALT			X				
CBC/Differential/Platelets			X				
Serum Creatinine (POCT creatinine allowed)			X				
Bilirubin			X				
Alk Phos			X				
HER-2 status	X						
Hormone Receptor status	X						
X-RAYS AND SCANS							
Mammogram				X£	X£	X£	
SPECIMENS †							
Submission for Oncotype DX Breast Cancer Assay #		X					
Tissue for correlatives		X§				X ∑	
Blood specimens for correlatives		X§			X ∑	X ∑	
TREATMENT							
Radiation %							
ARM 1 - chemotherapy followed by endocrine therapy			X	X ∫	X ∫	X ∫	
ARM 2 - endocrine therapy alone				X ∫	X ∫	X ∫	

Calendar continued on next page. Click here for footnotes.
Note: Forms are found on the protocol abstract page of the SWOG website (www.swog.org). Data submission guidelines are found in Section 14.0.

NOTE: Unless indicated otherwise in the protocol, scheduled procedures and assessments (treatment administration, toxicity assessment for continuous treatment, disease assessment, specimen collection and follow-up activities) must follow the established SWOG guidelines as outlined in outlined in the SWOG Best Practices document accessible from the “Best Practices” link at: https://www.swog.org/clinical-trials/protocol-workbench.

FOOTNOTES:

√ Physical exam to include breast examination.
May have already been performed prestudy, see Section 5.1e.
£ All patients with an intact breast will have an annual (± 14 days) mammogram thereafter until after time of invasive recurrence/progression/relapse. See also Section 7.1h for extended allowable windows for recurrence assessments in event of a COVID-19 extenuating circumstance.
* Clinic visits for labs and physical exams should be scheduled as clinically indicated based on the selected chemotherapy regimen.
† See Section 15.0 for specimen submission requirements and specimen processing instructions.
% Radiation is recommended per institutional and NCCN guidelines and may be given after chemotherapy and during endocrine therapy.
Ω Whether or not patient is receiving endocrine therapy, patients will be followed for recurrence/progression/relapse every 3 months for the first year, every six months (± 7 days) from Years 2-5 and then annually (± 14 days) until 15 years after randomization or after time of invasive recurrence/progression/relapse. See also Section 7.1h for extended allowable windows for recurrence assessments in event of a COVID-19 extenuating circumstance.
∫ Treatment with endocrine therapy will continue for at least five years.
^ After recurrence/progression/relapse, patients will be followed annually for additional recurrence endpoints, including second primary and for survival.
§ Specimens for correlatives must be collected after Step 2 consent, but prior to start of treatment (see Section 14.4f).
¿ The Health Related Quality of Life (HRQL) forms are to be completed by patients at U.S. institutions only and at the timepoints outlined in the HRQL schema.
∑ For patients registered to Step 3, blood must be submitted within 28 days after patient registration to Step 3, then at 2-3 years after patient registration to Step 3 (if prior to invasive recurrence), and at time of invasive recurrence. See Section 15.3b for sample collection kit ordering instructions and allow sufficient time for kit shipments. Tissue must also be submitted at time of invasive recurrence. See Sections 15.3 and 15.4.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 Invasive Recurrence

a. Appearance of any new invasive lesion(s) during or after protocol treatment. Whenever possible, recurrences should be documented histologically. Invasive recurrence includes local, regional, or distant recurrence with an invasive component. A new diagnosis of ipsilateral or contralateral DCIS without an invasive component is not considered to be a recurrence.

10.2 Sites of first invasive recurrence

All sites of invasive disease documented within 30 days of first documentation of invasive recurrence.

10.3 Invasive Disease-Free Survival

We use the STEEP definition (Hudis, et.al., JCO 2007) of invasive disease-free survival. Time from date of randomization (2nd Registration) to date of first invasive recurrence (local, regional or distant), second invasive primary cancer (breast or not), or death due to any cause. Patients last known to be alive who have not experienced recurrence or second primary cancer are censored at their last contact date. We use the acronym DFS for invasive disease-free survival in this protocol.

10.4 Distant Disease-Free Survival

Time from date of randomization (2nd Registration) to date of invasive distant disease recurrence, second invasive primary cancer (breast or not) or death due to any cause. Patients last known to be alive who have not experienced distant recurrence, or second primary cancer are censored at their last contact date. This secondary outcome requires continuing to follow the patient after local recurrence in order to ascertain subsequent distant recurrence.

10.5 Local Disease-Free Interval

Time from date of randomization (2nd Registration) to date of invasive local or regional recurrence. Patients last known to be alive without recurrence are censored at their last contact date. Patients with distant recurrence, second primary cancer or death are censored at the time of that event.

10.6 Overall Survival

Time from date of randomization (2nd Registration) to date of death due to any cause. Patients last known to be alive are censored at their last contact date.

10.7 Invasive Breast Cancer-Free Survival

We use the STEEP 2.0 definition (Tolaney, et.al., JCO 2021) of invasive breast cancer-free survival (IBCFS). Time from date of randomization (2nd Registration) to date of first invasive recurrence (local, regional or distant), second invasive breast cancer, or death due to any cause. Patients last known to be alive who have not experienced recurrence or second breast cancer are censored at their last contact date. This is similar to IDFS except that new non-breast primary cancers are not included as events. *(58)*

10.8 Performance Status

Patients will be graded according to the Zubrod performance status scale.

POINT	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

11.0 STATISTICAL CONSIDERATIONS

11.1 Accrual Goal

Women who have been diagnosed with node-positive (1-3 positive nodes), HER2-negative, endocrine-responsive breast cancer who meet the eligibility criteria in Section 5.0 will undergo testing by the 21-gene Recurrence Score assay (Oncotype DX®). It is anticipated that 10,000 women will be tested as part of the study or will already have been tested. Based on the distribution of RS scores in **SWOG-8814** for women with 1-3 positive nodes, 7,000 women would be expected to have RS ≤ 25. Of this group, 5,000 women are expected to accept randomization. The formal sample size goal for the randomized trial is 5,000 which will require approximately 10,000 women to be screened for inclusion. The 5,000 randomized patients will include the original accrual goal of 4,000 NCTN patients plus an additional 1,000 patients accrued by UNICANCER in France and combined for a joint analysis.

11.2 Accrual Rate

Originally we expected accrual of 4,000 randomized patients to take 6 years, giving a monthly accrual rate of 56 patients per month. The formal study completion date was five years after the last patient was enrolled to provide sufficient power for analyses. Thus, the original trial duration was expected to be 11 years. In this revised sample size calculation the actual monthly enrollment rates are used from study start in February 2011 to July 2014 which showed accrual not reaching the target goal until February 2012. Subsequently, accrual exceeded the goal considerably and the trial goal of 4,000 would be expected to be reached by August 2015 or 4.5 years of total accrual which is 1.5 years earlier than expected. If the trial used the original stopping rule of 5 years after the last patient, then power would be reduced considerably by the shorter follow-time for events to accrue. We can either extend the follow-up period and/or increase the accrual by combining with the UNICANCER group. SWOG has decided to do both as explained below.

Accrual from September 2014 to August 2015 is assumed to be 98 randomized patients per month until the NCTN accrual reaches 4,000 patients. We assume UNICANCER will accrue approximately 83.3 patients per month for an additional 12 months. This will result in a total sample size of 5,000 patients over a 5.5 year accrual period. After the last patient is accrued, then there will be 5.5 more years of follow-up before the study will have final analysis. The total trial duration will be 11 years which is the original length of the trial. However, individual patients will be followed for a minimum of fifteen years regardless of the date of enrollment to assess long-term effects of treatment.

11.3 Randomization and Stratification

A 1:1 parallel randomization is used with dynamic balancing on the three stratification factors: (1) RS 0-13 vs. RS 14-25; (2) menopausal status; and (3) axillary nodal dissection vs. sentinel node biopsy. Randomization is performed separately for NCTN and UNICANCER patients with randomized accrual goals of 4,000 and 1,000, respectively.

11.4 Summary of Analytic Plan

The complete statistical plan is presented in Appendix 18.4, but a brief summary is provided here. The primary question is to test whether chemotherapy benefit (if it exists) depends on the Recurrence Score. Thus the underlying hypothesis is that there is an interaction of chemotherapy and RS. This interaction is tested in a Cox regression model of DFS. If the interaction of chemotherapy and the linear RS term is statistically significant (two-sided α) and there is a point of equivalence between the two randomized treatments for some RS value in the range 0-25, then additional steps are undertaken. Based on simulation work, power to find a significant interaction with an equivalence point is 81%. Assuming there is a significant predictive effect of RS on chemotherapy benefit, then a clinical cutpoint for recommending chemotherapy will be estimated. This estimated cutpoint is the upper bound of the 95% confidence interval on the point of equivalence. Kaplan-Meier curves comparing randomized arms will be generated separately for RS values below and above this cutpoint and tested with stratified log-rank tests. Additionally, Kaplan-Meier curves will be generated for specific RS values (possibly grouped due to sparseness), and modeled 5-year and 10-year estimates by RS and treatment will be provided. If there is no statistical interaction between linear RS and chemotherapy, then chemotherapy will be tested in a Cox model adjusting for RS, but without an interaction term. This test will be conducted at a 1-sided $\alpha=0.025$ since chemotherapy would be expected to improve outcomes.

11.5 Compliance and Dropout

Despite the required discussion between patient and physician prior to randomization, some patients will still be noncompliant after randomization. The estimated sample size includes a 5% crossover rate and assumes that noncompliance depends on RS. For patients randomized to chemotherapy, the assumption is that 5% do not receive chemotherapy and that a patient with RS 0-11 is twice as likely to refuse as one who has RS 12-25. For patients randomized to not receive chemotherapy, the assumption is 5% do receive chemotherapy and that a patient with RS 18-25 is twice as likely to receive chemotherapy as one who has RS 0-17. The assumption is that the noncompliant patients remain in the study and provide follow-up. Thus, in the intent to treat analysis 5% of patients in each treatment group have a treatment opposite to their randomized assignment. This crossover rate was incorporated into the simulations which used a sample size of 4,750. This was increased to 5,000 to also accommodate dropout, ineligibility, or withdrawal of consent.

11.6 Early Termination for Futility

Apart from statistical testing of outcomes, low accrual and crossovers post-randomization affect the viability of the trial. It is also possible that differential acceptance of randomization across the range of RS from 0 to 25 may affect the statistical power of this study. With respect to accrual the study will employ the usual CTEP guideline to judge whether accrual is within expectations and whether the protocol needs to be amended. After two years of accrual, a committee of five statisticians (one SWOG, two from other cooperative groups, and two from CTEP) will meet and discuss viability of the trial based on accrual, acceptance of randomization, and crossover rate. The result of this discussion will be presented to CTEP and the Data and Safety Monitoring Committee (DSMC) for action. With respect to crossover rate (randomized participants receiving the opposite treatment), the upper limit is set at 15% in the first year and 12% after 2 years. The trial will terminate if the crossover rate exceeds this unless NCI determines that the high crossover rate has been corrected and that the trial remains viable. Crossover rates between 5% and the upper limit can be addressed by longer follow-up without increasing accrual. The target crossover rate is less than 5% total. As of August 2014, the actual crossover rate has been just less than 5%.

11.7 Summary of Revised Analytic Plan

As described in Section 11.2, accrual started slowly, but then improved dramatically so that now accrual of the original sample size goal is expected to finish 1.5 years early. Since the original design called for a final analysis 5 years after the last patient was enrolled, the number of events is considerably reduced due to the shorter time window. Using the observed sample size accrual, but keeping other design elements constant, then power to find a significant interaction would drop to 68% due to the shorter window for events. If we increase the window to 6.5 years after the last patient is accrued then power can be restored to 82.7% without adding 1,000 new patients. However, that provides no margin against some violation of the statistical assumptions of this important signature trial of the NCTN. For example, if the event rate is lower than predicted we would not have power to address the event rate accurately (and it depends on the intervention), but we can look at the population in terms of prognostic risk factors compared to what was expected. There has been a strong shift in the number of positive nodes compared to **SWOG-8814**. The older trial had a distribution of positive nodes for 1-3 of 48%, 32%, and 20%, respectively while the current trial has a distribution of 68%, 24%, and 8%. However, because of sentinel node biopsy there could have been an apparent shift downwards that does not reflect the true distribution of the number of positive nodes. Furthermore, the current trial has a distribution of Recurrence Scores that is the same as in **SWOG-8814** when restricted to ≤ 25 and in agreement with the expected distribution used to plan the trial. For trial

planning we used an overall 5-year DFS rate of 92.4%, but we would still like to have sufficient power if the event rate is less than expected.

The second reason to increase the sample size is to provide a validation that the results apply to an external population to NCTN patients. We are including these patients in the overall trial, but will conduct planned subset analyses of the two cohorts separately. If we retain the same design parameters with a 5,000 patient trial of the same total duration (11 years), then power increases to 86.3% and the expected number of events increases from 731 to 832. When the interaction is significant we determine the estimated cutpoint for using chemotherapy and its upper confidence bound. This cutpoint can be better determined when there are more events. Finally, the larger sample size allows some protection against lower event rates. For example, if the actual 5-year DFS rate is 93.9%, then power is still 80% to detect a significant interaction.

To guarantee that the trial will be well powered under the assumptions but have sufficient power under minor violation of the assumptions, we are increasing the sample size to 5,000 with a 5.5 year follow-up so that the total trial duration remains the same as in the original protocol.

11.8 Interim Analysis

Under the assumptions above, we would expect 832 events for the primary analysis of the interaction of RS and chemotherapy. The first interim analysis would be after 24% of the events have been observed or approximately 6.6 years after initiation of the study. This would correspond to the end of accrual if accrual is uniform and at the expected level. There would be subsequent annual interim analyses thereafter with 37%, 53%, 72%, and 92% with the final analysis at Year 11. The analyses will use the Lan-Demets spending function with a truncation bound. To achieve a cumulative 0.025 1-sided significance level, the interim test α's will be 0.0005, 0.0005, 0.00149, 0.00741, 0.01673, respectively, and the final α= 0.01871 so there is little loss of power due to the interim analyses. All of these analyses are expected to be after accrual has finished so a decision to publish early would be based on the interim analysis. We also want to monitor the upper RS group of 14-25 to avoid harming patients if there is early evidence of efficacy in this group. An analysis will be conducted at 4 years to evaluate efficacy of chemotherapy in patients with RS 14-25 to determine if there is a potential significant benefit of chemotherapy early in the trial. If this comparison is statistically significant at p=0.05 (2-sided) then further randomization in patients with RS 14-25 would be suspended. A similar comparison would then also be performed in the RS 0-13 group to determine if the trial should suspend accrual completely. Otherwise, all other analyses would occur after accrual is complete.

11.9 Translational Medicine

The statistical considerations for the translational medicine studies, quality of life and costs are described in their respective sections in the appendix.

11.10 Data and Safety Monitoring Committee

A Data and Safety Monitoring Committee will oversee the conduct of the study. The Committee consists of four members from outside of SWOG, 3 SWOG members, 3 non-voting representatives from the National Cancer Institute (NCI), and the Group Statistician (non-voting). The members of this Committee will receive confidential reports every 6 months from the SWOG Statistics and Data Management Center, and will meet at the Group's bi-annual meetings as necessary. The Committee will be responsible for decisions regarding possible termination and/or early reporting of the study.

12.0 DISCIPLINE REVIEW

This is not applicable to this study.

13.0 REGISTRATION GUIDELINES

13.1 Registration Timing

a. STEP 1 - Initial Registration (Oncotype DX® Screening)

Patients must meet the eligibility criteria in the Step 1 Registration criteria in Section 5.0. Patients who have not yet undergone Oncotype DX® Screening must be registered prior to initiation of treatment no later than 56 days from the date of definitive surgery.

Patients whose Oncotype DX® Recurrence Score is already known may undergo Step 1 and Step 2 registration at the same time (within 84 days after definitive surgery).

b. STEP 2 - Randomization (Chemotherapy plus Endocrine Therapy vs Endocrine Therapy alone)

Patients whose Recurrence Score is ≤ 25 and who meet the eligibility criteria as stated in the Step 2 Registration criteria in Section 5.0 will be registered to STEP 2 – Randomization. This registration must be performed within 84 days after the patient's date of definitive surgery.

Patients must be randomized prior to the initiation of treatment (no more than fourteen working days prior to the planned start of chemotherapy or endocrine treatment). For patients randomized to endocrine therapy only, who would receive radiation therapy prior to the start of endocrine therapy, radiation therapy must be planned to start within fourteen working days after randomization.

c. STEP 3 – Circulating Biomarker Assessment for Late Relapse Translational Medicine Substudy

U.S. patients must be registered to Step 3 Registration no more than 8 years after randomization (Step 2 Registration) and initial CBALR TM blood samples must be drawn within 28 days after registration. See also eligibility criteria indicated in Section 5.3.

13.2 Investigator/Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Prior to the recruitment of a patient for this study, investigators must be registered members of a Cooperative Group. Each investigator must have an NCI investigator number and must maintain an "active" investigator registration status through the annual submission of a complete investigator registration packet (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature and Financial Disclosure Form with original signature) to the Pharmaceutical Management Branch, CTEP, DCTD, NCI. These forms are available on the CTSU Web site (https://ctep.cancer.gov/investigatorResources/investigator_registration.htm). Questions should be directed to the CTEP Investigator Registration Help Desk by e-mail at pmbregpend@ctep.nci.gov.

Each investigator or group of investigators at a clinic site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU member web site by entering credentials at https://www.ctsu.org.

Requirements for site registration:

- CTSU IRB Certification
- CTSU IRB/Regulatory Approval Transmittal Sheet

NOTE: Sites participating on the NCI CIRB initiative and accepting IRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. This information will be provided to the CTSU Regulatory Office from the CIRB at the time the site's Signatory Institution accepts the CIRB approval. The Signatory site may be contacted by the CTSU Regulatory Office or asked to complete information verifying the participating institutions on the study. Other site registration requirements (i.e., laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

13.3 OPEN Registration Requirements

The individual registering the patient must have completed the appropriate SWOG Registration Worksheet for each Registration Step. The completed form must be referred to during the registration but should not be submitted as part of the patient data.

The individual registering the patient must have completed the appropriate SWOG Registration Worksheet for each Registration Step. The completed form must be referred to during the registration but should not be submitted as part of the patient data.

OPEN will also ask additional questions that are not present on the SWOG Registration Worksheet. For the Step 1 Registration, the individual registering the patient must be prepared to provide answers to the following questions:

a. Institution CTEP ID

b. Protocol Number

c. Registration Step

d. Treating Investigator

e. Cooperative Group Credit

f. Credit Investigator

g. Patient Initials

h. Patient's Date of Birth

i. Patient SSN (SSN is desired, but optional. Do not enter invalid numbers.)

j. Country of Residence

k. ZIP Code

l. Gender (select one):
- Female Gender
- Male Gender

m. Ethnicity (select one):
- Hispanic or Latino
- Not Hispanic or Latino
- Unknown

n. Method of Payment (select one):
- Private Insurance
- Medicare
- Medicare and Private Insurance
- Medicaid
- Medicaid and Medicare
- Military or Veterans Sponsored NOS
- Military Sponsored (Including Champus & Tricare)
- Veterans Sponsored
- Self Pay (No Insurance)
- No Means of Payment (No Insurance)
- Other
- Unknown

o. Race (select all that apply):
- American Indian or Alaska Native
- Asian
- Black or African American
- Native Hawaiian or other Pacific Islander
- White
- Unknown

13.4 Registration Procedures

a. All site staff (SWOG and CTSU Sites) will use OPEN to enroll patients to this study. OPEN is a web-based application that is integrated with the CTSU Enterprise System for regulatory and roster data and can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org, or from the OPEN Patient Registration link on the SWOG CRA Workbench.

b. Prior to accessing OPEN site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes (or each Registration Step) and the affirmation of eligibility on the Registration Worksheet has been signed by the registering investigator or another investigator designate. Site staff should refer to Section 5.0 to verify eligibility.

- All patients have signed an appropriate consent form for each Registration Step and HIPAA authorization form (if applicable).

- The study site is listed as "approved" in the CTSU RSS.

c. Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user ID and password) used for the CTSU members' web site. Additional information about obtaining a CTEP-IAM account can be found at http://ctep.cancer.gov/branches/pmb/associate_registration.htm. Questions should be directed to the CTEP Associate Registration Help Desk by e-mail at ctepreghelp@ctep.nci.nih.gov.

- To perform registrations, the site user must have been assigned the 'Registrar' role on the SWOG or CTSU roster:

1. If you are a SWOG member, to perform registrations on SWOG protocols you must have an equivalent 'Registrar' role on the SWOG roster. Role assignments are handled through SWOG.
2. If you are not a SWOG member, to perform registrations on SWOG protocols you must have the role of Registrar on the CTSU roster. Site and/or Data Administrators can manage CTSU roster roles via the new Site Roles maintenance feature under RSS on the CTSU members' web site. This will allow them to assign staff the "Registrar" role.

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

d. Further instructional information is provided on the OPEN tab of the CTSU members' side of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

13.5 Exceptions to SWOG registration policies will not be permitted.

a. Patients must meet all eligibility requirements.

b. Institutions must be identified as approved for registration.

c. Registrations may not be cancelled.

d. Late registrations (after initiation of treatment) will not be accepted.

14.0 DATA SUBMISSION SCHEDULE

14.1 Data Submission Requirement

Data must be submitted according to the protocol requirements for **ALL** patients registered, whether or not assigned treatment is administered, including patients deemed to be ineligible. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

14.2 Master Forms

Master forms can be found on the protocol abstract page of the SWOG website (www.swog.org) and (with the exception of the sample consent form and the Registration Worksheet) must be submitted on-line via the Web; see Section 14.3a for details. Exceptions to online data submission are source documents (e.g. pathology/operative/lab reports).

14.3 Data Submission Procedures

a. SWOG institutions must submit data electronically via the Web by using the SWOG CRA Workbench. To access the CRA Workbench, go to the SWOG Web site (http://swog.org) and logon to the Members Area. After you have logged on, click on the *CRA Workbench* link to access the home page for CRA Workbench website. Next, click on the *Data Submission* link and follow the instructions. For new users, the link to a "Starter Kit" of help files may be found by clicking on the **Starter Kit** link at the Members' logon page.

To submit data via the web the following must be done (in order):

1. You are entered into the SWOG Roster and issued a SWOG Roster ID Number,
2. You are associated as an investigator or CRA/RN at the institution where the patient is being treated or followed, and
3. Your Web User Administrator has added you as a web user and has given you the appropriate system permissions to submit data for that institution.

For assistance with points 1 and 2 call the Operations Office at 210/614-8808. For point 3, contact your local Web User Administrator (refer to the "Who is my Web User Administrator?" function on the swog.org Members logon page). For other difficulties with the CRA Workbench, please email technicalquestion@crab.org.

b. If you need to submit data that are not available for online data submission, the only alternative is via facsimile. Should the need for this occur, institutions may submit data via facsimile to 800/892-4007 or 206/342-1680 locally. Please do not use cover sheet for faxed data. Please make sure that each page of all faxed data includes the SWOG patient number, study ID and patient initials.

c. Data Submission Instructions for non-SWOG institutions (CTSU): See the CTSU participation table on Page 5.

14.4 Data Submission Overview and Timepoints

a. WITHIN 7 DAYS AFTER INITIAL REGISTRATION (STEP 1):

Submit a copy of the following:

S1007 Prestudy Form

All pre-registration breast cancer pathology reports.

b. (For all patients who have not yet undergone Oncotype DX® screening) WITHIN 7 DAYS AFTER INITIAL REGISTRATION (STEP 1):

Submit specimens for the Oncotype DX® test directly to Genomic Health as outlined in Sections 15.1a and 15.2.

c. (For all patients who do not continue on the randomized study and do not register to Step 2) WITHIN 14 DAYS AFTER DECISION NOT TO CONTINUE ON THE RANDOMIZED STUDY:

Submit the **S1007** Off Protocol Notice – Step 1.

d. AFTER RANDOMIZATION (STEP 2), BUT PRIOR TO START OF TREATMENT:

Submit blood specimens as outlined in Section 15.2b.

e. WITHIN 7 DAYS AFTER RANDOMIZATION (STEP 2):

Submit the **S1007** Prestudy Form – Randomized Study.

f. WITHIN 14 DAYS AFTER RANDOMIZATION (STEP 2)

Submit tissue specimen as outlined in Section 15.2a.

g. (For the subset of patients at U.S. INSTITUTIONS participating in the Quality of Life and Economic Analysis substudy) WITHIN 7 DAYS AFTER RANDOMIZATION, AT 6 MONTHS, 12 MONTHS, AND 3 YEARS AFTER RANDOMIZATION:

Submit a copy of the following:

S1007 Cover Sheet for Patient Completed Questionnaires

S1007 Health-Related Quality of Life Questionnaire: Randomized Study Form.

h. EVERY SIX MONTHS FOR THREE YEARS, AND THEN AT 4 YEARS AND 5 YEARS AFTER RANDOMIZATION:

Submit the **S1007** Treatment Form

i. AT 6 MONTHS, 12 MONTHS, 2 YEARS AND 3 YEARS AFTER RANDOMIZATION (if on treatment):

Submit the **S1007** Adverse Event Summary Form

j. For patients who receive chemotherapy, regardless of assigned treatment arm, AT ONE YEAR AFTER RANDOMIZATION:

Submit the **S1007** Chemotherapy Form.

k. AT ONE YEAR AFTER RANDOMIZATION:

Submit the **S1007** Radiation Therapy Form.

l. WITHIN 28 DAYS AFTER STEP 3 REGISTRATION:

Submit specimens as outlined in Sections 15.3 and 15.4.

m. WITHIN 2-3 YEARS AFTER STEP 3 REGISTRATION (IF PRIOR TO INVASIVE RECURRENCE):

Submit specimens as outlined in Sections 15.3 and 15.4.

n. AT FIVE YEARS AFTER RANDOMIZATION (IF PRIOR TO INVASIVE RECURRENCE/PROGRESSION/RELAPSE):

Submit the following:

Final **S1007** Treatment Form for current reporting period

Submit the **S1007** Follow Up Form

Off Treatment Notice

o. WITHIN 14 DAYS OF INVASIVE RECURRENCE/PROGRESSION/RELAPSE:

Submit the following:

- **S1007** Follow Up Form documenting date, site, and method for determining invasive recurrence/progression/relapse.
- If the patient was still on protocol treatment, final **S1007** Treatment Form for current reporting period.
- If patient was still on protocol treatment and if during the first three years (36 months) after randomization, final **S1007** Adverse Events Summary Form.
- Off Treatment Notice, if during the first five years after randomization.
- If patient was registered to Step 3, submit specimens as outlined in Sections 15.3 and 15.4.

p. AFTER OFF TREATMENT (STEP 2) – EVERY 6 MONTHS UNTIL YEAR 5, AND THEN ANNUALLY UNTIL YEAR 15 OR UNTIL DEATH, WHICHEVER COMES FIRST:

Submit the **S1007** Follow Up Form.

Note: Follow-up data submission will be entered under Step 2 registration for all patients whether or not they register to Step 3 for TM substudy participation.

q. WITHIN 4 WEEKS OF KNOWLEDGE OF SECOND MALIGNANCY (2nd Primary):

Submit the **S1007** Follow Up Form documenting date, site, and method of determining malignancy.

r. WITHIN 4 WEEKS OF KNOWLEDGE OF DEATH:

Submit the Notice of Death documenting death information.

15.0 SPECIAL INSTRUCTIONS

15.1 Oncotype DX® Testing Requirements

a. For patients who have not had the Oncotype DX® Breast Cancer Assay performed at the time of Step 1 Registration, submission of materials for the Oncotype DX® Breast Cancer Assay is required. Collection and submission instructions are outlined in Section 15.1b.

b. The clinical investigator and the submitting pathologist have the responsibility for submitting materials for the purposes of genomic profiling. Request kits and submit materials as outlined on the Oncotype DX® web site for "Submitting a Sample" under the "Healthcare Professionals" menu located at http://www.oncotypedx.com/en-US/Breast/HealthcareProfessional/submittingsample.aspx.

NOTE: A sample Oncotype DX® requisition form with instructions for completion is available at www.swog.org on the **S1007** abstract page. Sites that submit the Oncotype DX® sample via the Genomics Health website must enter "**S1007**" in the "Study Name" field.

Sites that do not submit samples via the Genomics Health website must complete the Oncotype DX® Submission Form [available on the **S1007** abstract page at www.swog.org] with the Oncotype DX® Requisition Form. Both of these forms should accompany the sample to Genomic Health.

15.2 Correlative Studies and Banking

a. Submission of tissue (for prognostic and predictive indices of breast cancer outcomes) is required, will be collected prior to starting treatment and will be shipped within 14 days after Step 2 Registration to the SWOG Biospecimen Bank – Solid Tissue, Myeloma, and Lymphoma Division, Lab #201.

- Paraffin block, punch biopsy or 20 unstained slides from the primary tumor
- Positive lymph node block, punch biopsy or 20 unstained slides
- Negative lymph node block, punch biopsy or 20 unstained slides

NOTE: Each type of tissue should be submitted, but the patient will not be made ineligible if the tissue is not available. Documentation of why incomplete submission took place must be noted in the patient's medical record.

Specimen collection kits are not being provided for this submission; sites will use institutional supplies.

Any leftover tissue not consumed by testing will be banked for future use according to the patient's selections on the "Consent Form for Use of Specimens for Research".

b. Submission of blood (for pharmacogenomic studies) is required. Blood will be collected prior to starting treatment and will be shipped within 24 hours of collection to the SWOG Biospecimen Bank – Solid Tissue, Myeloma, and Lymphoma Division, Lab #201.

- 7.5 mL whole blood collected in lavender top, EDTA, Vacutainer® tube
- 10 mL whole blood collected in red-top or serum separator tube (SST), Vacutainer® tube

Specimen collection kits are not being provided for this submission; sites will use institutional supplies.

Any blood specimens that remain after testing is performed will be banked for future use according to the patient's selections on the "Consent Form for Use of Specimens for Research".

c. Specimen collection and submission instructions

All specimen submissions for this study must be entered and tracked using the SWOG online Specimen Tracking System. Complete specimen collection and submission instructions can be accessed on the SWOG Specimen Submission webpage (https://www.swog.org/member-resources/biospecimen-resources). If collection/submission instructions differ from those in the protocol, the protocol instructions should be followed; otherwise, the website instructions should be followed.

