Protocol Update #10
01/15/2015

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO CALGB 40601

RANDOMIZED PHASE III TRIAL OF PACLITAXEL +TRASTUZUMAB + LAPATINIB VERSUS PACLITAXEL + TRASTUZUMAB AS NEOADJUVANT TREATMENT OF HER2-POSITIVE PRIMARY BREAST CANCER

Investigational agent supplied and distributed for this trial by the NCI:
Lapatinib (GW572016 NSC #727989, IND [redacted])
Companion study CALGB 70501

[X] **Update:**	[ ] **Status Change:**
[ ] Eligibility changes	[ ] Activation
[ ] Therapy / Dose Modifications / Study Calendar changes	[ ] Closure
[ ] Informed Consent changes	[ ] Suspension / temporary closure
[ ] Scientific / Statistical Considerations changes	[ ] Reactivation
[ ] Data Submission / Forms changes	
[X] Editorial / Administrative changes	
[ ] Other :	

No IRB approval of this amendment is required.
Please follow you local IRB guidelines.

UPDATES TO PROTOCOL:

- In keeping with new CTEP PIO requirements, the name of the lead group on the title page of the protocol has been updated from "Cancer and Leukemia Group B" to "Alliance for Clinical Trials in Oncology."
- The NCTN participating groups have been added to the lower left of the protocol cover page.

UPDATES TO THE MODEL CONSENT:

No changes have been made to the model consent form.

A replacement protocol document and model consent for have been issued.
This study remains closed to new patient accrual.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

Activation Date: December 1, 2008
Includes Update #10

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

CALGB 40601

RANDOMIZED PHASE III TRIAL OF PACLITAXEL +TRASTUZUMAB + LAPATINIB VERSUS PACLITAXEL + TRASTUZUMAB AS NEOADJUVANT TREATMENT OF HER2-POSITIVE PRIMARY BREAST CANCER

Investigational agent supplied and distributed for this trial by the NCI:
Lapatinib (GW572016 NSC #727989, IND #[redacted])
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This study is supported by the NCI Cancer Trials Support Unit (CTSU).
Institutions not aligned with CALGB will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix.

- 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
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to the CTSU logistical appendix for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to the CTSU logistical appendix for specific instructions and forms to be submitted.
- Data management will be performed by the CALGB. **Case report forms** (with the exception of patient enrollment forms), **clinical reports, and transmittals** must be sent to CALGB unless otherwise directed by the protocol. Do not send study data or case report forms to the CTSU Data Operations.
- **Data query and delinquency reports** will be sent directly to the enrolling site by CALGB. (generally via email but may be sent via fax or postal mail). Please send query responses and delinquent data to CALGB and do not copy the CTSU Data Operations. Query responses should be sent to CALGB via postal mail (no transmittal form needs to accompany response). Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP IAM account contact information current. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

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The pharmacogenomic component of this study is conducted as part of the NIH Pharmacogenomics Research Network, which is funded through a separate U01 mechanism (see http://www.nigms.nih.gov/initiatives/pgrn for details).

RANDOMIZED PHASE III TRIAL OF PACLITAXEL + TRASTUZUMAB + LAPATINIB VERSUS PACLITAXEL + TRASTUZUMAB NEOADJUVANT TREATMENT OF HER2-POSITIVE PRIMARY BREAST CANCER

Investigational Agent(s): Lapatinib (NSC#727989, IND#[redacted] will be supplied and distributed by CTEP, DCTD, NCI)

Patient Eligibility

Pathologic confirmation of invasive breast cancer; patients with inflammatory breast cancer are not eligible.
Clinical stage II-III invasive breast cancer with intent to perform surgical resection after neoadjuvant therapy. Staging to rule out metastatic disease is recommended for clinical stage III patients.
Multicentric or bilateral disease is eligible if target lesion meets eligibility criteria (see Section 4.2).
Tumors must be HER2 positive as defined in Section 4.3.
ER/PgR status must be known.
Target lesion in breast must be ≥ 1 cm by either clinical or radiographic measurement. Patients with axillary disease as only site are not eligible to participate (see Section 4.5).
Patient consents to undergo pretreatment biopsies*
No prior chemotherapy, hormone therapy, biologic or radiation therapy with therapeutic intent for this cancer.
Cardiac ejection fraction ≥ 50% by echocardiogram or MUGA scan
Women or men at least 18 years of age.
ECOG (Zubrod) Performance Status 0-1.
Patients must not be pregnant or nursing.

Required Initial Laboratory Values

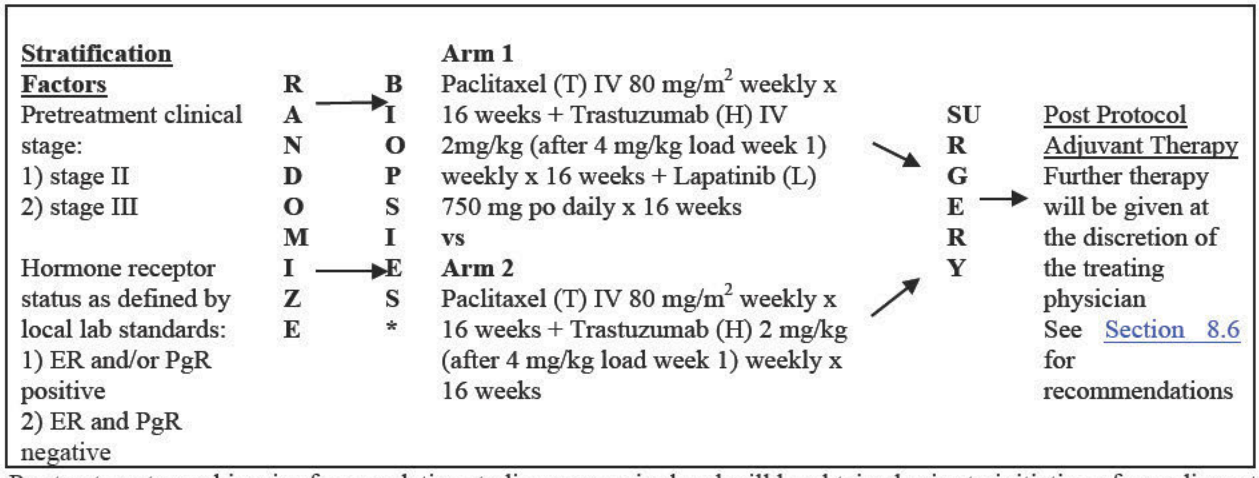
ANC ≥ 1,000/µl

Platelet count ≥ 100,000/µl
Total bilirubin ≤1.5x ULN

AST and AST ≤ 2.5 x ULN

Negative serum βHCG (see Section 4.12)

Update #5
06/15/11



* Pre-treatment core biopsies for correlative studies are required and will be obtained prior to initiation of neoadjuvant chemotherapy (see Section 6.2.1). **It is strongly suggested that these core biopsies be image guided and obtained at the time of clip placement.** Samples obtained at the time of diagnosis before registration to 40601, for other studies, may be submitted for this correlative analysis if they were collected according to the tissue acquisition instructions in Section 6.2.1.

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1.0 INTRODUCTION

1.1 Rationale for neoadjuvant approach

The neoadjuvant (preoperative) approach to chemotherapy has become more commonly used in operable breast cancer over the past two decades based upon randomized trials suggesting no difference in relapse-free or overall survival whether chemotherapy is administered before or after surgery [1], [2],[3]. Unlike postoperative chemotherapy, neoadjuvant chemotherapy has the appeal of in vivo chemosensitivity testing and provides the prognostic relevance of the extent of residual disease after neoadjuvant chemotherapy, which serves as an early marker of outcome. Tumor size diminishes in most patients; this effect is observed in both the primary tumor and regional lymph nodes. This change increases the opportunity for breast-conserving surgery for patients with large or inoperable primary tumors. In addition, the neoadjuvant approach provides a vastly improved framework for correlative science studies since measurable tumor remains in place during therapy and it provides the opportunity for quick and efficient evaluation of new regimens. Standard of care for high risk HER2-overexpressing breast cancer includes anthracycline- and taxane-based chemotherapy combined with the anti-HER2 monoclonal antibody trastuzumab (Herceptin®, H). Despite the effectiveness of this regimen, relapse occurs in 15% within the first 2-3 years, and clinical cardiac toxicity occurs in 4% [4] [5], so we still need strategies to enhance efficacy and diminish toxicity of therapy. The HER1/HER2 dual kinase inhibitor lapatinib has documented efficacy in trastuzumab-refractory disease [6] and has improved time to disease progression when combined with chemotherapy versus chemotherapy alone in the Stage IV setting [7]. Lapatinib has been combined without notable toxicity with both paclitaxel and trastuzumab, making it a natural candidate for a combined HER2 blockade approach.

Update #5
6/15/11

This study was initiated as a 3 arm randomized phase III trial examining the pathologic response to neoadjuvant HER2-directed therapy plus paclitaxel in HER2-positive breast cancer. The investigational arms were trastuzumab + lapatinib + paclitaxel and lapatinib + paclitaxel. The control arm was, and remains standard trastuzumab + paclitaxel. In 2011, based on emerging data, the trial was amended to compare paclitaxel + trastuzumab + lapatinib versus paclitaxel + trastuzumab. Details of the rationale for this amendment are found in Section 1.5. Planned correlative science studies that exploit the neoadjuvant approach will, among other things, examine molecular pathways implicated in trastuzumab and lapatinib resistance and whether CYP3A4 and 2C8 polymorphisms are associated with toxicity or efficacy since both paclitaxel and lapatinib are known substrates for these enzymes.

1.2 Rationale for examining lapatinib

Recent reports suggest striking improvements in relapse-free and overall survival with trastuzumab incorporation into adjuvant therapy [4] [5] [8]; for the patient with a high risk HER2-positive tumor, trastuzumab is standard of care. Unfortunately, an estimated 15% of patients will still relapse despite AC-paclitaxel/trastuzumab during the first 4 years of follow-up [5]. The small molecule HER1/HER2 dual kinase inhibitor lapatinib has been examined as monotherapy in trastuzumab-resistant HER2-positive breast cancers. In a recently reported Phase III study of lapatinib plus capecitabine versus capecitabine alone in HER2-positive metastatic breast cancer patients who had progressed on trastuzumab, the primary endpoint of time to disease progression was significantly increased from approximately 20 weeks to 37 weeks with the addition of lapatinib [7]. Two putative mechanisms of trastuzumab resistance, PTEN deficiency and IGF-1R expression, do not appear to affect response to lapatinib [9]. Another potential mechanism of resistance to HER2 blockade is via HER1; as a small molecule inhibitor of the ATP binding site of both HER1 and HER2, lapatinib inhibits

downstream signaling of HER1 homodimers, HER2 homodimers, and HER1/HER2 heterodimers. HER2-targeted small molecules and antibodies work by different mechanisms and are not cross-resistant, supporting combination of these biologic agents [10]. Lapatinib has been combined with paclitaxel (EGF 10009, EGF 30001) and with trastuzumab (EGF 10023) previously [11]. Each was well tolerated, although there is a suggestion of more frequent adverse events with the weekly rather than the once per three weeks paclitaxel schedule. The recommended dose as single HER2 blockade plus paclitaxel is 1500 mg. When combined with standard weekly trastuzumab, the recommended dose of lapatinib is 1000 mg. However, two recent reports suggest that lapatinib 1000 mg combined with trastuzumab and paclitaxel results in excessive diarrhea [12, 13]. For this reason, lapatinib will be dosed at 750 mg in the triplet combination, a dose modification that is also being performed in the ongoing adjuvant trial ALTTO)/N063D. The most common adverse events include diarrhea, nausea, vomiting, constipation, anorexia, rash, and fatigue. Lapatinib alone or the addition of lapatinib to trastuzumab does not appear to augment cardiac toxicity. The risk of symptomatic cardiac toxicity with single agent lapatinib appears to be under 1% even in patients previously treated with anthracyclines and trastuzumab [14]. Of 393 patients treated with lapatinib plus trastuzumab in the metastatic setting, only 2 experienced symptomatic cardiotoxicity, and 10 suffered asymptomatic declines in LVEF [15]. In this study, all patients are recommended to receive trastuzumab adjuvantly for 1 year in a similar regimen to that used in the HERA trial [4]. In this way, none of the patients will be deprived of the known benefit of trastuzumab.