15.3 Specimens for Circulating Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) Substudy (***REQUIRED IF PATIENT CONSENTS***):

At time of distribution of Revision #16 (Version Date 03/24/21), all U.S. patients who:

a) are disease free, with no prior invasive recurrence AND
b) have been on protocol for up to 8 years after time of randomization to **S1007** (Step 2 registration) must be offered participation in the Circulation Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) substudy, as indicated in Section 5.3.

SUBMISSION SUMMARY TABLE:

	CBALR Visit #1: Within 28 days after patient registration to Step 3	**CBALR Visit #2: 2-3 years after patient registration to Step 3**	**CBALR Visit #3: At time of Invasive Recurrence**
Metastatic Tissue (if applicable and where available)			FFPE block or 20 unstained slides **Ship to SWOG Biospecimen Bank (Lab #201)**
10 mL whole blood [a]	One 10 mL SST: **Ship to SWOG Biospecimen Bank (Lab #201)** [b]	One 10 mL SST: **Ship to SWOG Biospecimen Bank (Lab #201)** [b]	One 10 mL SST: **Ship to SWOG Biospecimen Bank (Lab #201)** [b]
20 mL whole blood [a]	Two 10 mL CellSave® tubes: [b] **Ship to Menarini Silicon Biosystems Lab # 122**	Two 10 mL CellSave® tubes: [b] **Ship to Menarini Silicon Biosystems Lab # 122**	Two 10 mL CellSave® tubes: [b] **Ship to Menarini Silicon Biosystems Lab # 122**
40 mL whole blood [a]	Four 10 mL Streck cfDNA tubes: • First 2 Streck cfDNA tubes collected (if both are collected at first draw attempt) [b, c]: **Ship to Epic Sciences Lab #236** • **2 Streck cfDNA tubes: Ship to SWOG Biospecimen Bank (Lab #201)**	Four 10 mL Streck cfDNA tubes: • First 2 Streck cfDNA tubes collected (if both are collected at first draw attempt): [b, c] **Ship to Epic Sciences Lab #236** • **2 Streck cfDNA tubes: Ship to SWOG Biospecimen Bank (Lab #201)**	Four 10 mL Streck cfDNA tubes: • First 2 Streck cfDNA tubes collected (if both are collected at first draw attempt): [b, c] **Ship to Epic Sciences Lab #236** • **2 Streck cfDNA tubes: Ship to SWOG Biospecimen Bank (Lab #201)**

[a] Important: See Section 15.3c.1: Order of Sample Collection. SST should be collected under fasting conditions.

[b] If the minimum blood volume for one 10 mL SST, **two** 10 mL CellSave® tubes, and *at least* **two** 10 mL Streck cfDNA tubes (being shipped to Epic Sciences) cannot be collected during a visit, a subsequent second attempt should be scheduled for another time to collect the rest of the samples. The subsequent draw time can be scheduled for later the same day (if appropriate and site has collection tubes on hand) or for another date. See Section 15.3c.1. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint. If the first draw attempt fails and the site does not have tubes on hand, then the site will need to: 1) reschedule the patient for a subsequent blood draw, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for that patient. Both CellSave® tubes must be collected on the same day. See Sections 15.3c.3c and 15.3c.4b for more information.

[c] If the second attempt at blood draw is unsuccessful in obtaining the minimum blood volume for **two** 10 mL CellSave® tubes, the patient will be deemed not evaluable for the CBALR TM substudy. If the patient is deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood (SST, CellSave, or Streck) or tissue samples for the CBALR TM substudy.

a. With the patient's consent, the following blood, and tissue samples must be drawn and submitted at the following 2-3 time points:

1. CBALR Visit # 1: **Within 28 days after patient registration to Step 3:**

   a. 10 mL whole blood collected in one 10 mL (red top) SST, Vacutainer;

   b. 20 mL whole blood collected in two 10 mL Cellsave® tubes;

   c. 40 mL whole blood collected in four 10 mL Streck tubes.

2. CBALR Visit #2: **If no invasive recurrence at time of 2-3 years** after patient registration to Step 3, then draw:

   a. 10 mL whole blood collected in one 10 mL (red top) SST, Vacutainer;

   b. 20 mL whole blood collected in two 10 mL Cellsave® tubes;

   c. 40 mL whole blood collected in four 10 mL Streck tubes.

   Note: Samples should be collected at the regular patient visit 2 years after registration to Step 3, however they may be collected and submitted at any time between 2 and 3 years from registration if not drawn at the 2-year visit. If invasive recurrence occurs prior to the collection of the 2-year timepoint, the 2-year timepoint specimens are no longer required.

3. CBALR Visit #3: **At time of invasive recurrence (if applicable)**:

   a. FFPE tissue block, punch biopsy, or 20 unstained slides from metastatic site at invasive recurrence (where tissue from the standard of care biopsy is available);

   b. 10 mL whole blood collected in one 10 mL (red top) SST, Vacutainer;

   c. 20 mL whole blood collected in two 10 mL Cellsave® tubes;

   d. 40 mL whole blood collected in four 10 mL Streck tubes.

b. Sample Collection Kits

Specimen collection kits (Streck cfDNA and CellSave tubes) must be ordered IMMEDIATELY after patient registration to Step 3 and prior to each collection timepoint. Sites should allow 5-7 days to receive kits. Note: Patient samples must be <u>drawn within 28 days after patient registration to Step 3.</u> Please note that **<u>two separate collection kits are provided</u>**, and both must be ordered to obtain the blood collection tubes needed for submissions to all three labs.

Streck cfDNA and CellSave® kits may be ordered by using the SWOG Biospecimen Bank Kit Management Application at: https://kits.bpc-apps.nchri.org.

The Streck cfDNA Collection Kit provides the following for a single collection timepoint:

- Two Streck cfDNA tubes for the collection and shipment of blood to the SWOG Biospeicmen Bank and includes an ambient gel pak, insulated shipper and packaging supplies.
- A shipping label (e.g. Exempt Human Specimen) is provided in Kit Management for the shipment of Streck cfDNA tubes to the SWOG Biorepository.

The Streck cfDNA and CellSave Collection Kit provides the following for a single collection timepoint:

- Two Streck cfDNA tubes and an ambient pak for blood submitted to Epic Sciences Lab.
- Two CellSave tubes plus an ambient pak for blood submitted to Menarini Silcon Biosystems.
- Shipping supplies are not provided and sites must use their own shipping accounts for the cost of shipments to Epic and Menarini.

Supplies (kits) are not being provided for tissue and SST sample collection. Sites will use institutional supplies to collect and ship tissue and whole blood in SSTs.

c. Sample Collection and Submission Instructions

All sample submissions for this study must be entered and tracked using the SWOG online Specimen Tracking System. Complete sample collection and submission instructions can be accessed on the SWOG Specimen Submission webpage (https://www.swog.org/member-resources/biospecimen-resources). If collection/submission instructions differ from those in the protocol, the protocol instructions should be followed; otherwise, the website instructions should be followed.

1. **Order of Sample Collection:**

Substudy samples <u>must</u> be collected in the following order. **If samples are *not* collected in the following order, then one (or more) of the samples may need to be discarded**.

<u>First scheduled draw for each annual visit:</u>

Under fasting conditions:

- First, collect: Whole blood in a Red-Top, SST, Vacutainer tube

- Then, collect: Two 10-mL CellSave® tubes.

  NOTE: The 10 mL red-top tube must be obtained prior to filling the CellSave® tube using the same needle stick. This decreases the chance of contamination of the CTC sample with skin epithelial cells, which may occur when the needle enters the skin.

  Only the SST should be collected under fasting conditions. After collection of the SST, patients may eat or drink something.

- Then, collect: Four 10-mL Streck cfDNA tubes.

<u>If the first scheduled draw of the two 10 mL CellSave® tubes was NOT successful:</u>

If the SST was collected at the first scheduled draw, and then the minimum blood volume for <u>two</u> 10 mL CellSave® tubes was <u>not</u> subsequently collected at the same time (after the SST was collected), then **for the subsequent attempt to draw one or both CellSave® sample(s),** collect samples in the following order:

- First, collect: One 10-mL Streck tube (prior to filling the CellSave® tube using the same needle stick). If this happens, this first Streck cfDNA tube sample will be shipped to the SWOG Biospecimen Bank (unless site is only able to collect two Streck cfDNA samples, in which case both Streck cfDNA samples would be shipped to Epic Sciences).

- Then, collect: The remaining (one or two) 10-mL CellSave® tube(s). **NOTE:** While CellSave® tubes can be collected at different times throughout the day, both CellSave® tubes must be collected on the <u>same</u> day.

- Then, collect: three 10-mL Streck tubes. The last 10-mL Streck cfDNA tube collected will also be shipped to the SWOG Biospecimen Bank.

If there is a deviation from protocol order of collection, contact the study chair for guidance on which samples should still be submitted and document the order of collection in the "comments" section of the Specimen Tracking System.

2. Whole blood (submitted to the SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201):

   a. **Seat the participant for at least five minutes prior to blood collection.**

   b. The SST sample should be collected under fasting conditions.

   c. Collect in a 10 mL (red-top) SST, Vacutainer tube and label as indicated in Section 15.4.

      NOTES TO AVOID HEMOLYSIS *(59,60,61)*

      Do not use small-bore needles.
      Do NOT mix. Invert filled tubes gently.
      Do not keep tourniquet on too long.
      Allow the cleaned venipuncture site to dry completely before skin puncture.
      Do not expose to extreme heat or cold.

   d. In the event that 10 mL whole blood volume in (red-top) SST cannot be collected, **a subsequent second attempt at collection should be scheduled for another time.** (Note: the subsequent draw time can be scheduled for later the same day, if deemed appropriate or can be rescheduled for another date.)

   e. Place the tube in a rack at room temperature for at least one hour and not more than two hours.

   f. See Section 15.4 for Specimen Labelling and Shipping Instructions. Blood in SST tubes may be shipped with the Streck cfDNA tubes to the SWOG Biospecimen Bank. Samples should be shipped the same day as collection or if not possible, then sample should be shipped on the next working day to the SWOG Biospecimen Bank (Lab #201).

   g. Specimen collection supplies are not being provided for whole blood in SST samples; sites will use institutional SST, Vacutainer supply.

   h. If the patient is subsequently deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

3. CellSave® Tubes (Two Cellsave® tubes at each time point, ship directly to Menarini Silicon Biosystems – Lab #122 for CellSearch CTC testing)

   a. Required materials for blood collection:

      i. Two (2), 10 mL purple/yellow top CellSave® blood collection tubes,

      ii. Vacutainer brand adapter, and

      iii. Needles.

   b. See Section 15.3b for CellSave® tube collection kit ordering instructions. The kit does not include an adapter or needles.

   c. Collection Instructions:

      i. See Section 15.3c.1: Order of Sample Collection.

         Note: To prevent contamination of the CellSave® tube samples with epithelial cells, another tube must be collected *prior to* collection of the CellSave® tubes, so that the CellSave® tubes may be drawn from the same needle stick as the prior sample (either the SST at first draw attempt or a Streck cfDNA tube if a second attempt at drawing the CellSave® tube) .

      ii. **Use the same needle stick as the prior tube drawn to collect the CellSave® sample.** This decreases the chance of contamination of the CellSave® sample with skin epithelial cells, which may occur when the needle enters the skin.

      iii. For each patient, perform a venous puncture using a Vacutainer brand adapter and needle and fill each of the blood collection tubes (**minimum blood volume of 9 mL for each tube**). Alternatively, blood samples may be obtained from a port or other central venous catheter using appropriate access needles and techniques. Invert each tube a minimum of eight (8) times to ensure proper mixing of the additives contained in each tube.

      iv. **Important Note:** Both (two) 10 mL CellSave® tubes are required for analysis. In the event that the 18 mL minimum blood volume (9 mL in each) in the **two** 10 mL tubes CellSave® tubes cannot be collected, **a subsequent second attempt at collection should be scheduled for another time.**

         While CellSave® tubes can be collected at different times throughout the day, both CellSave® tubes must be collected on the same day. See Section 15.3c.1. Do not submit either a single CellSave® tube or **two** CellSave® tubes that were collected on different days to Menarini Silicon Biosystems Labs.

Note: The subsequent draw time can be scheduled for later the same day, if deemed appropriate and site has additional collection tubes on hand, or can be rescheduled for another date. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint for each patient registered to Step 3. If the first draw attempt fails, and the site does not have tubes on hand, then the site will need to: 1) reschedule the patient for another day, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for the second draw attempt

v. **<u>If at time of second attempt at blood draw</u>**, the blood draw is unsuccessful in obtaining the minimum 18 mL blood volume (9 mL in each) in the **<u>two</u>** 10 mL CellSave® tubes, then the patient will be deemed not evaluable for the CBALR TM substudy. If this occurs and the patient is deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood or tissue samples for the CBALR TM substudy.

vi. **The filled CellSave® tubes must be maintained at ambient (15–30°C) temperature, avoiding extremes of heat and cold, at all times**.

vii. Label the CellSave® tubes with:

- Number 1 and Number 2 (respectively, in order of collection). Record the lot number and expiration date for each corresponding tube in the Specimen Tracking System.
- SWOG patient number (patient ID)
- Patient initials
- Substudy visit number (time point) [Visit number = one, two, or three; i.e. one=initial blood draw on substudy, two= next blood draw 2-3 years later, three=invasive recurrence].
- Collection date and time (date and time the specimen was collected from the patient)
- Initials of the phlebotomist
- Specimen type (i.e., whole blood)

viii. The following information must be **entered into the SWOG Specimen Tracking System prior to shipment**:

- Site identification number;
- SWOG patient number (same number as written on the filled blood tubes);
- Site comments (i.e. phlebotomy problems; and any additional comments);
- Collection date and time of blood draw (if all blood samples were not drawn at the same time; specify time of draw of each sample);
- Order of blood sample collection (SST, CellSave® tubes, Streck cfDNA tubes). For CellSave® tubes, record the order of collection of (Number 1 or Number 2) as well as the lot number and expiration date for each corresponding CellSave® tube in the Specimen Tracking System;
- Lot number and expiration date of each corresponding CellSave® tube (Number 1 and Number 2).

d. Packaging and Shipping Instructions: **Ship directly to Menarini Silicon Biosystems (Lab #122)**

i. Cellsave® tubes must be shipped **ambient** (with an ambient gel pack) the same day as collected, via overnight delivery to: Menarini Silicon Biosystems (Lab # 122). If possible, collect and ship samples Monday-Thursday. **Packages shipped on a Friday, must be sent via Fed-Ex Saturday Delivery, with no signature required. Do not collect samples the day prior to a holiday.**

ii. Wrap the CellSave® tubes in the shipping blanket. This gives added thermal protection during shipment. **Place ambient gel packs in the box to stabilize the temperature at 15-30ºC.** Place the Styrofoam lid, and seal the Styrofoam box.

iii. **All shipments must include a requisition form (Packing list) generated by the SWOG Specimen Tracking System.** See Section 15.4c. Place the completed Packing List generated from the SWOG Specimen Tracking System into the shipper box.

e. Questions pertaining to Cellsave® collection or shipping should be directed to:

Menarini Silicon Biosystems Labs (Lab #122)
msb-labservicesus@siliconbiosystems.com
Phone: 215/346-8499
Fax: 215/560-3730
Customer Service Hours of Operation: M-F, 8:00am - 5:00pm ET

4. Streck Cell-Free DNA Collection Tubes (2 Streck cfDNA Tubes will be submitted to the SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201 and 2 Streck cfDNA Tubes will be submitted to Epic Sciences (Lab #236)):

   a. Prior to Collection

      - Patients are not required to fast prior to Streck cfDNA blood draw. Patients may eat or drink something prior to blood draw into Streck cfDNA tubes.
      - See Section 15.3b for ordering instructions for Streck cfDNA tube collection kits.
      - Confirm blood tubes are not expired. Expired tubes should not be used for blood collection.
      - Schedule courier for same-day sample pick-up prior to collection.

   b. Instructions for handling Streck cfDNA tubes:

      Prevention of Backflow: Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube. To guard against backflow, observe the following precautions:

      - Keep patient’s arm in the downward position during the collection procedure.

      - Hold the tube with the stopper uppermost.

      - Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.

      - Tube contents should not touch stopper or the end of the needle during the collection procedure.

   c. Additional Blood Collection Instructions:

      - Draw whole blood samples into four (4), 10 mL Streck Cell-Free DNA BCT tubes. Fill tube until blood flow stops.

      - **IMPORTANT NOTE:** Fill each tube completely (10 mL), when possible.

      - For the 2 tubes being shipped to the Epic Sciences (Lab # 236), a minimum of 4 mL blood per tube is required (at least 8 mL total volume collected in two 10 mL Streck tubes – subsequent to SST and CellSave® draws). In the event that 8 mL blood volume cannot be collected, do not submit Streck cfDNA tube samples to Epic Sciences. (The SST and CellSave® Tubes must still be submitted as indicated in Sections 15.3c.2 and 15.3c.3.) For the two Streck Tube samples being sent to the Epic Sciences (Lab # 236), **a subsequent second attempt at collection should be scheduled for another time.** (Note: the subsequent draw time can be scheduled for later the same day, if deemed appropriate and site has tubes on hand, or can be rescheduled for another date.)

- If the patient is subsequently deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

- Approximate volumes are illustrated below. Each red arrow indicates the level to which the blood collection tube should be filled to achieve the corresponding volume in red, yellow, or blue. As a reference, a volume of 6-mL would fill the Streck tube to just below the first “7” in the blood tube lot number “72750315” on the blood tube label.

- Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. Inadequate or delayed mixing may result in inaccurate test results.

- **After collection, blood in Streck cfDNA tubes should never be refrigerated or frozen**, as this will compromise the specimen. Blood collected in Streck cfDNA tubes is stable at room temperature.

d. Labelling and Shipping Instructions

i. **Two (2) Streck cfDNA tubes (preferably first and second Streck cfDNA tubes drawn – if first draw attempt is successful) will be shipped to Epic Sciences (Lab # 236)** per the following instructions:

a. Specimens must be shipped with an **ambient pack** (provided in the Streck tube kit).

b. See Section 15.4 for Specimen Labelling Instructions and Section 15.4c for requirements for specimen entry, tracking, and generation of a Packing List via the SWOG Specimen Tracking System.

c. All shipments must include requisition form (Packing list) generated by the SWOG Specimen Tracking System. Print a copy of the Packing List in the online SWOG Specimen Tracking System. Place the Packing List in the pocket of the specimen bag if it has one, or in a separate resealable bag.

d. **IMPORTANT NOTE:** If a collection time is not provided, Epic Sciences will default to sample collection at 8:00 am (local time) on the date of collection.

SWOG Authorized Document

e. Do not place "Infectious Substance" sticker on shipper, as this can result in a delay of shipment. If possible, include a scanned copy of the completed sample requisition form.

f. For questions pertaining to the **two Streck cfDNA tubes being shipped to Epic Sciences Lab**, contact:
Lab #236: Epic Sciences
Email: partners@epicsciences.com / Attn: **S1007**
Phone: 858/356-6610

ii. **Two (2) Streck cfDNA tubes will be shipped to the SWOG Biospecimen Bank (Lab #201)**.

a. If blood in Streck cfDNA tube cannot be shipped the day of collection, then it must be kept at **room temperature** and shipped on the next working day to the SWOG Biospecimen Bank (Lab #201). Do not process.

b. See Section 15.4 for Specimen Labelling and Shipping Instructions.

c. For questions pertaining to the the **two Streck cfDNA tubes being shipped to the SWOG Biospecimen Bank,** contact:
Lab #201: SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201
Phone: 614/722-2865
E-mail: bpcbank@nationwidechildrens.org

5. Metastatic Tumor tissue (SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201)

a. **At time of invasive recurrence (where tissue is available),** submit FFPE tissue block, punch biopsy, or 20 unstained slides from standard of care biopsy of metastatic site at invasive recurrence.

b. See Section 15.4 for Specimen Labelling and Shipping Instructions. It is preferred that samples are shipped within 48 hours of collection, where possible. **Samples must be shipped within 28 days after collection**.

c. Specimen collection kits are not being provided for tissue submission; sites will use institutional supplies.

d. NOTE: Correlative biomarker testing of specimens banked for planned future research (not otherwise specified in Sections 18.6, 18.7, or 18.8) will not occur until an amendment to this protocol (or separate correlative science protocol) is reviewed and approved in accordance with National Clinical Trials Network (NCTN) policies.

15.4 SPECIMEN LABELING AND SHIPPING

a. Label blood tubes with the following:

- SWOG patient number (patient ID)
- Patient initials
- Substudy visit number (time point) [Visit number = one, two, or three; i.e. one=within 28 days after patient registration to Step 3, two= 2-3 years after patient registration to Step 3, three=invasive recurrence].
- Collection date and time (date and time the specimen was collected from the patient)
- Initials of the phlebotomist (Epic Sciences Streck cfDNA and Menarini Silicone Biosystems CellSave® blood tubes only)
- Specimen type (i.e., whole blood)

b. Include the following on FFPE tissue labels:

- SWOG patient number
- Patient initials
- Collection date (date the specimen was collected from the patient)
- The Surgical Pathology ID # (Accession#) and block number (e.g., A2, 3E, 2-1, B, etc.) that corresponds with the pathology report

Note: if submitting slides, then also include the thickness (in μm).

c. SHIPPING SAMPLES

1. SWOG Specimen Tracking System (STS)

All specimen submissions for this study must be entered and tracked using the SWOG online Specimen Tracking System. SWOG members may log on the online system via the CRA Workbench. To access the CRA Workbench, go to the SWOG Web site (http://swog.org) Non- SWOG users may log into SpecTrack using their CTSU UserID and password on the SpecTrack login page located at https://spectrack.crab.org (select the option "SWOG – SWOG – CTSU"). SpecTrack start-up instructions (both written and demo) are available after signing in to SpecTrack.

A copy of the Shipment Packing List produced by the online Specimen Tracking System should be printed and placed in the pocket of the specimen bag if it has one, or in a separate resealable bag. The Specimen Submission Form is NOT required when the online system is used.

ALL SPECIMENS MUST BE LOGGED VIA THIS SYSTEM; THERE ARE NO EXCEPTIONS.

To report technical problems with Specimen Tracking, such as database errors or connectivity issues, please send an email to technicalquestion@crab.org. For procedural help with logging and shipping specimens, there is an introduction to the system on the Specimen Tracking main page (https://spectrack.crab.org/Instructions); or contact the SWOG Statistics and Data Management Center at 206/652-2267 to be routed to the Data Coordinator for further assistance.

In the online Specimen Tracking System, the appropriate SWOG laboratory for submission of diagnostic tissue samples for SWOG Biospecimen Submission and Pathology review is identified for each specimen type and timepoint.

For questions pertaining to the the **SST, two Streck cfDNA tubes, or FFPE tissue being shipped to the SWOG Biospecimen Bank**, contact:

Lab #201: SWOG Biospecimen Bank – Solid Tissue, Myeloma and Lymphoma Division, Lab #201
Phone: 614/722-2865
E-mail: bpcbank@nationwidechildrens.org

For questions pertaining to the **two Cellsave® tubes being shipped to Menarini Silicon Biosystems** contact:

Lab #122: Menarini Silicon Biosystems Labs (Lab #122)
msb-labservicesus@siliconbiosystems.com
Phone: 215/346-8499
Fax: 215/560-3730
Customer Service Hours of Operation: M-F, 8:00am - 5:00pm ET

For questions pertaining to the **two Streck cfDNA tubes being shipped to Epic Sciences Lab** contact:

Lab #236: Epic Sciences
Email: partners@epicsciences.com / Attn: **S1007**
Phone: 858/356-6610

2. Complete instructions for packaging and shipping specimens are located on the SWOG Specimen submission webpage (https://www.swog.org/clinical-trials/biospecimen-resources/biospecimen-processing-and-submission-procedures).

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

S1007 Remote Consent Procedures for the CBALR TM substudy:

For U.S. sites utilizing a local IRB of record, the Study Chair has determined that the CBALR TM substudy presents no more than minimal risk to participants, and that an alteration of the informed consent process to allow for remote consent to be obtained in accordance with the following NCI CIRB requirements for remote consent would not adversely affect the rights and welfare of participants.

Procedures that must be utilized for the **S1007** Remote Consent Process for the CBALR TM substudy (for sites utilizing the NCI CIRB as the IRB of record) are as follow:

a. The participant or their legally-authorized representative (LAR) must receive a copy of the NCI CIRB-approved informed consent document (e.g., via mail, fax or email) in advance of discussion regarding the study.
   - If mailed, two copies must be mailed so the participant or LAR is able to retain a copy for reference when their signed document is returned to the site and they are waiting to receive the final copy with all necessary signatures back from the site.

b. The investigator or designee must discuss the study with the potential participant either via telephone or video conferencing.
   - The investigator/designee must have the same consent discussion via telephone/video conferencing that they would have had with the participant or LAR during an in-person meeting.
   - Important: The investigator/designee must also implement a method to ensure the identity of the participant or LAR (such as: verification of state identification or other identifying documents or use of personal questions or visual methods).

c. A witness must be present during the telephone/videoconferencing consent process.
   - There are no restrictions on who can serve as a witness and the witness does not need to be impartial. The witness must be able to hear both sides of the conversation (e.g., speaker phone, conference line).
   - Requirements for social distancing may dictate that the witness is in a different location than both the potential participant and/or the investigator/designee obtaining consent. Any arrangement is acceptable if the witness can listen to both parties in the informed consent discussion.

d. If the potential participant or LAR agrees to participation, they sign the consent form and return it to the investigator (e.g., via mail, fax or email). If postal mail is used, a pre-paid, self-addressed envelope should be provided to the participant or LAR to mail the signed consent form back to the investigator.

e. Once the research team receives the signed informed consent document from the participant or LAR, the investigator/designee who conducted the consent process must sign and date the document using the current date.
   - Under the signature line, the investigator/designee must document whether consent was obtained over the telephone or video conferencing, the date of the telephone/video conference, and the date the signed consent was received. For example, "Discussed with [participant or LAR name] via [telephone or videoconferencing] on [insert date] and received signed consent form on [insert date]." Include a brief reason for performing the informed consent discussion over the telephone/videoconferencing.

f. If the participating site has an informed consent policy that requires the witness to sign the consent document, the witness signs the informed consent. If the participating site does not have an informed consent policy that requires the signature of the witness on the consent document, then the name of the witness along with the date of the original consenting phone call is recorded in the research records to document the participation of the witness.

g. The date the investigator/designee signs the informed consent document, not the date the consent discussion with the participant or LAR took place, is the official date of informed consent for the participant on the trial.

h. The final informed consent document must be filed in the designated investigator/site regulatory file location. A copy of the final informed consent document, signed by the participant or LAR, the investigator, and the witness (if applicable), must be sent back to the participant via email/scan, fax, or postal mail.

i. No research activities related to the study can begin until all steps of the informed consent process are complete.

j. Participating site utilization of an e-signature with the remote consent process: For sites utilizing the NCI CIRB as the IRB of record, eSignatures, may be permitted to be implemented on a site-by-site basis. Sites must submit the required information on Study Specific Worksheets (SSW) or the Signatory Institution Worksheet (SIW) worksheets before using an e-signature with the remote consent process. For sites utilizing the NCI CIRB as the IRB of record, questions regarding NCI CIRB approval process for participating site utilization of e-signatures are to be directed to the NCI CIRB.
   - An e-signature is defined as: an electronic sound, symbol, or process, attached to or logically associated with a contract or other record and executed or adopted by a person with the intent to sign the record.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31.

GEICAM is responsible for auditing their own institutions according to SWOG and NCI-CTMB Guidelines. Audit data will be provided in English to the SWOG QA Department who will enter the audit data into the NCI database.

Confidentiality

Please note that the information contained in this protocol is considered confidential and should not be used or shared beyond the purposes of completing protocol requirements until or unless additional permission is obtained.

16.1 Adverse Event Reporting Requirements

a. Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. (Directions for routine reporting are provided in Section 14.0.) Additionally, certain adverse events must be reported in an expedited manner to allow for more timely monitoring of patient safety and care. The following guidelines prescribe expedited adverse event reporting for this protocol. See also Appendix 18.4 for general and background information about expedited reporting.

b. Reporting method

This study requires that expedited adverse events be reported using the CTEP Adverse Event Reporting System (CTEP-AERS). The NCI's guidelines for CTEP-AERS can be found at http://ctep.cancer.gov. A CTEP-AERS report must be submitted to the SWOG Operations Office electronically via the CTEP-AERS web-based application located at http://ctep.cancer.gov/protocoldevelopment/electronic_applications/adverse_events.htm.

In the rare event when internet connectivity is disrupted an electronic report MUST be submitted immediately upon re-establishment of internet connection.

c. When to report an event in an expedited manner

When the adverse event requires expedited reporting, submit the report within 10 calendar days of learning of the event.

d. Other recipients of adverse event reports

The SWOG Operations Office will forward reports and documentation to the appropriate regulatory agencies and drug companies as required.

Adverse events determined to be reportable to the Institutional Review Board responsible for oversight of the patient must be reported according to local policy and procedures.

e. Expedited reporting for commercial agents

Commercial reporting requirements are provided in Table 16.1. If there is any question about the reportability of an adverse event or if on-line CTEP-AERS cannot be used, please telephone or email the SAE Program at the Operations Office, 210/614-8808 or adr@swog.org, before preparing the report.

Table 16.1 Expedited reporting requirements for adverse events experienced by patients within 30 days of the last administration of the commercial agent.

ATTRIBUTION	**Grade 4**		**Grade 5**[a]	
	Unexpected	Expected	Unexpected	Expected
Unrelated or Unlikely			**CTEP-AERS**	**CTEP-AERS**
Possible, Probable, Definite	**CTEP-AERS**		**CTEP-AERS**	**CTEP-AERS**

CTEP-AERS: Indicates an expedited report is to be submitted via CTEP-AERS within 10 calendar days of learning of the event. [b]

[a] This includes all deaths within 30 days of the last dose of treatment with a commercial agent(s), regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent(s) and is attributed (possibly, probably, or definitely) to the agent(s) and is not due to cancer recurrence must be reported according to the instructions above.

[b] Submission of the on-line CTEP-AERS report plus any necessary amendments generally completes the reporting requirements. You may, however, be asked to submit supporting clinical data to the Operations Office in order to complete the evaluation of the event. If requested, the specified data should be sent within 5 calendar days by fax to 210-614-0006.

f. ***Reporting Pregnancy, Fetal Death, and Death Neonatal***

1. **Pregnancy** Study participants who become pregnant while on study; that pregnancy should be reported in an expedited manner via CTEP-AERS as **Grade 3 "Pregnancy, puerperium and perinatal conditions – Other (pregnancy)"** under the **Pregnancy, puerperium and perinatal conditions** SOC.

   Additionally, the pregnancy outcome for patients on study should be reported via CTEP-AERS at the time the outcome becomes known, accompanied by the same Pregnancy Report Form used for the initial report.

2. **Pregnancy Loss** Pregnancy loss is defined in CTCAE as "Death in utero." Pregnancy loss should be reported expeditiously as **Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC**.

   A Pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.

3. **Death Neonatal** Death neonatal is defined in CTCAE as "Newborn death occurring during the first 28 days after birth. A neonatal death should be reported expeditiously as Grade 4 "Death neonatal" under the General disorders and administration SOC.

Neonatal death should **NOT** be reported as a Grade 5 event under the General disorders and administration SOC as currently CTEP-AERS recognizes this event as a patient death

NOTE: When submitting CTEP-AERS reports for "Pregnancy, "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should also be completed and faxed with any additional medical information to 301-897-7404. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

The Pregnancy Information Form is available at:
http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm.

g. COVID-19 Adverse Event Reporting Requirements:

Per the NCI "Guidance for Collection of Adverse Events Related to COVID-19 Infection," accessible from:
https://ctep.cancer.gov/content/docs/Adverse_Event_Guidance_COVID-19_Final_3-25-20.pdf, any known COVID-19 infection should be reported as an adverse event (and if applicable via CTEP-AERS).

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18.0 APPENDIX

18.1 Comparative Effectiveness and Health Related Quality of Life

18.2 Prospective Validation of Other Prognostic/Predictive Indices of Breast Cancer Outcomes and Evaluation of Pre-treatment Hormone Levels

18.3 Statistical Analysis Plan

18.4 Determination of Expedited Adverse Event Reporting Requirements

18.5 Participation Procedures for the International Collaborating Institutions

18.6 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy (U.S. INSTITUTIONS ONLY) - **CELLSEARCH ANALYSES (Menarini Silicon Biosystems)**

18.7 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy (U.S. INSTITUTIONS ONLY) - **EPIC SCIENCES CTC DETECTION PLATFORM**

18.8 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy (U.S. INSTITUTIONS ONLY) - **NON-CTC ANALYSES**

18.9 Instructions for the SWOG Biospecimen Bank – Lab #201, Solid Tissue, Myeloma and Lymphoma Division

18.1 Comparative Effectiveness and Health Related Quality of Life **(U.S. INSTITUTIONS ONLY) (Permanently closed to accrual effective 12/1/12.)**

A. Health-Related Quality of Life

Background and Significance: HRQOL& Oncotype DX® Testing/Effects of Chemotherapy

While it is recognized that the majority of the acute effects of chemotherapy (e.g. nausea, vomiting, diarrhea, stomatitis, alopecia and neutropenia) will resolve, sub-acute effects and long-term sequelae may have a lasting impact on the quality of life of survivors. Other symptoms such as fatigue, depression, and pain are slow to resolve and may have long-term consequences for women. *(1)*

Anxiety associated with the breast cancer diagnosis, anticipatory distress prior to treatment, and fear of recurrence has been documented for women with breast cancer. *(2,3,4)* A central question is whether women will accept a recommendation not to receive chemotherapy based on the results of Oncotype DX®, given the findings of benefit for adjuvant therapy in this population.

Recent studies in doctor/patient decision making suggest that women are likely to accept adjuvant chemotherapy for little or minimal benefits. *(5)* Thus, it is possible that a woman will experience anxiety about the idea of forgoing chemotherapy, even if the RS predicts that chemotherapy is unlikely to provide benefit. Such anxiety might be debilitating or lead to a decision to proceed to chemotherapy despite a low RS for those randomized to no treatment. Lo et al reported a significant decrease in anxiety for women receiving adjuvant treatment for breast cancer but there was only 1 patient who received observation only and one who received observation and then endocrine treatment. *(6)* Patients were not randomized to chemotherapy versus no chemotherapy as in this trial. This study will include one general measure of anxiety for cancer patients (primary measure) plus a measure of concerns about cancer recurrence and diagnostic testing.

We will measure anxiety with the Patient-Reported Outcomes Measurement Information System (PROMIS) short form Emotional Distress-Anxiety scale. *(7)* The 8-item anxiety measure was developed and received preliminary validation in a cancer population (Personal Communications, Sofia Garcia and David Cella, January 2010).

We expect that the "reach" of the various treatment-related side effects discussed above will be generally captured by the ratings of health status provided by the EQ-5D along with the utilities associated with these ratings. *(8)* The EQ-5D descriptive ratings for different health states and the single utility index value will be compared for women with RS ≤ 25 who receive chemotherapy versus those who do not. However, given that the quality of life study will be conducted with the randomized sample where one arm receives chemotherapy and the other arm does not, this study offers an opportunity to examine a placebo-controlled chemotherapy effect over a three-year period on patient report of both fatigue and cognitive dysfunction. Therefore, we decided to include two single-item measures of these important symptom problems.

However, two symptoms are of particular interest because of their prevalence and association with cancer therapy: fatigue and cognitive dysfunction. While the acute effects of chemotherapy on fatigue has long been recognized, a number of studies have reported problems with fatigue for lasting months or even years after adjuvant chemotherapy. *(9)* Such fatigue was associated with a decrease in daily functioning. *(10)* We will measure fatigue with the 7 item PROMIS Fatigue Short Form. *(Personal Communication, S. Garcia, January 2007; 2)* In addition there has been an increased awareness of cognitive dysfunction associated with adjuvant

chemotherapy in women with breast cancer. *(11)* Schagen et al evaluated 39 women at approximately 2 years following 6 cycles of chemotherapy compared to 34 women who had received local therapy only; 28% of the patients treated by CMF compared to 12% of control groups showed evidence of cognitive dysfunction characterized by difficulty with concentration, memory, and word finding. *(12)* van Dam et al suggested that such symptoms were worse in patients with high dose chemo-therapy. *(13)* While these studies suffer from lack of good controls and poor association between reports of cognitive dysfunction and scores on formal testing, they remain concerning. Studies conducted by Jacobsen and colleagues have failed to document the association between adjuvant chemotherapy and cognitive dysfunction. *(14)* Vardy et al. summarized the evidence for the association based on the second international workshop on cognitive function in terms of effects of the cancer and its treatment and conclude that "the characterization of cognitive impairment in terms of its nature, course over time, underlying mechanisms and impact on subject's lives is still limited". *(15)* Anecdotally many breast cancer patients complain of forgetfulness, difficulty concentrating or "chemo brain". These effects are likely to have significant long-term consequences for women's quality of life. The current study with its randomized design offers an opportunity to contribute to the understanding chemotherapy's impact on cognitive function. We will use the PROMIS Perceived Cognitive Function Concerns Short Form (8 items) to measure cognitive function.

The impact that chemotherapy has on HRQL is key and central for patients deciding whether or not to receive adjuvant chemotherapy in order to prevent further recurrence. **S1007** provides a unique opportunity to prospectively and quantitatively evaluate the impact of modern chemotherapy on the HRQL of women with early breast cancer. It is critical now to take the opportunity to integrate HRQL assessment into this unique randomized trial. While it is hypothesized that chemotherapy is unlikely to have an effect on cancer outcomes in those with an RS ≤ 25, it is possible that a subset of patients with RS between 11 and 25 may achieve small benefits in reduction in recurrence. If so, the impact of chemotherapy on HRQL will be particularly relevant for this group of patients. Such information is also likely to be useful to all women with breast cancer and their physicians who are considering adjuvant chemotherapy.

HRQL Study Design

This protocol addresses the impact of two treatment issues on patient HRQL: the impact of testing and receiving information about risk of recurrence (Phase I); and the impact on HRQL of being randomized to chemotherapy vs. no chemotherapy (Phase II).

SCHEMA: HRQL Sample (U.S. INSTITUTIONS ONLY)

The following Schema describes the process for administering Health-Related Quality of Life (HRQL) forms to patients participating in the two HRQL Studies for S1007.

Node-positive (1-3 nodes) HR-positive and HER2-negative breast cancer

Left path:

Patients consent to Recurrence Score (RS) testing, discussion of potential randomization on this trial.

Step 1 — Step 1 Registration and tumor tissue submission for RS testing 1000 of 8800 approached for HRQL Study
1st HRQL Time point: Enrollment Form

Recurrence Score

- RS > 25: Discuss alternative trials for high risk patients 40% of 1000=400 → (Step 1) 2nd HRQL Time point Observational Study Form No further follow-up
- RS ≤ 25: Physician and patient discuss randomization knowing the RS N=600
  - Refuse → (Step 1) 2nd HRQL Time point Observational Study Form No further follow-up
  - Accept → Step 2 Registration/Randomization

Right path:

RS already available and ≤ 25. Physician and patient discuss randomization knowing the RS 10% of Full Sample = 60

Step 1 Registration

Step 2 Registration/ Randomization stratified by:

1. RS 0-13 vs. 14-25
2. Menopausal Status
3. Axillary node Dissection vs. Sentinel node Biopsy

HRQL sample=440+60 =500

→ Chemotherapy; endocrine therapy
→ No Chemotherapy; endocrine therapy

Step 2 — 2nd HRQL Time Point/1st for women who enter with Recurrence Score: Randomized Study Form HRQL Follow-up: Months 6, 12, 36

Step 1 Study: Impact of Oncotype DX® Testing on Patient HRQL

A. Step 1 data collection for assessments will be supported with NIH funding: Comparative Effectiveness Evaluation of Oncotype DX® for Women with Node-positive, Hormone-responsive and HER2-negative Breast Cancer. NIH RC2CA148570-01 (Scott Ramsey, MD, PhD, Principal Investigator)

B. At Enrollment (prior to Oncotype DX® testing), 1000 women (of the 8800 tested) will be asked to complete the ***S1007*** *Health-Related Quality of Life Questionnaire: Enrollment* that includes the following scales:

1. Patient Pre-Oncotype (RS Assay) Questionnaire [10 items] (Lo et al., 2010);

2. Decisional Conflict Scale [16 items] (O'Connor, 1995; O'Connor, 1993/1999/2005);

3. EQ-5D [6 items], a measure of patient health state preference (Krabbe et al., 2004; Pickard, Wilke, et al., 2007; Pickard, Neary, et al., 2007);

4. a measure of anxiety (Patient-Reported Outcomes Measurement Information System [PROMIS] short form, Emotional Distress-Anxiety [9 items]) (Cella, 2009; Yost et al., submitted);

5. Assessment of Survivor Concerns [3 items] that addresses concern about cancer recurrence (Gotay, 2007).

Anxiety is the primary outcome measure for Step 1 enrollment assessment.

C. The same scales will be completed a second time by different subsets of the 1,000 women in Step 1 above (Recurrence Score Known/Treatment Decision Made Assessment), all of whom will have their Recurrence Score (RS) results and will have made a treatment decision. The **S1007** *Health-Related Quality of Life Questionnaire: Observational Study* will be administered to women with RS value > 25 (estimated % of 1,000 = 40%; n = 400) and to those women whose RS was ≤ 25 but who refused randomization (estimated refusal rate of 27%, n = 160). Thus, the total number of women in the observational QOL study is estimated to be 560. **Women in these two groups will not receive any additional follow-up assessments**. These women will answer the items after the recurrence results have been received (week to 10 days after enrollment) and after a discussion regarding treatment options has resulted in a treatment decision. The scales at this second-time point will be the same as those in the Enrollment Questionnaire with the exception of the RS Assay items. These Post-Oncotype RS questions [13 items] address the patient's evaluation of the RS result. Those who refuse to be randomized will also be asked to indicate reasons for their refusal to accept a treatment based on randomization and to rate the importance of the reasons deemed true for that patient. Anxiety is the primary outcome for women who receive a second assessment but will not be participating in the randomized study.

Step 2 Randomized Trial: Impact of Chemotherapy on HRQL

A. Women whose RS is ≤ 25 and consent to participate in the randomized trial will complete the **S1007** Health-Related Quality of Life Questionnaire: Randomized Study. A subsample (n=440) of the women hemotherapy and who were enrolled in Step 1 will be asked to complete additional forms as part of Step 2 of the HRQL study. In addition, women who already have RS results (i.e., did not participate in Step 1 of the HRQL study) will enter the randomized phase of the study at this point. We estimate that 6% of the 1000 patients assessed in Step 1 or 60 patients will enter Step 2 with their RS from another source. These women will answer the items after the recurrence results have been received and after a discussion regarding treatment options has resulted in a consent to randomization and a treatment assignment. For the patients who consent to the randomized trial after Enrollment, the time from Oncotype DX® testing to receipt of RS results will be approximately 3 weeks after Enrollment; we estimate that discussion of treatment options and the randomized trial will require another 3 weeks. Assessment 2 (baseline assessment for the randomized trial) will occur prior to randomized treatment initiation. Women who have an RS not obtained through Step 1 will complete the randomized trial baseline assessment after discussion with a physician about the RS score, the consent to being randomized, and the knowledge about the treatment assignment but prior to initiation of the randomized treatment.

B. The randomized trial has two primary HRQL outcomes: anxiety (PROMIS short form Emotional Distress-Anxiety); and a utility or health state preference score (EQ-5D). Additional HRQL outcomes include the following: PROMIS short form measures of Fatigue (7 items) and Perceived Cognitive Function-Cognitive Impairments (8 items). The following scales will be administered at the baseline assessment for the randomized trial:

1. Post-Oncotype (RS Assay) baseline items [13 items];
2. Post-Oncotype (RS Assay) follow-up items months 6, 12, & 36 [2 items]
3. Decisional Conflict Scale [16 items];
4. EQ-5D [6 items];
5. PROMIS Emotional Distress-Anxiety Short Form [9 items];
6. Assessment of Survivor Concerns [3 items] that addresses concern about cancer recurrence (Gotay, 2007.
7. PROMIS Fatigue Short Form [7 items] (Garcia et al., 2007; Yost et al., submitted; Personal Communications, D Cella, S Garcia, Jin-Shei Lai, 2010 and draft project summaries for the Cancer PROMIS Supplement)
8. PROMIS Cognitive Function Concerns [8 items] (Personal Communications, D Cella, S Garcia, Jin-Shei Lai, 2010 and draft project summaries for the Cancer PROMIS Supplement and the Development of PROMIS-cancer Perceived Cognitive Function (PCF) Item Bank)

In addition, three follow-up HRQL post-randomization assessments will occur at six months, one year, and three years.

HRQL Study Calendar

REQUIRED STUDIES	PRE Oncotype DX®	POST Oncotype DX®	**RANDOMIZED STUDY**			
			Pre Tx	6 months	1 year	3 years
STEP 1						
Enrollment Form	X					
Observational Study Form		X				
STEP 2						
Randomized Study Form			X	X	X	X

<u>Outcome Measures</u>

Primary Outcomes: Two primary HRQL outcomes will be used to compare patients receiving chemotherapy versus those not receiving chemotherapy: anxiety and a utility value for the patient's selfreport of health status.

PROMIS Anxiety Short Form: Anxiety will be assessed by one short form measure of Emotional Distress-Anxiety. This 9-item scale was developed as part of the NIH-sponsored Patient-Reported Outcomes Measurement Information System (PROMIS) initiative. *(16,17)* We will use the anxiety measure validated for cancer patients (Personal Communication, Sofia Garcia, January 2010). Higher scores reflect more anxiety.

EQ-5D: Patients will rate their health status with the EuroQol-5D [EQ-5D], which includes population adjustments for health state preferences or utilities for different health states as well as a visual analogue scale (VAS) measure of perceived value for that status (degree of good versus bad) for those patients who receive chemotherapy versus those who do not. *(18,19,20)* The utility index weighting the health state descriptions with values that were obtained from other samples of individuals with the VAS or time trade-off techniques. The index can be used to adjust years of life. *(21)* Higher scores reflect the person's perceived health state as a better/more preferred state of health. We will use the EQ-5D Index scores

Secondary Outcomes: In addition, we will assess the degree to which patients worry about their cancer using the Assessment of Survivor Concerns (ASC) as a secondary, exploratory HRQL outcome. *(22)* The psychometric properties of this measure have been documented. We will use the 3-item Cancer Worry subscale (coefficient alpha reliability for this subscale is 0.93), which the authors suggest can be used alone if the research context is cancer. Two prevalent symptom measures, cognitive dysfunction and fatigue, will be assessed at the four-time points with PROMIS short forms developed as part of the PROMIS item bank project. The fatigue short form was developed as part of the PROMIS item bank initiative and was further tested in cancer patients. *(23,24,25,26)* The cognitive disabilities or concerns short form was also part of the PROMIS item bank initiative

(Unpublished project summaries: Development of PROMIS-Cancer Perceived Cognitive function (PCF) Item Bank; The Cancer PROMIS Supplement (CaPS).

Decision making issues regarding recurrence risk testing and selection of treatment were measured with two sets of items. The Decisional Conflict Scale (DCS) (O'Connor, 1995; O'Connor, 1993/1999/2005) has 16 items addressing uncertainty associated with choosing a health care option, factors contributing to this uncertainty, and the evaluation of the decision. We are using the Traditional Decisional Conflict Scale that has items in the form of statements (versus questions); reliability has been estimated at ≥ 0.78 for the various versions of the scale.

A second set of decision making items specifically addresses the Oncotype DX® testing context. *(27; Personal Communication: S Lo, P Mumby)*. These items were developed to report as single items with no overall scaled score. **S1007** uses items that were slightly revised from those reported by Lo et al. based on the investigators' experience with the items in the published study. *(28, Personal Communication, Lo SS, Mumby P, August 2010)* Some of these single items have been further revised to reflect specific research questions in **S1007**. The Phase I Enrollment form items address current choice of treatment [1 item], perceived risk for recurrence [1 item], factors affecting a treatment decision [4 items], and perceptions about the Oncotype DX® test [4 items]. The Phase I Observational Study form addresses current choice of treatment [1 item], perceived risk of recurrence [1 item], factors affecting a treatment decision [4 items], yes/no question regarding the influence of the test on a treatment decision, and evaluation of the Oncotype DX® test and the patient's treatment decision [6 items].

For patients who do not consent to be randomized, the Observational study questionnaire contains a list of 8 possible reasons (plus an Other option with space to provide a specific reason) for not agreeing to participate in a randomized trial for cancer treatment. Items used in **S0316**, Barriers to Accrual to Clinical Trials in older (≥ 65 years) Cancer Patients, were selected for **S1007** based on frequency of endorsement in that study. *(*Javid *SH, Unger JM, Gralow JR, et. al. manuscript in preparation).* The Phase II Randomized Study form at baseline has the same Oncotype DX® testing and treatment decision making items as the Phase I Observational Study but the three follow-up forms for the randomized trial include two additional questions (satisfaction with treatment decision; if not satisfied, reasons for non-satisfaction).

Statistical design

Step 1. The primary comparison for Step 1 testing will be the change in the PROMIS anxiety short form scores between the Enrollment and second assessments (as obtained for patients who do not participate in the randomize trial as well as those who do consent to the trial). We will select the first 1000 patients enrolled in **S1007** at either Community Clinical Oncology Program (CCOP) or non-CCOP sites participating in the trial. This provides a sufficient sample size for the HRQL component of the randomized trial (see support for randomized n=500 below).

Hypothesis:

Comparisons based on the full sample.

H1: Women who receive an RS that is > 25 will have greater anxiety than those whose RS is ≤ 25 when compared at the second assessment when RS results and treatment decisions are known.

H2: Women whose RS is ≤ 25 but do not consent to randomization will have lower anxiety scores than those who do agree to be randomized when compared at the second assessment when

H3: EQ-5D Index scores will be worse for women whose RS is > 25 compared to women whose RS is ≤ 25 at the second assessment when RS and treatment decisions are known.

Comparisons based on the randomized group.

H4: Anxiety for women who are randomized to no chemotherapy will be greater than for those who are randomized to receive chemotherapy when compared at the second assessment and at all follow-up assessments.

H5: Patients receiving adjuvant chemotherapy will report worse EQ-5D utility or Index scores than will patients receiving no chemotherapy.

Step 1 Analyses: The primary analyses for the QOL follow-up measures adjust for baseline if one exists. In general, analysis of covariance of the follow-up measured on a continuous scale will benefit from adjustment from baseline by taking advantage of internal consistency within an individual. Analysis of covariance includes "change score" as a special case where the coefficient of the baseline variable is -1. If baseline and follow-up are weakly correlated, then simple analyses at follow-up are more powerful. For ordinal or binomial outcomes, adjustment for baseline is rarely helpful. For some outcomes measured at time of the second registration (choice to enter the randomized trial or not), there is not an appropriate baseline from the initial battery. Standard regression (linear and logistic) will be used for these analyses. Note that even for a binomial outcome from 1,000 patients (600 RS ≤ 25 versus 400 RS>25) we can detect a 10% difference with power 85% or greater.

Step 2 Analyses: The primary randomized trial HRQL treatment arm comparisons for anxiety and health utility will occur at 6 months but additional analyses of the HRQL measures will use longitudinal methods that can address loss-to-follow-up which may be associated with survival. The proposed sample size for the HRQL randomized trials analyses is 500 women randomized to receive chemotherapy or not. Projected accrual for the trial is 56 patients per month. CCOP sites account for approximately 25% of patient registrations to SWOG trials (Report of Studies, April 2010). In addition, we will also accrue from non-CCOP sites.

The PROMIS Emotional Distress-Anxiety standard deviation (SD) for 101 cancer patients was 7.5 for the first assessment and 7.7 for the second assessment; the longitudinal SD was 4.6. As an index of a clinically significant difference between the two study groups (chemotherapy versus no chemotherapy), we would be interested in SD differences between 1/3 (1/3 SD's were 2.5 and 2.6 were reported for two sequential assessments) to ½ (1/2 SD's were 3.7 and 3.9 for 2 sequential assessments2): longitudinal SD's were smaller (1/3 SD=1.5; ½ SD=2.3). [Personal Communication, Kathleen Yost, January, 2010). HRQL score differences of 1/3 SD have previously been shown to be clinically meaningful in terms of their association with change in other important clinical indicators such as performance status, pain levels, and patient self-perceived change. *(29)* With a sample size of

500, α = 0.025 (two primary HRQL outcomes: anxiety and utility scores) and a two-sided test, power is .93 for detecting a 0.33 SD difference in anxiety and utility scores between the two arms.

Sample size calculations for the EQ-5D are based on published identification of minimally important differences for its utility scores. *(30)* Pickard et al. estimated that a minimally important difference for a EQ-5D utility score was .06 (US cancer patients, mixed cancer sites); the SD for the U.S. sample (mixed cancer sites including breast cancer) was 0.15. Therefore the 0.33 effect we are basing our sample size on is consistent with the minimally important difference identified for the ED-5D utility scores.

The primary HRQL comparisons will occur at 6 months to evaluate the difference in anxiety and utility scores for patients receiving versus not receiving adjuvant chemotherapy. However, we will also use linear mixed model analysis to estimate change in anxiety and EQ-5D scores, but will monitor the influence of non-random missing data. *(31)* Should cohort plots and other diagnostic techniques suggest the presence of non-random missing data, we will use more appropriate analysis techniques such as pattern mixture models. *(32)* Secondary, exploratory analyses will address the relationship between patient anxiety scores and several other outcomes: relationships with continuous measures such as the Decisional Conflict Scale scores and the Survivorship Concerns scores will be examined with correlations; relationships between a dichotomous form of the anxiety outcome and single items from the Oncotype DX® testing questions and items addressing barriers to randomization will be examined with chi-square tests.

C. Comparative Effectiveness Analysis: Backgound and Analysis Plan

<u>Background and Significance: Cost</u>

Direct expenditures on breast cancer were estimated to be about $6 billion in 1996 (the last year such estimates were made), and are surely higher today. Gene expression profile (GEP) tests are expensive, costing approximately $4,000 per patient, yet adjuvant chemotherapy is much more expensive, costing $20,000 – $26,000 (upper ranges are closer to $50,000) (2003 dollars) (Oestreicher et al. Cancer 2005;104:2054-62). The immediate impact of GEP on breast cancer expenditures will depend on the degree to which the test spares women from undertaking costly chemotherapy. Based on current evidence regarding test outcomes, GEP could reduce initial breast cancer treatment costs by hundreds of millions of dollars. The long-term budget impact, however, will depend on the ability of the test to distinguish those who ultimately would experience breast cancer recurrence from those who would not. If GEP is a poor predictor of recurrence, the testing strategy could be more expensive than current practice while at the same time producing poorer outcomes. On the other hand, if GEP can better target women who will recur, risk profiling will substantially improve the cost-effectiveness of adjuvant therapy. The successful use of adjuvant chemotherapy in a highly targeted population thus represents a paradigm shift, both clinically and from an economic value standpoint. An accurate understanding of the changing economic value of adjuvant chemotherapy will be essential to ensure appropriate reimbursement policies.

Given the proliferation of GEP tests, their potential role in clinical practice, and the national clinical and economic burden of breast cancer, quantitative evaluations of the economic outcomes associated with GEP are warranted.

There have been two published cost-effectiveness evaluations of GEP for women with localized breast cancer. Both used simulation modeling and available data, but came to very different conclusions. One analysis, basing its estimates on the performance characteristics of Oncotype DX®, found that risk stratification using

GEP would reduce cancer care costs and increase quality adjusted survival (Hornberger et al. Am J Manag Care 2005;11:476). The other based its analysis on the MammaPrint assay, and found that GEP increased costs and reduced quality-adjusted survival (Oestreicher et al. Genet Med 20057:380-9). While some might conclude that these analyses support the superiority of Oncotype DX® vs. MammaPrint, differences in the model structures and other input parameters in these studies make such conclusions tentative at best. Moreover, no study has evaluated costs and outcomes of GEP tests in the management of node positive breast cancer.

Clinical trials evaluating medicines, medical devices and procedures now commonly assess economic value of these interventions. "Piggybacking" cost-effectiveness analyses alongside clinical trials offers many advantages over 'pure' modeling approaches: combining studies is efficient; the internal validity of both studies is maximized, and; economic data is made available alongside clinical data in a timely fashion (Ramsey et al. Value Health 2005;8:521-33). A trial-based economic analysis will provide the most accurate estimates of the cost-effectiveness of GEP. Decision makers in many countries now consider clinical and economic evidence together for formulary and insurance coverage policies, as will surely be the case for GEP.

1. OBJECTIVES

The primary objectives of the comparative effectiveness analyses are as follows:

1) Determine short term (1 year) and longer term (3 years) direct medical care costs for women with 1-3 positive nodes, HR-positive HER2, negative breast cancer with low Oncotype Recurrence Scores

   a) managed with chemotherapy

   b) managed without chemotherapy

2) Determine health state utilities for three health states related to the study (see Quality of Life section for details):

   a) Breast cancer, Recurrence Score unknown (one time point)

   b) Breast cancer, Recurrence Score known, no chemotherapy (multiple time points)

   c) Breast cancer, Recurrence Score known, chemotherapy (multiple time points)

   Using data from 1) and 2) and overall survival estimates from the trial, estimate the lifetime cost-effectiveness of Oncotype DX®-directed management vs. best alternative care (NIH guidelines) for women with 1-3 positive nodes, HR-positive HER2, negative breast cancer. Cost-effectiveness will be **measured as cost per life-year gained** and cost per quality-adjusted life year (QALY) over a time horizon of 3 years (within-trial analysis) and a lifetime (projections of cost and outcome modeled from the observed trial.

   A **planned subgroup analysis** is to estimate costs, QALYs and cost-effectiveness for two subgroups: (1) women for whom Medicare is their primary insurer (2) Women under age 65 with commercial insurance.

2. RESEARCH DESIGN AND METHODS

Conceptual framework and primary research hypothesis. The study will be conducted as cost-effectiveness analysis using life years gained (LYG) and quality-adjusted life years (QALYs) as measures of effectiveness. QALYs are an appropriate measure of outcome for this study because they capture most important health dimensions of the effects of an intervention, reflect preferences for health states under uncertainty, and facilitate comparison with other health care interventions whose effects have been evaluated using QALYs. *(33,34)* The Public Health Service's Panel on Cost-Effectiveness in Health and Medicine recommends using QALYs as the measure of outcome for cost-effectiveness analysis. *(35)* The methods that will be used to estimate QALYs are described below.

Costs and outcomes will be estimated using the health insurer perspective (secondary). Health insurers, who make the bulk of health care resource allocation decisions in the United States, generally consider only costs that they are directly accountable for when making resource allocation decisions for their insured populations. To address a primary objective of the study, the cost-effectiveness of OncotypeDX versus Usual Care will be calculated as follows:

$$(C_{ODx} - C_{UC})/(QALY_{ODx} - QALY_{UC})$$

Here, C denotes the direct health care costs, and QALY denotes quality-adjusted life years for Oncotype DX® (ODx) and Usual Care (UC), respectively. Costs and QALYs will be calculated for a 3-year time horizon (the maximum observation period possible for **S1007**). Future costs and QALYs will be discounted to their present value on the date of the intervention.

Cost-effectiveness alongside clinical trials. Prospective measurement of economic and health outcome data alongside randomized clinical trials (RCTs) to support has become increasingly common in recent years. There are two main advantages. First, this procedure is an efficient and timely way to obtain clinical, economic, and health-related quality of life, health state preference data simultaneously. Timely economic data will be particularly useful to those who are responsible for health care budgets, since as mentioned above, rapid and extensive growth in the use of Oncotype DX® can be expected if the results are positive. Second, a randomized, controlled trial has high internal validity and low potential for bias. On the other hand, RCTs have disadvantages for cost-effectiveness analysis. First, RCTs tend to have low external validity due to their restrictive inclusion/exclusion criteria and carefully selected settings. Second, artificially close monitoring and many protocol-induced procedures are part of the study. These factors may distort the economic and health outcome data relative to what one may expect under real-world conditions. Finally, limited follow-up periods that are common in a clinical trial may not be sufficient to directly estimate outcomes that are important for the cost-effectiveness analysis (e.g., a lifetime). Because of these issues, the estimates of cost-effectiveness obtained from the **S1007**, will represent a best-case scenario.