1.3 Rationale for chemotherapy regimen

A recent preliminary report of BCIRG 006, a randomization of AC-docetaxel (AC-T), AC-docetaxel/trastuzumab (AC-TH), and docetaxel/carboplatin/trastuzumab (TCH), revealed a reduction in disease recurrence of 39% for AC-docetaxel/trastuzumab and 33% for docetaxel/carboplatin/trastuzumab, which were not significantly different [8]. The cardiotoxicity of the two regimens was markedly different, with clinical congestive heart failure in 1.9% in the AC-TH arm versus 0.4% in the TCH arm. While the anthracycline/trastuzumab and nonanthracycline/trastuzumab arms did not significantly differ from one another in efficacy, the suggestion of a higher recurrence risk reduction and clearly higher toxicity when anthracyclines are included has renewed efforts to identify subsets of patients for whom the anthracycline is not needed. While the proposed study cannot answer this question directly, it will add clinical and translational information about response to a nonanthracycline regimen in addition to information about combined HER2 blockade. Both weekly and every 3 week paclitaxel has been combined with lapatinib, and was generally well-tolerated, although diarrhea was frequent (92%, 50% grade 3) among 12 patients treated on the weekly schedule [11]. This toxicity may relate to a pharmacokinetic interaction between the two drugs, both of which inhibit CYP3A4 [16] and resulted in institution of aggressive diarrhea management protocols in the phase III study [17].

In this study, we will administer the taxane plus biologic therapy in the preoperative setting followed by surgery. In this way, we will obtain accurate clinical data and facilitate correlative studies examining effects of combined and single agent HER2-targeting. Since the primary endpoint of the study is pathologic response to neoadjuvant therapy, further adjuvant chemotherapy is not protocol-directed; however post-surgical adjuvant dose-dense AC followed by trastuzumab will be recommended. Post-surgical treatment will be tracked and long-term disease outcomes will be followed.

There are several important issues to consider regarding the planned regimens. First, concerns have been raised about the potential for augmented cardiotoxicity of the recommended anthracycline after trastuzumab-based therapy. Three sources of data reassure us on this point. A recently reported study from M.D. Anderson Cancer Center administered trastuzumab

concurrently with FEC(75) after paclitaxel/trastuzumab. In that study, no clinical cardiotoxicity was seen in the first 23 patients. Seven of the 23 had asymptomatic LVEF declines, compared to 5 of 19 patients receiving the same chemotherapy without trastuzumab [18]. Epirubicin is less cardiotoxic at the same doses, with a dose ratio for toxicity of 1:1.8 [19]. In the proposed study, AC will be given, however will not be given concurrently with trastuzumab. Second, there was no evidence of enhanced cardiac toxicity in an ECOG pilot using a similar regimen [20]. In that study, 234 patients received paclitaxel/trastuzumab followed immediately by standard every-three-week dose and schedule AC. Only four patients (1.7%) developed congestive heart failure. Median left ventricular ejection fractions (LVEF) were 63% pretreatment, 61% after paclitaxel/trastuzumab, and 59% after AC. Abnormal LVEF was seen in 3% of patients after paclitaxel/trastuzumab and 6% after AC, which is similar to the 4% expected rate of LVEF decline with AC alone [5]. Finally, the AC will be administered adjuvantly, so there will be approximately 4 weeks between completion of trastuzumab and initiation of AC. For these reasons, we believe that the proposed reversed schedule should be reasonably safe in a population of women with early breast cancer.

Another consideration is the timing of the surgical intervention, which is proposed to be after the taxane/HER2-targeted therapy and before initiation of AC. The benefits of this approach are listed in Table 1.

Table 1

Benefits of sequence of Taxane/biologic → Surgery → AC → Trastuzumab
1. Interpretability of primary and correlative endpoints examining biologic effect
2. Cardiac safety of increased interval between trastuzumab and anthracycline
3. Flexibility in determination of need for adjuvant therapy based upon pathologic endpoints.

From a clinical and correlative research standpoint, this approach is superior to the inclusion of the AC neoadjuvantly. Several other neoadjuvant studies have administered one component of therapy prior to surgery, with the remainder given in the adjuvant setting [1] [21]. While some might argue that growth kinetics of breast cancer suggest that chemotherapy, once initiated, should be given without dose delays, it is not clear that this approach is important at the time of crossover to a non-cross resistant regimen. NSABP B-27 compared neoadjuvant AC alone (group 1) with neoadjuvant AC followed by either neoadjuvant docetaxel (group 2) or adjuvant docetaxel (group 3) [1]. In spite of a near doubling of pathologic complete response in the breast in group 2, there was no difference in breast conservation rates and no significant difference in outcome among the three arms. There was a suggestion of a better outcome in group 2 compared with group 3, however these differences (HR of relapse 0.85 vs 0.91) were not significant. Moreover, chemotherapy effectiveness may have been blunted by the administration of tamoxifen concurrently [22]. This may have particularly affected group 3; in those patients tamoxifen would have been on board for over 22 weeks at the time of docetaxel. There was a long interval between completion of AC and initiation of docetaxel in group 3, on average 62 days. This interval will be minimized in this study given the non-myelosuppressive weekly taxane regimen, which permits surgical intervention within 1-2 weeks after the last chemotherapy. Finally, the trial was not conducted exclusively in women with HER2 positive disease who are also receiving potent HER2-directed therapy. For these reasons and given the potential to identify patients and profiles of sensitivity to nonanthracycline-based approaches to HER2-positive disease, we believe that surgery after the well-delivered taxane/biologic component is not only reasonable, but preferable.

We have deliberately extended the preoperative therapy from 12 to 16 weeks with the hope of increasing pathologic complete response rates. There is ample evidence to suggest that duration of preoperative therapy is associated with pathologic complete response rate. In the

GEPARTRIO study, clinical nonresponders after two cycles of TAC were randomized to capecitabine/vinorelbine versus continued TAC [23] without significant improvement in outcome. Notably, over 20% of patients that were nonresponders after 6 weeks of TAC chemotherapy became clinical complete responders by the 18th week of continued TAC. This impact of duration may be even more important in biologic therapy. A variety of neoadjuvant regimens including trastuzumab combined with chemotherapy have generally produced pathologic complete response rates in the breast and axilla less than 25% with 12 weeks of therapy [24] [21] [25] [26]. One potential contributor to the extraordinarily high pCR rate of 67% in the recent MDACC experience was the prolonged duration of therapy to 24 weeks [18]. For these reasons, we will administer neoadjuvant treatment for 16 weeks with the taxane/HER2-targeted therapy.

Finally, the protocol specifies the taxane/biologic component of the therapy and the primary surgery. Management of the axilla will not be protocol-directed since there is considerable controversy about this. Data regarding surgical practices will be studied as a secondary endpoint. In addition, adjuvant dose-dense AC and one year of adjuvant trastuzumab is recommended but not mandated. We believe this is reasonable given the primary endpoint of pCR and the controversies regarding the utility of further toxic chemotherapy in the setting of pCR and the optimal duration of trastuzumab.

1.4 Rationale for use of pathologic complete response (pCR) as primary endpoint

Response to neoadjuvant therapy has proven a good surrogate for subsequent disease-free survival [2] [27] [28]. Since it can be assessed within months rather than years, pathologic response is a particularly attractive endpoint in the rapidly evolving area of breast cancer therapeutics, where it allows agility in trial development and adaptation. The best validated measure of outcome is pathologic complete response in the breast [2], which has been used as an endpoint of randomized phase III neoadjuvant trials such as NSABP B-27 [29]. Although significant improvements in pathologic response do not always translate into significant improvements in outcome, the value of pathologic response as an intermediate endpoint has been amply demonstrated. Recent studies provide us with greater ability to interpret pCR improvements. In NSABP B-27, why was the improvement in pathologic complete response so significant while the improvement in relapse-free survival was not? In fact, the modest improvement in outcome suggested by the NSABP trial is consistent with the improvement in pCR if the benefit is primarily limited to those patients who became pathologic complete responders. In the MDACC randomized trastuzumab neoadjuvant study, the addition of neoadjuvant trastuzumab to chemotherapy improved pCR by over 40% [18], which is also approximately the relapse benefit seen in the adjuvant trials [4] [5]. One argument about the use of pCR as an endpoint relates to the emerging understanding that different breast cancer subtypes have significantly different pCR rates to the same chemotherapy [30] [31]. The association of pCR with survival has been demonstrated within different subtypes of breast cancer [32]. Since this study is limited to HER2-overexpressing tumors, who usually fall in the HER2 subtype, this should be less of an issue in this trial.

Previous neoadjuvant studies including various chemotherapy/trastuzumab regimens have resulted in pathologic complete response rates of at least 15% even with short durations of therapy. These include weekly trastuzumab combined with every third week paclitaxel, which demonstrated 18% pathologic complete response after 12 weeks of therapy [21]; weekly docetaxel/trastuzumab, which demonstrated 12% pathologic complete response after 14 weeks of therapy [24]; weekly vinorelbine/trastuzumab, which demonstrated 19% pathologic complete response after 12 weeks [26]; a combination of every second week docetaxel and vinorelbine with weekly trastuzumab, which demonstrated 42% pathologic complete response in the breast after 12 weeks of therapy [33]; and the sequence of every third week AC

followed by weekly paclitaxel/trastuzumab, which demonstrated pathologic complete response of 19% [25]. More recently, the randomized trial of paclitaxel followed by fluorouracil/epirubicin/ cyclophosphamide (T-FEC) with or without trastuzumab revealed a 67% pathologic complete response after 24 weeks of therapy (95% CI 41-87%) [18].

Since the current study uses a shorter duration of trastuzumab, we estimate that the baseline pathologic complete response rate will be 30%, which is higher than the 12 week regimens but lower than the Buzdar study. Pathologic response categories other than pCR also carry prognostic information [27] and will be noted. Since there are large variations in management of the axilla across institutions, pathologic nodal status will be noted as a secondary endpoint, the primary endpoint will be based upon the in-breast response only.

1.5 Rationale for the change in study design (protocol update #5)

Update #5
6/15/11

CALGB 40601 was activated as a 3-arm study (paclitaxel + trastuzumab + lapatinib [THL], paclitaxel + trastuzumab [TH] and paclitaxel + lapatinib [TL]). In December 2010, two neoadjuvant studies were presented at the Cancer Therapy and Research Center-American Association for Cancer Research (CTRC-AACR) San Antonio Breast Cancer Symposium that affected the scientific and practical enthusiasm for the TL arm. For this reason the CALGB 40601 has been amended to omit the TL treatment arm (arm 3). In the Geparquinto trial, patients with HER2-positive breast cancer received epirubicin/cyclophosphamide followed by docetaxel combined either with trastuzumab (EC-DOC-H) or lapatinib (EC-DOC-L) neoadjuvantly, then trastuzumab for a total of 12 months adjuvantly [34]. Pathologic complete response (pCR) in breast and axilla was the primary endpoint. Among 620 patients randomized, there was a higher pCR rate (31% vs 22%) and lower toxicity with fewer discontinuations (10% vs 16%) among patients on the trastuzumab arm than the lapatinib arm. The first results of the NeoALTTO trial were similar [35]. Approximately 450 women with HER2-positive breast cancer received a lead-in phase of 6 weeks of biologic therapy with lapatinib (L), trastuzumab (H), or both, then paclitaxel was added for an additional 12 weeks prior to surgery. All drugs were given at doses similar to CALGB 40601. Postoperative adjuvant therapy included additional chemotherapy and biologic therapy, however, the presentation focused solely on the primary endpoint of pCR in the breast at surgery; no long-term outcomes were presented. There were more grade $\geq$ 3 adverse events in the lapatinib arm compared with the trastuzumab arm, including diarrhea (23% vs 2%), hepatic abnormalities (13% vs 1%), and neutropenia (16% vs 3%). The only death occurred in the combined biologic (LH) arm. Failure to complete treatment as planned was higher in the L (34%) and LH arms (39%) that in the H arm (u%). PCR was highest with LH-paclitaxel (51%), followed by H-paclitaxel (30%), and L-paclitaxel (25%).