Estimating direct medical care costs. Direct health care costs include the value of all health care resources, non-health care resources, informal caregiver time, and the value of patient treatment time utilized over the time horizon of interest. The cost of care is determined as the product of the number of units of each item consumed and the unit value assigned to that product or service. Detailed elements of each cost category are described below. Note that these equations do not include the cost of working time lost to treatment. Cost of working time will be excluded from the primary analysis.

Total Medical Care Costs. The elements included in this category include all hospital, physician, laboratory, durable medical equipment, medications, home health and skilled nursing facility (SNF) care. Services will be valued using based on nationally standardized Medicare reimbursement rates. Sources and methods for collecting these costs are detailed in this section.

Medicare enrollees: The Medicare files will be the main source of data on the utilization of health care resources for trial enrollees ages 65 and higher. Medicare files provide data on hospital, physician, laboratory, and home health care services, durable medical equipment, infused or injected pharmaceuticals, outpatient medications and some skilled nursing facility (SNF) care. The Medicare Claims History Files contain patient identifying information, date of service, and complete utilization information for every Medicare-covered service submitted for payment by providers or patients under the Medicare traditional fee-for-service program. CMS's Bureau of Data Management and Strategy has established methods for creating analytic files on individual beneficiaries. Files will be created annually covering all enrollees and Medicare registrants in the trial from the day of registration.

Hospital Inpatient: Expenditures for hospitalizations will be valued according to DRG codes assigned on the patient's discharge abstract. Site-specific urban/rural, and teaching/nonteaching multipliers for the year 2011 will be applied, as defined by the CMS.

Outpatient Technical Services: Each service provided (coded by revenue center on the UB-92) will be mapped into a Medicare cost-reporting department. The Medicare Hospital Cost Reporting Information System (HCRIS) files for each year will be used to convert charges into costs using department-level cost-to-charge ratios reported annually by every Medicare-participating hospital. The Medicare cost-reporting data are available in public use tapes approximately 2 years after the cost-reporting period ends. Therefore, to avoid delay in publication after the final study year, department-level cost-to-charge ratios will be estimated from the historical average over the first 3 years of the study.

Physician Services: The Resource-Based Relative Value System (RBRVS) Medicare fee schedule value in the year 2011 for each CPT-4 code under which physician's bill will be the basis for unit cost of each specific billed physician service.

Laboratory and Other Part B Services: National 2011 Medicare average allowed amounts recorded for each Medicare-covered service (HCPCS Level I and Level II coding system) in the Medicare Part B file will be used to estimate the cost of all Medicare Part B services except physicians.

Skilled Nursing Facilities: National 2011 average allowed daily rate for payment of nursing home care will be used to assign SNF unit costs.
Home Health and Hospice Services: National 2011 Medicare fee schedules for payment of home health visits will be used to estimate these costs.

Outpatient Prescription Drugs: National average Medicare Part D 2011 reimbursements for outpatient medicines will be used for this analysis.

Long-Term Institutional Nursing Home Care: Medicare covers skilled nursing facilities for a limited period post-hospitalization, but long-term residence in a nursing home is not

covered. The history and physical form administered to trial enrollees during the follow-up period will contain a checklist item for current residence as follows: private home; retirement home; nursing home; hospital. If the patient was a nursing home resident at the previous follow-up visit, it will be assumed that he or she was a nursing home resident for the entire period. If not, then it will be assumed that the patient entered the nursing home at the midpoint of the follow-up period. Medicare SNF claims will be compared with patient responses to avoid double counting of costs. The unit (daily) cost of nursing home care will be valued at the Medicare SNF daily rate.

Commercially Insured Patients
Due to the large number of commercial insurance plans in the United States and the national scope of this study, it is not feasible to obtain health insurance records for all commercially insured patients. Rather, we will identify the most common commercial insurance carriers for trial enrollees and obtain claims records directly from those plans. Four health insurance groups—Blue Cross/Blue Shield Association Plans, Aetna, UnitedHealth, and CIGNA—insure more than 70% of all working age individuals and their families in the United States. We expect that approximately the same proportion of trial enrollees will have one of these plans as their primary insurer.

Obtaining Health Insurance Records:
Medicare and commercial enrollees will be asked to provide their Medicare identification (HIC) or commercial plan identification information as part of study enrollment and consent. Identifiers for those who provide consent will be used to obtain insurance claims for a period from 1 year prior to study enrollment through death or end of the follow-up period. We will exclude patients enrolled in Medicare Advantage (capitation) or commercial HMO plans because individual-level service claims will not be available for these individuals.

Measurement of quality-adjusted life years (QALYs). The **S1007** clinical protocol calls for measurement of health-related quality of life using the EQ-5D questionnaire. The EQ-5D is a validated measure with several domains of health status. *(36,37)* Population-based preference weights have been derived for each EQ-5D domain that permit translation of functional status scores to utility weights that may be used to derive QALYs. *(38,39)*

Survival estimates for patients in each trial arm will be combined with utility weights to determine QALYs for the arms of the study. To create uniform periods of utility and survival, **S1007** trial patient survival rates will be computed for each observation period covered the EQ-5D. The method for estimating QALYs from survival and utility data is discussed below.

Statistical Methods and Data Analysis.
Estimating Mean Costs for OncotypeDX+nochemotherapy and OncotypeDX+ chemotherapy. In order to minimize bias in the cost estimates, two important issues must be addressed: (1) the problem of censoring; and (2) that a number of patients will incur extremely high costs of care, resulting in skewed data. Ideally, one would measure lifetime costs for patients in each study arm, but the finite observation period and continuous enrollment throughout the study will result in the cost histories being truncated (censored) for many patients. Censoring and skewedness can be addressed by using the Kaplan-Meier sample average estimation (KMSA) technique described by Etzioni and colleagues. Using monthly cost histories from the patients in each study arm, the KMSA technique determines the mean cost over the time period of interest as:

$$M = \sum_i S_i \, c_i$$

where S_i denotes the probability of the event occurring in the time period *i* and c_i is the average cost among patients experiencing the event in time period *i*. The probability of the event occurring is a function of the probability of surviving to the start of the time period *i,*

as determined by Kaplan-Meier survival analysis. Lin et al demonstrate that the KMSA estimator is unbiased and consistent so long as (1) censoring is independent in time and (2) the time intervals for the cost analysis are sufficiently narrow. The design of the treatment trial is consistent with independent censoring. Monthly cost records are available from Medicare, thus providing appropriately narrow time intervals. Lin et al also show that the KMSA estimator is asymptotically normal with easily estimated variances, permitting standard two-sample parametric testing. Thus, the KMSA estimator also has the advantage of overcoming the problems of statistical testing of skewed data.

Using KMSA, 1-year and 3-year average cumulative costs with confidence intervals for each treatment arm will be computed. Future costs will be adjusted for inflation to constant dollars and discounted to net present value using a discount rate of 3 percent prior to calculating ***M.*** Cumulative costs will be plotted for 1- and 3-year time intervals for each study arm, with standard error bars for each interval. The Bonferroni method will be used to adjust for multiple comparisons among the treatment arms. Confidence intervals for incremental costs (i.e., absolute differences in the mean 3-year cost) will also be reported.

Estimating QALYs.
Quality-adjusted life years can be estimated in a manner similar to that which is used to described costs (see above). To estimate QALYs (), utility weights are combined with survival at each point of measurement across the period of interest using the following formula, as described in Ramsey et al.

$$Q = \sum_i \frac{[\![\omega_i (S]\!]_i \, \overline{x_i} + S_{i+1} \, \overline{x_{i+1}})}{2}$$

Where ω_i is the width of the interval *i,* x_i and x_{i+1} and the sample average utility weights, and S_i and S_{i+1} are the estimated survival probabilities at *i* and *i+1*, respectively. As with costs, future QALYs will be discounted to their present value at a rate of 3% per annum.

Estimating Incremental Costs Per QALY.
Using the 3-year estimates for cumulative costs and QALYs in each treatment arm derived as described above, we will compute the incremental cost per QALY for the all study participants and the specified subgroups

Uncertainty Analysis.
One-way uncertainty analyses will be conducted to identify model parameters that have the greatest impact on the results presented as a tornado diagram. Multi-way uncertainty analysis will be conducted to represent overall uncertainty around the estimates of clinical and economic effectiveness using probabilistic sensitivity analysis (PSA). The PSA will be completed using a second-order Monte Carlo simulation with 50,000 runs and appropriate parameter distributions. ICER results will be presented on the cost-effectiveness plane and as acceptability curves with willingness-to-pay (WTP) thresholds of $25,000, $50,000, $75,000 and $100,000 marked for reference. The uncertainty of cost per unit of outcome results for other endpoints (e.g. lives saved) will be presented as 95% confidence intervals. Again, threshold sensitivity analyses will also be completed to identify specific conditions under which the overall cost-effectiveness result would change.

Missing Data.
As with all clinical trials, missing data can be expected for **S1007** trial participants. Although Medicare claims records are expected to be complete for all participants, it is likely that some patient records will have incomplete health care utilization data (for services not covered by Medicare) and quality of life estimates. Imputation methods used for the primary clinical endpoints will be used to generate inputs for missing data elements. The method of imputation will vary depending on whether or not the missing data are believed to occur at random. This is difficult to know *ex ante*. It seems likely, however, that the

likelihood of missing quality of life data may be a function of the patients health status (i.e., sicker patients will be less likely to complete the questionnaires). Logistic regression will be used to test the correlation between missing parameters and patient characteristics [dependent variable: missing response (Y/N)].

Mid-study Analysis
To ensure that the complex task of analyzing the cost-effectiveness data runs smoothly and quickly at the end of the study, a mid-study "mock analysis" will be conducted where claims, EQ-5D, and survival data are merged and analyzed to produce an incremental analysis. To protect from revealing the results of the study at this point, SWOG analysts will remove actual patient assignment information and randomly assign enrollees to treatment and control arms prior to analysis.

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18.2 Prospective Validation of Other Prognostic/Predictive Indices of Breast Cancer Outcomes and Evaluation of Pre-treatment Hormone Levels

a. Objectives

1. To perform other molecular assays or test other signatures that measure prognosis and potential benefit of chemotherapy and compare them to Oncotype DX®.

2. To determine the role of other assays as predictors of DFS, DDFS and LDFI for patients randomized to chemotherapy versus no chemotherapy.

3. To compare clinically reported menopausal status with status categorized by serum hormone levels determined from baseline serum in women under age 55 years and to assess subsequent association with outcomes.

b. Background and Rationale for Additional Biomarker Analysis

Gene Expression Based Prognostic and Predictive Markers in Hormone Receptor Positive Breast Cancer

Although in **S1007** the 21-gene RS was used for patient stratification, several other gene expression signatures are commonly used as prognostic/predictive markers including 70-gene MammaPrint signature and PAM50 gene intrinsic subtype, SET index, Endopredict and Breast cancer index. Some of these expression profiles are described below.

While comparison amongst genomic assays was performed in the TransATAC trial, generalizability concerns of these analyses include the study was limited to postmenopausal patients and patients did not receive chemotherapy *(1, 2)*. There remains an unmet need to identify predictors of chemotherapy benefit within premenopausal as well as postmenopausal patients. Molecular analysis will allow for testing of prognostic and predictive biomarkers in patients who received chemotherapy on the same platform across the entire patient cohort, and will determine the underlying biological differences between premenopausal and postmenopausal patients.

Brief description of additional assays and signatures

70-gene MammaPrint signature: MammaPrint is a 70-gene signature which classifies tumors into groups that are associated with a good or poor prognosis on the basis of distant metastasis-free survival (DMFS) at 5 years and at 10 years. *(3)* Among the 658 women with HR+/HER2-, N1 breast cancers in the MINDACT (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy) trial who had clinical high but genomic low risk as determined by the 70-gene MammaPrint assay (Agendia) there was a 2.6% improvement in 8-year DMFS with chemotherapy. *(4, 5)* An exploratory subgroup analysis demonstrated an age-dependent effect of chemotherapy, in which the magnitude of chemotherapy benefit reached 5% in women age ≤50 and <1% benefit if age >50.

PAM50 signature: Gene expression profiling classifies breast cancer into "intrinsic subtypes" based on the biology of the underlying disease pathways. *(6)* This has been developed as Prediction Analysis of Microarray 50 (PAM50) Risk of Recurrence (ROR) score (Veracyte Technologies, previously Prosigna). The ROR Score was validated to determine the risk of recurrence of disease in HR+ breast cancer after surgery and treatment with 5 years of endocrine therapy. The ROR score depends on the intrinsic subtype, proliferation score of the tumor, and the tumor size. *(7, 8, 9)*

SET ER/PR and SET2,3 index: Sensitivity of endocrine therapy (SET) ER/PR index was developed to measure gene expression microarray probe sets that associate with hormone receptors (ESR1 and PGR). Higher SET ER/PR index in MBC samples predicted improved PFS and OS when patients received endocrine therapy as next treatment, even after adjustment for clinical-pathologic risk factors (PFS: HR 0.534, 95% CI 0.299 to 0.955, p = 0.035; OS: HR 0.315, 95% CI 0.157 to 0.631, p = 0.001). *(10)* SET2,3 index was proposed as a test for sensitivity to adjuvant endocrine therapy for patients with stage II-III breast cancer by measuring transcription related to estrogen and progesterone receptors (SET ER/PR index) adjusted for a baseline prognostic index combining clinical tumor and nodal stage with molecular subtype by RNA4 (ESR1, PGR, ERBB2, and AURKA). In HR+ patients who underwent neoadjuvant therapy, SET2,3 index was found to add independent prognostic information in addition to residual cancer burden in two separate cohorts *(11).*

EndoPredict (EP; Myriad Genetics, Cologne, Germany): EndoPredict (EP) is an RNA based multigene test that predicts the likelihood of distant recurrence in patients with HR+ breast cancer being treated with adjuvant endocrine therapy. In the GEICAM 9906 trial, EP was an independent prognostic parameter in node-positive, HR+ breast cancer patients treated with adjuvant chemotherapy followed by hormone therapy *(12, 13).* The EP assay is based on the quantification of eight cancer-related genes of interest and three reference genes.

Breast Cancer Index (BCI; Biotheranostics,San Diego, CA): The Breast Cancer Index test analyzes the activity of seven genes to help prognosticate the risk of recurrence in patients with HR+ breast cancer 5 to 10 years after diagnosis. BCI can be used for prediction with the benefit of extended adjuvant endocrine therapy *(14, 15).*

Genomic Alteration in Breast Cancer as Prognostic Markers

Genomic characterization of breast cancer has become standard of care for metastatic breast cancer (MBC) patients with HR+ cancer. There is already one therapy FDA-approved linked to a genomic biomarker for MBC: PI3K inhibitor alpelisib in combination with endocrine therapy for *PIK3CA* mutant HR+ breast cancer. There are several other genomically matched therapies under investigation in MBC, with expected increase in clinical utility of genomic testing in MBC.

Although genomic testing is not standard of care in non-metastatic breast cancer, we and others have already demonstrated that several key genomic alterations are associated with an increased risk of relapse and/or endocrine resistance in HR+ breast cancer including *TP53* mutations, *(16)* and alterations in MAPK pathway such as *NF1* loss *(17, 18, 19).* Notably, *ESR1* mutations have also been associated with endocrine resistance but this has been primarily found in metastatic tumors, as a mechanism of acquired resistance *(20).*

Taken together, there are several different prognostic signatures already developed for HR+ breast cancer and many genomic features associated with recurrence. We *hypothesize* that three established prognostic signatures (21-gene signature, breast cancer intrinsic subtype and 70-gene signature) based on RNAseq are associated with prognosis in premenopausal and postmenopausal patients with 1-3 LN+. Prognostic endpoints include IDFS, DDFS, LDFS, and OS. We also hypothesize that these prognostic signatures alone or integrated together will predict chemotherapy benefit in premenopausal patients with 1-3 LN+. The prognostic and predictive value may be further enhanced with integration of additional gene expression sets (*e.g., SET2,3*, RNA4 index, *MKI67* gene expression) and breast cancer genomics and proteomics.

Baseline serum hormone levels

In addition to clinical characteristics, serum hormone levels may be able to further discriminate menopausal status. Beyond self-reporting of menopausal status, serum levels can offer an objective measure. The mean age at onset of menopause is 51 years in Western countries, and by age 55 approximately 85% of women have undergone menopause, whereas less than 10% of women experience menopause at or before age 45 *(21, 22).* In clinical practice, estradiol, luteinizing hormone, and follicle-stimulating hormone are often evaluated for determination of whether a patient is pre- or post-menopausal. Anti-Müllerian Hormone (AMH), also called Müllerian inhibiting factor (MIF) is an additional indicator available as to whether a woman is approaching or is likely to have reached her final menstrual period. Given that there is a significant interaction between menopausal status, as determined by clinical characteristics, and IDFS and DDFS in RxPONDER, we propose evaluating hormone levels in pre-treatment baseline samples to assess whether menopausal status is further refined and whether an interaction term remains statistically significant based upon menopausal status per serum hormone levels.

c. Tumor Tissue

For all of the translational medicine studies specified below, specimens will be collected and banked at the SWOG Biospecimen Bank – Lab #201, Solid Tissue, Myeloma and Lymphoma Division. Integrated DNA/RNA will be performed for available tissue samples. Matching blood or normal tissue will be analyzed to facilitate identifying somatic vs germline variants on whole exome sequencing.

1. Prospective validation of other prognostic/predictive indices of breast cancer outcomes

   We will collect one paraffin block of the primary tumor, one positive lymph node block, and one negative lymph node block in all patients. Slides will be obtained when blocks are not available or feasible to obtain.

   A prospective clinical trial of these characteristics is the perfect setting to validate other available molecular signatures that have been previously associated with prognosis or therapy benefit. Although performing different assays has some advantages, it is tissue intensive and will limit the number of different signatures that can be tested. Therefore, we will perform integrated analysis with RNAseq and whole exome sequencing, and will test different signatures and their prognostic/predictive role.

   a. Prognostic and predictive role of three established breast cancer prognostic signatures

      Prognostic and predictive role of three established breast cancer prognostic signatures (21-gene signature, breast cancer intrinsic subtype, and 70-gene signature) based on RNAseq are associated with prognosis and chemotherapy benefit. Though assessment of alternate gene expression signatures is a pre-planned secondary objective, tissue limitations will not allow us to perform each individual assay, thus we will perform transcriptional profiling on archival primary tumor tissue using RNAseq (GEM Extra assay). We will estimate the 21-gene recurrence score (RS), 70-gene MammaPrint signatures, and the PAM50 subtype using data from the RNAseq and assess whether these signatures are associated with invasive breast cancer–free survival (IBCFS) and DDFS. We will also compare the 21-gene signature estimated through RNAseq with that of Oncotype DX® RS score.

We use the STEEP 2.0 definition (Tolaney, et.al., JCO 2021) of invasive breast cancer-free survival (IBCFS). Time from date of randomization (2nd Registration) to date of first invasive recurrence (local, regional or distant), second invasive breast cancer, or death due to any cause. Patients last known to be alive who have not experienced recurrence or second breast cancer are censored at their last contact date. This is similar to IDFS except that new non-breast primary cancers are not included as events *(23).*

b. Prognostic/predictive value of other available signatures

We will assess whether other established models and signatures are prognostic and predictive of chemotherapy benefit. The following signatures will be evaluated: SET2,3, RNA4 index, MKI67 gene expression, the hypoxia signature, 12-gene EndoPredict signature, and 7-gene breast cancer index. The prognostic and predictive role of these signatures will be assessed alone and in combination with the intrinsic subtype. In addition, we will assess the association of genomic alterations (TP53 mutations, PIK3CA mutations, MAPK pathway alterations) with IBCSF and DDFS in order to determine the role of integrated DNA/RNA analysis.

Notably, we will assess the prognostic and predictive role of different gene sets rather than derive formal scores corresponding to different commercial assays. Please see below for description of use of principal component analysis to derive “pseudoscores” with different gene sets.

c. Description of assay

We will perform RNAseq and whole exome sequencing with the Exact Sciences Corporation’s GEM ExTra assay. The GEM ExTra assay interrogates exonic sequences from 19,396 genes and 169 introns known to harbor cancer-related translocations. Using KAPA Hyper library construction and an IDT bait-capture solution, these sequences (collectively referred to as the exome, herein) are selected, amplified and prepared for sequencing on an Illumina NovaSeq 6000 instrument. Importantly, both DNA from the tumor tissue and an accompanying germline sample (saliva or blood) are sequenced, enabling the discovery of polymorphisms that are somatic in nature. In parallel, KAPA RNA libraries are constructed from the cancer’s total RNA and sequenced on the same Illumina platform. To preserve tissue, DNA and RNA are co-extracted from the same material, using Covaris’ truXTRAC kits, which we have found to improve our ability to extract high quality RNA from FFPE specimens.

GEM ExTra is validated for use with FFPE or freshly frozen tissue specimens. We anticipate using FFPE tissue sections that will be assessed by a board-certified anatomic pathologist and marked for manual microdissection if there is less than 50% tumor compared to metabolically active tissues per the Genomic Health, Inc SOP used to handle all RxPONDER samples used for Recurrence Score testing.

d. Statistical Plan

1. Clinical Endpoints and the biomarker measurements involved in the analysis.

   The primary outcome of the trial was invasive disease-free survival (IDFS) with distant disease-free survival (DDFS) as secondary. We propose to use invasive breast cancer–free survival (IBCFS) as described in STEEP 2.0 which excludes non-breast new primaries as events, but includes all other IDFS event types. This sharpens the evaluation of chemotherapy benefit. Each biomarker is constructed from the sequencing analysis.

2. Case selection method if only a subset of patients will be included in the biomarker evaluation.

   Patients from UNICANCER (n=1014) are excluded since tissue samples were not submitted to the Nationwide repository. Tissues from GEICAM patients (n=762) have been submitted to Nationwide recently, but have not yet been integrated into the inventory so we cannot specify yet how many patients are included. Of the remaining 3,071 eligible patients, 2,959 (96.4%) have available tissue.

3. Numbers of patients to be studied and biomarker assays/tests to be performed.

   All patients with tissue are included. Case-cohort approaches could reduce the number of assays, but events continue to occur so it is wiser to include all from the start. Only the GEM ExTra assay is performed and all signatures are computed across that common platform.

4. Statistical analysis methodology and underlying assumptions.

   For this restricted sample the number of IBCFS events are currently 96 and 222 for premenopausal and postmenopausal women, respectively. (In contrast, for IDFS there are 106 and 274 events, showing that many IDFS events in postmenopausal women were other cancers and equally distributed by treatment group.) Median follow-up time is approximately 6.1 years in this cohort. IBCSF hazard ratios (HRs) in favor of chemotherapy are 0.69 (95% CI 0.46-1.04) and 0.99 (95% 0.76-1.29), for premenopausal and postmenopausal patients respectively.

   All analyses use IBCSF or DDFS measured from randomization in ITT Cox regression analyses adjusting for tumor size, tumor grade, and number of positive nodes. All analyses maintain the expected overall HRs given above. Prognostic analyses include the biomarker with and without the original Recurrence Score (RS) based on expression, to test whether the marker can add prognostic value beyond commercial RS, and conversely whether the original RS adds prognostic power given the new marker. Prediction analyses include an interaction term between continuous marker and randomized treatment assignment. If the interaction is statistically significant then a cutpoint for chemotherapy benefit is estimated using the methods established in the original protocol for the expected interaction of RS and treatment assignment. For dichotomous markers an interaction test will be conducted as well as separate analyses by marker positive and negative subgroups to test qualitative interactions.

In terms of prediction for premenopausal women there may be a small fraction of patients who do not benefit from chemotherapy despite the strong results in **S1007**. Similarly, for postmenopausal women there may be an even smaller fraction who do benefit from chemotherapy and the goal of this research is to identify those groups. Ultimately, we may determine an optimal signature though any signature would need validation (internal or external with the GEICAM/UNICANCER patients).

Continuous RS based on gene expression is a highly significant prognostic factor for both premenopausal and postmenopausal women in this sample. The Interquartile Range (IQR) of 7 points (RS 11-18) has a HR of 1.53 ($p<0.003$) for premenopausal women and 1.42 ($p<0.001$) for postmenopausal women. It is likely that replacing RS with values based on sequencing will improve prognostic ability. For 1-sided $\alpha=0.05$ and 80% power, the magnitude of the HR for the IQR must exceed 1.43 and 1.30 for premenopausal and postmenopausal women, respectively. Consequently, there is sufficient power for testing prognosis.

Testing prediction will be with limited power. We will use a continuous marker by treatment interaction to signify a potential predictive marker. However, using a dichotomous marker has more limited power. For example, for premenopausal women a 50% negative marker with HR of 1.00 and 50% positive with a computed HR maintaining the overall hazard ratio at 0.69 would have only 54% power at 1-sided $\alpha=0.05$ and 68% power at 1-sided $\alpha=0.10$ (commonly used for interactions). This decreases to 33% and 47% power, respectively, if the negative marker has 30% prevalence. Since the postmenopausal HR is 0.985 there is room only to identify a small subset who may benefit. For example, if 5% benefit considerably but the remainder have HR=1.00 then there is only 21% power at 1-sided $\alpha=0.05$ and 33% power at 1-sided $\alpha=0.10$, respectively, to find a significant interaction. Consequently, while an interaction of a continuous marker and treatment may suggest prediction it may be difficult to demonstrate with a dichotomized marker.

5. Additional Bioinformatics Considerations

The bioinformatics analyses will guide selection of the gene expression signatures (21-gene signature, PAM50 and 70-gene signature) that are predictive of prognosis and chemotherapy (CT) benefit. The median normalized mRNA expression data will be analyzed with both supervised and unsupervised techniques to identify the molecular correlates/predictors of CT responses. We will also implement user-friendly scripts and tools for rapid data access and visualization using the Next-Generation clustered heatmaps and Oncoprint suites *(24, 25).* The tools will enable automated selection of patient groups carrying aberrant expression or mutations in genes of interest.

a. **Unsupervised detection of genes signatures.** The analysis will inform on which gene signatures and subsets of genes within the signatures are most informative of CT benefit. With a multi-step unsupervised approach on mRNA expression data, we will detect the gene signatures (and gene subsets within) that can best classify patients as responders and non-responders. First, through a two-way unsupervised hierarchical clustering, we will map the distribution of gene expressions across the patient groups. The patient clusters will be selected with the dynamictree-cutting algorithm applied on the hierarchical patient trees. *(26)* To test whether distinct clusters

correlate with CT benefit, we will compare the IDFS, IBCFS, and DDFS differences between the clusters with Kaplan-Meier curves and log-ranked tests. Next, to detect the most predictive subsets of genes within the signatures, we will quantify the median gene expression differences between the clusters. With a Wilcoxon rank-sum test, the genes with significant differential expression between responder and non-responder cohorts will be selected. The analysis will be applied to each gene signature of interest (21-gene, PAM50, 70-gene signature). The signatures will be ranked for their predictive power of patient-classification for CT-benefit based on the survival differences between clusters. Through Fisher's exact test, we will also determine the mutations that are significantly enriched in responder or non-responder groups. All statistical analyses will be corrected for multiple hypothesis testing using Benjamini-Hochberg procedure.

b. **Supervised detection of gene signatures.** To go beyond the correlative analysis and select most predictive gene-sets, we will perform a Cox regression with elastic-net penalty (R-package: Regularized Cox Regression). The regression analysis will be applied to each signature and mRNA expression data. The survival data will serve as the dependent variables and mRNA expression values for signature genes are the independent variables. The predictive power of each signature will be quantified with a 10-fold cross validation. The models will be built using a training set (90% of patients selected with boot-strapping). Resulting models will be used to predict the CT-response of remaining patients that were hidden in the model building. The gene signatures will be ranked for their predictive power based on the cross-validation errors. The subsets of the most predictive gene subsets from most predictive signatures will be determined based on regression-predicted weights.

c. **Improvement of gene signatures through differential analysis of responder and non-responder groups.** We will perform a differential analysis of genomic alterations across whole genome to identify other genes (not-sampled in the signatures) that are enriched in patients with CT-benefit. First, we will stratify the patients to responders vs. non-responders based on the survival distributions. Next, we will perform a differential RNA expression analysis using the Deseq2 R package. The differentially expressed genes in responder or non-responder groups will be included to the pre-defined gene signatures. The supervised and unsupervised analyses will be iterated using the expanded gene list. The improvements in predictive powers will be quantified and improved gene-signatures for CT-benefit will be reported. This iterative approach will prevent loss of predictive power due to pre-existing bias in selection of gene signatures.

d. **Test of prediction for derived signatures.** To actually determine whether we have significant predictive abilities with the derived signatures we intend to use the following methods.

6. Discovery Analysis Methods

Genes considered will be all the constituent genes (except reference genes, where present) from the MammaPrint, PAM50, SET Index, Endopredict, Breast Cancer Index and Recurrence Score tests. For each set of genes examined, a score will be constructed using the first principal component of the gene set.

For assessment of chemotherapy effect prediction, a Cox proportional hazards regression model will be fit with endpoint iDFS and terms for the gene set score, treatment and the interaction of the gene set score with treatment. The log standardized hazard ratio for interaction *(27)* and its variance will be computed for each gene set score.

For assessment of prognosis, a Cox model will be fit with a single term for the gene score using the patients who were randomized to endocrine therapy alone. For assessment of residual risk, the same procedure will be used for patients randomized to chemo-endocrine therapy.

False discovery rates *(28)* and log standardized hazard ratios with correction for regression to the mean *(29, 30)* will be calculated using model space sampling considering the universe of gene set scores selected from all genes under consideration and gene sets from 1 to 40 genes.

If prognostic gene sets are discovered at FDR 10%, their prognostic efficacy will be described using predictiveness curves *(31)* corrected for regression to the mean.

If predictive gene sets are discovered at FDR 10%, their predictive efficacy will be described using treatment effect predictiveness curves, that is, predictiveness curves applied to the distribution of estimated treatment hazard ratio with correction for regression to the mean. Potential gene set score cut-points for identifying patients with substantial treatment benefit versus no substantial benefit will be assessed based on these curves.

These discovery analyses will be conducted separately for pre-menopausal patients and post-menopausal patients.

Pseudoscores were previously constructed using RNASeq of the SWOG 8814 study and the constituent genes of the MammaPrint®, Prosigna®, EndoPredict®, Genomic Grade Index, Breast Cancer Index® and Sensitivity Endocrine Treatment (SET) scores as well as the Oncotype DX Recurrence Score®. Each pseudoscore was constructed using the coefficients of the first principal component of the constituent genes. These pseudoscores will be evaluated and compared as continuous numeric biomarkers for prognosis of iDFS and prediction of the effect of chemotherapy using the RxPONDER data set and standardized hazard ratios. Categorical analyses for both prognosis and prediction will use equivalent cut-points using population quantiles. Since SWOG 8814 included only post-menopausal women, the pseudoscores will be re-derived separately using pre-menopausal and post-menopausal women in RxPONDER and the scores compared between pre- and post-menopausal women. If it is concluded that the premenopausal pseudoscores are sufficiently different from the postmenopausal pseudoscores, then it may require regeneration of the first principal component weights using five-fold cross-validation. Overall, it is

recognized that the pseudoscores are not an exact match for the actual gene signatures, as they use different coefficients and analytical platforms, so that the performance of the actual signatures might be different. This analysis seeks to generally evaluate the information content of the constituent genes in each gene list.

Because of the restriction of the S1007 study population to patients with Recurrence Score result 0 – 25, it is recognized that the estimated prediction of effects of genes from the Recurrence Score and other genes substantially correlated with these genes will be biased downward.

2. Additional Molecular characterization of node-positive, HR-positive and HER2-negative breast cancer and association with patient outcome

   We will collect one paraffin block of the primary tumor, one positive lymph node block, and one negative lymph node block (if available) in all patients. Blocks will be stored at the SWOG Biospecimen Bank – Solid Tissue, Myeloma, and Lymphoma Division, Lab #201. We plan to use the samples for evaluations at the protein levels and immune profile. By evaluating the tumors of patients in this trial we will be able to discover potential markers of response to commonly used and novel therapies as well as new potential targets for future therapies in this population.

   Samples will not be used until the proposed research platforms to be used at the time of analyses have been validated.

3. Pharmacogenomic studies of the effects of inherited, germline polymorphisms on toxicity and efficacy of aromatase inhibitors and chemotherapy.

   By evaluating germ-line DNA we will be able to discover potential pharmacogenomic markers of outcome and toxicity to commonly used and novel therapies.

   Samples will not be used until the proposed research platforms to be used at the time of analyses have been validated *(32, 33, 34, 35, 36, 37, 38, 39, 40, 41).*

d. Baseline Serum

1. Serum Hormone Level

   Given that there is a significant interaction between menopausal status, as determined by clinical characteristics, and IDFS and DDFS in RxPONDER, we propose evaluating hormone levels in pre-treatment baseline samples to assess whether menopausal status is further refined and whether an interaction term remains statistically significant based upon menopausal status per serum hormone levels.

2. Description of Assay

   We will analyze baseline serum estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-müllerian hormone (AMH)/Müllerian inhibiting factor (MIF). These analyses will be performed at the University of Kansas Medical Center through the Kansas Institute for Precision Medicine (KIPM) COBRE (P20 GM130423, PI: Godwin, A.K.) Biobanking and Biomarker Validation Core (BBV). We will use fluorescent bead-based immunoassays (Luminex assays) to measure circulating levels of all 4 markers. These will be performed via standard laboratory procedures following manufacturer's protocol.

3. Statistical Plan

   a. Clinical Endpoints and the biomarker measurements involved in the analysis.

      The primary outcome of the trial was IDFS, with secondary outcomes including DDFS and now IBCFS.

   b. Case selection method if only a subset of patients will be included in the biomarker evaluation.

      Patients from UNICANCER (n=1,014) are excluded since samples were not submitted to the Nationwide repository. At this time, there 3,255 patients with baseline samples available. Of these, 1,039 were reported as premenopausal. Of the 3,255 patients, 1,364 were age < 55.

   c. Numbers of patients to be studied and biomarker assays/tests to be performed.

      We will focus on the premenopausal patient population with available baseline serum samples – and analyze patients age < 55, as these are most likely to be premenopausal.

   d. Statistical analysis methodology and underlying assumptions.

      For this restricted sample the number of IBCFS events are currently 96 and 222 for premenopausal and postmenopausal women, respectively. (In contrast, for IDFS there are 106 and 274 events, showing that many IDFS events in postmenopausal women were other cancers and equally distributed by treatment group.) Median follow-up time is approximately 6.1 years in this cohort. IBCFS hazard ratios in favor of chemotherapy are 0.69 (95% CI 0.46-1.04) and 0.99 (95% 0.76-1.29), for premenopausal and post-menopausal patients respectively. In Section 18.3, the statistical analysis plan is defined. In this biomarker analysis, we will evaluate whether an interaction term with menopausal status and clinical outcome remains when menopausal status, as defined by serum hormone levels.

   e. Funding

      The Biobanking and Biomarker Validation Core (part of the Kansas Institute for Precision Medicine COBRE) will analyze baseline serum samples from pre-menopausal patients under age 55 for estradiol, luteinizing hormone, follicle-stimulating hormone, and anti-mullerian hormone/Mullerian inhibiting factor, with labor and equipment fee provided in-kind (at no cost to the study). The reagent costs will be offset via Hematology and Medical Oncology-associated funds provided by the Winship Cancer Institute of Emory University in support of biomarker analyses to further inform whether invasive disease-free survival benefit remains in premenopausal patients.

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18.3 Statistical Analysis Plan

1.0 SCIENTIFIC OVERVIEW AND SUMMARY OF THE STATISTICAL PLAN

1.1 SPECIFIC HYPOTHESES

a. We hypothesize that the 21-gene recurrence score (RS) will predict the benefit of chemotherapy in node positive (1-3 nodes), hormone receptor (HR)-receptor positive breast cancer patients with RS ≤ 25 treated with state-of-the-art endocrine therapy. Chemotherapy benefit (if it exists) will increase as the RS increases.

b. We hypothesize that chemotherapy is not beneficial for some patients in the range of RS 0-25 and that the point of equivalence between chemotherapy and no chemotherapy can be identified in this range. Above this point, patients begin to benefit from the addition of chemotherapy. We will identify a RS cutpoint for which there is clinically significant benefit of chemotherapy for all RS values above this cutpoint.

1.2 PRIMARY OBJECTIVE

To determine the effect of chemotherapy in patients with node positive breast cancer who do not have high Recurrence Scores; In patients with 1-3 positive nodes, and HR-positive, HER2-negative breast cancer with RS ≤ 25 treated with state-of-the-art endocrine therapy we will test whether the difference in disease-free survival for patients treated with optimal chemotherapy compared to no chemotherapy depends directly on the magnitude of RS. If benefit depends on the RS score, the trial will determine the optimal cutpoint for recommending chemotherapy or not.

1.3 OVERVIEW OF ANALYSIS FOR THE PRIMARY OBJECTIVE

The primary analysis will be a test of interaction of Recurrence Score with randomized treatment assignment. If this test is statistically significant, then it will be determined whether there is a point of equivalence between the two treatments within the range of RS from 0 to 25. The upper limit of the 95% confidence interval on this equivalence point determines the cutpoint for determining that chemotherapy is effective for all values at that cutpoint or above. If there is no significant interaction, then the overall effect of chemotherapy will be tested adjusting for a common prognostic effect of RS on both treatment groups.

1.4 SECONDARY OBJECTIVES

- To compare overall survival (OS), distant disease-free survival (DDFS) and local disease-free interval (LDFI) by receipt of chemotherapy or not and its interaction with RS.
- To compare the toxicity across the treatment arms
- To perform other molecular assays or test other signatures that measure prognosis and potential benefit of chemotherapy and compare them to Oncotype DX®.
- To determine the impact of management with Oncotype DX® on patient-reported anxiety (co-primary Health-Related Quality of Life [HRQL] outcome) prior to screening, after disclosure of test results, and during the randomized trial.
- To determine the impact of Oncotype DX® on the initial management cost of node-positive, HR-positive, HER2-negative breast cancer.
- To compare patient-reported utilities (e.g., QOL) outcomes for those randomized to chemotherapy versus no chemotherapy.
- Using modeling and DFS information from the trial, to estimate the cost-effectiveness of management with Oncotype DX® vs. usual care.
- To determine the role of other assays as predictors of DFS, DDFS and LDFI for patients randomized to chemotherapy versus no chemotherapy.
- To determine the impact of treatment with chemotherapy versus no chemotherapy on patient-reported fatigue and cognitive concerns (secondary HRQL outcomes).
- To determine the impact of management with Oncotype DX® on patient-reported decision conflict, perceptions regarding Oncotype DX® testing, and survivor concerns prior to screening, after disclosure of test results, and during the randomized trial (secondary HRQL outcomes).
- The presence of circulating tumor cells (CTC+) using two CTC platforms will be assessed at up to two time points to assess late recurrence in those still at risk for the primary outcome. Invasive disease-free survival (IDFS) will be compared between CTC+ versus CTC-, incorporating use of endocrine therapy.
- To compare clinically reported menopausal status with status categorized by serum hormone levels determined from baseline serum in women under age 55 years and to assess subsequent association with outcomes.

2.0 BACKGROUND

2.1 Results of the S8814 trial

Until recently, there was no information on the potential value of the RS assay in patients with positive axillary nodes and HR-positive disease from a study that contains a similar tamoxifen-alone control arm since today these patients are routinely treated with chemotherapy as well as endocrine adjuvant therapy. **SWOG-8814** was a practice-changing phase III trial for postmenopausal women with node-positive, ER-positive breast cancer that demonstrated that CAF chemotherapy added survival benefit to tamoxifen, especially in the sequential setting, with CAF preceding the initiation of tamoxifen therapy. The study had optional specimen banking which yielded tumor specimens for RS determination by the Oncotype DX® gene assay. When comparing the tamoxifen and the sequential CAF-T arms in tissues from 367 patients, the RS was prognostic for DFS in the tamoxifen-alone arm (p=0.006). In this study we used the RS grouping defined by Paik et al. *(1,2)* There was no apparent CAF benefit in the low RS (0-

17) group (p=0.97) or the intermediate RS (18-30) group (p=0.48), but a significant DFS improvement was detected for the high RS (31-100) subset (p=.03). Due to failure of the proportional hazards assumption, separate analyses were performed for the first five years of follow-up and the period beyond five years. The RS-by-treatment interaction was significant in the first 5 years for DFS (p=0.029), with no additional prediction of CAF benefit beyond 5 years (p=0.58). No impact of CAF was observed in the lowest RS, regardless of nodal status. Results were similar for OS. *(3)*

In the proposed trial, only women with RS ≤ 25 and 1-3 positive nodes would be included. We reexamined the **S8814** data using this cutoff and 10 years of survival using a standard Cox model. The Kaplan-Meier graph below shows little difference between the two groups (Figure 1) even if we restrict attention to those with RS ≥ 14 (Figure 2). However, the Kaplan-Meier graphs may obscure a possible difference since they do not use the continuous RS. If one fits a more complex model using continuous RS and its interaction with chemotherapy, then a pattern emerges even though there is still no significant interaction of RS and treatment in this subset with RS ≤ 25. The Cox model gives an estimate of the log hazard ratio (relative failure rates) by RS with high hazard ratios indicating worse DFS. The hazard ratios apply at any time point (e.g. 5 or 10 years) which is why we prefer to illustrate them here. In the simplest case we use a Cox model and allow for a linear interaction of RS and treatment (Figure 3). The hazard ratios cross indicating there may be a point of equivalence where a chemotherapy benefit may emerge, but of course the difference would have to be large enough to be both clinically and statistically significant. For nodes 1-3 this point of equivalence was about RS = 19. Based on this model the estimated hazard ratio at RS=22 would be 0.84 for chemotherapy versus no chemotherapy, but the 95% CI is 0.28-2.49 due to the small sample size. Note that the effect in very low RS scores may be exaggerated due to the sparseness of data. Pepe et al. recommends using quantiles in the regression so that scores are distributed equally along the x-axis. *([4])* We used a linear quantile model in (Figure 4), but then graphed it on the original RS axis. This mutes the effect of sparse scores, but the pattern is similar. These models are based on sparse data and are perhaps too simple, but they do indicate that using continuous RS may provide more insight and power than simple categorization. However, interaction alone is also not sufficient and needs to be supplemented by a clinically useful cutoff.

Figure 1

All patients with RS = 25

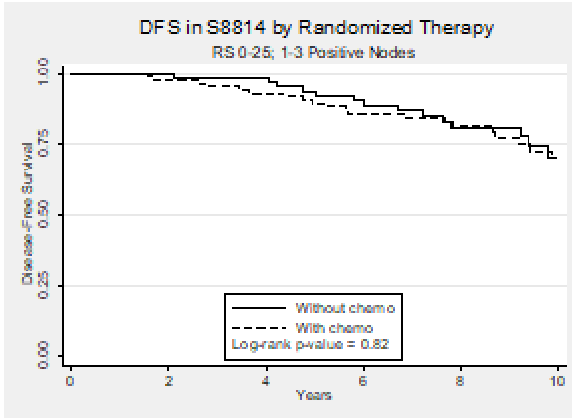


Figure 2

Patients with 14-25

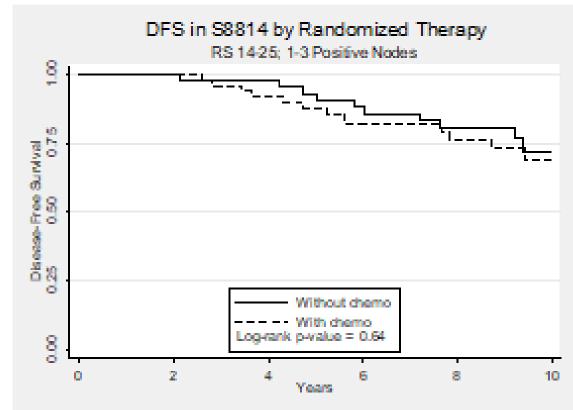


Figure 3

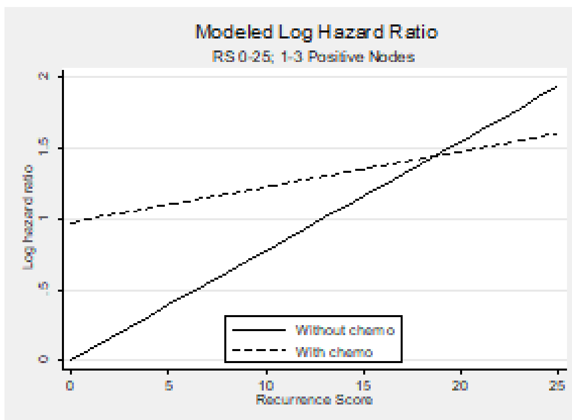
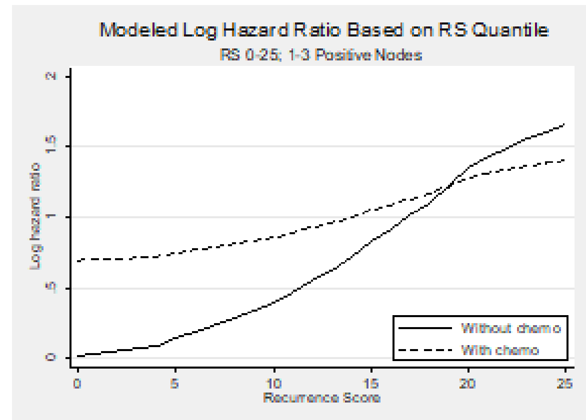


Figure 4



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Based on **S8814** data alone it is difficult to conclude that there is benefit of chemotherapy in patients with RS ≤ 25. We recognize that the sample size is small, there are some trends in the data that support chemotherapy at the higher ends of the RS range, and that chemotherapy has improved since **S8814**. There have also been improvements in hormonal therapy, surgery, and radiation therapy, though these would apply equally to the two randomized groups. How representative of current outcomes are the **S8814** data given that patients were randomized in the early 1990's? SEER does not provide DFS, but does provide some information about overall survival (OS). Using SEER, one can examine some of this improvement by tumor stage. For Stage II/III disease there have been improvements in OS (Figure 5), while in Stage I disease (Figure 6) there has been little improvement in OS. From SEER one can derive the estimated overall survival of 52,592 women aged 55-74 diagnosed with receptor positive, node positive breast cancer in the years 1996-2003. In this group overall survival was 83% at 5 years and 64% at 10 years. Note that **S8814** had an overall survival of 82% at five years and 64% at ten years, so the outcomes are almost identical to current population results. Nonetheless, it seems likely that better chemotherapies are available. This would strengthen the importance of this trial since we would have a randomized comparison with modern chemotherapy, rather than observational data based on outdated chemotherapy.

While the failure rate in **S8814** seems high, we show that the overall survival is exactly what one would expect in a comparable population. We have also compared **S8814** recurrence results with those of TRANS-ATAC at 5 years. Their primary outcome is disease-free interval (DFI) which censors deaths that are not associated with a recurrence. Ten deaths in **S8814** occurred within 5 years and had no evidence of recurrence so are treated as censored in this analysis, but would be considered as failures for DFS. The TRANS-ATAC investigators kindly provided comparison data, but collapsed over treatment group. Recurrence rates (events/person years) suggest little difference between ATAC and **S8814**. Adjusting for number of nodes and RS risk group (< 18, 18-30, > 30) showed no statistically significant difference in DFI between ATAC and **S8814**. There are not appreciable differences in the TRANS-ATAC and **S8814** outcomes when RS groupings and number of nodes are considered. Thus, the **S8814** data still provide an excellent reference point for the proposed trial.

Figure 5

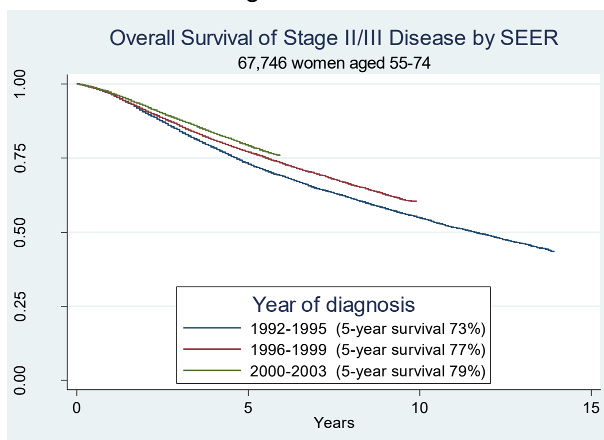
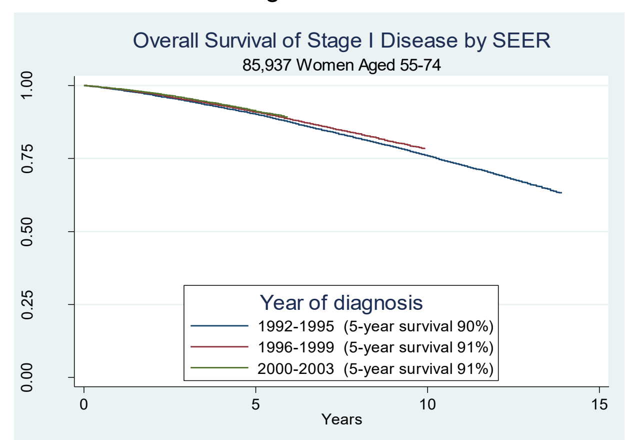
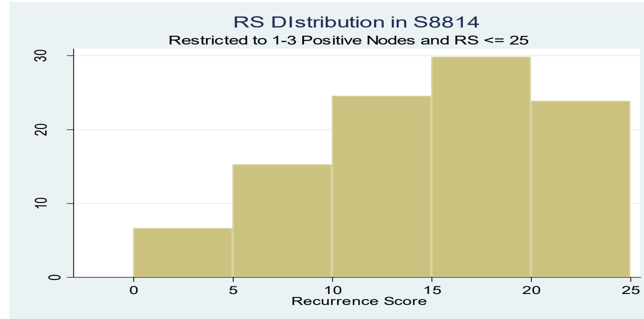


Figure 6



The percentage of patients in **S8814** with > 3 positive nodes was 43%. This proposed trial will restrict eligibility to patients with 1-3 positive nodes. In women with 1-3 positive nodes, RS was ≤ 25 in 67%. The overall distribution is shown below. Since the point of equivalence is unknown it is necessary to have a wider range of RS scores to distinguish who may need chemotherapy from those who do not.

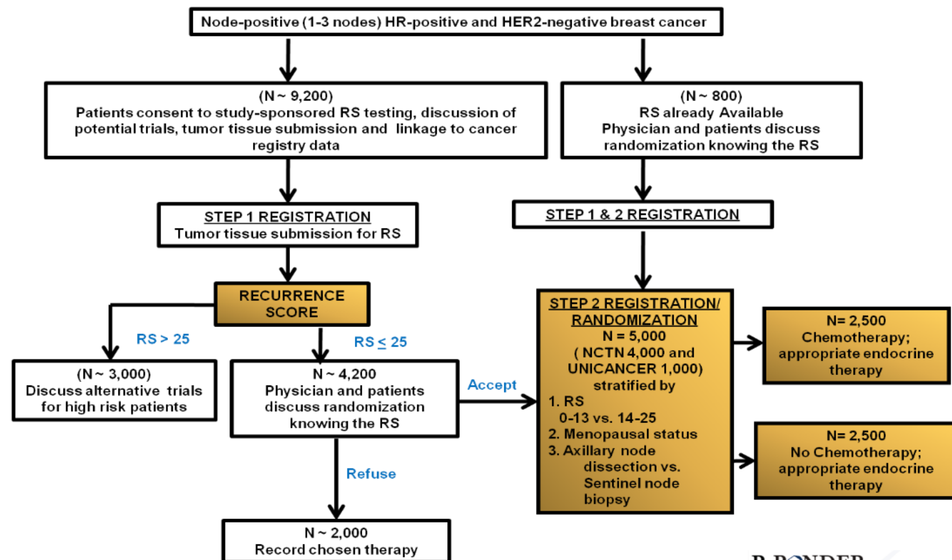
Figure 7



To summarize, **S8814** provides general support for chemotherapy in patients with receptor-positive, node-positive disease, but no strong evidence of benefit in the subset with RS ≤ 25. Looking more carefully using the actual value of RS, does reveal that treatment effects may start to diverge at higher values of RS, but this would need to be supported by data using modern chemotherapy and many more patients. We predict that continuous RS will be directly associated with the degree of benefit. We also predict there may be an equivalence point between RS 0 to 25 after which a benefit to chemotherapy may emerge. When categorized we expect no benefit below that cutpoint, but a clinically significant benefit above the cutpoint.

3.0 TRIAL OVERVIEW

3.1 TRIAL DESIGN SCHEMATIC



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NUMBER OF PATIENTS SCREENED AND NUMBER RANDOMIZED

The schema shows the patient flow from screening to randomization. Most patients who meet eligibility (1-3 positive nodes; HR-positive and HER2-negative disease) will need to be screened. We expect to perform the 21-gene assay on approximately, 10,000 patients but the number could vary to achieve the target accrual in the randomized trial. A small number of eligible patients (~800) will already have RS scores available from having ordered the test commercially. Among those with an obtained RS score (~10,000), patients with RS ≤ 25 (N~7,000) will be eligible for the randomized trial while those with RS>25 (N~3,000) will be ineligible. Upon receipt of the RS, patients with RS > 25 will be informed of their RS score, its interpretation, and other available trials. Patients with RS ≤ 25 will undergo discussion of this trial in consultation with their oncologist considering known RS value. We expect about 29% (~2,000) will not accept randomization. Thus, the target accrual goal is 5,000 patients randomized equally to chemotherapy or no chemotherapy with stratification by Recurrence Score (0-13 vs. 14-25), menopausal status, and complete nodal dissection versus sentinel node biopsy. NCTN will be limited to 4,000 randomized patients and UNICANCER to 1,000 patients. The randomized accrual targets are fixed, but all other stated sample sizes are approximate and depend on the distribution of RS scores and the number accepting randomization.

3.2 STRATIFICATION FACTORS

Patients are randomized separately for NCTN and UNICANCER. Patients are also stratified at time of randomization on three factors:

1. RS value 0-13 versus RS value 14-25
2. Pre-menopausal versus post-menopausal
3. Type of nodal dissection: axillary lymph node dissection (with or without sentinel node mapping) versus sentinel node biopsy without axillary lymph node dissection.

A dynamic balancing scheme is used to assure that randomization is balanced across all stratification factors.

3.3 ENDPOINTS

PRIMARY ENDPOINT
DFS: Invasive disease-free survival (DFS) - time from the second registration (randomization) to local, regional, or distant recurrence, new invasive primary, or death due to any cause. The STEEP definition of invasive disease-free survival (IDFS) is used, although it is referred to here by the more common acronym DFS. Survival times are censored at time of last follow-up for individuals who did not have any event meeting the above definition.

SECONDARY ENDPOINTS
OS: Overall survival (OS) - time from the second registration (randomization) to death due to any cause. Survival times are censored at time of last follow-up for individuals who are not known to have died.

DDFS: Distant disease-free survival (DDFS) - time from second registration to distant recurrence, new invasive primary, or death due to any cause. Patients who have local or regional recurrence are continued to be followed for a distant event or death. Survival times are censored at time of last follow-up for individuals who are not known to have died and have not had a distant recurrence or new primary.

LDFI: Local-regional disease-free interval (LDFI) - time from second registration to local/regional recurrence. 3 Patients who have distant recurrence or a new primary or who die without recurrence are censored at time of this event. The analysis of this endpoint must account for informative censoring using a competing risk framework. Survival times are also censored at time of last follow-up for individuals who are not known to have died and have had a recurrence or new primary.

Toxicity: Toxicities using standard NCI-CTCAE criteria (CTCAE Version 4.0).

Other endpoints including QOL, costs, and correlative outcomes are described elsewhere.

4.0 SAMPLE SIZE AND POWER

4.1 ASSUMPTIONS

We propose to randomize 5,000 patients over a 5.5-year accrual period with 5.5 additional years of follow-up after the last randomized patient. Since the accrual target has changed, we are using actual enrollment per month through July 2014 and the estimated enrollment thereafter until August 2016. All statistical tests use an overall 2-sided $\alpha = 0.05$ except where specified.

Estimated survival is based on disease-free survival in **S8814**. In this study, only patients with RS ≤ 25 and 1-3 positive nodes are included. In that subgroup in **S8814** the overall 5 year DFS rate was 91% and was 70% at 10 years. In this case the overall 5-year DFS rate is 92.4% reflecting improvement in modern rates.

Compliance is discussed in detail below, but in order to accommodate random dropout we increased the sample size by 5%. Simulations used a sample size of 4,750 which is increased by 5.3% to 5,000 for the overall accrual goal. Power was obtained by simulating a population (n=4,750; 2,375 per group) that had relative results similar to **S8814**. A Weibull model provided a reasonable fit to the **S8814** data and gave hazard ratios comparable to those of the Cox model. Since the model data were sparse, we assumed that the observed interaction in **S8814** (β = -0.052) may be an overestimate. We used a more conservative value of -0.042 (20% reduction) as the primary simulation model. The interaction parameter was fixed at that value and the other parameters re-estimated from **S8814** so that the estimated model would be consistent with observed data. The primary simulation model used an equivalence point of 19 and a hazard ratio of 0.78 in favor of chemotherapy at RS=25. Alternatives are considered below. Ten thousand simulations were performed to determine power. The simulation was performed in SAS.

4.2 OVERVIEW OF POWER CALCULATIONS

Power is based on a stepped analytic plan. It seeks to establish the following:

1) There is a statistically significant interaction (2-sided $p \le 0.05$) of treatment and RS. That leads to three subsequent steps:
   a) There is a point of equivalence in the range 0-25 for a model that incorporates a linear interaction of continuous RS and treatment randomization.
   b) If (a) is true, the upper limit of a one-sided 95% confidence interval for the point of equivalence marks the RS for which there is a significant benefit of chemotherapy. If this upper limit ≤ 25 then we can establish a clinically meaningful cutoff for recommending chemotherapy. If the upper limit exceeds 25, then we can only suggest chemotherapy for RS > 25.
   c) If (b) gives a cutoff in the range 0-25, then the trial population will be divided at this cutoff. At the cutoff or above, we expect to see a clinically and possibly statistically significant effect of chemotherapy. Below the cutoff, we will find no statistically significant benefit of chemotherapy.

2) If there is no statistically significant interaction of treatment and RS, then the overall effect of chemotherapy will be tested adjusting for RS as a prognostic factor.
   a) If a significant benefit of chemotherapy is observed, then it will suggest all patients in the range RS 0 to 25 should receive chemotherapy.
   b) If no significant effect of chemotherapy is observed, then power is sufficiently high to conclude that it is unlikely a benefit exists and no patients with RS 0-25 and 1-3 positive nodes would be recommended for chemotherapy.

PREDICTION OF CHEMOTHERAPY BENEFIT

The first hypothesis to be tested is whether continuous RS predicts chemotherapy benefit. In this case we are predicting an interaction of randomized treatment with the linear RS score. Even if the log hazard ratios are not perfectly linearly related to RS, the test of interaction will measure divergence in chemotherapy benefit. We tested the significance of the interaction in the Cox model:

$$\lambda(\text{t; chemo, RS}) = \lambda_0(\text{t}) \exp(\beta_1 \text{chemo} + \beta_2 RS + \beta_3 \, chemo * RS)$$

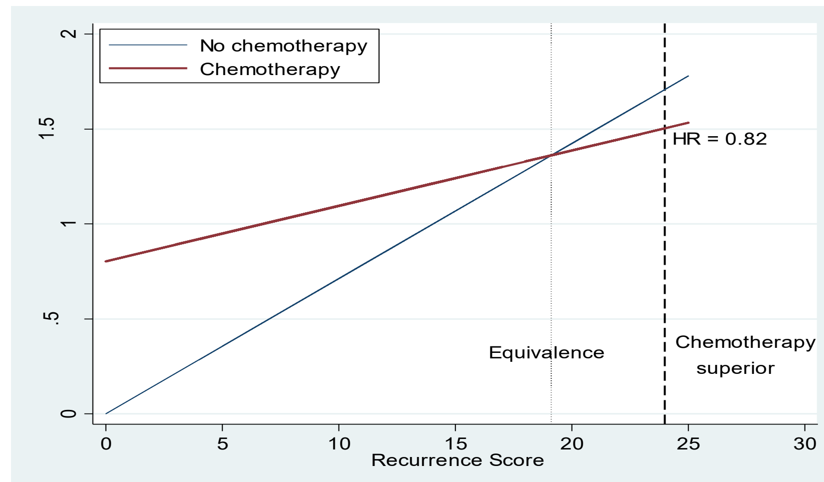
Using an ITT analysis, the power for the primary simulation was 86.3% to detect a significant interaction (predictive ability of the RS score). The interaction was marginal ($0.05 < p \le 0.20$) in another 10% of the cases, but these are not included as a significant interaction. For the 14% of simulations which showed no significant interaction, we fit a model without the interaction term. In the simulations without a significant interaction, not one simulation showed a statistically significant benefit of chemotherapy. Furthermore, 88% of these simulations showed that the lower 95% CI for the hazard ratio (chemo versus no chemo) exceeded 0.90, thus ruling out a protective effect of chemotherapy.