Based on the inferior results and higher toxicity of the investigational lapatinib arms of these studies and in discussion with CTEP, in January 2011 it was decided to amend CALGB 40601 to omit Arm 3 (TL). Those patients on this arm will complete protocol therapy as per the original study design. In CALGB 40601, it is recommended that all patients receive trastuzumab adjuvantly for 1 year, hence all of the patients including those previously randomized to the TL neoadjuvant arm, will be able to receive the known benefit of trastuzumab. After local IRB approval of Update #5, the remainder of the patients will be enrolled and randomized to Arm 1 (THL) and Arm 2 (TH) in a 2:1 fashion. Given that TH is the standard regimen, and is also clearly the best tolerated, we will continue that arm of the study as the comparator, with THL as the only investigational arm.

1.6 Inclusion of women and minorities

Given the focus upon breast cancer, it is expected that this study will primarily include women, however eligible men will be included. Breast cancer patients of all ethnic backgrounds will be included. There are no expected racial/ethnic differences in the intervention effort.

Accrual Targets						
Ethnic Category	**Sex/Gender**					
	Females		**Males**		**Total**	
Hispanic or Latino	15	+	0	=	15	
Not Hispanic or Latino	285	+	0	=	285	
Ethnic Category: Total of all subjects	300 (A1)	+	0 (B1)	=	300	C1)
Racial Category						
American Indian or Alaskan Native	1	+	0	=	1	
Asian	7	+	0	=	7	
Black or African American	36	+	0	=	36	
Native Hawaiian or other Pacific Islander	1	+	0	=	1	
White	255	+		=	255	
Racial Category: Total of all subjects	300 (A2)	+	0 (B2)	=	300	(C2)
	(A1 = A2)		(B1 = B2)		(C1 = C2)	

2.0 OBJECTIVES

2.1 Primary objective

Update #5 6/15/11

To determine if the pathologic complete response (pCR) in the breast to neoadjuvant weekly paclitaxel with trastuzumab plus lapatinib (THL) is 20% greater than the pCR to weekly paclitaxel with trastuzumab alone (TH).

2.2 Secondary objectives

Update #5 6/15/11

2.2.1 To determine the pathologic complete response in the breast and axilla, using AJCC TMN criteria (Version 6), to neoadjuvant weekly paclitaxel plus HER2-targeted therapy in patients with HER2-positive operable breast cancer.

2.2.2 To evaluate residual cancer burden (RCB) as a predictor of long term relapse free survival (RFS) and overall survival (OS).

2.2.3 To document the toxicity of all chemotherapeutic regimens (THL, TH).

2.2.4 To determine the correlation between clinical, radiographic and pathologic response.

2.2.5 To compare overall survival (OS), relapse free survival (RFS) and time to first failure (TFF) among the treatment groups. OS and TFF will be measured for all patients from study registration. RFS will be measured from definitive surgery for those patients who undergo definitive surgery.

2.2.6 To obtain blood, fresh frozen and fixed tumor tissue to test specific hypotheses for which biomarker data exist and to evaluate biomarkers in blood, serum and tissue that are likely to influence response to and toxicity of trastuzumab alone or trastuzumab plus lapatinib, when given with paclitaxel.

2.2.7 To determine the surgical practice patterns for breast conservation and sentinel lymphadenectomy in patients undergoing neoadjuvant chemotherapy.

2.2.8 To determine the radiotherapy practice patterns for post-mastectomy and regional nodal irradiation in patients undergoing neoadjuvant chemotherapy.

2.2.9 To evaluate pharmacogenomic determinants of toxicity.

3.0 ON-STUDY GUIDELINES

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate. Although they will not be considered formal eligibility (exclusion) criteria, physicians should recognize that the following may seriously increase the risk to the patient entering this protocol:

- Psychiatric illness which would prevent the patient from giving informed consent.
- Medical condition such as uncontrolled infection (including HIV), uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and considered by their physician to be at less than 30% risk of relapse.
- Women and men of reproductive potential should agree to use an appropriate method of birth control throughout their participation in this study due to the teratogenic potential of the therapy utilized in this trial. Appropriate methods of birth control include abstinence, oral contraceptives, implantable hormonal contraceptives (Norplant), or double barrier method (diaphragm plus condom).

4.0 ELIGIBILITY CRITERIA

All questions regarding eligibility criteria should be directed to the CALGB Study Chair. Please note that the Study Chair cannot grant waivers to eligibility requirements.

4.1 Histologic documentation

Pathologic confirmation of invasive breast cancer. Patients with inflammatory breast cancer are not eligible.

4.2 Stage

Clinical stage II-III operable invasive breast cancer with intent to perform surgical resection after neoadjuvant therapy.

Patients with multicentric or bilateral disease are eligible as long as the target lesion meets the eligibility criteria for this study.

Staging to rule out metastatic disease is recommended for clinical stage III patients.

4.3 HER2 status

Tumors must be HER2 positive defined as HER2 3+ by immunohistochemical (IHC) assays or gene amplification by FISH with a ratio $\geq$ 2 on invasive tumor.

4.4 ER and PgR

Status must be known.

4.5 Measurable disease

The target lesion in the breast must be ≥ 1 cm on physical examination or by radiographic measurement. Palpable axillary adenopathy will be documented but not serve as measurable disease for the primary endpoint. Patients with axillary disease only are not eligible to participate.

4.6 Tissue acquisition

Patient agrees to provide pretreatment biopsies.

4.7 Prior Treatment

No prior chemotherapy, hormone therapy, biologic, or radiation therapy with therapeutic intent for this cancer.

4.8 Cardiac ejection fraction

Must be ≥ 50% by echocardiogram or MUGA scan.

4.9 Age requirement

Women or men age ≥ 18 years of age.

4.10 ECOG (Zubrod) Performance Status

PS = 0-1.

4.11 Non-pregnant non-nursing

Patients must not be pregnant or nursing as the chemotherapy drugs used in this study may cause harm to a fetus or a newborn.

4.12 Required Initial Laboratory Values:

ANC	≥1,000/µl
Platelet count	≥100,000/µl
Bilirubin	≤ 1.5 x upper limit of normal
ALT and AST	≤ 2.5 X ULN
Serum β-HCG	Negative (in female patients unless S/P hysterectomy or menopausal or no menses for 24 consecutive months). Assay must have a sensitivity of at least 50 mIU/mL.

5.0 REGISTRATION/RANDOMIZATION AND STRATIFICATION

5.1 Registration requirements

- Informed consent: the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the multiple procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks and discomforts. If local guidelines and regulations permit the enrollment of persons not able to fulfill all of these requirements, the registering institution is responsible for complying with all local regulations and requirements for obtaining consent from these persons. Modification of the model consent form would be required for this purpose. Human protection committee approval of this protocol and a consent form is required.

5.2 Patient registration/randomization

This study uses the CALGB Web-based Patient Registration system. Randomization will be accepted only through CALGB Main Member Institutions, selected affiliate institutions and CCOPs using the Web-based Patient Registration system. Registration must occur prior to the initiation of therapy. Confirm eligibility criteria (Section 4.0). Complete the Registration Worksheet. Access the Web-based Patient Registration system via the Patient Registration tab on the CALGB Member Website at www.calgb.org. If the study does not appear on the list of

studies in the Patient Registration system, the registration must be performed by the CALGB Registrar via phone or fax. If the registering CRA requires assistance, he/she may consult the on-line help file at the bottom of the screen or call the IS Help Desk at 1-888-44CALGB. If further assistance is required, the registering CRA may call the CALGB Registrar (919)-668-9396, Monday-Friday, 9 AM - 5 PM, Eastern Time. Enter the following information:

CALGB patient ID #, if applicable

Study

Name of group (CALGB)

Name of institution where patient is being treated

Name of treating physician

Name of person in contact with the patient record (responsible contact)

Protocol IRB approval date

Date of signed consent

Treatment Start Date

Date [of]

HIPAA authorization signed by the patient

Patient's initials

Patient's Social Security #, date of birth, hospital ID #, and survival status

Patient's gender

Patient's race

Patient's ethnicity

ECOG performance status

Patient's height (cm) and weight (kg)

Type of insurance (Method of Payment)

Patient's postal code

Disease, type and stage, if applicable

Eligibility criteria met (no, yes)

When the patient is registered, a CALGB patient identification number will be generated. Please write the number in your records. After registration is complete, the patient may be randomized. The patient is randomized according to the stratification factors, which must be entered to obtain a treatment assignment. Once the randomization is complete, note the patient's treatment assignment in your records.

The Main Member Institution and registering institution will receive a Confirmation of Registration and a Confirmation of Randomization. Please check both confirmations for errors. Submit corrections in writing to the data coordinator at the CALGB Statistical Center, Data Operations, 2424 Erwin Rd, Suite 802 Hock Plaza, Durham, NC 27705, or fax to 919-668-9397.

5.3 Registration to companion studies

There are two substudies, CALGB 150702 and CALGB 60701, within 40601. These substudies do not require separate IRB approval and are described in Appendices I and II of the 40601 protocol.

- CALGB 70501, "Collection of patient reported symptoms and performance status via the internet", is a separate companion protocol available to CALGB institutions. All patients enrolled or enrolling to CALGB 40601 should be approached and invited to participate in CALGB 70501. Registration should occur simultaneously with the CALGB or CTSU registration/randomization to CALGB 40601; however, registrations to CALGB 70501 may take place later. Please note the CALGB 70501 participants must be registered prior to receiving therapy on scheduled clinic visit #2 (week 4 of visit) of 40601.

5.3.1 Registration to CALGB 150702

All participants in 40601 will have pre-treatment tissue specimens submitted for the correlative studies described in Appendix I Part A. Please see Section 6.2 for specimen submission instructions.

5.3.1.1 All patients entered on 40601 must be registered to 150702 due to the required submission of pre-treatment tissue. In addition, there are four questions for optional studies included in 150702, 1) for blood specimens, 2) for tissue core biopsy at surgery 3) for tissue core biopsy after completion of study treatment if surgery is not performed and 4) for DNA sequencing. These questions must be asked of all patients enrolled on CALGB 40601 (although patients may opt to not participate).

If a patient answers "yes" to "My blood specimens may be used for the research described above," question #1 in the model consent, they have consented to participate in the substudy described in Appendix I, Part B. Samples should be submitted per Sections 6.2, and 6.2.4 and 6.2.5.

If a patient answers "yes" to "Samples of my tissue may be collected at the time of surgery to evaluate the effects of the study treatment," question #2 or question #3 "I agree that an additional biopsy of my tumor tissue may be collected for research purposes if I do not have surgery after completing study treatment," in the model consent, they have consented to this future research.

5.3.2 Registration to CALGB 60701

CALGB 60701 is an optional pharmacogenomic substudy that must be offered to all patients enrolled on CALGB 40601 (although patients may opt to not participate).

If a patient answers "yes" to "My blood specimen may be used for the genetic research described above," question #7 in the model consent, they have consented to participate in the substudy described in Appendix II, Part A. We strongly encourage the collection of a pre-therapy sample and registration to CALGB 60701 at the same time the patient is registered to the treatment trial (40601); however, patients may be registered to 60701 within 60 days of registration to 40601. At that time the whole blood sample may be collected after consent has been obtained, and submitted per Section 6.2 and 6.2.3.

If registering to the 60701 within 60 days of registration to 40601, proceed to the online registration screen. Select "register a patient". Enter the patient number from CALGB 40601. Confirm the selection. Choose the companion "CALGB 60701" and continue the process as indicated. Once the sequence is completed and the patient is registered, you will receive a confirmation of registration to CALGB 60701.

If the patient says "yes" to "I agree to provide my tissue for the DNA sequencing project. I understand that my coded genetic information and coded medical information will be placed in databases accessible by the Internet," question #8 in the model consent, they have consented to participate in the substudy described in Appendix II, Part B. No

additional sample submission is required. For patients who answer "yes" to this question, the pre-treatment tissue will be used for this purpose.

5.4 Stratification factors

Pretreatment clinical stage:

1) stage II

2) stage III

Hormone receptor status as defined by local laboratory standards:

1) ER and/or PgR positive

2) ER and PgR negative

5.5 Surgical requirements

This study is intended to target HER2-positive clinical stage II-III operable invasive breast cancer patients with the intent to perform surgical resection after neoadjuvant therapy. Patients are randomized to preoperative chemotherapy with the intent that it will reduce the tumor size allowing for breast conserving surgery and the opportunity to develop correlative science studies to evaluate the extent of residual disease.