However, a significant interaction alone is not sufficient for prediction. One also needs to demonstrate a qualitative interaction as well, i.e. there is a group who benefits from chemotherapy and a group who does not.

PREDICTION OF THE POINT OF EQUIVALENCE AND ITS CONFIDENCE INTERVAL

When the interaction is statistically significant, the second hypothesis to be tested is whether a specific range of RS values can be specified as showing equivalent results for chemotherapy versus no chemotherapy. Estimation of this value can be done by several methods. One method is to test all possible RS integer values between 0-25 to determine maximum discrimination after accounting for multiple comparisons. However, this method may not guarantee a single consistent answer. Instead, we consider the point of intersection of the two therapies based on the model in (1). The interaction model yields estimates that allow estimation of an estimated equivalence point $\theta = (-\beta_1 / \beta_3)$ with a standard error for the estimate of θ determined by the delta method. If θ is not within the range (0, 25) then one treatment dominates the other and would always be preferred. If θ is in the range 0 to 25, then a 95% one-sided CI would be obtained on the upper bound. The upper limit of this confidence interval ("c") would indicate that chemotherapy can be assumed to be more efficacious above this point. This is illustrated below. In the example, we identify that the two treatments are estimated to be equivalent at RS=19.3, but that the upper limit of the 95% CI extends to 24.0. At this value, the estimated benefit of chemotherapy would be about a 18% relative reduction in the failure rate, which would be in the range of clinical benefit. The actual hazard ratio at the upper limit would vary, but the power calculations assumed that the hazard ratio is 0.78 at RS=25. The expected 5 year DFS survival rates under the simulation model are the following:

Recurrence Score	No Chemotherapy	Chemotherapy	Difference in favor of chemotherapy
15	93.2%	92.0%	-1.2%
20	90.5%	90.8%	0.3%
25	86.7%	89.4%	2.7%



With the current sample size the estimated θ would have a standard deviation of approximately 2.44. In the simulations finding a statistically significant interaction, the point of equivalence (θ) was in the range of 0-25 in 99.34% so the crossover point almost certainly will be found in the range of the data. In 81.6% of the simulations, the upper bound of the confidence interval for the estimate of θ (c) was in the range of 0-25. So we have sufficient power to declare a value at which chemotherapy is clinically superior if the underlying model is correct. In the remaining simulations the upper limit exceeded 25 so the clinical recommendation would be to use chemotherapy when RS > 25. Therefore, there is strong evidence that the linear interaction model will provide evidence for a clinically useful cutpoint.

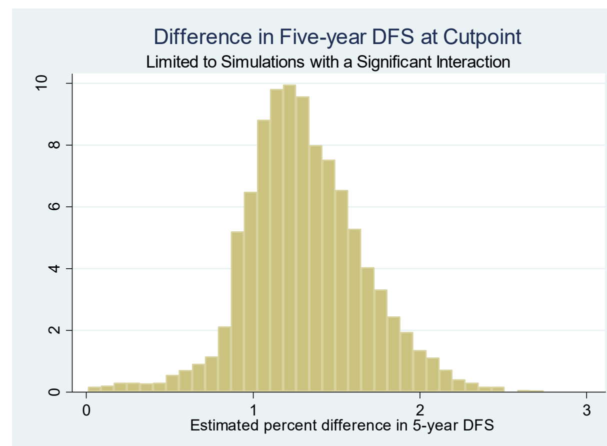
If there is a significant interaction and the point of equivalence is in the range 0-25, then we expect there could be a slight difference in DFS at the cutpoint. The following table shows the results of the simulations and the expected DFS and difference at 5 and 10 years at the cutpoint:

5-year and 10-year DFS at the upper limit of the equivalence region

Conditional on a significant interaction and estimable equivalence point

			Percentile				
Outcome	Mean	SD	5	25	50	75	95
5-yr Chemo DFS	90.0%	2.01%	86.3%	88.8%	90.3%	91.4%	92.9%
5-yr No Chemo DFS	88.7%	2.27%	84.6%	87.3%	89.0%	90.3%	91.9%
5-yr Difference	1.31%	0.36%	0.81%	1.08%	1.28%	1.51%	1.92%
10-yr Chemo DFS	66.1%	5.72%	55.9%	62.5%	66.6%	70.2%	74.6%
10-yr No Chemo DFS	62.4%	6.17%	51.6%	58.5%	63.0%	66.8%	71.7%
10-yr Difference	3.67%	0.81%	2.41%	3.23%	3.68%	4.17%	4.91%

The expected difference in 5-year DFS at the cutpoint is shown below:



SWOG Authorized Document

PREDICTION OF CHEMOTHERAPY BENEFIT IN PATIENTS ABOVE AND BELOW THE CUTPOINT

Having established this upper cutpoint (c) inside the range 0-25, we would then like to show that for observations above c, that there is a significant benefit to chemotherapy collapsing across RS above this cutpoint. However, because this cutoff is likely to be very close to 25 if it exists then there may not be sufficient data to show a statistically significant benefit. Power for this analysis is likely to be low (estimated to be 23%), but we will also show the Kaplan-Meier graph for this group which should indicate an advantage to chemotherapy. Similarly, one would want to show no significant benefit of chemotherapy below the cutpoint c. When there was a significant statistical interaction, the estimated lower bound for the HR of chemo versus no chemotherapy for RS values below the cutpoint exceeded 0.90 in 97% of the simulations. Thus, there is sufficient power for data below the cutpoint to show no clinical benefit of chemotherapy.

The analysis of the overall chemotherapy effect (collapsed over RS) and then separately by groups below and above the cutpoint will provide further supportive evidence of the approach since it does not directly require that RS be linearly related to outcome. Patients above the cutpoint will have clear evidence in favor of chemotherapy while patients below the cutpoint might want to balance the risks against the benefits. As discussed above the cutpoint should divide the population into two groups. One might expect a qualitative interaction, i.e. that there would not be a benefit in the lower group, but there would be a significant benefit in the upper group. The size of these two groups would depend on the location of the cutpoint. Kaplan-Meier survival curves will be used to illustrate the comparison of chemotherapy versus no chemotherapy for the group below the cutoff and the group above the cutoff.

EFFECT OF COMPLIANCE ON POWER

Our proposed trial enrollment is a two-step method intended to minimize non-compliance. We hope to eliminate most potentially non-compliant patients from the randomized trial prior to randomization and thus improve the power of the ITT analysis. For patients with a higher RS score, the patient may be unwilling to be randomized to no chemotherapy. Therefore, we intend to provide the RS value prior to randomization. Similarly, patients with a very low RS may not want to receive chemotherapy so these patients may elect not to be randomized. All patients will be fully informed prior to randomization and will provide informed consent after knowledge of the RS value.

While we hope that the above procedures eliminate likely noncompliant patients prior to randomization, we know that some will still be noncompliant after randomization. Dr. Gray, statistician for the TAILORx trial, has suggested that among patients with known RS value prior to enrollment, about 10% in each group were noncompliant with randomized assignment and this may depend on the RS values. We believe the procedures instituted above can reduce this to about 5% in each group. Consequently, the estimated sample size above includes this 5% noncompliance rate and assumes that noncompliance depends on RS. For patients randomized to chemotherapy, we assume 5% do not receive chemotherapy and that a patient with RS 0-11 is twice as likely to refuse as one who has RS 12-25. For patients randomized to not receive chemotherapy, we assume 5% do receive chemotherapy and that a patient with RS 18-25 is twice as likely to receive chemotherapy as one who has RS 0-17. We further assume that the noncompliant patients remain in the study and provide follow-up. Thus, in the ITT analysis 5% of patients in each treatment group have a treatment opposite to their randomized assignment. This results in a huge increase in the necessary

sample size. Based on the original design if all patients were compliant, then the necessary sample size would be 3,200. With just 5% of patients expected to be noncompliant, then the sample size increases by 800 patients. Clearly, eliminating potentially noncompliant patients may result in more screens, but ultimately provides a much smaller randomized group.

The 5% noncompliance rate was incorporated into the simulations. However, we also added an additional 5% to the simulation sample size (n=4,750) to allow for dropout and ineligible patients so the final accrual goal is 5,000.

5.0 STATISTICAL ANALYSIS PLAN

5.1 STATISTICAL MODEL

All analyses will use Cox regression models for DFS. The model will be stratified by group (NCTN or UNICANCER) so that the baseline hazard rate will reflect the different populations. The primary hypothesis about interaction of RS and treatment will be tested using the model:

$$\lambda(\text{t; chemo, rs, menopause}) = \lambda_0(\text{t}) \exp(\beta_1 \text{ chemo} + \beta_2 \text{ RS} + \beta_3 \text{ chemo*RS} + \beta_4 \text{ menopause})$$

where menopause is an indicator for the stratification factor of menopausal status. The power calculations assume no interaction of prediction by RS and menopausal status or number of nodes, but those assumptions will be tested. If the interaction of treatment and RS is statistically significant then the second step is to estimate the equivalence point. The two log hazard ratios will intersect at

$$\hat{\theta} = -\hat{\beta}_1 / \hat{\beta}_3$$

indicating the point of equivalence. Variance for the estimate of θ is derived from the delta method as

$$Var(\hat{\theta}) = \frac{1}{\hat{\beta}_3^2}\left[V_{11} + 2\,\hat{\theta}\,V_{13} + \hat{\theta}^2\,V_{33}\right]$$

where V indicates the covariance matrix. Then the 95% CI for the estimate of θ can be derived assuming normality. In this case we are interested only in a 1-sided 95% CI for the upper limit. We assume that this estimate of θ is in the range 0-25, otherwise either chemotherapy is universally better or worse for all RS values in the range. To establish the cutpoint for efficacy we use the upper limit

$$c = \left(\hat{\theta} + 1.645 * \sqrt{Var(\hat{\theta})}\right)$$

If c > 25, then we need to recommend chemotherapy for all RS>25. If c ≤ 25, then we can determine the cutpoint where chemotherapy is superior to no chemotherapy.

Power was computed first on the probability of finding a significant interaction and secondly on the upper confidence interval of the cutpoint being less than 25.

These methods assume that there is at least a monotonic increase in benefit of chemotherapy as RS increases. This will be further established with secondary analyses including tests of departure from linearity and dependence of the cutpoint on other factors such as menopausal status or number of positive nodes. Simulations using a misspecified model that was nonlinear showed that the liner model provided a reasonable fit to the data and gave an accurate representation of the cutpoint. Furthermore, the trial data will be dichotomized at the cutpoint in order to perform a stratified (by menopausal status) log-rank test of chemotherapy in the lower RS group and the upper RS group as determined by the cutpoint. The

expectation is that the lower group will not show a significant benefit of chemotherapy while the upper group may show a statistically significant benefit after collapsing RS values within the subgroup. This will further confirm that there is an optimal RS value to consider chemotherapy.

The assumption of proportional hazards in all models will be tested. If the proportional hazards assumption is not satisfied ($p<0.05$) we will split the time axis at 5 years and perform separate analyses.

Should a significant interaction not be found, the next step is to assume that the underlying model is the following:

$$\lambda(\text{t; chemo, rs, menopause}) = \lambda_0(\text{t}) \exp(\beta_1 \text{ chemo} + \beta_2 \text{ RS} + \beta_3 \text{ menopause})$$

The main effect of chemotherapy will be tested against the reduced (null) model:

$$\lambda(\text{t; chemo, rs, menopause}) = \lambda_0(\text{t}) \exp(\beta_1 \text{ RS} + \beta_2 \text{ menopause})$$

The null model assumes that RS is prognostic, and that menopause is included as a stratification variable.

Planned secondary analyses include adjustment for sentinel node biopsy and group NCTN/UNICANCER. Test of the interactions of these stratification variables with treatment will also be conducted. If there is a significant interaction, separate models will be fit for that stratum to determine if it is a quantitative or qualitative interaction.

5.2 INTERIM ANALYSIS

Under the assumptions above, we would expect 832 events for the primary analysis of the interaction of RS and chemotherapy. The first interim analysis would be after 24% of the events have been observed or approximately 6.6 years after initiation of the study. This would correspond to the end of accrual if accrual is uniform and at the expected level. There would be subsequent annual interim analyses thereafter with 37%, 53%, 72%, and 92% with the final analysis at Year 11. The analyses will use the Lan-Demets spending function with a truncation bound. To achieve a cumulative 0.025 1-sided significance level, the interim test α's will be 0.0005, 0.0005, 0.00149, 0.00741, 0.01673, respectively, and the final $\alpha=0.01871$ so there is little loss of power due to the interim analyses. All of these analyses are expected to be after accrual has finished so a decision to publish early would be based on the interim analysis.

5.3 SAFETY ANALYSIS

We also want to monitor the upper RS group of 14-25 to avoid harming patients if there is early evidence of efficacy in this group. An analysis will be conducted at 4 years to evaluate efficacy of chemotherapy in patients with RS 14-25 to determine if there is a potential significant benefit of chemotherapy early in the trial. If this comparison is statistically significant at $p=0.05$ (2-sided) then further randomization in patients with RS 14-25 would be suspended. A similar comparison would then also be performed in the RS 0-13 group to determine if the trial should suspend accrual completely. Otherwise, all other analyses would occur after accrual is complete.

5.4 SECONDARY OUTCOMES ANALYSIS

Analyses for secondary survival outcomes (OS and DDFS) will be analyzed in a similar manner to DFS, though power will be lower than DFS, due to fewer events. Local disease-free interval will use a competing risk framework to accommodate informative censoring due to distant recurrence or death. Analysis of toxicity will be compared between the two arms using logistic regression.

5.5 OVERALL TYPE 1 ERROR

Power is based on the alternative hypothesis being true. We have a two-step testing procedure. The first step is a test of the interaction. If that interaction is not statistically significant, then we test for a main effect of chemotherapy adjusting for RS as a linear variable. Thus, we consider the following model as the null hypothesis for the simulations:

$$\lambda(\mathrm{t};\mathrm{RS}) = \lambda_0(\mathrm{t})\exp\left(\beta_1\ \mathrm{RS}\right)$$

As expected the first stage had a Type 1 error rate of 5.08%. If there was not a significant interaction, then the effect of chemotherapy was tested. This added 2.46% to the error rate so the Type 1 error rate overall is about 7.5% for the two-stage testing.

5.6 ACCRUAL RATE (ORIGINAL)

Years 1-6, 48 patients per month for the randomized trial.

6.0A ACCRUAL (ORIGINAL SECTION)

We intend to accrue 4,000 randomized patients over 6 years. The power calculations assume uniform accrual over the six years. In practice, however, accrual in the first year is typically slower due to IRB approvals and lack of familiarity with the trial. We allow for 5 additional years of follow-up after the last patient has been entered. TAILORx has accrued well, but targets node-negative women which comprises a greater percentage of women with breast cancer. Using SEER incidence rates from 2004 we computed the expected number of incident breast cancer cases for women aged 35-74 using age-specific incidence to be 148,686 invasive cases in the U.S. Of this number 38,671 would have stage II/IIIA and would be ER positive. Based on the S8814 distribution of RS, 23,589 per year would have RS ≤ 5. To reach our goal of 667 per year (56/month) we would have to enroll about 2.8% of patients in the U.S. We believe this trial will be attractive to insurers and HMO's since there is the possibility of costs reduction with the expected outcome. With the support of advocacy groups and the assistance of NCIC and other international partners the monthly accrual goal seems feasible.

Another issue concerns the number needed to screen to identify a population of 1-3 node-positive patients with RS ≤ 25. We have estimated the number of 8,800 screenees to yield 5,600 possibly eligible patients based on the **S8814** distribution. The Oncotype DX® test has been used in clinical practice for node positive disease since 2008, and there is experience in reporting Recurrence Score results in more than 5,000 patients. For both the node negative and node positive cases submitted in clinical practice, there has had a somewhat smaller proportion of very high and very low Recurrence Scores compared to the distribution in the clinical trials. Approximately 80% of the tested cases in the Genomic Health clinical laboratory have had RS ≤ 25 (32% with RS 0 – 13 and 48% with RS 14-25) (Dr. Shak, personal communication). Based on these numbers, it is estimated that randomization of 4,000 patients with RS ≤ 25 would require screening with Oncotype DX®

approximately 7,200 patients. To allow some protection we have specified this as 8,800, but believe this to be an overestimate. One area of concern with our proposed recruitment strategy is that patients eligible for the trial may not enroll after receiving their RS value. We have estimated that the proportion accepting randomization will be 69%.

To reach our goal of 667 per year (56/month) we would have to enroll about 2.8% of patients in the U.S. With the support of advocacy groups and the assistance of NCIC and other international partners the monthly accrual goal seems feasible.

6.0B ACCRUAL (REVISED)

Originally we expected accrual of 4,000 randomized patients to take 6 years, giving a monthly accrual rate of 56 patients per month. The formal study completion date was five years after the last patient was enrolled to provide sufficient power for analyses. Thus, the original trial duration was expected to be 11 years. In this revised sample size calculation we use the actual monthly enrollment rates from study start in February 2011 to July 2014 which showed accrual not reaching the target goal until February 2012. Subsequently, accrual exceeded the goal considerably and the trial goal of 4,000 would be expected to be reached by August 2015 or 4.5 years of total accrual which is 1.5 years earlier than expected. If the trial used the original stopping rule of 5 years after the last patient, then power would be reduced considerably by the shorter follow-time for events to accrue. We can either extend the follow-up period and/or increase the accrual by combining with the UNICANCER group. We have elected to do both as explained below.

Accrual from Sept 2014 to August 2015 is assumed to be 98 randomized patients per month until the NCTN accrual reaches 4,000 patients. We assume UNICANCER will accrue approximately 83.3 per month for 12 additional months. This will result in a total sample size of 5,000 patients over a 5.5 year accrual period. After the last patient is accrued, then there will be 5.5 more years of follow-up before the study will have final analysis. The total trial duration will be 11 years which is the original length of the trial. However, individual patients will be followed for a minimum of fifteen years regardless of the date of enrollment to assess long-term effects of treatment.

Through July 2014, we have found 50% of patients who enroll in the trial are subsequently randomized. Originally, we expected that to be only 43%. Therefore, to achieve 5,000 randomized patients we expect to screen 10,000 possible candidates.

7.0 INCREASE IN SAMPLE SIZE FROM 4,000 TO 5,000

In September 2014, we amended the sample size from 4,000 to 5,000. This was in part due to accrual that was more rapid than expected. Since the analysis was to be performed at 5 years after the last accrual, then total follow-up time would be shortened and there would not be enough events. While one could lengthen the follow-up time without increasing accrual, this increased power back to the original level but would provide no protection against lower than expected event rates or violation of the statistical assumption. Instead we had an opportunity to partner with UNICANCER in France and increase the sample size by 1,000 randomized patients. The combined data will be the basis of the analysis with planned subset analyses in each of the populations intended to show similar results. Below we explain the implications in changing the accrual target.

As described in Section 11.2 accrual started slowly, but then improved dramatically so that now accrual of the original sample size goal is expected to finish 1.5 years early. Since the original design called for a final analysis 5 years after the last patient was enrolled, the number of events is considerably reduced. Using the observed sample size accrual, but keeping other design elements constant then power to find a significant interaction would drop to 68% due to the shorter window for events. If we increase the window to 6.5 years after the last patient is accrued then power can be restored to 82.7% without adding 1,000

new patients. However, that provides no margin against some violation of the statistical assumptions of this important signature trial of the NCTN. For example, if the event rate is lower than predicted we would not have power to address this important question. It is too early in the trial to assess the event rate accurately (and it depends on the intervention), but we can look at the population in terms of prognostic risk factors compared to what was expected. There has been a strong shift in the number of positive nodes compared to **SWOG-8814**. The older trial had a distribution of positive nodes for 1-3 of 48%, 32%, and 20%, respectively while the current trial has a distribution of 68%, 24%, and 8%. However, because of sentinel node biopsy there could have been an apparent shift downwards that does not reflect the true distribution of number of positive nodes. Furthermore, the current trial has a distribution of Oncotype DX® scores that is the same as in **SWOG-8814** when restricted to ≤ 25 and in agreement with the expected distribution used to plan the trial. For trial planning we used an overall 5-year DFS rate of 92.4%, but we would still like to have sufficient power if the event rate is less than expected.

The second reason to increase the sample size is to provide a validation that the results apply to an external population to NCTN patients. We are including these patients in the overall trial but will conduct planned subset analyses of the two cohorts separately. If we retain the same design parameters with a 5,000 patient trial of the same total duration (11 years), then power increases to 86.7% and the expected number of events increases from 731 to 832. When the interaction is significant we determine the estimated cutpoint for using chemotherapy and its upper confidence bound. This cutpoint can be better determined when there are more events. Finally, the larger sample size allows some protection against lower event rates. For example, if the actual 5-year DFS rate is 93.9%, then power is still 80% to detect a significant interaction.

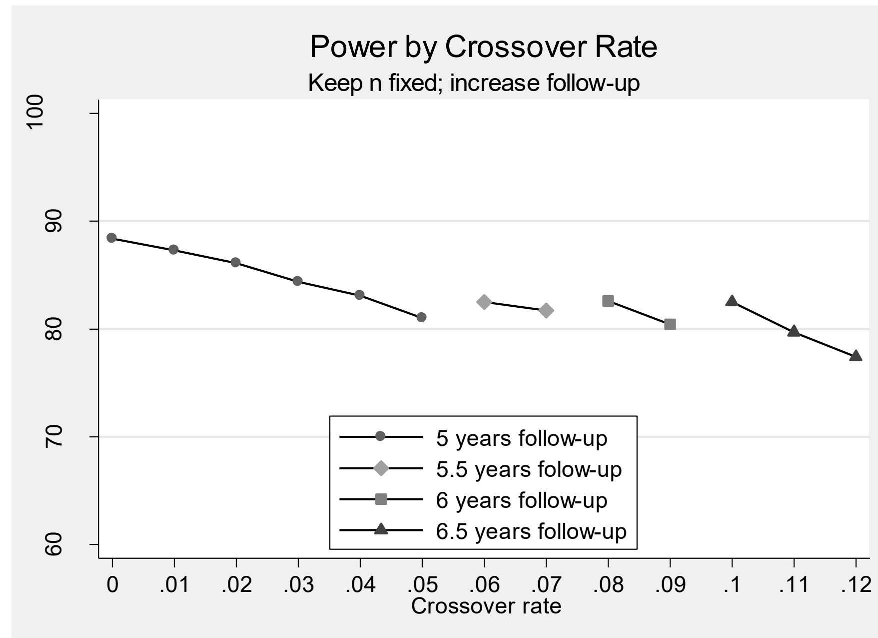
To guarantee that the trial will be well powered under the assumptions but have sufficient power under minor violation of the assumptions, we are increasing the sample size to 5,000 with a 5.5 year accrual and a 5.5 year follow-up so that the total trial duration remains the same as in the original protocol.

8.0 MONITORING TRIAL ACCRUAL AND COMPLIANCE

Accrual will always be a potential concern in every trial and it is often difficult to assess how successful a trial will be. CTEP has an existing rule at one year that mandates an assessment of accrual. Even after passing this test, the success of such a trial will depend on accrual projections, percentage eligible, percentage accepting randomization, and compliance. We propose that a committee of five statisticians (1 SWOG, 2 NCI, and 2 others from the cooperative groups) jointly review the data (excluding outcomes) after two full years of accrual to determine viability of the trial and/or changes to be made in accrual projections.

Compliance is a threat to statistical power since the intention-to-treat analysis classifies patients by treatment assignment regardless whether they received the assigned treatment. This trial has been powered with an expectation that 5% of patients are noncompliant, i.e. receive the alternative treatment, but remain in the analysis and are classified by treatment assigned, not by treatment received. Accordingly, if compliance among randomized patients exceeds 15% in the first year of accrual, consideration will be given to stopping the trial unless the problem has been corrected. After two years, the cumulative crossover rate cannot exceed 12%.

As long as the crossover rate is 12% or less, we can maintain power by keeping the sample size constant, but increase the follow-up period to ensure sufficient power to find a significant interaction. For the standard model we assumed a 5% crossover rate and had 81% power. If crossover is lower, than power increases. If crossover is 6-7%, then we increase follow-up by 6 months to regain 80% or greater power. If crossover is 8-9%, then we increase follow-up by 12 months to regain 80% or greater power. If crossover is 10-12%, then increasing follow-up to 18 months provides sufficient power as shown in the graph below.



9.0 REFERENCES

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18.4 Determination of Expedited Adverse Event Reporting Requirements

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. (Directions for routine reporting are provided in Section 14.0.) Additionally, certain adverse events must be reported in an expedited manner to allow for more timely monitoring of patient safety and care. Expedited adverse event reporting principles and general guidelines follow; specific guidelines for expedited adverse event reporting on this protocol are found in Section 16.1.

All serious adverse events determined to be reportable to the Institutional Review Board responsible for the oversight of the patient must be reported according to local policy and procedures. Documentation of this reporting should be maintained for possible inspection during quality assurance audits.

Steps to determine if an adverse event is to be reported in an expedited manner (*This includes all events that occur while on treatment or within 30 days of the last dose of protocol treatment.*)

Step 1: Determine whether the patient has received an investigational agent, commercial agent, or a combination of investigational and commercial agents.

An investigational agent is a protocol drug administered under an Investigational New Drug Submission (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.
When a study includes both investigational and commercial agents, the following rules apply.

- ***Concurrent administration***: When an investigational agent(s) is used in combination with a commercial agent(s), the combination is considered to be investigational and expedited reporting of adverse events would follow the guidelines for investigational agents.
- ***Sequential administration***: When a study includes an investigational agent(s) and a commercial agent(s) on the same study arm with sequential administration all expedited reporting of adverse events should follow the guidelines for the type of agent being given. For example, if the patient begins the study on the investigational agent(s), then all expedited reporting of adverse events should follow guidelines for the investigational agent(s). Once the patient begins receiving the commercial agent(s) then all expedited reporting of adverse events should follow the guidelines for commercial agent(s).

Step 2: Identify the type of event using the NCI Common Terminology Criteria for Adverse Events (CTCAE). The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov). Additionally, if assistance is needed, the NCI has an Index to the CTCAE that provides help for classifying and locating terms.

Step 3: Grade the event using the NCI CTCAE version specified in the protocol for reporting serious adverse events.

Step 4: Determine if the adverse event is Expected or an Exception to Expedited Reporting. **Expected** events are those that have been previously identified as resulting from administration of the agent and are listed in one of the following:

- The current NCI SPEER (Specific Protocol Exceptions to Expedited Reporting) for treatments using agents provided under an NCI-held IND, or an equivalent listing for treatments using agents provided under a Non-CTEP-held IND; located in Section 3.0 of the protocol.
- For treatments using commercial agents, the current CAEPR (Comprehensive Adverse Event and Potential Risks), ASAEL (Agent Specific Adverse Event List), or other list of expected toxicities located in Section 3.0 of the protocol, or the drug package insert.
- Exception to Expedited reporting located in Section 16.1f of the protocol.

An adverse event is considered **unexpected,** for expedited reporting purposes only, when either the type of event or the severity of the event is **not** listed in one of the areas outlined above.

Step 5: Determine whether the adverse event involved hospitalization or a prolongation of hospitalization (≥ 24 hours).

Step 6: Additionally, for commercial drugs, determine whether the adverse event is related to the protocol therapy. Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite. Consult the appropriate table for expedited reporting criteria for commercial agent(s).

NOTE: Any event that occurs more than 30 days after the last dose of study agent and is attributed (possible, probable, or definite) to the study agent(s) must be reported according to the instructions above and as outlined in the appropriate table in Section 16.1.

18.5 Participation Procedures for the International Collaborating Institutions

SWOG

A PHASE III, RANDOMIZED CLINICAL TRIAL OF STANDARD ADJUVANT ENDOCRINE THERAPY +/- CHEMOTHERAPY IN PATIENTS WITH 1-3 POSITIVE NODES, HORMONE RECEPTOR-POSITIVE AND HER2-NEGATIVE BREAST CANCER WITH RECURRENCE SCORE (RS) OF 25 OR LESS. RXPONDER: A CLINICAL TRIAL RX FOR POSITIVE NODE, ENDOCRINE RESPONSIVE BREAST CANCER

EudraCT 2012-000174-37

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CTEP – IND — NOTE: The CTEP IND is crossfiled against the docetaxel IND. However, the investigational aspect of the study is in regard to the use of the Oncotype DX® testing.

Appendix Overview

International sites should follow the main protocol except as detailed below.

Subject	Protocol Section	Appendix Section
Study Monitoring	**16.0**	**1.0**
Data Submission	**13.0 and 14.0**	**2.0**
Handling of Samples	**15.0**	**3.0**
Independent Ethics Committee	**16.0**	**4.0**
Archiving	**Not mentioned in protocol**	**5.0**
Compliance with National Cancer Institute (NCI) Guidelines for International Collaborations	**Not mentioned in protocol**	**6.0**

Treatment questions from Spanish sites should be directed to Emilio Alba Conejo, M.D., (oncologia98@yahoo.com, 34 951 032000). Non-treatment questions from Spanish sites should be directed to Ruth Campo (rcampo@geicam.org).

Treatment questions from French sites should be directed to Suzette Delaloge M.D., M. Sc., (suzette.delaloge@gustaveroussy.fr, 01 42 11 42 11). Non-treatment questions from French sites should be directed to Jerome Lemonnier (j-lemonnier@unicancer.fr).