Pathologic confirmation of invasive tumor by core needle or incisional biopsy is required.

Pre-treatment tissue, core biopsies as described in Section 6.2.1, will be sent to the PCO for correlative science studies.

After neoadjuvant therapy and prior to surgery, the surgeon will assess the patient and decide whether the patient is a candidate for breast conserving surgery. This is at the discretion of the surgeon and the patient, and it is required that patients who undergo a mastectomy have the reasons documented on the C-1719 form, and documented in the operative report. This form must be submitted within 2 weeks of surgery.

Surgery should be performed within 42 days of the last dose of paclitaxel. For patients undergoing breast conserving therapy, documentation of negative margins is required. It is strongly recommended that the tumor be excised to free surgical margins of both invasive carcinoma and DCIS, with the exception noted of posterior margins if this margin is pectoral major fascia or the anterior margin or dermis.

For patients who have a tumor-involved partial mastectomy margin (except the posterior margin at the pectoralis fascia or anterior margin at the dermis) re-excision is strongly recommended. This may be accomplished with a re-operative partial mastectomy or with a total mastectomy. This choice will be at the discretion of the treating surgeon.

It is strongly recommended that all patients with pre-treatment T4 or N1-2 disease have at least a level I and II axillary node dissection done at the time of surgery.

Sentinel lymphadenectomy, prior to or following neoadjuvant chemotherapy, is at the surgeons discretion in clinical N0.

It is strongly recommended that patients with pre- or post-treatment sentinel node with N1 microscopic disease or greater should undergo a post treatment level I and level II axillary node dissection.

For consenting patients, tissue obtained at the time of surgery should be submitted for correlative studies (see Section 6.2.2).

6.0 DATA AND SPECIMEN SUBMISSION

6.1 Data submission

Forms should be submitted to the CALGB Statistical Center, Data Operations in compliance with the Data Submission schedule below. There are three options for submitting forms that use the Teleform barcode and cornerstones:

- The preferred method is to submit the forms electronically using the "Submit to CALGB" button located at the bottom of the last page of each form. Forms submitted electronically should not be submitted by fax or mail.
- The forms may be faxed at 919-416-4990. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.
- The forms may be mailed to the CALGB Statistical Center, Data Operations, Hock Plaza, 2424 Erwin Rd, Suite 802, Durham, NC 27705. Please note that the four cornerstones and the form id ("bitmap") must appear on the form. Copies must be 100% of the original form size.

For the most up-to-date data forms, please visit the CALGB website at www.calgb.org.

Forms		Submission Schedule
	CALGB 40601 Registration Worksheet 40601 Eligibility Checklist	
C-1709	CALGB: 40601 On study Form	
C-1443	CALGB: HER-2/neu Testing Form	
C-816	CALGB: Baseline Solid Tumor Evaluation Form	
Report*	Pathology report from original diagnosis	
Report*	MRI, mammogram or ultrasound reports	
C-1718	CALGB: 40601 Pre-Neoadjuvant Chemotherapy Surgical Evaluation Form	Submit prior to beginning chemotherapy
C-1712	CALGB: 40601 Treatment Form	Submit every 4 weeks and when patient completes or discontinues study treatment
C-1713	CALGB: 40601 Adverse Event Form	
S-055	CALGB: 40601 Lapatinib Medication Calendar	
C-817	CALGB: Follow Up Solid Tumor Evaluation Form	Submit prior to surgery
Report*	MRI, mammogram or ultra sound report(s)	
C-1714	CALGB 40601 Treatment Summary (All Patients)	Submit at the end of all chemotherapy treatment
Report*	MRI, mammogram or ultrasound report(s)	
C-1715	CALGB: 40601 Follow Up Form	Submit every 6 months post chemo-therapy for 2 years, then annually for 10 years from registration; submit at progression and at death
C-1719	CALGB: 40601 Post-Neoadjuvant Chemotherapy Surgical Evaluation Form	
C-1717	CALGB: 40601 Post Surgery Histopathology Review Form	Submit within 2 weeks post surgery
Report*	Post chemotherapy surgical pathology and operative reports	
C-1716	CALGB: 40601 Radiation Therapy Form	Submit upon completion of RT
C-260	CALGB Remarks Addenda	As needed
C-1001	CALGB New Malignancy Form	Submit for any new or secondary malignancy
C-1742	Confirmation of Lost to Follow-up Form	Follow form directions

* Submit copies of all required reports to confirm eligibility and response.

Common Terminology Criteria for Adverse Events: This study will utilize the Common Terminology Criteria for Adverse Events version 3.0 for routine toxicity reporting on study forms.

6.1.1 Patient follow up

Follow all patients enrolled on this study, including those who do not receive any protocol therapy for: First locoregional (that is, ipsilateral in-breast or regional invasive breast recurrence) and first distant recurrence and death. In the event of first locoregional recurrence prior to first distant recurrence, continue to follow for first distant recurrence and death. In the event of first distant recurrence prior to first locoregional recurrence, continue to follow for first locoregional recurrence and death. After both first locoregional and first distant recurrence, follow for death for a maximum of 10 years from registration. During this 10-year follow up period, report any DCIS and any new primary of invasive cancer regardless of site.

Note that lobular carcinoma *in situ* (LCIS) is not considered a breast recurrence. Except for DCIS, do not report as a new primary any *in situ* carcinoma or squamous/basal cell skin cancer.

6.2 Specimen submission for correlative studies:

Correlative Study	Sample Type	Volume and Tube	Prior to Treatment	Follow-up Samples	Ship To
Circulating Tumor Cells (CTC)	Blood	10 mL x 3 CellSave	Yes	Yes (see Section 6.2.5)	Georgetown University
	Blood	10 mL x 1 lavender top tube (EDTA)	Yes		
HER2 extracellular domain (ECD)	Blood	8.5 mL (red/grey top serum separator no additive)	Yes	Yes (see Section 6.2.4)	PCO
Pharmacogenomics	Blood	10 mL Lavender top tube (EDTA)	Yes	No	PCO
Tissue markers	Core biopsies (see Sections 6.2.1-6.2.2.1 for details	-	Yes	Obtain at surgery for consenting patients	PCO
	Core biopsies (see Sections 6.2.1-6.2.2.1 for details	-	Yes	Obtain at surgery for consenting patients	PCO

CALGB Specimen Tracking System (STS) specimen submission instructions:

These instructions apply to all specimens submitted on CALGB trials.

All submitted specimens must be labeled with the protocol number (40601), CALGB patient number, patient's initials and date and type of specimen collected (e.g., serum, whole blood).

Specimens for patients registered on this study must be logged and shipped using the online CALGB Specimen Tracking System. All institutions may access this system via the CALGB Web site, http://www.calgb.org.

A copy of the Shipment Packing Slip produced by the CALGB Specimen Tracking System must be printed and placed in the shipment with the specimens.

USE OF THE SPECIMEN TRACKING SYSTEM IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

For procedural help in logging and shipping specimens, please refer to the Specimen Tracking System User Guide, which can be accessed via the Help link within the Specimen Tracking System.

To report technical problems with the CALGB Specimen Tracking System, such as login issues or application errors, and/or for further assistance using the application, please contact the CALGB Help Desk at 877-44CALGB or calgb-support@calgb.duhs.duke. edu.

Instructions for the collection and shipping of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary. Ship only on Monday-Friday by overnight service to assure receipt. If Shipping on Friday, FedEx or UPS must be used and the airbill must be marked "For Saturday delivery." Do not ship specimens on Saturdays.

All specimens (except the blood for circulating tumor cells, see Section 6.2.5) should be sent to the following address:

CALGB Pathology Coordinating Office
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

Note: Specialized tissue specimen collection kits will be used for this study. Several starter kits should be ordered from the CALGB Pathology Coordinating Office, by calling the phone number above, at the time that the 40601 protocol is submitted for local IRB approval. Additional kits may be ordered by calling the CALGB PCO at the phone number above. Details about the types of kits that are available can be found in the "Supplemental Materials" section of the 40601 study page on the CALGB website. In general, the kits will contain all the materials that are needed for the specimen collection on this trial including all the tubes for the pharmacogenomic, ECD and CTC studies (lavender top EDTA, red/grey top serum separator and CellSave tubes). The only options to choose from will be whether a biopsy gun is to be supplied and whether the kit is for the pretreatment biopsies only or if kits are needed for both pretreatment and surgical specimens (if the patient has consented to those).

Additional information on the guidelines for collection, handling, and storage of specimens from the National Cancer Institute (NCI) Cooperative Group and Breast International Group (BIG) Breast Cancer Clinical Trials may be accessed at: http://ctep.info.nih.gov/investigatorResources/tbci/trials.htm.

Unused kit components should be returned to the PCO.

6.2.1 Pre-treatment core biopsy submission

Samples will be collected from all patients prior to initiating protocol chemotherapy using the specimen collection/shipping kits provided. It is strongly suggested that core biopsies be image guided and obtained at the time of clip placement.

The required biopsies include two core biopsies in RNA Later and two core biopsies in 10% neutral buffered formalin to be used for the correlative analyses described in Appendix I; the order of specimen collection will be 1st biopsy into RNA Later, 2nd into formalin, 3rd into RNA Later, 4th into formalin.

If your site is able to procure frozen specimens and has dry ice available to ship them, it is requested that two additional core biopsies be obtained and placed in OCT. The order of specimen collection for sites that are able to procure all 6 biopsies will be: 1st biopsy into RNA later, 2nd into formalin, 3rd frozen and OCT embedded, 4th into RNA Later, 5th into formalin and the 6th core frozen and OCT embedded.

Reimbursement is available for the following tissue procurement procedures:

- The procedure to obtain pre-treatment core biopsies for research purposes that are not taken at the time of clip placement.
- Charge by the institution for protocol-specified biopsies that are obtained at the time of clip placement (or other standard of care biopsy).
- If a patient does not undergo surgery and consents to have post-treatment core the biopsy procedure is eligible for reimbursement.

Details regarding reimbursement, including the maximum allowable reimbursement amount, can be found on the 40601 Institutional MUGA/Echocardiogram and Research Biopsy Procedure Reimbursement Form, in the Supplemental Materials section of the 40601 study page of the CALGB website.

Note: Samples obtained at the time of diagnosis for other studies, prior to enrollment on CALGB 40601, may be submitted for this correlative analysis if they meet the protocol requirements. Samples should be labeled with the institutional surgical pathology number, CALGB study number and CALGB patient ID number.

6.2.2 Post-treatment core biopsy submission

Submission of the Post-Surgery Histopathology Review Form (C-1717) by the institutional study pathologist is required. This form captures the pathologic details of residual invasive carcinoma, DCIS and lymph node status and will be used to calculate residual cancer burden.

For patients who have consented, at the time of surgery core biopsy specimens will collected precisely as described in Section 6.2.1. These biopsies from the residual tumor (or from the tumor bed, if no gross tumor can be identified) may be performed by the surgeon prior to resection of the tumor/tumor bed. Alternatively, the site pathologist can remove the tumor samples during the dissection of the surgical specimen (using a 5-mm skin punch biopsy device) as long as the samples are processed preferably within 1 hour, but may be processed up to 2 hours of removal from the patients as described in Section 6.2.1.

In the case of disease progression, if the patient does not undergo immediate surgery, but will receive further non-protocol chemotherapy, the biopsies are requested prior to the initiation of chemotherapy in consenting patients (see Section 6.2.2.1).

Samples should be labeled with the institutional surgical pathology number, CALGB study number and CALGB patient ID number.

6.2.2.1 Post-treatment core biopsy submission for patients not undergoing surgery

For consenting patients, who do not undergo surgery after completing study treatment, core biopsy specimens will collected precisely as described in Section 6.2.1.

Samples should be labeled with the institutional surgical pathology number, CALGB study number and CALGB patient ID number.

6.2.3 Whole blood submission for pharmacogenomics

For patients who have agreed to participate, draw a total of 10 mL of peripheral venous whole blood into an EDTA (purple-top) Vacutainer tube(s). Samples should be refrigerated until shipped. The samples should be placed in a biohazard bag and shipped with a cold refrigerant pack by overnight carrier to the CALGB PCO. The sample should be shipped the same day that the blood is drawn.

It is strongly encouraged that the sample be collected prior to the initiation of study treatment, however sample collection and registration to this pharmacogenomic substudy may take place within 60 days of registration to the treatment trial (see Section 5.3.2).

6.2.4 Whole blood submission for serum HER2 extracellular domain (ECD)

For patients who have agreed to participate, peripheral whole blood will be used for the evaluation of HER2 extracellular domain described in Appendix I, Part B.

Collect 8.5 mL in one red/grey top serum separator tube (no additive) Vacutainer tube prior to the initiation of study therapy, then on day 15 (just prior to third chemotherapy infusion) and before the final (week 16) infusion of paclitaxel has been started. The samples should be placed in a biohazard bag and shipped with a cold refrigerant pack by overnight carrier to the CALGB PCO. The samples should be shipped the same day that the blood is drawn.

6.2.5 Whole blood submission for circulating tumor cells (CTC)

For patients who have agreed to participate, serial samples of peripheral whole blood will be obtained at three time points and used for the circulating tumor cell analyses described in Appendix I, Part B.

Collect four tubes prior to the initiation of study therapy, four tubes on day 15 (just prior to third chemotherapy infusion), and four tubes before the final (week 16) infusion of paclitaxel has been started.

Draw 10 mL of peripheral whole blood into each of the three CellSave tubes and 10 mL into one EDTA tube (it is critical that each tube be completely filled with the required volume). The samples should be placed in a biohazard bag and shipped at ambient temperature by overnight carrier the same day that the blood is drawn. All four samples will be shipped to Georgetown University. For samples drawn on Friday, please ship for "Monday Delivery".

Prior to shipping each sample alert:
Shiva Krishnan, Rong Shen and Jeena Mathew
•ssk5@georgetown.edu, rs263@georgetown.edu, jm722@georgetown.edu OR
FAX 202-784-3034
Ship the four samples, with the 40601 Blood Specimen Submission Form (C-1711) to:
Minetta C. Liu, MD
C/O Shiva Krishnan
Lower Level, Room S170
Lombardi Comprehensive Cancer
Georgetown University Medical Center
3800 Reservoir Road NW
Washington, DC 20057
Tel: 202-687-1022

7.0 REQUIRED DATA

Pre-Study Testing Intervals

To be completed within 16 DAYS before registration:

- All bloodwork
- History and physical

To be completed within 28 DAYS before registration:

- Breast MRI (recommended), mammogram or ultrasound.
- MUGA scan or echocardiogram
- Pregnancy test (blood) for women of child-bearing potential.

	Prior to Registration	Prior to neoadjuvant therapy	Every 4 wks during neo-adjuvant therapy	Pre-operative	Post-Treatment Follow-up
Tests & Observations					
History & Progress Notes	X		X		K
Physical Examination	X		X	X	K
Pulse, Blood Pressure	X			X	
Height	X				
Weight/Body Surface Area●	X		X		
Performance Status	X		X	X	X
Clinical Tumor Measurements	X		X	X	
Drug Toxicity Assessment			X		
MUGA scan or echocardiogram	X		I	I	
Laboratory Studies					
CBC, Differential, Platelets	A		B		
AST, ALT, T Bilirubin	A		X	X	
Serum Beta HCG★	X				
Radiographic Tumor Measurements					
MRI, mammogram or ultrasound	C			C	
Correlative Studies					
Whole Blood for pharmacogenomic studies	D				
Whole blood for ECD		E			
Whole blood for CTC		F			
Image guided core biopsies		G			H
Neoadjuvant chemotherapy surgical evaluation		J		J	

A Pre-registration labs may be used for day 1 of cycle 1 tests if obtained within 16 days prior to registration. For subsequent cycles labs may be obtained within 48 hours prior to day of treatment.

B Obtain weekly, may be obtained within 48 hours prior to day of treatment.

C Breast exam and MRI will be performed prior to beginning study therapy and again before surgery (16 weeks). Only one radiographic modality may be used to follow each patient. MRI is strongly recommended, although other imaging modalities (mammogram, ultrasound) will be permitted if practical or financial considerations preclude MRI.

D Collect whole blood sample for pharmacogenomics from consenting patients prior to the initiation of study therapy per Section 6.2.3.
E Collect whole blood sample for ECD from consenting pts prior to the initiation of study therapy, day 15 and week 16 per Section 6.2.4.
F Collect whole blood sample for CTC from consenting pts prior to the initiation of study therapy day 15 and week 16 per Section 6.2.5.
G Required for all patients prior to beginning study therapy (see Sections 6.2 and 6.2.1). It is strongly suggested that these core biopsies be image guided and be obtained at the time of clip placement. See Section 6.2.1 for information regarding reimbursement the of pre-treatment core biopsy procedure.
H Post-treatment surgical specimen from consenting patients (see Sections 6.2 and 6.2.2).
I Repeat measurement of LVEF should be performed, using the same method as the baseline evaluation, at 8 weeks and prior to surgery. See Section 9.5 for dose modifications based on LVEF/cardiac toxicity. See Section 7.1 for information regarding reimbursement of the Week 8 research MUGA/echocardiogram.
J Required prior to neoadjuvant chemotherapy and prior to surgery (see Section 6.0).
K After completion of all study therapy patients should be followed every 6 months for the first two years, than annually for 10 years from registration. Follow-up should be of sufficient frequency to allow for accurate submission of the study follow-up form per Section 6.0.
★ For women of childbearing potential.
● The dose of paclitaxel, trastuzumab or lapatinib need not change unless the calculated dose changes by > 10%.

7.1 MUGA Scan/echocardiogram reimbursement

The baseline screening MUGA or echocardiogram, the assessment after completion of HER2 targeted therapy (prior to surgery) and any assessments required for toxicity monitoring (see Section 9.5) are considered standard of care. The MUGA/echocardiogram during treatment (Week 8) will be obtained for research purposes and will be eligible for reimbursement. To obtain reimbursement complete the 40601 Institutional MUGA/Echocardiogram and Research Biopsy Reimbursement Form and submit per the instructions on the form.

The reimbursement form can be found on the CALGB web site under “Studies”, “40601”, “Supplemental Materials.”

8.0 TREATMENT PLAN

Questions regarding treatment should be directed to the CALGB Study Chair.

Protocol treatment is to begin within 14 days of registration/randomization to allow time for the study specific clinical supply of lapatinib to arrive at study sites.

8.1 Pre-chemotherapy surgical evaluation

Prior to beginning drug therapy, the patient must be seen and examined by the treating surgeon. This will include a clinical breast and lymph node examination and review of the imaging studies (MRI or other radiographic method) of the breast and axilla. After examining the patient and reviewing the pertinent radiologic studies, the surgeon will determine whether the patient is a breast conservation candidate. If the patient is not a breast conservation candidate, the reason(s) will be documented on the C-1718 form.

Some clinicians may choose to determine the pathologic axillary status prior to beginning drug therapy. This can be done by whatever means the surgeon feels is appropriate, including a sentinel node procedure in patients with a clinical N0 axilla. **For patients with pretreatment N1 or N2 disease, we recommend that the axillary metastasis is confirmed by FNA.**

8.2 Pre-chemotherapy primary tumor biopsy

Prior to beginning chemotherapy, all patients will undergo an image-guided core biopsy. It is strongly suggested that these biopsies be image guided and obtained at the time of clip placement. In patients with large breast cancer, physicians who obtain image-guided core biopsies have a higher rate of obtaining cancer within the core biopsy as compared to non image-guided biopsies [36]. Please see 6.2.1 for details of obtaining the core biopsies.

8.3 Chemotherapy

Update #5
6/15/11

Patients are to be randomized to receive weekly paclitaxel with a HER2 targeted agent (either trastuzumab weekly, or lapatinib daily or both) administered for a total of 16 weeks. Surgery must be performed within 42 days following completion of paclitaxel chemotherapy. Protocol-defined therapy ends at surgery, however adjuvant therapy with 4 cycles of AC either dose-dense or every 3 weeks followed by one year of every 3 week trastuzumab is recommended.

Patients enrolled to this trial after local IRB approval of Update #5 will be randomized to Arm 1(THL) or Arm 2 (TH) only.

Arm 1 (THL):

Trastuzumab 2 mg/kg IV over 30 minutes (after week 1 dose of 4 mg/kg IV over 90 minutes) weekly for 16 weeks plus

paclitaxel 80 mg/m^2 IV over 1 hour weekly for 16 weeks

Plus lapatinib 750 mg po daily administered for 16 weeks

Arm 2 (TH):

Trastuzumab 2 mg/kg IV over 30 minutes (after week 1 dose of 4 mg/kg IV over 90 minutes week 1) weekly for 16 weeks plus

paclitaxel 80 mg/m^2 IV over 1 hour, weekly for 16 weeks

Arm 3 (TL):

Paclitaxel 80 mg/m^2 IV over 1 hour weekly for 16 weeks

Plus lapatinib 1500 mg po daily for 16 weeks.

Update #5
6/15/11

Patients enrolled to this trial after local IRB approval of Update #5 will be randomized to Arm 1 or Arm 2 only.

Patients who are unable to complete neoadjuvant protocol therapy with TH, TL or THL should go to surgery if operable. If they remain inoperable patients may be treated with off-protocol neoadjuvant therapy or radiation therapy.

8.3.1 Premedication for paclitaxel

The following premedication regimen is recommended for the initial dose of paclitaxel. If the patient does not experience an allergic reaction, the premedication regimen may be altered at the discretion of the treating physician.

Diphenhydramine	12.5 - 50 mg IV or po	30-60 minutes pre-paclitaxel
Ranitidine	50 mg IV	30-60 minutes pre-paclitaxel
(can be replaced with cimetidine 300 mg, or famotidine 20 mg)		
Dexamethasone	10 mg IV	< 60 minutes pre-paclitaxel
OR		
Dexamethasone	10 mg po	> 60 minutes pre-paclitaxel
OR		
Dexamethasone	20 mg po	6 hrs and 12 hrs pre-paclitaxel

8.4 Pre-surgery breast examination and radiographic imaging

Following completion of paclitaxel/HER2-targeted therapy, all patients must undergo repeat breast and axillary examination with clinical tumor measurements; breast imaging (MRI or other radiographic method) for tumor extent; and a MUGA scan or Echocardiogram for left ventricular ejection fraction (see Section 7.0).

8.5 Surgery

Following the completion of neoadjuvant chemotherapy, patients will again be examined by the treating surgeon and will be assessed as to whether or not they are a breast conservation candidate. All patients should undergo appropriate surgical management unless they are inoperable or surgery is medically contraindicated. If they are not surgical candidates, the reasons will be documented on the C-1719. If the patient is a breast conservation candidate, but opts for a mastectomy this will also be documented.

Primary surgery (breast conservation or total mastectomy) will be performed with appropriate management of the axilla. The choice of breast conservation therapy (BCT) or mastectomy will be at the discretion of the surgeon and the patient's preference if applicable. For patients undergoing breast conservation, documentation of negative margins is required. It is strongly recommended that the tumor be excised to free surgical margins of both invasive carcinoma and DCIS, with the exception noted of posterior margins if this margin is pectoral major fascia or the anterior margin or dermis.

For patients who have a tumor-involved partial mastectomy margin (except the posterior margin at the pectoralis fascia or anterior margin at the dermis) re-excision is strongly recommended. This may be accomplished with a re-operative partial mastectomy or with a total mastectomy. This choice will be at the discretion of the treating surgeon.

It is strongly recommended that all patients with pretreatment T4 or N1-2 disease have at least a level I and II axillary node dissection at the time of the definitive breast cancer operation. The use and timing (pre-chemotherapy or post-chemotherapy) of sentinel lymphadenectomy in clinical N0 is at the surgeon's discretion according to the individual surgeon's practice.

It is strongly recommended that any patient with a pre- or post-treatment sentinel node with a N1 (mic) or greater should undergo a post-treatment level I and II axillary node dissection.

8.6 Post protocol adjuvant therapy

8.6.1 Chemotherapy and trastuzumab

Adjuvant therapy will be at the discretion of the treating oncologist, however adjuvant dose-dense AC (doxorubicin 60 mg/m^2 Day 1, cyclophosphamide 600 mg/m^2 Day 1 every 2 weeks x 4 cycles) or standard schedule AC is recommended. It is strongly recommended that adjuvant AC be followed by one year of adjuvant trastuzumab 6 mg/kg (after 8 mg/kg load week 1) every 3 weeks for 39 weeks (13 doses) unless contraindicated. It is recommended that adjuvant therapy begin within 4 weeks of surgery.