1.0 STUDY MONITORING

Prior to the first patient registration, an Initiation Visit will be conducted onsite or via teleconference and the local approved consent form will be submitted to SWOG for approval.

International Collaborating Institutions are responsible for auditing their own institutions according to SWOG and NCI-CTMB Guidelines. Audit data will be provided in English to the SWOG QA Department who will enter the audit data into the NCI database.

2.0 REGISTRATION AND DATA SUBMISSION

Sites will follow instructions in Sections 13.0 and 14.0 of the main protocol with the following exceptions:

Sites must submit original pathology reports along with the English Translation Summary which is located on the **S1007** abstract page (www.swog.org).

Eligibility data are due within7 days after randomization. Subsequent treatment, adverse event and follow-up data submission vary; adherence to protocol requirements is expected.
SWOG Policies apply to GEICAM and UNICANCER's participation in **S1007**. Policy 33 describes Institutional Performance Review metrics and expectations. Failure to submit data as specified in the protocol may result in corrective action as described in the policy,
(https://swog.org/visitors/Download/Policies/Policy33.pdf).

3.0 HANDLING OF SAMPLES

Institutions are required to collect specimens as outlined in Section 15.0 of the protocol.

Institutions will submit tissue directly to Genomics Health for the Oncotype testing as outlined in Section 15.1 of the protocol.

Spanish institutions will submit tissue and blood to the GEICAM repository (Fundation Jimenez Diaz located in Madrid, Spain) for correlative studies and banking as per Section 15.2 of the protocol. French institutions will submit tissue and blood to the Centre de Ressource Biologique (located in Lyon, France) for correlative studies and banking per Section 15.2 of the protocol.

The GEICAM and UNICANCER repositories will batch ship the specimens **every 6 months** to the SWOG Repository at the following address:

SWOG Biospecimen Bank
Solid Tissue, Myeloma and Lymphoma Division
Nationwide Children's Hospital
700 Children's Drive, WA 1340
Columbus, OH 43205

4.0 INDEPENDENT ETHICS COMMITTEE

The study will be performed in accordance with the protocol, ICH-GCP, Competent Authorities regulations and the national laws applicable for conduct of clinical trials. The Coordinating Investigator has the responsibility to apply and receive acceptance and applications for the study from the Regional Ethics Committee and the Competent Authority, and to forward such acceptance to these parties.

5.0 ARCHIVING

In accordance with the U.S. regulations, essential documents must be maintained for at least 3 years after completion of the research (see SWOG Record Retention Guidance available at www.swog.org).

6.0 COMPLIANCE WITH NATIONAL CANCER INSTITUTE (NCI) GUIDELINES FOR INTERNATIONAL COLLABORATIONS

Participation in this study will take place in compliance with the National Cancer Institute's "Cooperative Group Guidelines for the Development, Conduct and Analysis of Clinical Trials with International Collaborating Institutions, Version 2.0". The guidelines have been satisfied as detailed below.

Monitoring the trial:

The study is monitored by the SWOG Data and Safety Monitoring Committee (DSMC) as described in Section 11.9 of the main protocol. Sites will be notified of DSMC recommendations and actions through postings on the SWOG website. The NCI CTEP guidelines applied to Phase III studies regarding rate of accrual will be followed.

Clinical study data analysis:

Data will be transmitted directly from each site to the SWOG Statistics and Data Management Center in Seattle. Each site transmitting data has an FWA or other OHRP-approved assurance.

Auditing of participating institutions:

The Clinical Trials Monitoring Branch (CTMB) Cooperative Group Audit Guidelines will be followed; all scheduling, preliminary reports and final reports will be reported to the SWOG QA Department which will enter audit findings into the CTMB Audit Information System (AIS). Each single participating site will have a separate report submitted through the CTMB-AIS. SWOG will be responsible for obtaining follow-up information and monitoring/reporting any disciplinary action, if required.

Logistical issues related to international collaborative clinical trials:

GEICAM has established the following Federal-Wide Assurance (FWA) with the Office for Human Research Protections (OHRR):

Foundation for GEICAM FWA00018352

UNICANCER has established the following Federal-Wide Assurance (FWA) with the office of for Human Research Protections (OHRP):

UNICANCER FWA00020555

Each registering institution will need to provide a link to an active FWA. The following pages of this section include all of the approved institutions and registering investigators for GEICAM and UNICANCER.

Notification of international involvement in group trials (Department of State clearance): SWOG and the CTEP Program Specialist received State Department approval of GEICAM's participation **effective 6/5/12**.

1572 Registrations: All registering investigators have submitted 1572s. Please note that the FDA 1572 document must be submitted to the National Cancer Institute's Pharmaceutical Management Branch for final approval before registration privileges can be finalized for investigators.

Adverse event reporting: Adverse events will be transmitted to AdEERS by the sites as designated in Section 16.1 of the main protocol. SWOG will notify sites of adverse events via postings on the SWOG website. CTEP will receive a copy of all MedWatch Forms submitted to the FDA or international drug agencies for adverse events related to commercial drugs.

Biological specimens: The procedures for biological specimen collection and shipment meet local regulations for the international collaborative group.

Protocol and informed consent translation: The collaborative groups version of the protocol and the translated version of the informed consent form are accepted by the Regional Ethics Committee and Competent Authority (CA).

Logistical issues specific to international collaborative clinical trials performed with European Union member states:

Ethics committee: A lead ethics committee has been designated, and it has reviewed and approved the conduct of the trial within the country of the international collaborative group. This has been submitted to the CTSU-RSS.

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18.6 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy **(U.S. INSTITUTIONS ONLY) – CELLSEARCH ANALYSES (Menarini Silicon Biosystems)**

Although endocrine therapy remains standard of care for patients with operable hormone receptor-positive (HR+) breast cancers (BCs), pts with HR+ BC may have a recurrence as a result of acquired resistance. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for approximately 50% of all BC recurrences. For instance, in a meta-analysis of 62,923 women with HR+ BC who were disease-free after 5 years of endocrine therapy, distant recurrence risk associated with tumor size and lymph node status, ranging from 10-40% up to 20 years from diagnosis. *(1)* Identifying markers that can predict late recurrence, such as blood-based biomarkers, remains an unmet need in this population.

a. Objectives

1. Primary
The presence of circulating tumor cells (CTC+) using CELLSEARCH will be assessed at up to 8 years after randomization in those still at risk for the primary outcome. Invasive disease-free survival (IDFS) will be compared between CTC+ versus CTC-, incorporating endocrine therapy use prior to and during follow-up after the CTC evaluation.

2. Secondary
To evaluate changes in CTC over time, i.e. a) baseline at up to 8 years after randomization and b) 2-3 years later after the initial blood draw on the substudy in those who have not yet had a recurrence. We will explore whether those patients for whom CTC status becomes CTC+, or who have additional mutations develop over time, develop a late recurrence at a rate that is different from those who do not.

3. Exploratory
We will explore the degree of heterogeneity of the CTC population.

b. Background

1. Late Recurrence

Hormone receptor positive (HR+)/HER2- breast cancers account for approximately 2/3 of breast cancers. Although endocrine therapy, such as selective estrogen receptor modulators (e.g., tamoxifen) and aromatase inhibitors, remain standard of care in the operable setting for patients with HR+ breast cancers, these patients may have a recurrence of their cancer, as a result of acquired resistance to endocrine therapies. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for approximately 50% of all breast cancer recurrences. In a meta-analysis involving 62,923 women with ER+ breast cancer who were disease-free after 5 years of endocrine therapy, the risk of distant recurrence strongly correlated with original tumor size and lymph node status, ranging from 10-40% up to 20 years *(1)*. We propose creating a biospecimen repository, including plasma and serum, for those who have not recurred, and to evaluate for blood-based markers that associate with a risk of late recurrence.

2. Circulating Tumor Cells (CTCs)

The presence of CTCs may have prognostic significance in this setting. Sparano et al. have reported the prognostic implications of CTCs in patients with HER2 negative breast cancer enrolled to **E5103** *(2)*. CTCs were enumerated using CELLSEARCH. Of the nearly 5000 patients enrolled to **E5103**, 547 patients agreed to have blood collected who were disease free at 4.5-7.5 years from initial diagnosis. While the CTC+ rate was low in the HR+ population (23/353 pts: 5.1%), a positive CTC assay was associated with a 10.8-fold higher risk of recurrence ($p<0.001$). In the multivariate analysis adjusted for important clinical and pathologic features, including age, tumor size, node status, and grade, a positive CTC assay was associated with a 13.1-fold increased risk of recurrence in HR+ breast cancer. The median time to recurrence was 2.8 years (0.1-2.8 years) among the CTC-positive patients. These data are intriguing and worth assessing in a high-risk, node positive cohort, such as **S1007**.

CellSearch

The CellSearch System employs immunomagnetic separation technology in which the blood specimen is incubated with magnetic beads coated with antibodies directed against the epithelial cell adhesion molecule (EpCAM) *(3)*. A magnet is used to isolate the immunomagnetically labeled epithelial cells. After immunomagnetic isolation, the cells are stained with fluorescently labeled anti-cytokeratin antibodies and the fluorescent nuclear stain DAPI. A fluorescently labeled pan-leukocyte monoclonal antibody is included as a counter-stain to discriminate contaminating white blood cells. Sample processing is fully automated. After isolation and staining, the CTCs are placed in a proprietary chamber for viewing on a semi-automated fluorescence microscope. Image analysis software pre-selects specific objects based on fluorescence staining patterns and intensities. The outcome/report is a quantitative analysis of the CTC contained in the blood specimen.

The CellSearch System has been shown to be accurate and reproducible *(4)*. Blood samples spiked with standardized numbers of cultured human breast cancer cells demonstrate a linear recovery over a range of 5 to 1,142 cells (correlation coefficient R2=0.99), with an average recovery of > 85% at each level. There is also strong agreement between duplicate samples (correlation coefficient R2=0.975) and between independent operators reviewing the same digital images (correlation coefficient R2=0.994).

c. Rationale and Hypothesis

The above findings support the design of utilizing plasma repositories from large adjuvant trials of endocrine therapy. A number of critical questions remain in early-stage breast cancer. For instance, if CTCs are identified in a patient who is still on adjuvant therapy without a recurrence, should we consider switching the systemic therapy to decrease the risk for late recurrence? Establishing a biorepository in this node positive population after 5 years of endocrine therapy offers a unique opportunity to evaluate whether we can identify blood-based predictors of late relapse.

While there are commercially available tumor-tissue based genomic tests looking at rate of distant relapse, including breast cancer index, these are looking at

baseline, pre-treatment samples. This substudy will look at real-time, on-treatment predictors, which may be more reflective of current tumor biology due to selective treatment pressure, dormancy escape, etc.

In addition, other previously described blood-based markers, such as serum tumor markers, in patients with breast cancer can be unreliable, including in patients with metastatic breast cancer. CTC enumeration and the association with risk of late relapse was selected as the primary outcome, given that preliminary data with CELLSEARCH in the adjuvant setting has been previously described, allowing for appropriately power calculations in this concept[2]. Ultimately, the goal of this project is to identify early predictors of dormancy escape and late recurrence in patients with operable breast cancer, which can serve as the basis for future, randomized, interventional trials.

We may be able to identify 3 groups of patients: 1) those who have not escaped dormancy (and may not need continued hormonal or other therapy), 2) those who are escaping dormancy and will relapse in the near future (and may need to modify treatment immediately, identifying the highest risk group for future clinical trial approach considerations), and 3) those who still are in dormancy and may experience a later relapse (and may need to switch hormonal therapy and/or add a new targeted treatment: again a population for future trial selection).

We hypothesize that invasive disease-free survival will be poorer in CTC-positive patients compared to those patients who are CTC-negative.

d. Experimental Approach and Assays

1. Sample Collection and Timepoints

At time of distribution of Revision #16 (Version Date: 03/24/21), all U.S. patients who a) are disease free, with no prior invasive recurrence AND b) have been on protocol for up to 8 years after time of randomization to **<u>S1007</u>** (Step 2 registration) must be offered participation in the Circulation Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) substudy.

SUBMISSION SUMMARY TABLE:

	CBALR Visit #1: Within 28 days after patient registration to Step 3	**CBALR Visit #2: 2-3 years after patient registration to Step 3**	**CBALR Visit #3: At time of Invasive Recurrence**
20 mL whole blood [a]	Two 10 mL CellSave® tubes [b, c] Ship to Menarini Silicon Biosystems (Lab # 122)	Two 10 mL CellSave® tubes [b, c] Ship to Menarini Silicon Biosystems (Lab # 122)	Two 10 mL CellSave® tubes [b, c] Ship to Menarini Silicon Biosystems (Lab # 122)

[a] Important: See Section 18.6d.4: Order of Sample Collection.

[b] If the minimum blood volume for **two** 10 mL CellSave® tubes cannot be collected during a visit, a subsequent second attempt at collection should be scheduled for another time. The subsequent draw time can be scheduled for later the same day (if appropriate and site has collection tubes on hand) or for another date. See Section 18.6d.4. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint. If the first draw attempt fails and the site does not have tubes on hand, then the site will need to: 1) reschedule the patient for a subsequent blood draw, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for that patient. Both CellSave® tubes must be collected on the same day. See Section 18.6d.5 for more information.

[c] If the second attempt at blood draw is unsuccessful in obtaining the minimum blood volume for **two** 10mL CellSave® tubes, the patient will be deemed not evaluable for the CBALR TM substudy. If the patient is deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

2. Sample Collection Kits

Specimen collection kits (CellSave® tubes and ambient gel pack) must be ordered IMMEDIATELY after patient registration to Step 3 and prior to each collection timepoint. Sites should allow 5-7 days to receive kits. Note: Patient samples must be drawn within 28 days after patient registration to Step 3.

CellSave® kits may be ordered by using the SWOG Biospecimen Bank Kit Management Application at: https://kits.bpc-apps.nchri.org.

The kit provides: Two CellSave® tubes and an ambient pak for blood submitted to Menarini Silcon Biosystems for a single collection timepoint.

3. Sample Collection and Submission Instructions

All sample submissions for this study must be entered and tracked using the SWOG online Specimen Tracking System. Complete sample collection and submission instructions can be accessed on the SWOG Specimen Submission webpage (https://www.swog.org/member-resources/biospecimen-resources). If collection/submission instructions differ from those in the protocol, the protocol instructions should be followed; otherwise, the website instructions should be followed.

4. **Order of Sample Collection:**

Substudy samples must be collected in the following order. **If samples are *not* collected in the following order, then one (or more) of the samples may need to be discarded**.

First scheduled draw for each annual visit:

Under fasting conditions:

- First, collect: Whole blood in a Red-Top, SST, Vacutainer tube

- Then, collect: Two 10-mL CellSave® tubes.

  NOTE: The 10 mL red-top tube must be obtained prior to filling the CellSave® tube using the same needle stick. This decreases the chance of contamination of the CellSave® sample with skin epithelial cells, which may occur when the needle enters the skin.

  Only the SST should be collected under fasting conditions. After collection of the SST, patients may eat or drink something.

- Then, collect: Four 10-mL Streck cfDNA tubes for future analysis, such as cfDNA assessment.

If the SST was collected at the first scheduled draw, and then the minimum blood volume for two 10 mL CellSave® tubes was not subsequently collected at the same time (after the SST was collected), then **for the subsequent attempt to draw one or both CellSave® sample(s),** collect samples in the following order:

- First, collect: One 10-mL Streck cfDNA tube (prior to filling the CellSave® tube using the same needle stick). If this happens, ship the first Streck cfDNA tube sample to the SWOG Biospecimen Bank.

- Then, collect: The remaining (one or two) 10-mL CellSave® tube(s). **NOTE:** While CellSave® tubes can be collected at different times throughout the day, both CellSave® tubes must be collected on the same day.

- Then, collect: three 10-mL Streck cfDNA tubes.

- Streck cfDNA tubes will be collected for future analyses, such as cfDNA assessment.

If there is a deviation from protocol order of collection, contact the study chair for guidance on which samples should still be submitted and document the order of collection in the “comments” section of the Specimen Tracking System.

5. CellSave® Tubes (Two Cellsave® tubes at each time point, ship directly to Menarini Silicon Biosystems – Lab #122 for CellSearch CTC testing)

a. Required materials for blood collection:
   i. Two (2), 10 mL purple/yellow top CellSave® blood collection tubes,
   ii. Vacutainer brand adapter, and
   iii. Needles.

b. See Section 18.6d.2 for CellSave® tube collection kit ordering instructions. The kit does not include an adapter or needles.

c. Collection Instructions:

   i. See Section 18.6d.4: Order of Sample Collection.

   Note: To prevent contamination of the CellSave® tube samples with epithelial cells, another tube must be collected *prior to* collection of the CellSave® tubes, so that the CellSave® tubes may be drawn from the same needle stick as the prior sample (either the SST at first draw attempt or a Streck cfDNA tube if a second attempt at drawing the CellSave® tube).

   ii. **Use the same needle stick as the prior tube drawn to collect the CellSave® samples.** This decreases the chance of contamination of the CellSave® sample with skin epithelial cells, which may occur when the needle enters the skin.

   iii. For each patient, perform a venous puncture using a Vacutainer brand adapter and needle and fill each of the blood collection tubes (minimum blood volume of 9 mL for each tube). Alternatively, blood samples may be obtained from a port or other central venous catheter using appropriate access needles and techniques. Invert each tube a minimum of eight (8) times to ensure proper mixing of the additives contained in each tube.

   iv. **Important Note:** Both (two) 10 mL CellSave® tubes are required for analysis. In the event that the 18 mL minimum blood volume (9 mL in each) in the **two** 10 mL tubes CellSave® tubes cannot be collected, **a subsequent second attempt at collection should be scheduled for another time.**

   While CellSave® tubes can be collected at different times throughout the day, both CellSave® tubes must be collected on the same day. See Section 18.6d.4. Do not submit either a single CellSave® tube or **two** CellSave® tubes collected on different days to Menarini Silicon Biosystems Labs.

   Note: The subsequent draw time can be scheduled for later the same day, if deemed appropriate and site has additional collection tubes on hand or can be rescheduled for another date. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint for each patient registered to Step 3. If the first draw attempt fails, and the site does

not have tubes on hand, then the site will need to 1) reschedule the patient for another day, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for the second draw attempt.

v. **<u>If at time of second attempt at blood draw</u>**, the blood draw is unsuccessful in obtaining the minimum 18 mL blood volume (9 mL in each) in the **<u>two</u>** 10 mL CellSave® tubes, then the patient will be deemed not evaluable for the CBALR TM substudy. If this occurs and the patient is deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood or tissue samples for the CBALR TM substudy.

vi. **The filled CellSave® tubes must be maintained at ambient (15–30°C) temperature, avoiding extremes of heat and cold, at all times.**

vii. Label the CellSave® tubes with:

- Number 1 and Number 2 (respectively, in order of collection). Record the lot number and expiration date for each corresponding tube in the Specimen Tracking System.
- SWOG patient number (patient ID)
- Patient initials
- Substudy visit number (time point) [Visit number = one, two, or three; i.e. one=initial blood draw on substudy, two= next blood draw 2-3 years later, three=invasive recurrence].
- Collection date and time (date and time the specimen was collected from the patient)
- Initials of the phlebotomist
- Specimen type (i.e., whole blood)

viii. The following information must be **<u>entered into the SWOG Specimen Tracking System prior to shipment</u>**:

- Site identification number;
- SWOG patient number (same number as written on the filled blood tubes);
- Site comments (i.e. phlebotomy problems; and any additional comments);
- Collection date and time of blood draw (if all blood samples were not drawn at the same time; specify time of draw of each sample);
- Order of blood sample collection; For CellSave® tubes, record the order of collection of (Number 1 or Number 2) as well as the lot number and expiration date for each corresponding CellSave® tube in the Specimen Tracking System;
- Lot number and expiration date of each corresponding CellSave® tube (Number 1 and Number 2).

d. Packaging and Shipping Instructions: **Ship directly to Menarini Silicon Biosystems (Lab #122)**

i. Cellsave® tubes must be shipped **ambient** (with an ambient gel pack) the same day as collected, via overnight delivery to: Menarini Silicon Biosystems (Lab # 122). If possible, collect and ship samples Monday-Thursday. **Packages shipped on a Friday, must be sent via Fed-Ex Saturday Delivery, with no signature required. Do not collect samples the day prior to a holiday.**

ii. Wrap the CellSave® tubes in the shipping blanket. This gives added thermal protection during shipment. **Place the ambient gel packs in the box to stabilize the temperature at 15-30ºC.** Place the Styrofoam lid, and seal the Styrofoam box.

iii. **All shipments must include a requisition form (Packing list) generated by the SWOG Specimen Tracking System.** Place the completed Packing List (generated from the SWOG Specimen Tracking System) into the shipper box.

e. Questions pertaining to Cellsave® collection or shipping should be directed to:

Menarini Silicon Biosystems Labs (Lab #122)
msb-labservicesus@siliconbiosystems.com
Phone: 215/346-8499
Fax: 215/560-3730
Customer Service Hours of Operation: M-F, 8:00am – 5:00pm ET

6. CTC methods

a. CellSearch Systems

i. Operator Training: System Operators will be training at Menarini Silicon Biosystems. Follow-up examinations will be given to document operator proficiency. Training manuals and documentation of training will be maintained at Menarini Silicon Biosystems.

ii. Image interpretation and enumeration of CTCs: Determination of CTC counts will be made by trained operators. Circulating tumor cells are identified based on analysis of results from the CellTracks Analyzer II. Tumor cells will be identified by the instrument software, and objects will be confirmed visually by the operator.

iii. Quality Control of the AutoPrep and CTC analysis systems is maintained via Operator Training procedures, daily, weekly and monthly maintenance of systems, use of a two-level control cell sample for AutoPrep according to standard protocols as described in the Operator Manuals.

iv. Assay Procedures

a. Upon specimen arrival at the laboratory, the laboratory personnel will ensure that one of the

two tubes of blood collected will be tested and the other stored in case it is required due to any circumstances leading to the inability to test the first tube. This will mitigate the potential of being unable to perform a CTC count at each blood draw.

b. The sample will be processed within 96 hours of collection using the CellSearch® System.

c. All patient specimens are to be assayed using the CellSearch Assay as per the manufacturer's instructions. CTCs will be isolated using the CellTracks AutoPrep sample processor. A volume of 7.5 mL from the sample will be diluted with buffer and centrifuged for processing on the AutoPrep system. Plasma is withdrawn and additional buffer added. Epithelial cell-specific immunomagnetic particles (EpCAM-ferrofluid) is added and incubated for 30 minutes at room temperature. Unbound sample is then aspirated while the sample is in a magnetic field. Buffer is added and the sample is mixed and separated in a magnetic field. Supernatant is removed and a permeabilizing agent added, followed by a nucleic acid dye, anti-cytokeratin (a marker of epithelial cells), and anti- CD45. The specimen is mixed and incubated for 15 minutes. The sample is washed with buffer, magnetically separated, and labeled cells are fixed. Multiple lots of CellSearch® Reagents and Control materials will be used in the performance of this clinical trial.

e. Statistical Design

We anticipate that 8.9% of patients without a recurrence at substudy enrollment will develop a late recurrence in the follow-up period[1]. We will be collecting and analyzing blood samples on all patients, as we will otherwise not be able to prospectively predict who will develop a late recurrence. Furthermore, CTC evaluation by CELLSEARCH must be done in real time rather than use stored specimens once case/control status is known. Among the 1,951 U.S. patients still being followed and who do not have a recurrence, we assume that we may recruit 890 patients for at least a single blood submission. Allowing for a CTC assay failure of 10%, then 800 patients would have assay results. We anticipate conservatively that 3% of the 800 patients will be CTC+ (n=24) and 97% will be CTC- (n=776) post-endocrine therapy initiation[2]. The estimated number of invasive disease-free survival events after an additional median 4 years of follow-up (from time of blood collection) in 800 patients is 71 using an overall hazard rate of 0.231. With a two-sided alpha of 0.05, power is 80% to detect a hazard ratio of 3.3 for CTC-positive versus CTC-negative. This is a conservative estimate given that E5103 had a 5.1% positivity rate and a 13.1 hazard rate in HR+ breast cancer. This will still allow sufficient power for a lower positivity rate than 3% or greater difficulty in patient recruitment as long as the hazard ratio is high.

The actual analysis planned is a Cox regression on IDFS starting at the time of blood collection. We believe it is important to control for time since initial

randomization and initial treatment assignment. It is also important to include use of endocrine therapy as a time-dependent covariate, as there will some variability in terms of whether patients extend beyond 5 years of endocrine therapy. The ultimate goal is to determine who may have benefited from extended endocrine therapy given CTC determination, but power will be lower for making this assessment.

f. Data Analysis

The data will be analyzed by the SWOG Statistics and Data Management Center along with the investigators of **S1007**. The **S1007** primary endpoint must be published prior to reporting results of the Circulating Biomarker Assessment for Late Relapse TM substudy.

g. References

1. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017.
2. Sparano J, O'Neill A, Alpaugh K, et al: Association of Circulating Tumor Cells With Late Recurrence of Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol. 2018 Jul 26. doi: 10.1001/jamaoncol.2018.2574.
3. Zhang QX, Borg A, Wolf DM, et al: An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. Cancer Res 57:1244-9, 1997.
4. Segal CV, Dowsett M: Estrogen receptor mutations in breast cancer--new focus on an old target. Clin Cancer Res 20:1724-6, 2014.
5. Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, et al: Prognostic role of PIK3CA mutations of cell-free DNA in early-stage triple negative breast cancer. Cancer Sci 106:1582-9, 2015.

18.7 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy **(U.S. INSTITUTIONS ONLY) – EPIC SCIENCES CTC DETECTION PLATFORM**

Although endocrine therapy remains standard of care for patients with operable hormone receptor-positive (HR+) breast cancers (BCs), pts with HR+ BC may have a recurrence as a result of acquired resistance. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for approximately 50% of all BC recurrences. For instance, in a meta-analysis of 62,923 women with HR+ BC who were disease-free after 5 years of endocrine therapy, distant recurrence risk associated with tumor size and lymph node status, ranging from 10-40% up to 20 years from diagnosis. *(1)* Identifying markers that can predict late recurrence, such as blood-based biomarkers, remains an unmet need in this population.

a. Objectives

1. Primary

The presence of circulating tumor cells (CTC+) will be assessed at up to 8 years after randomization in those still at risk for the primary outcome. Invasive disease-free survival (IDFS) will be compared between CTC+ versus CTC-, incorporating endocrine therapy use prior to and during follow-up after the CTC evaluation.

2. Secondary

To evaluate changes in CTC over time, i.e. a) baseline at up to 8 years after randomization and b) 2-3 years later after the initial blood draw on the substudy in those who have not yet had a recurrence. We will explore whether those patients for whom CTC status becomes CTC+, or who have additional mutations develop over time, develop a late recurrence at a rate that is different from those who do not.

3. Exploratory

In addition, we will explore the degree of heterogeneity of the CTC population.

b. Background

1. Late Recurrence

Hormone receptor positive (HR+)/HER2- breast cancers account for approximately 2/3 of breast cancers. Although endocrine therapy, such as selective estrogen receptor modulators (e.g., tamoxifen) and aromatase inhibitors, remain standard of care in the operable setting for patients with HR+ breast cancers, these patients may have a recurrence of their cancer, as a result of acquired resistance to endocrine therapies. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for approximately 50% of all breast cancer recurrences. In a meta-analysis involving 62,923 women with ER+ breast cancer who were disease-free after 5 years of endocrine therapy, the risk of distant recurrence strongly correlated with original tumor size and lymph node status, ranging from 10-40% up to 20 years *(1)*. We propose creating a biospecimen repository, including plasma, for those who have not recurred, and to evaluate for blood-based markers that associate with a risk of late recurrence.

2. Circulating Tumor Cells (CTCs)

The presence of CTCs may have prognostic significance in this setting. Sparano et al. have reported the prognostic implications of CTCs in patients with HER2 negative breast cancer enrolled to **E5103** *(2)*. CTCs were enumerated using CELLSEARCH. Of the nearly 5000 patients enrolled to **E5103**, 547 patients agreed to have blood collected who were disease free at 4.5-7.5 years from initial diagnosis. While the CTC+ rate was low in the HR+ population (23/353 pts: 5.1%), a positive CTC assay was associated with a 10.8-fold higher risk of recurrence ($p<0.001$). In the multivariate analysis adjusted for important clinical and pathologic features, including age, tumor size, node status, and grade, a positive CTC assay was associated with a 13.1-fold increased risk of recurrence in HR+ breast cancer. The median time to recurrence was 2.8 years (0.1-2.8 years) among the CTC-positive patients. These data are intriguing and worth assessing in a high-risk, node positive cohort, such as **S1007**.

Epic Sciences
The Epic Sciences CTC detection platform has been analytically validated *(3)* and has the ability to assess clonality and cell morphology, to determine the degree of heterogeneity of the CTC population, and to apply downstream assays such as copy number variation (CNV), similar to gene status reporting via fluorescence in-situ hybridization (FISH) and biomarker assessment (ex. PD-L1 expression) at the single cell level *(4).* The technology falls under the selection free immunofluorescence-based assay category, in which the entire buffy coat containing peripheral blood WBCs and CTCs is spread onto slides, and all cells undergo immunofluorescence staining for the markers of interest. CTCs are identified based on staining patterns as well as morphology. CTC-based biomarker assays have subsequently been utilized in research trials. Additionally, the AR-V7 Nucleus Detect assay has been validated and is clinically accessible through Epic Sciences' commercial access partner, Exact Sciences. Using the Epic platform, Scher et al. recently showed that the degree of CTC phenotype heterogeneity can inform treatment decisions for metastatic castrate resistant prostate cancer patients, with differential responses in the low and high heterogeneity groups to taxane versus androgen receptor signaling inhibitor therapy *(5)*.

c. Rationale and Hypothesis

The above findings support the design of utilizing liquid biopsy repositories from large adjuvant trials of endocrine therapy. A number of critical questions remain in early-stage breast cancer. For instance, if CTCs are identified in a patient who is still on adjuvant therapy without a recurrence, should we consider switching the systemic therapy to decrease the risk for late recurrence? Establishing a biorepository in this node positive population after 5 years of endocrine therapy offers a unique opportunity to evaluate whether we can identify blood-based predictors of late relapse.