8.6.2 Radiotherapy

Adjuvant radiation therapy should be provided if the breast is conserved; the use of post-mastectomy adjuvant radiotherapy is at the judgment of the treating radiation oncologist. However, post-mastectomy radiation therapy is encouraged for patients with presenting or residual T3/4 tumors or persistent involved lymph nodes despite chemotherapy. Regional nodal irradiation is at the discretion of the treating physician, and may include supraclavicular, axillary and internal mammary node irradiation or any combination of these regions. Partial breast irradiation techniques may be used but are discouraged. Radiotherapy may be delivered concurrently with adjuvant trastuzumab.

The following are the recommended radiation therapy guidelines:

Target volume: The ipsilateral breast should be irradiated in all patients who receive breast conserving surgery. In patients receiving post-mastectomy radiotherapy, the chest wall should be treated. Elective supraclavicular or axillary fields may be added to either breast or chest wall treatment at the discretion of the treating physician. Internal mammary nodal irradiation is discouraged.

Timing: Irradiation should begin 3-6 weeks following breast conserving surgery, 3-6 weeks following mastectomy or 3-6 weeks following post protocol adjuvant chemotherapy, with the exception of extenuating circumstances such as wound infection.

Technique: Treatment planning on a simulator or CT-simulation is recommended in all patients. The intact breast or chest wall should be treated with opposed tangential fields. Gantry angulation or half-beam block should be used to achieve coplanarity of the deep margins, and blocks may be required to avoid divergence into the supraclavicular field. IMRT is allowed in the context of standard field arrangements. In general, the medial border will be at midline, the lateral border at the mid-axillary line, the superior border at the matchline of the supraclavicular field at the inferior aspect of the clavicular head, and the inferior border 2 cm below the inframammary fold of the intact breast, or the contralateral breast in the case of chest wall irradiation. The maximal amount of lung tissue to be included within the tangential fields is 3 cm, as defined along the transverse plane of the central axis.

For left-sided primary tumors, care should be taken to exclude as much of the heart as possible from the tangential fields using CT to plan beam arrangements to minimize cardiac irradiation. If used, the supraclavicular fields should be treated through an anterior photon field angled into the ipsilateral side at approximately 10-15 degrees to avoid irradiation of the trachea, esophagus, and spinal cord. In general, the superior border is to the level of the cricothyroid groove, the inferior border matches with the tangents, generally at the clavicular head. A half beam block technique should be used to avoid beam divergence into the tangential fields. The medial border should be at least to the anterior midline, and a block and can be added, if necessary, to shield the trachea, esophagus, and spinal cord. The lateral border is to the medial aspect of the humeral head.

If the axilla requires treatment, the lateral border should be extended to the mid-humeral head and a posterior axillary boost is used to supplement dose delivered from the extended supraclavicular field, with the superior border to the coracoid process, the inferior border to coincide with the inferior margin of the supraclavicular field, and the medial border to the midclavicle as it crosses the shadow of the lung apex. A block may be used to shield the humeral head.

Dose: The ipsilateral remaining breast or chest wall should be treated to a dose of 5000 cGy in 2 Gy fractions or 5040 cGy in 1.8 Gy fractions using 4-6 MV photons.

For the intact breast, a boost to the tumor bed of 1000-1600 cGy in 180-200 Gy fractions is strongly recommended. For the chest wall, bolus (tissue equivalent material) should be applied to provide full skin dose, typically 1.0 cm every other day or 0.5 cm daily. An occasional patient with an increased separation between the medial and lateral tangent entrance points may be treated with a > 6 MV linear accelerator if necessary to comply with dose homogeneity requirements, but this may require beam spoilers or additional bolus depending on the dose delivered to the skin. The maximum dose in homogeneity should be +10% and -5% at the central transverse plane. It is expected that wedge filters, tissue compensation devices or IMRT will be used to maximize dose homogeneity.

The supraclavicular field should be treated to a dose of 4500-5040 cGy in 1.8 – 2 Gy fractions to a depth of 3 cm. If a posterior axillary boost field is used to treat the axilla, the dose should be calculated so that the mid-plane of the axilla at the central axis received a total cumulative dose of 4500-5040 cGy delivered both from the anterior-oblique supraclavicular and posterior axillary fields.

Treatment modifications: Treatment breaks are discouraged, but may be necessary secondary to treatment-related toxicities. Breaks are used at the discretion of the patient's physicians, and treatment interruptions should be carefully indicated in the treatment record.

8.6.3 Endocrine therapy for hormone receptor positive patients

For premenopausal patients that are ER or PgR positive, tamoxifen 20 mg/day for 5 years is recommended following completion of chemotherapy and radiation therapy. It may be given concurrently with adjuvant trastuzumab. Physicians are also encouraged to enroll appropriate patients onto adjuvant endocrine therapy trials such as SOFT or TEXT. For postmenopausal patients that are ER or PgR positive either tamoxifen or an aromatase inhibitor is recommended for up front therapy. Patients receiving up front tamoxifen should be considered for aromatase inhibitor therapy after 2-5 years of tamoxifen. The total duration of endocrine therapy is expected to be from 5-10 years.

9.0 DOSE MODIFICATIONS AND MANAGEMENT OF TOXICITY

Arm 1 (THL) Dose Levels

Dose level	Paclitaxel	Lapatinib	Trastuzumab
0 (starting dose)	80 mg/m2	750 mg/day	2 mg/kg
-1	60 mg/m^2	500 mg/day	No changes
-2	40 mg/m^2	250 mg/day	No change

Arm 2 (TH) Dose Levels

Dose level	Paclitaxel	Trastuzumab
0 (starting dose)	80 mg/m^2	2 mg/kg
-1	60 mg/m^2	No changes
-2	40 mg/m^2	No change

Arm 3 (TL) Dose Levels

Dose level	Paclitaxel	Lapatinib
0 (starting dose)	80 mg/m^2	1500 mg/day
-1	60 mg/m^2	1250 mg/day
-2	40 mg/m^2	1000 mg/day

Update #5
6/15/11

For patients enrolled to this trial BEFORE local approval of Update #5.

9.1 Hematologic

The following dose modifications should be made for blood counts obtained within 48 hours prior to each weekly dose of paclitaxel, after the first dose.

9.1.1 Neutropenia

For all arms

- **For ANC < 800/µl:** skip paclitaxel. Resume paclitaxel at the same dose when neutropenia resolves to ≥ 800/µl.
- **For ANC < 100/µl or febrile neutropenia:** skip paclitaxel. Resume paclitaxel when infection and neutropenia resolve to ≥ 800/µl, with one dose level reduction for all subsequent doses.
- If ANC < 800/µl is obtained > 24 hours before a scheduled dose, the CBC should be repeated on the day of treatment. In this instance the ANC on the day of treatment should be used for dose modifications.
- GCSF or GMCSF may be used between days 2-6 according to patient need, following an ANC < 800/µl. Pegfilgrastim may not be used in this study.
- If paclitaxel is skipped for 3 weeks in a row, discontinue paclitaxel.
- There are no dose reductions of paclitaxel below dose level -2. If a dose reduction below dose level -2 is required, discontinue paclitaxel.
- Continue lapatinib and/or trastuzumab if paclitaxel is skipped or discontinued for neutropenia.

9.1.2 Thrombocytopenia

For all arms

- **Grade 2 thrombocytopenia:** skip paclitaxel. Resume paclitaxel when platelets recover to ≥ 75,000/µl with one dose level reduction in dose for all subsequent doses.
- **Grade 3 or grade 4 thrombocytopenia:** skip paclitaxel. Resume paclitaxel when platelets recover to ≥ 75,000/µl with a two-dose level reduction in dose for all subsequent doses.
- If paclitaxel is skipped for 3 weeks in a row for thrombocytopenia, discontinue paclitaxel.
- Continue lapatinib and/or trastuzumab if paclitaxel is skipped or discontinued for thrombocytopenia.

9.2 Hepatic dysfunction

9.2.1 Grade 2 bilirubin

Arm 1 (THL)

- Decrease lapatinib by one dose level for all subsequent doses.
- Skip paclitaxel. Resume when bilirubin resolves to < grade 1, at the previous dose. If bilirubin remains at grade 2 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- If a dose reduction below dose level -2 is required for paclitaxel and/or lapatinib, discontinue paclitaxel and or lapatinib.
- Continue trastuzumab if lapatinib or paclitaxel is skipped or dose reduced or discontinued for bilirubin.

Arm 2 (TH)

- Skip paclitaxel. Resume when bilirubin resolves to ≤ grade 1, at the previous dose. If bilirubin remains at grade 2 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- If a dose reduction below dose level -2 is required for paclitaxel, discontinue paclitaxel.
- Continue trastuzumab if paclitaxel is skipped or discontinued for bilirubin.

Arm 3 (TL) Update #5 6/15/11

- Decrease lapatinib by one dose level for all subsequent doses.
- Skip paclitaxel. Resume when bilirubin resolves to < grade1, at the previous dose. If bilirubin remains at grade 2 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- If a dose reduction below dose level -2 is required for paclitaxel and/or lapatinib, discontinue paclitaxel and/or lapatinib.

9.2.2 Grade ≥ 3 bilirubin

For all arms

Discontinue all protocol therapy.

9.2.3 Grade 3 AST or ALT

Arm 1 (THL)

- Decrease lapatinib by one dose level for all subsequent doses.
- Skip paclitaxel. Resume when AST/ALT resolves to < grade 3, at the previous dose. If AST/ALT remains at grade 3 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- If a dose reduction below dose level -2 is required to paclitaxel and/or lapatinib, discontinue paclitaxel and or lapatinib.
- Continue trastuzumab if lapatinib or paclitaxel are skipped or decreased of discontinued for AST/ALT.

Arm 2 (TH)

- Skip paclitaxel. Resume when AST/ALT resolves to < grade 3, at the previous dose. If AST/ALT remains at grade 3 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- Continue trastuzumab if paclitaxel is skipped or discontinued for bilirubin.

Arm 3 (TL) Update #5 6/15/11

- Decrease lapatinib by one dose level for all subsequent doses.
- Skip paclitaxel. Resume when AST/ALT resolve to < grade 3, at the previous dose. If AST/ALT remain at grade 3 after skipping one dose of paclitaxel, resume paclitaxel with one dose level reduction for all subsequent doses.
- If a dose reduction below dose level -2 is required for paclitaxel and/or lapatinib, discontinue paclitaxel and/or lapatinib.

9.2.4 Grade 4 AST or ALT

For all arms

Discontinue all protocol therapy.

9.3 Neurologic toxicity

9.3.1 Neuropathy

For all arms

- Grade 2: reduce paclitaxel by one dose level for all subsequent doses.
- Grade > 3: skip paclitaxel until neuropathy improves to < grade 1. Then resume at one dose level reduction for all subsequent doses.
- Continue lapatinib and/or trastuzumab if paclitaxel is skipped or discontinued for neuropathy.
- If paclitaxel is skipped for 3 weeks in a row for neuropathy, discontinue paclitaxel.
- If a dose reduction below dose level -2 is required for paclitaxel, discontinue paclitaxel.

9.4 Gastrointestinal toxicity

9.4.1 Diarrhea

Diarrhea of any grade should be managed aggressively with loperamide and supportive care as described in Section 13.4. In addition, patients should be monitored closely per the treating physician.

Arm 1 (THL)
Grade ≥ 2 diarrhea

- Interrupt lapatinib. Resume lapatinib when diarrhea resolves to < grade 2, with one dose level reduction for all subsequent doses.
- If lapatinib is interrupted for > 3 weeks for diarrhea, discontinue lapatinib.
- Continue paclitaxel and trastuzumab if lapatinib is interrupted or discontinued for diarrhea.

Arm 2 (TH)
No dose modification are required for diarrhea.

Arm 3 (TL) Update #5 6/15/11

- Grade > 2 diarrhea: interrupt lapatinib. Resume lapatinib when diarrhea resolves to < grade 2, with one dose level reduction for all subsequent doses.
- If lapatinib is interrupted for > 3 weeks for diarrhea, discontinue lapatinib.
- Continue paclitaxel if lapatinib is interrupted or discontinued for diarrhea.

9.5 Cardiac toxicity

Arm 1 (THL)

- For > grade 2 left ventricular systolic dysfunction or LVEF at 8 weeks that has decreased to less than institution LLN: interrupt lapatinib and skip trastuzumab for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is WNL and left ventricular systolic dysfunction is < grade 1, resume lapatinib and trastuzumab at the same dose. If LVEF is less than LLN or left ventricular systolic dysfunction remains > grade 1, discontinue lapatinib and trastuzumab therapy.

  For LVEF at 8 weeks that has decreased by > 15 percentage points from baseline, but is still above LLN, interrupt lapatinib and skip trastuzumab for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is < 15 percentage points from baseline, but remains above institutional LLN, resume lapatinib and trastuzumab at the same dose. If repeat LVEF is > 15 percentage points below baseline, discontinue trastuzumab and lapatinib.
- For symptomatic (NYHA Class (III-IV) congestive heart failure, discontinue lapatinib and trastuzumab.
- If trastuzumab and lapatinib are interrupted/skipped or discontinued for decreased LVEF, continue paclitaxel.

Arm 2 (TH)

- For > grade 2 left ventricular systolic dysfunction or LVEF at 8 weeks that has decreased to less than institution LLN: skip trastuzumab for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is WNL and left ventricular systolic dysfunction is < grade 1, resume trastuzumab at the same dose. If LVEF is less than LLN or left ventricular systolic dysfunction remains > grade 1, discontinue trastuzumab.

  For LVEF at 8 weeks that has decreased by > 15 percentage points from baseline, but is still above LLN, skip trastuzumab for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is < 15 percentage points from baseline, but remains above institutional LLN,

resume trastuzumab at the same dose. If repeat LVEF is > 15 percentage points below baseline, discontinue trastuzumab.

- For symptomatic (NYHA Class III-IV) congestive heart failure, discontinue trastuzumab.
- If trastuzumab is skipped or discontinued for decreased LVEF, continue paclitaxel.

Arm 3 (TL) Update #5 6/15/11

- For > grade 2 left ventricular systolic dysfunction or LVEF at 8 weeks that has decreased to less than institution LLN: interrupt lapatinib for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is WNL and left ventricular systolic dysfunction is < grade 1, resume lapatinib at the same dose. If LVEF is less than LLN or left ventricular systolic dysfunction remains > grade 1, discontinue lapatinib.

  For LVEF at 8 weeks that has decreased by > 15 percentage points from baseline, but is still above LLN, interrupt lapatinib for 4 weeks. Repeat LVEF in 4 weeks. If repeat LVEF is < 15 percentage points from baseline, but remains above institutional LLN, resume lapatinib at the same dose. If repeat LVEF is > 15 percentage points below baseline, discontinue lapatinib.
- For symptomatic (NYHA Class III-IV) congestive heart failure, discontinue lapatinib.
- If lapatinib is interrupted or discontinued for decreased LVEF, continue paclitaxel.

9.6 Hypersensitivity due to paclitaxel

All Arms

- Mild symptoms (Grade 1) (e.g., transient flushing, rash or fever): Complete paclitaxel infusion, no treatment required, but observe patient at least until symptoms have resolved.
- Moderate symptoms (Grade 2) (e.g., rash, flushing, urticaria, dyspnea, chest discomfort): Interrupt paclitaxel infusion. Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg (or equivalent). Resume paclitaxel at a slower rate after recovery of symptoms to ≤ grade 1. If no recurrence of symptoms, the planned rate may be resumed. If symptoms recur after re-challenge, stop paclitaxel infusion. Discontinue paclitaxel. Continue lapatinib and/or trastuzumab.
- Severe or life-threatening symptoms (Grade 3 or 4) (e.g., hypotension angioedema, respiratory distress or anaphylaxis) Stop paclitaxel infusion and discontinue paclitaxel. Continue lapatinib and/or trastuzumab. Manage the reaction according to institutional guidelines.

9.7 Trastuzumab infusion reactions (occurring during trastuzumab infusion)

Arms 1 and 2

- **Grade 2 infusion reactions:** interrupt trastuzumab and treat symptomatically according to institutional guidelines. Resume infusion at a slower rate after reaction has resolved to < grade 1.
- **Grade 3 or 4 infusion reaction:** stop trastuzumab and manage the reaction according to institutional guidelines. Discontinue trastuzumab therapy, continue paclitaxel (and lapatinib).

9.8 Skin toxicity

Arm 1 (THL)

- For > grade 3 rash, interrupt lapatinib. Resume lapatinib and reduce by one dose level when rash improves to < grade 1.
- If lapatinib is interrupted for rash for > 3 weeks, discontinue lapatinib.
- If lapatinib is interrupted or discontinued for rash, continue paclitaxel and trastuzumab.

Arm 2 (TH)

No dose modifications required for rash.

Arm 3 (TL) Update #5 6/15/11

- For > grade 3 rash, interrupt lapatinib. Resume lapatinib and reduce by one dose level when rash improves to < grade 1.
- If lapatinib is interrupted for rash for > 3 weeks, discontinue lapatinib.
- If lapatinib is interrupted or discontinued for rash, continue paclitaxel.

9.9 Interstitial lung disease

In the event of symptomatic interstitial pneumonitis or symptomatic pulmonary edema, all protocol therapy should be interrupted during evaluation. If no other source is found and the pulmonary disease is thought to be at least possibly drug related, discontinue protocol therapy.

9.10 Other grade 3 non-hematologic toxicity attributed to paclitaxel

All arms

- Skip paclitaxel until toxicity improves to < grade 2, then resume paclitaxel with one dose level reduction.
- If paclitaxel is skipped for > 3 weeks, discontinue paclitaxel.
- If paclitaxel is skipped or discontinued, continue lapatinib and/or trastuzumab for all arms.

9.11 Other grade 3 non-hematologic toxicity attributed to lapatinib

For arms 1 (THL) and Arm 3 (TL) Update #5 6/11/15

- Interrupt lapatinib until toxicity improves to < grade 2, then resume lapatinib with one dose level reduction.
- If lapatinib is interrupted for > 3 weeks, discontinue lapatinib.
- If lapatinib is interrupted or discontinued, continue paclitaxel (and trastuzumab).

9.12 Other grade 4 non-hematologic toxicity

Discontinue all protocol therapy.

9.13 Dose modifications for obese patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all dosing is to be determined solely by (1) the patient's BSA as calculated from actual weight or (2) actual weight without any modification unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation. Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on CALGB protocols.

10.0 SURGICAL PRACTICE PATTERNS FOR USE OF BREAST CONSERVATION AND SENTINEL LYMPHADENECTOMY IN PATIENTS TREATED WITH NEOADJUVANT CHEMOTHERAPY

10.1 Background

This surgical sub-study examines surgical practice patterns for use of breast conservation and sentinel lymphadenectomy in patients treated with neoadjuvant chemotherapy. Several studies suggest that breast conservation after cytoreduction of larger tumors by neoadjuvant chemotherapy is safe and feasible [37, 38], however other studies have raised concern about higher than expected rates of in-breast recurrence [39-41]. Current surgical practice patterns are not known and likely vary widely. Similarly, lymphatic mapping and sentinel lymphadenectomy (LM/SL) is an accepted axillary staging procedure for patients with T1 or T2 breast cancers. However, the use and timing of LM/SL and breast conservation in patients with large breast cancers treated with neoadjuvant chemotherapy varies considerably even among academic institutions. Some argue for the sentinel node procedure to be performed prior to any drug therapy so that the true histopathologic status of the axilla is known prior to initiating systemic therapy [42, 43]. Others advocate its use following neoadjuvant chemotherapy arguing that it is accurate and that the most important axillary status is the post-neoadjuvant chemotherapy tumor status [44]. The only multi-institutional trial data available is from NSABP B27, which reported a false-negative rate of 11% in patients who received the LM/SL after neoadjuvant chemotherapy [45], clearly not approaching the <5% recommended in the ASCO Position Statement. An observational surgical trial will be added to the randomized neoadjuvant trial to determine a) the current practice patterns of the timing of LM/SL and conservation of the breast after cytoreduction in patients treated with neoadjuvant chemotherapy and b) the identification and accuracy rates of performing LM/SL before or after neoadjuvant chemotherapy. These data will guide decisions in future surgical neoadjuvant studies.

10.2 Objectives

10.2.1 To examine the practice patterns and use of sentinel lymphadenectomy (pre-chemotherapy or post-chemotherapy) in patients with T2 or T3 breast cancer.

10.2.2 To examine the proportion of patients who presented with T2 or T3 cancers who undergo mastectomy despite cytoreduction adequate for breast conservation.

10.3 Methods

Data regarding surgical practice decisions during neoadjuvant therapy will be collected across both this and the CALGB 40603 HER2-negative neoadjuvant trial using the C-1718 CALGB Pre- Neo-adjuvant Chemotherapy Surgical Evaluation Form and the C-1719 CALGB Post Neo-adjuvant Chemotherapy Surgical Evaluation Form. These data include the use and timing of sentinel lymphadenectomy as well as the use of breast conservation in patients deemed initially ineligible for BCT.

11.0 ADJUVANT RADIOTHERAPY PATTERNS OF CARE AND LOCOREGIONAL FAILURE PATTERNS IN THE SETTING OF NEOADJUVANT CHEMOTHERAPY

11.1 Background

While radiotherapy is routinely recommended after breast conserving therapy, the pivotal trials that established this practice were based on treatment regimens that included treatment to the breast but not the regional lymph nodes [46-48]. Radiotherapy practice has evolved to include regional nodal treatment under certain circumstances, particularly elective treatment of the supraclavicular fossa. However, there is currently no consensus as to when to include

treatment to the supraclavicular fossa [49, 50]. Indeed, an ongoing trial is directly testing the utility of adjuvant regional nodal treatment (NCIC MA20). While awaiting the results of this study, regional nodal treatment is typically driven by estimates of recurrence based on pathologic axillary nodal stage. In the neoadjuvant setting, there is even less information available regarding risk of regional nodal failure to facilitate informed decision-making with regard to adjuvant nodal treatment. Furthermore, the use of elective post-mastectomy radiotherapy remains controversial, particularly in the setting of intermediate risk disease [51]. Nodal stage is the primary factor influencing treatment decisions, as the risk of locoregional recurrence based on nodal stage is well established. However, there are limited data correlating failure patterns with nodal stage after neoadjuvant chemotherapy [52]. This trial offers an opportunity to query practice patterns in a setting of protocol-stipulated neoadjuvant treatment. Our hypothesis is that patients with increased nodal burden of metastatic disease after neoadjuvant chemotherapy will be more likely to receive adjuvant regional radiotherapy and post-mastectomy radiotherapy.

The surgical companion to this trial investigates sentinel lymph node practice patterns in the setting of neoadjuvant chemotherapy. Knowledge of nodal status prior to initiating chemotherapy may be preferentially used by radiation oncologists, as risk rates have traditionally been based on pre-chemotherapy nodal stage. However, total nodal staging (i.e., total number of involved lymph nodes) is not determined by sentinel nodal examination. There is currently no information available related to how radiation oncologists use sentinel node data in the neoadjuvant setting to decide radiotherapy treatment volumes. Our hypothesis is that regional nodal irradiation and postmastectomy radiotherapy are more likely to be delivered in patients with involved sentinel lymph nodes than in cases where the sentinel node is not involved.

Since radiotherapy is associated with increased toxicity (particularly pulmonary and cardiac toxicity and lymphedema), and since both lapatinib and trastuzumab are radiosensitizers in preclinical studies, it is also relevant to obtain regional radiotherapy treatment data in order to evaluate radiation-related toxicity between arms [53, 54]. Finally, while disease free survival is not a primary endpoint of the study, collection of failure pattern data will be highly valuable in the long term. An assessment of local failure patterns in patients who do or do not receive radiotherapy will be highly informative with regard to identifying the role of radiotherapy in the setting of novel regimens of neoadjuvant radiotherapy. Ultimately, joint analysis of locoregional failure patterns from this and neoadjuvant studies in other breast cancer subtypes will provide an initial assessment of potential subtype-specific differences in locoregional control. These analyses will require collection of radiotherapy treatment data.

11.2 Objective

To determine the radiotherapy practice patterns for post-mastectomy and regional nodal irradiation in patients undergoing neoadjuvant chemotherapy.