While there are commercially available tumor-tissue based genomic tests looking at rate of distant relapse, including breast cancer index, these are looking at baseline, pre-treatment samples. This substudy will look at real-time, on-treatment predictors, which may be more reflective of current tumor biology due to selective treatment pressure, dormancy escape, etc.

In addition, other previously described blood-based markers, such as serum tumor markers, in patients with breast cancer can be unreliable, including in patients with metastatic breast cancer. Ultimately, the goal of this project is to identify early predictors of dormancy escape and late recurrence in patients with operable breast cancer, which can serve as the basis for future, randomized, interventional trials.

We may be able to identify 3 groups of patients: 1) those who have not escaped dormancy (and may not need continued hormonal or other therapy), 2) those who are escaping dormancy and will relapse in the near future (and may need to modify treatment immediately, identifying the highest risk group for future clinical trial approach considerations), and 3) those who still are in dormancy and may experience a later relapse (and may need to switch hormonal therapy and/or add a new targeted treatment: again a population for future trial selection).

We hypothesize that invasive disease-free survival will be poorer in CTC-positive patients compared to those patients who are CTC-negative.

d. Experimental Approach and Assays

1. Sample Collection and Timepoints

At time of distribution of Revision #16 (Version Date 03/24/21), all U.S. patients who a) are disease free, with no prior invasive recurrence AND b) have been on protocol for up to 8 years after time of randomization to **S1007** (Step 2 registration) must be offered participation in the Circulation Biomarker Assessment for Late Relapse Translational Medicine (CBALR TM) substudy.

SUBMISSION SUMMARY TABLE:

	CBALR Visit #1: **Within 28 days after patient registration to Step 3**	**CBALR Visit #2:** **2-3 years after patient registration to Step 3**	**CBALR Visit #3:** **At time of Invasive Recurrence**
20 mL whole blood [a]	Two 10 mL Streck cfDNA tubes Ship 2 tubes to Epic Sciences Lab #236 [b, c]	Two 10 mL Streck cfDNA tubes Ship 2 tubes to Epic Sciences Lab #236 [b, c]	Two 10 mL Streck cfDNA tubes Ship 2 tubes to Epic Sciences Lab #236 [b]

[a] See Section 18.7d.4a. Patients may eat or drink something prior to blood draw for the Streck cfDNA tubes.

[b] If the minimum blood volume for two 10 mL Streck cfDNA tubes (being shipped to Epic Sciences) cannot be collected during a visit, a subsequent second attempt at collection should be scheduled for another time. The subsequent draw time can be scheduled for later the same day (if appropriate and site has collection tubes on hand) or for another date. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint. If the first draw attempt fails and the site does not have tubes on hand, then the site will need to: 1) reschedule the patient for a subsequent blood draw, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for that patient. See Section 18.7d.4c for more information.

[c] If the patient is subsequently deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

Streck tubes will be collected for future analyses, such as cfDNA assessment.

2. Sample Collection Kits

Specimen collection kits (Streck cfDNA tubes and ambient gel packs) must be ordered IMMEDIATELY after patient registration to Step 3 and prior to each collection timepoint.

Sites should allow 5-7 days to receive kits. Note: Patient samples must be drawn within 28 days after patient registration to Step 3.

Streck cfDNA kits may be ordered by using the SWOG Biospecimen Bank Kit Management Application at: https://kits.bpc-apps.nchri.org.

The kit provides: Two Streck cfDNA tubes and an ambient pak for blood submitted to Epic Sciences Lab for a single collection timepoint.

3. Sample Collection and Submission Instructions

All sample submissions for this study must be entered and tracked using the SWOG online Specimen Tracking System. Complete sample collection and submission instructions can be accessed on the SWOG Specimen Submission webpage (https://www.swog.org/member-resources/biospecimen-resources). If collection/submission instructions differ from those in the protocol, the protocol instructions should be followed; otherwise, the website instructions should be followed.

4. Streck Cell-Free DNA Collection Tubes (2 Streck cfDNA Tubes will be submitted to Epic Sciences (Lab #236)):

a. Prior to Collection

- Patients are not required to fast prior to Streck cfDNA blood draw. Patients may eat or drink something prior to blood draw into Streck cfDNA tubes.
- See Section 18.7d.2 for ordering instructions for Streck cfDNA tube collection kits.
- Confirm blood tubes are not expired. Expired tubes should not be used for blood collection.
- Schedule courier for same-day sample pick-up prior to collection.

b. Instructions for handling Streck cfDNA tubes during collection: Prevention of Backflow: Since Streck Cell-Free DNA BCT tubes contain chemical additives, it is important to avoid possible backflow from the tube.

To guard against backflow, observe the following precautions:
- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper uppermost.
- Release tourniquet once the blood starts to flow into the tube, or within 2 minutes of application.
- Tube contents should not touch stopper or the end of the needle during the collection procedure.

c. Additional Blood Collection Instructions:

- Draw whole blood sample into four (4), 10 mL Streck Cell-Free DNA BCT tubes. Fill tube until blood flow stops.

- **IMPORTANT:** Fill each tube completely (10 mL), when possible.

- For the 2 tubes being shipped to Epic Sciences (Lab # 236), a minimum of 4 mL blood per tube is required (at least 8 mL total volume collected in two 10 mL Streck cfDNA tubes). In the event that 8 mL blood volume (4 mL blood per tube) cannot be collected, do not submit Streck cfDNA tube samples to Epic Sciences. **A subsequent second attempt at collection should be scheduled for another time.**

- Note: The subsequent draw time can be scheduled for later the same day, if deemed appropriate and site has additional collection tubes on hand or can be rescheduled for another date. Due to funding restrictions and tube expiration dates, the SWOG Biospecimen Bank will only ship sufficient tubes for a single collection timepoint for each patient registered to Step 3. If the first draw attempt fails, and the site does not have tubes on hand, then the site will need to 1) reschedule the patient for another day, allowing 5-7 days for collection kit shipment, and 2) re-order tubes for the second draw attempt.
- If the patient is subsequently deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

- Approximate volumes are illustrated below. Each red arrow indicates the level to which the blood collection tube should be filled to achieve the corresponding volume in red, yellow, or blue. As a reference, a volume of 6-mL would fill the Streck cfDNA tube to just below the first "7" in the blood tube lot number "72750315" on the blood tube label.

- Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Tube inversion prevents clotting. **<u>Inadequate or delayed mixing may result in inaccurate test results</u>.**

- **After collection, do <u>not</u> refrigerate or freeze blood in Streck cfDNA tubes**, as this will compromise the specimen. Blood collected in Streck cfDNA tubes is stable at room temperature.

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d. Labelling and Shipping Instructions

1. Label blood tubes with the following:

    - SWOG patient number (patient ID)
    - Patient initials
    - Substudy visit number (time point) [Visit number = one, two, or three; i.e. one=within 28 days after patient registration to Step 3, two= 2-3 years after patient registration to Step 3, three=invasive recurrence].
    - Collection date and time (date and time the specimen was collected from the patient)
    - Initials of the phlebotomist (Epic Sciences tubes only)
    - Specimen type (i.e., whole blood)

2. Two (2) Streck cfDNA tubes will be shipped to Epic Sciences (Lab # 236) per the following instructions:

    - Specimens must be shipped with an **ambient pack** (provided in the Streck cfDNA tube kit).
    - All shipments must include a requisition form (Packing list) generated by the SWOG Specimen Tracking System. Print a copy of the Packing List in the online SWOG Specimen Tracking System. Place the Packing List in the pocket of the specimen bag if it has one, or in a separate resealable bag.
    - **IMPORTANT NOTE:** If a collection time is not provided, Epic Sciences will default to sample collection at 8:00 am (local time) on the date of collection.
    - Do not place “Infectious Substance” sticker on shipper, as this can result in a delay of shipment. If possible, include a scanned copy of the completed sample requisition form.

e. For questions pertaining to the **two Streck cfDNA tubes being shipped to Epic Sciences Lab** contact:

Lab #236: Epic Sciences
Email: partners@epicsciences.com / Attn: **S1007**

5. CTC methods

Epic Systems

a. The Epic Sciences CTC platform is a multi-component system utilizing automated staining and fluorescent scanning instrumentation to detect and characterize nucleated cells isolated from patient blood samples. Peripheral blood samples are collected via standard butterfly needle into Streck Cell-Free DNA blood collection tubes containing protein fixatives. Upon sample receipt, plasma is isolated and nucleated cells are isolated from whole blood via red blood cell lysis and subsequently deposited onto functionalized glass slides. Plasma and slides can be either immediately stained or banked in -80 C for future testing.

b. Immunofluorescent staining of deposited cells utilizes a primary antibody cocktail enabling the detection of all nucleated cells with DAPI staining, the detection and distinction of cytokeratin (CK)-positive CTCs from CD45-positive white blood cells, and the identification of a fourth protein biomarker as specified by project (e.g., ER, PR, HER2). Appropriate anti-species secondary antibodies conjugated to fluorescent dyes allow for multiplexed visualization of DAPI, CK, CD45 and an additional biomarker.

c. All cells on a slide are imaged by automated fluorescent scanners, and the images are subsequently fed into proprietary software algorithms that quantify fluorescence intensities and morphologic features to distinguish and characterize rare cells from normal WBCs. WBCs are categorized by CD45 positivity and associated morphologic features, while rare cells and CTCs are categorized by cytokeratin positivity and/or fourth channel biomarker positivity and CD45 negativity. Trained operators review the algorithmic classifications to produce a final report that includes total cell counts, CTC count, fluorescent staining intensities and various morphologic features including nuclear and cytoplasmic areas. If desired, each cell of interest can be relocated and individually recovered via a micromanipulator for single-cell genomic analysis.

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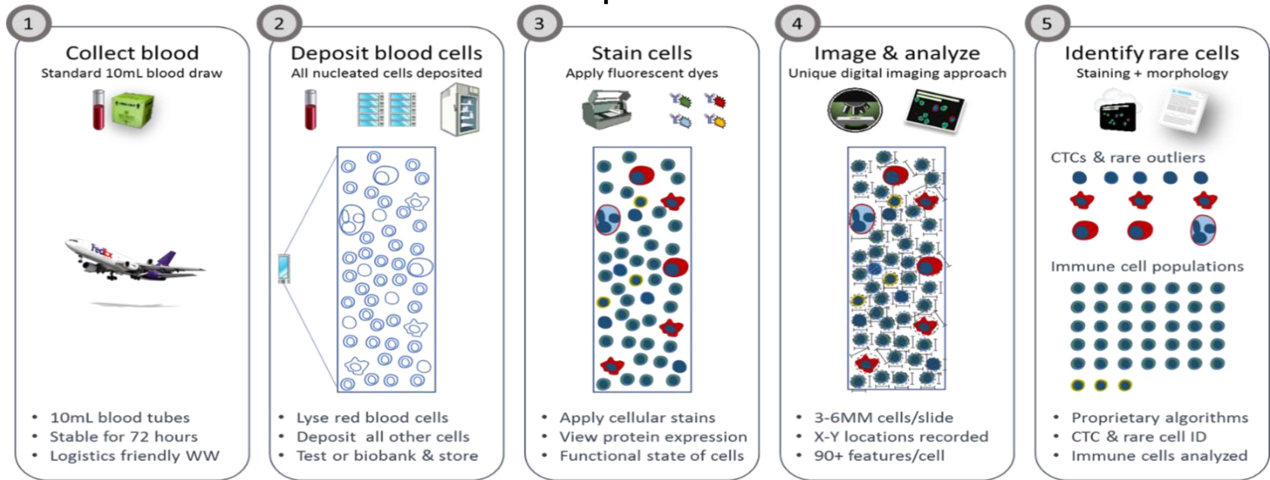
Overview of Epic Sciences Process for CTC Identification



e. Statistical Design

We anticipate that 8.9% of patients without a recurrence at substudy enrollment will develop a late recurrence in the follow-up period[1]. We will be collecting and analyzing blood samples on all patients, as we will otherwise not be able to prospectively predict who will develop a late recurrence. Among the 1,951 U.S. patients still being followed and who do not have a recurrence, we assume that we may recruit 890 patients for at least a single blood submission. Allowing for a CTC assay failure of 10%, then 800 patients would have assay results. We anticipate conservatively that 3% of the 800 patients will be CTC+ (n=24) and 97% will be CTC- (n=776) post-endocrine therapy initiation[2]. The estimated number of invasive disease-free survival events after an additional median 4 years of follow-up (from time of blood collection) in 800 patients is 71 using an overall hazard rate of 0.231. With a two-sided alpha of 0.05, power is 80% to detect a hazard ratio of 3.3 for CTC-positive versus CTC-negative. This is a conservative estimate given that E5103 had a 5.1% positivity rate and a 13.1 hazard rate in HR+ breast cancer. This will still allow sufficient power for a lower positivity rate than 3% or greater difficulty in patient recruitment as long as the hazard ratio is high.

The actual analysis planned is a Cox regression on IDFS starting at the time of blood collection. We believe it is important to control for time since initial randomization and initial treatment assignment. It is also important to include use of endocrine therapy as a time-dependent covariate, as there will some variability in terms of whether patients extend beyond 5 years of endocrine therapy. The ultimate goal is to determine who may have benefited from extended endocrine therapy given CTC determination, but power will be lower for making this assessment.

The kappa statistic will be used to assess agreement between the two systems when dichotomized into positive and negative.

f. Data Analysis

The data will be analyzed by the SWOG Statistics and Data Management Center along with the investigators of **S1007**. The **S1007** primary endpoint must be published prior to reporting results of the Circulating Biomarker Assessment for Late Relapse TM substudy.

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g. References

1. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017.
2. Sparano J, O'Neill A, Alpaugh K, et al: Association of Circulating Tumor Cells With Late Recurrence of Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol. 2018 Jul 26. doi: 10.1001/jamaoncol.2018.2574.
3. Segal CV, Dowsett M: Estrogen receptor mutations in breast cancer--new focus on an old target. Clin Cancer Res 20:1724-6, 2014.
4. Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, et al: Prognostic role of PIK3CA mutations of cell-free DNA in early-stage triple negative breast cancer. Cancer Sci 106:1582-9, 2015.
5. Diehn M, Alizadeh AA, Adams H-P, et al: Early prediction of clinical outcomes in resected stage II and III colorectal cancer (CRC) through deep sequencing of circulating tumor DNA (ctDNA). Journal of Clinical Oncology 35:3591-3591, 2017.

18.8 Circulating Biomarker Assessment for Late Relapse in Patients with Node-Positive, Hormone-Receptor Positive, Her2 Negative, Operable Breast Cancer Translational Medicine Substudy **(U.S. INSTITUTIONS ONLY) – NON-CTC ANALYSES – FUTURE ANALYSES**

Although endocrine therapy remains standard of care for patients with operable hormone receptor-positive (HR+) breast cancers (BCs), pts with HR+ BC may have a recurrence as a result of acquired resistance. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for approximately 50% of all BC recurrences. For instance, in a meta-analysis of 62,923 women with HR+ BC who were disease-free after 5 years of endocrine therapy, distant recurrence risk associated with tumor size and lymph node status, ranging from 10-40% up to 20 years from diagnosis. *(1)* Identifying markers that can predict late recurrence, such as blood-based biomarkers, remains an unmet need in this population. Blood specimens for future cfDNA analyses will be collected at the same time as CTC specimens. The analytic plan for these specimens has not yet been developed and will be submitted to CTEP for approval in the future. Below we outline the steps needed for future analysis.

a. Primary Objective

To collect and store blood specimens so that we can assess whether subsequent invasive disease-free survival depends on cfDNA status. The primary objective is to bank blood specimens from the blood draw at a baseline that is up to 8 years post-randomization and then 2-3 years later to be used in future TM proposals.

b. Future objectives to be submitted to CTEP (Secondary to analyses, specified above and CTC analyses in Sections 18.6 and 18.7)

1. To determine the frequency of serial cell free DNA (cfDNA) mutation when assessed at the same time as CTC and whether it is associated with subsequent IDFS.

2. To determine the frequency of mutations, including ESR1, PIK3CA, AKT1 (E17K), and p53, and other alterations as measured by cfDNA and subsequent association with IDFS.

3. To assess the molecular evolution of mutation status by comparing the mutations identified with the following: a) primary tumor, b) cfDNA at the time of recurrence, and c) recurrence tumor tissue.

4. To assess for associations between serum-based markers of the insulin growth factor pathway and subsequent IDFS. Other markers, such as inflammatory markers (c-reactive protein), will be explored. We note that pre-randomization blood specimens are available as well.

c. Background

1. Late Recurrence

Hormone receptor positive (HR+)/HER2- breast cancers account for approximately 2/3 of breast cancers. Although endocrine therapy, such as selective estrogen receptor modulators (e.g., tamoxifen) and aromatase inhibitors, remain standard of care in the operable setting for patients with HR+ breast cancers, these patients may have a recurrence of their cancer, as a result of acquired resistance to endocrine therapies. Recurrences that occur 5 or more years after diagnosis (i.e., late relapse) account for

approximately 50% of all breast cancer recurrences. In a meta-analysis involving 62,923 women with ER+ breast cancer who were disease-free after 5 years of endocrine therapy, the risk of distant recurrence strongly correlated with original tumor size and lymph node status, ranging from 10-40% up to 20 years *(1)*. We propose creating a biospecimen repository, including plasma and serum, for those who have not recurred, and to evaluate for blood-based markers that associate with a risk of late recurrence.

2. Cell-free DNA (cfDNA)

Several other assays are available that may detect occult tumor burden. These include collecting plasma and assessing for cfDNA. The majority of mutation detection work with cfDNA has been in the metastatic setting. As an example, mutations in the ESR1 gene, first reported in 1997 *(2)*, have been reported in ~ 20% of recurrent breast cancer patients previously treated with endocrine therapy. These mutations are infrequently detected in the primary breast tumor (~ 2%) *(3)*. Four hot-spot mutations contribute to ~ ¾ of ESR1 acquired mutations identified in the recurrent setting (D538G, Y537S/N/C)4. In a small study in early-stage triple negative breast cancer, the rate of PIK3CA mutations in cfDNA was 24.4% *(4)*. In other cancer types, including colorectal cancer, the identification of cfDNA in Stage II (5% prevalence) and stage III (16% prevalence) colorectal cancer has prognostic implications6. In fact, there is a proposed NRG adjuvant colorectal study (CR1643), randomizing patients with Stage II colorectal cancer to chemotherapy (Folfox) vs. observation based upon cfDNA status.

Numerous recent reports have demonstrated the detection of mutant DNA alleles as tumor-specific markers in cfDNA, including a number of prospective-retrospective analyses of ESR1 mutations in randomized-controlled trials. Bolero-2 was a double-blind, placebo-controlled metastatic trial in patients who previously progressed on a non-steroidal aromatase inhibitor (NSAI) who were randomized to the steroidal AI exemestane with or without everolimus. The presence of ESR1 D537S and D538G mutations had a significantly worse prognosis (20 months) compared to ESR1 wild type tumors (32 months) *(5)*. Patients with D538G ESR1 mutations experienced clinical benefit from everolimus, while Y537S mutation did not. While the overall rate of ESR1 mutation detection at the time of study entry was ~ 30%, the rate was higher in those who had previously received AI for treatment in the metastatic setting (33%) compared to those who went onto study after receiving AI therapy only in the adjuvant setting (11%). In the SoFEA trial, women whose cancer had progressed after a period of sensitivity to an NSAI, defined as recurrence after at least 12 months of adjuvant NSAI or disease progression after at least 6 months of first-line metastatic treatment with an NSAI, were randomly assigned to be administered exemestane, fulvestrant 250 mg, or the combination of anastrozole and fulvestrant. Patients with ESR1 mutations (~40% of those evaluated) had an improved progression free survival (PFS) receiving fulvestrant compared to exemestane (p=0.02). ESR1 wild type did just as well on either arm5. These data demonstrate the potential advantage of evaluating liquid biopsies.

3. Rationale and Hypothesis

The above findings support the design of utilizing plasma repositories from large adjuvant trials of endocrine therapy. A number of critical questions remain in early-stage breast cancer. Is there a role for detection of ESR1 mutations, or other frequent mutations such as PIK3CA mutations, during adjuvant AI therapy? Will the ESR1 mutation status results observed in the metastatic Bolero-2 trial (endocrine therapy +/- everolimus) be recapitulated in the operable setting (endocrine +/- everolimus)? If ESR1 mutations are identified, should these patients be switched to an alternative adjuvant therapy, such as a selective estrogen receptor downregulator (SERD)? Perhaps, switching strategies or combination of hormone therapy with a SERD should be utilized to overcome endocrine resistance in operable breast cancer? Establishing a biorepository in this node positive population after 5 years of endocrine therapy offers a unique opportunity to evaluate whether we can identify blood-based predictors of late relapse.

While there are commercially available tumor-tissue based genomic tests looking at rate of distant relapse, including breast cancer index, these are looking at baseline, pre-treatment samples. This substudy will look at real-time, on-treatment predictors, which may be more reflective of current tumor biology due to selective treatment pressure, dormancy escape, etc.

In addition, other previously described blood-based markers, such as serum tumor markers, in patients with breast cancer can be unreliable, including in patients with metastatic breast cancer. Ultimately, the goal of this project is to identify early predictors of dormancy escape and late recurrence in patients with operable breast cancer, which can serve as the basis for future, randomized, interventional trials.

We may be able to identify 3 groups of patients: 1) those who have not escaped dormancy (and may not need continued hormonal or other therapy), 2) those who are escaping dormancy and will relapse in the near future (and may need to modify treatment immediately, identifying the highest risk group for future clinical trial approach considerations), and 3) those who still are in dormancy and may experience a later relapse (and may need to switch hormonal therapy and/or add a new targeted treatment: again a population for future trial selection).

d. Experimental Approach and Assays

1. Sample Collection Timepoints

SUBMISSION SUMMARY TABLE:

	CBALR Visit #1: Within 28 days after patient registration to Step 3	**CBALR Visit #2: 2-3 years after patient registration to Step 3**	**CBALR Visit #3: At time of Invasive Recurrence**
Metastatic Tissue (if applicable and where available)			FFPE block or 20 unstained slides Ship to SWOG Biospecimen Bank
10 mL whole blood [a]	One 10 mL SST Ship to SWOG Biospecimen Bank	One 10 mL SST Ship to SWOG Biospecimen Bank	One 10 mL SST Ship to SWOG Biospecimen Bank
20 mL whole blood [a]	Two 10 mL Streck cfDNA tubes: Ship 2 Streck cfDNA tubes to SWOG Biospecimen Bank (Lab #201) [b, c]	Two 10 mL Streck cfDNA tubes: Ship 2 Streck cfDNA tubes to SWOG Biospecimen Bank (Lab #201) [b, c]	Two 10 mL Streck cfDNA tubes: Ship 2 Streck cfDNA tubes to SWOG Biospecimen Bank (Lab #201) [b, c]

[a] Important: See Section 15.3c.1: Order of Sample Collection. Samples should be collected into the SST under fasting conditions.

[b] If one 10 mL SST cannot be collected, a subsequent second attempt at collection should be scheduled for another time (Note: the subsequent draw time can be scheduled for later the same day, if deemed appropriate or can be rescheduled for another date.) If the two 10 mL Streck cfDNA tubes being shipped to the SWOG Biospecimen Bank (most likely the third and fourth Streck cfDNA tube draws) cannot be collected, a second attempt at collection of the two Streck cfDNA tubes being shipped to the SWOG Biospecimen Bank is not required. See Section 15.3c.4b for more information on Streck cfDNA requirements.

[c] If the patient is subsequently deemed not evaluable for the CBALR TM substudy, do not submit any subsequent blood samples for the CBALR TM substudy.

2. Correlative biomarker testing of above-referrenced specimens banked for planned future research will not occur until an amendment to this protocol (or separate correlative science protocol) is reviewed and approved in accordance with National Clinical Trials Network (NCTN) policies.

e. Data Analysis

These future analyses of cfDNA are planned. Given the tumor-based analyses being conducted by Exact Sciences Corporation (Section 18.2), including whole exome sequencing, the plan will be to conduct cfDNA analyses with a bespoke assay in collaboration with Exact Sciences Corporation. These analyses will not take place until the forthcoming statistical plan is reviewed and approved by CTEP.

f. References

1. Pan H, Gray R, Braybrooke J, et al: 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 377:1836-1846, 2017.
2. Zhang QX, Borg A, Wolf DM, et al: An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. Cancer Res 57:1244-9, 1997.
3. Segal CV, Dowsett M: Estrogen receptor mutations in breast cancer--new focus on an old target. Clin Cancer Res 20:1724-6, 2014.
4. Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, et al: Prognostic role of PIK3CA mutations of cell-free DNA in early-stage triple negative breast cancer. Cancer Sci 106:1582-9, 2015.
5. Chandarlapaty S, Chen D, He W, et al: Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial. JAMA Oncol 2:1310-1315, 2016.

18.9 Instructions for the SWOG Biospecimen Bank – Lab #201, Solid Tissue, Myeloma and Lymphoma Division

a. **Formalin-Fixed Paraffin-Embedded (FFPE) Tissue**: The SWOG Biospecimen Bank will receive FFPE specimens as either blocks, punch biopsy or slides/sections at time of invasive recurrence (if recurrence occurs and where tissue is available from standard of care biopsy of metastatic site) (Registration Step 3). Upon receipt of FFPE tissue block, punch biopsy, or 20 unstained slides, Bank will accession and store at room temperature.

b. **Whole Blood in SST (Vacutainer):** The SWOG Biospecimen Bank will receive one 10mL (red top) SST at up to 3 timepoints. Upon receipt, the Bank will accession and process serum from the whole blood collected in the SST and store in aliquots in a -80°C freezer for future analysis.

c. **Whole Blood in Streck Tubes:** The SWOG Biospecimen Bank will receive 20mL ambient peripheral blood in two 10 mL Streck cfDNA tubes at up to 3 timepoints. Upon receipt, the Bank will accession and process for plasma and buffy coat. Plasma is processed using the following double-centrifugation protocol: 1,500xg for 10 minutes at room temperature with brake (buffy coat is removed at this point); plasma is removed and centrifuged again at 3,000xg for 10 minutes at room temperature with brake. Plasma will be stored in 1-mL aliquots, and buffy coat will be stored as 1 aliquot; both plasma and buffy coat will be banked in a -80°C freezer until distribution. Planned future correlatives (cfDNA analysis) are planned for incorporation with a forthcoming revision.

d. **Distribution Instructions for Translational Medicine Objectives: Prospective validation of other prognostic/predictive indices of breast cancer outcomes:**

1. Type and Volume of Samples to be Shipped

a. Tissue

The SWOG Biospecimen Bank will retrieve, perform QA pathology review, and ship the following for each patient with available tissue (FFPE slides): 11 unstained 5-micron slides from the primary tumor at baseline submission timepoint along with the accompanying de-identified pathology report.

Exact Sciences Corporation will receive two shipments of tissue. The first will consist of samples for 60 patients. It is estimated that a minimum of 6 unstained slides sectioned at 5-micron thickness are required for testing (1 x 5 microns for H&E to guide assessment and dissection; 5 x 5 microns for dissection and extraction of nucleic acids; sections will be mounted on charged glass slides and unbaked, and numbered sequentially from 1 to 6 to indicate the order in which they were sectioned from the tumor block). Patients with less than 5 unstained slides will be recorded as "insufficient tissue available for testing". Exact Sciences Corporation will conduct run-in testing on approximately 50 patient samples, then report out the process statistics on the run-in testing. In the randomized run-in, Exact Sciences Corporation will confirm estimated yields with "preferred" vs. "minimum" number of slides, make any minor adjustments as appropriate for the analyses and convey to the SWOG Biospecimen Bank prior to the second shipment of tissue.

If an FFPE block or a punch biopsy was submitted in lieu of slides, then the SWOG Biospecimen Bank will ship the FFPE block or the punch biopsy. Exact Sciences will be responsible for slide preparation and return (including return shipping cost) of all remaining tissue to the SWOG Biospecimen Bank.

All tissue will be packaged in biohazard bags and shipped according to the SWOG Biospecimen Bank procedures.

b. Blood (serum hormone studies)

One 1 mL aliquots of serum processed and/or cryopreserved by the SWOG Biospecimen Bank (*i.e.,* the Biopathology Center at Nationwide Children's Hospital) will be provided for all patients that submitted baseline blood samples for analysis of serum estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-müllerian hormone (AMH)/Müllerian inhibiting factor (MIF). Due to funding and logistical considerations, the samples to be shipped and tested will be limited to samples drawn from pre-menopausal patients under age 55.

The SWOG Biospecimen Bank will retrieve and send a 1-mL aliquots from each of the estimated 1,364 eligible serum samples currently stored at -80°C. These samples will be shipped on dry ice to Dr. Godwin (in care of Dr. Harsh Pathak) at the University of Kansas Medical Center). For each of these patient samples the serum estradiol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and anti-müllerian hormone (AMH)/Müllerian inhibiting factor (MIF) levels will be measured.

It is estimated that ~300 to 400 µL of each serum sample will be consumed to measure serum hormone levels (if the specimens are run in duplicate then ~600 to 800 µL will be required). Any remaining residual serum will be destroyed in accordance with laboratory procedure. All data derived from processing will be forwarded to the SWOG Statistics and Data Management Center.

2. Laboratory Conducting the Analyses

a. Tissue

Exact Sciences Corporation: Attention Kevin Chew
701 Galveston Dr.
BMR Room 104
Redwood City, CA 94063
Ph: 650-569-2402
Email: kchew@exactsciences.com

b. Blood (serum hormone studies)

Andrew K. Godwin, PhD
Professor and Division Director, Genomic Diagnostics
c/o Harsh Pathak, PhD
Biospecimen Repository Core Facility
2106 Olathe Blvd
Kansas City, KS 66160
Wahl Hall East Rm. 4005
Ph: 913-945-6378
Fax: 913-945-6327
Email: hpathak@kumc.edu

3. Materials and Data Management

   a. Tissue

      All tissue will be returned to the SWOG Biospecimen Bank upon completion of processing.

      Exact Sciences Corporation will forward all marker data (raw and analyzed) to the SWOG Statistics and Data Management Center.

   b. Blood

      Any remaining residual serum will be destroyed in accordance with KUMC laboratory procedures. KUMC will forward all data derived from processing to the SWOG Statistics and Data Management Center.