11.3 Methods

Breast radiotherapy is required after breast conserving therapy. Post-mastectomy radiotherapy is left to investigator discretion. Radiotherapy data collection will include the following: breast radiotherapy (yes or no), elective postmastecomy radiotherapy delivery (yes or no), regional nodal treatment (yes or no), if yes, which regional nodal volumes (supraclavicular, axillary, internal mammary lymph nodes). Dose per fraction, total dose, and total treatment duration will be collected. Acute (skin), and subacute (pulmonary, fibrosis, cardiac and lymphedema) radiation related toxicity will be prospectively captured.

Treatment delivery will be examined with regard to post-neoadjuvant chemotherapy nodal status. The proportion of patients receiving adjuvant post-mastectomy radiotherapy will be determined by nodal disease burden (0, 1-3, 4+) and T stage. Likewise the proportion of

patients receiving elective nodal irradiation will be evaluated by nodal disease burden. Rates of grade 3 or greater toxicity will be determined in patients treated with regional nodal irradiation vs. no regional nodal irradiation, and with concomitant trastuzumab vs. no concomitant trastuzumab.

Use of up-front sentinel lymph node dissection is being evaluated in the surgical substudy. We will also evaluate post-mastectomy radiotherapy and regional nodal irradiation use with regard to up-front sentinel lymph node dissection results. The proportion of patients receiving adjuvant post-mastectomy radiotherapy or regional nodal irradiation will be determined in patients with involved sentinel lymph nodes vs. non-involved sentinel lymph nodes.

A joint analysis of the patterns of radiotherapy delivery based on nodal status will be performed between this study and 40603.

12.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

12.1 Preparation and handling

Qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment.

12.2 Unused drugs

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

12.3 Dose rounding

The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

12.4 Dose changes

It is not necessary to change the dose of paclitaxel or trastuzumab due to changes in weight unless the calculated dose changes by ≥10%.

12.5 Lapatinib (GW572016, Tykerb®)

Please refer to the FDA-approved package insert for lapatinib for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

Lapatinib (NSC 727989/IND [redacted]) is an investigational agent in this study and will be provided for treatment Arms 1 and 3 by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

A supply of lapatinib may be ordered by completing the form NIH-986 "Clinical Drug Request" and faxing it to the Pharmaceutical Management Branch at:

301-480-4612

Questions should be directed to 301-496-5725.

Lapatinib is supplied as 250 mg oval, biconvex, orange, film-coated tablets for oral administration. The lapatinib tablets have one side plain and one side embossed with "FG HLS" or "GS XJ6". Each tablet contains 405 mg of lapatinib ditosylate monohydrate, equivalent to 250 mg of lapatinib free base per tablet, with povidone (K29-32), sodium starch glycolate, and magnesium stearate. The film coat contains FD&C yellow #6, hypromellose,

polyethylene glycol 400, polysorbate 80, and titanium dioxide. Lapatinib will be supplied in bottles of 90 tablets.

Drug Accountability for Lapatinib

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record available on the CTEP home page (http://ctep.cancer.gov) or by calling the PMB at 301-496-5725.

Storage and Stability

Lapatinib tablets should be stored at controlled room temperature (15ºC to 30ºC, 59ºF to 86ºF) and protected from light. Shelf-life studies with lapatinib are continuing and investigators will be notified when lots have expired.

Administration Update #5 6/15/11

For this study, the starting dose of lapatinib in Arm 3 will be administered at a dose of 1500 mg (6 X 250 mg tablets) orally once daily. The staring dose for Arm 1 will be administered at a dose of 750 mg (3 x 250 mg) daily for 16 weeks. Lapatinib should be taken on an empty stomach (either 1 hour before or 1 hour after meals). In the event of vomiting, the dose of lapatinib may be repeated if the time from administration is $\leq$ 1 hour and the total number of tablets taken can be identified in the emesis.

Patients enrolled to this trial after local IRB approval of Update #5 will be randomized to Arm 1 or Arm 2 only.

Whenever possible, whole tablets should be administered. Lapatinib tablets have not been deliberately formulated to be dispersible tablets; however, in circumstances where dosing of whole tablets is not possible, see the procedure below. **Tablet crushing is not recommended.**

For patients unable to swallow tablets, a suspension preparation in water or Kool-Aid® can be made using the following procedure:

1. Prepare Lemonade or Tropical Punch Kool-Aid® as directed on package.
2. Place 2 or 4 ounces of water or Kool-Aid® in a glass container, then add the required number of lapatinib tablets for dose (up to six tablets per 2 to 4 ounces) to the container.
3. Cover the container, let it stand for 5 minutes, and the stir the mixture intermittently for 15 minutes or until it is fully dispersed.
4. Stir the container for 5 seconds then administer.
5. Rinse the container with 2 ounces of water or Kool-Aid® and repeat the administration process.

(The lemonade mixture appears somewhat like orange juice whereas the tropical punch mixture appears like carrot juice.)

Toxicity

The most common adverse events reported to date with lapatinib include nausea, anorexia, diarrhea, acneiform rash and fatigue.

Hepatotoxicity has been observed in lapatinib studies sponsored by GlaxoSmithKline. The most frequent presentation is elevated liver enzymes; however, elevated liver enzymes, associated with signs of impaired hepatic function such as elevated direct bilirubin have also been reported. Elevated liver enzymes generally returned to normal levels when patients stopped taking lapatinib. Rarely, liver failure causing death has been reported.

Because liver enzyme elevations and liver failure have occurred in patients several months after the initiation of treatment, liver function (transaminases, bilirubin and alkaline

phosphatase) in patients should be monitored before initiation of treatment, at monthly intervals thereafter, or more frequently as clinically indicated. All patients on and off protocol therapy who develop signs and symptoms suggestive of hepatotoxicity should be thoroughly evaluated, closely monitored and supported as clinically indicated. Exclusion of alternate causes of hepatic dysfunction including viral hepatitis A, B, or C; alcoholic or autoimmune hepatitis, biliary disorders, and circulatory disorders as causes of liver injury should be considered and review of concomitant medication, herbal or dietary supplement, nonprescription medication, such as acetaminophen, or exposure to occupational chemical agents should be undertaken and results documented.

Also reported on lapatinib trials but with the relationship to lapatinib still undetermined: leukocytes (total WBC).

Based on effects seen with trastuzumab and with EGFR inhibitors, the potential exists for lapatinib (which inhibits both HER2 and EGFR) to cause left ventricular dysfunction or pneumonitis.

Drug Interactions

Lapatinib is metabolized primarily by CYP3A4 and 3A5. Thus there is the potential for inhibition of metabolism (possibly increased toxicity) by 3A4 inhibitors (e.g., itraconazole, ketoconazole) or increased metabolism (potential decreased effect) by 3A4 inducers (e.g., phenytoin or carbamazepine).

Specifically, AUC increased 3.5 fold with co-administration of ketoconazole in healthy volunteers, and decreased approximately 72% with co-administration of carbamazepine, also in healthy volunteers.

Where possible, drugs that are known inducers or inhibitors of CYP3A4 should be avoided.

See the table below for a list of CYP3A4 Inducers and Inhibitors.

Examples of Inducers and Inhibitors of CYP3A4

INDUCERS	INHIBITORS		
Carbamazepine	Amiodarone	Fluoxetine	Norfloxacin
Dexamethasone	Azithromycin	Grapefruit Juice	Quinine
Ethosuximide	Cimetidine	Indinavir	Ritonavir
Phenobarbital	Clarithromycin	Itraconazole	Roxithromycin
Phenytoin	Ciprofloxacin	Ketoconazole	Saquinavir
Primidone	Clotrimazole	Metronidazole	Sertraline
Rifabutin	Cyclosporine	Mibefradil	Telithromycin
St. John's Wort	Delaviridine	Miconazole	Troleandomycin
	Dirithromycin	Mifepristone	Verapamil
	Erythromycin	Nefazodone	Voriconazole
	Fluconazole (doses >	Nelfinavir	Zafirlukast
This is not a comprehensive list of inducers and inhibitors of CYP3A4 and is meant only to be a guide.			

In vitro studies with human liver microsomes also indicate that lapatinib may inhibit CYP3A4 at clinically achievable concentrations. Since many drugs are metabolized by 3A4, their use in combination with lapatinib should be carefully monitored. There are no available data as of yet regarding such interactions in humans.

12.6 Paclitaxel

Please refer to the FDA-approved package insert for paclitaxel for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

Paclitaxel is commercially available in a concentration of 6 mg/ml in 5 mL, 16.7 mL, 25 mL and 50 mL multidose vials. Each mL of solution also contains 527 mg of polyoxyethylated castor oil and dehydrated alcohol, USP. Please refer to the FDA-approved package insert for complete product information.

Preparation

Paclitaxel must be diluted prior to administration with 0.9% sodium chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers due to leaching of DEHP [di-(2ethylhexyl)phthalate] plasticizer from polyvinyl chloride (PVC) containers. Non-PVC containing tubing and connectors should also be used. Paclitaxel should be administered through an in-line $\leq$ 0.22 micron filter.

Storage and Stability

Intact vials should be stored at controlled room temperature (20° - 25°C; 68° - 77°F) in the original package to protect from light, and remain stable until the expiration date on the label. Refrigeration does not adversely affect stability. Upon refrigeration components in the paclitaxel vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. Diluted 0.3 – 1.2 mg/mL solutions are stable for up to 27 hours at room temperature under normal room lighting. Although solutions may be chemically stable for longer periods in some situations, precipitation may occur unpredictably. The mechanism of this precipitation has not been determined.

Administration

Paclitaxel, 80 mg/m^2/week for 16 weeks, will be administered as an IV infusion over 1 hour. Please see Section 8.3.1 for premedication regimen.

Toxicities

Hypersensitivity reactions are common. They are thought to be due, at least in part, to the Cremaphor EL® vehicle. Reactions generally occur within the first hour of administration. The most common symptoms observed in severe reactions include dyspnea, flushing, chest pain and tachycardia. Patients should be pretreated to prevent hypersensitivity reactions (see Section 8.3.1).

Cardiovascular events observed with paclitaxel include hypotension and bradycardia; typically neither discontinuation of paclitaxel nor specific therapy for the event is required.

The frequency and severity of neurologic events are dose-dependent. Peripheral neuropathy is rarely severe and may be the cause of paclitaxel discontinuation in 1% of patients. Sensory symptoms usually improve or resolve within several months of completion of treatment. Serious neurologic events such as grand mal seizures, syncope, ataxia and neuroencephalopathy are rare. Pre-existing neuropathies are not a contraindication to treatment with paclitaxel.

The most common GI toxicities, which include nausea, vomiting, diarrhea and mucositis, are typically mild or moderate in severity. Mucositis occurs more frequently with a 24 hour infusion than with shorter infusion schedules.

Although 60% of all patients experience arthralgia and myalgia, there is no consistent relationship between the dose or schedule of paclitaxel and the frequency of these events. The symptoms, which usually begin 2 or 3 days after paclitaxel treatment, are generally transient. Infusion site reactions are more common with 24 hour infusions. They are typically mild, consisting of erythema, tenderness, skin discoloration or swelling at the infusion site. Pacliltaxel is generally considered to be an irritant, but isolated cases of more severe tissue damage following extravasation have been reported. Almost all patients receiving paclitaxel experience alopecia. Nail changes are uncommon. Occasionally edema is seen, usually of mild severity.

12.7 Trastuzumab

Please refer to the FDA-approved package insert for trastuzumab for product information, extensive preparation instructions, and a comprehensive list of adverse events.

Availability

Trastuzumab is commercially available and is available as a powder for reconstitution in vials containing 440 mg of drug. Each vial is accompanied by a 20 mL vial of Bacteriostatic Water for Injection, USP, 1.1% benzyl alcohol.

Storage and Stability

Unopened vials of trastuzumab should be stored in a refrigerator. Vials reconstituted with the diluent supplied are intended for multiple use and are stable of 28 days under refrigeration. Solutions further diluted in 0.9% sodium chloride are stable for 24 hours. Reconstituted or further diluted solutions should not be frozen.

Preparation

The vial of trastuzumab should be reconstituted with the supplied diluent, resulting in a concentration of 21 mg/ml. (In the event of a known allergy to benzyl alcohol, trastuzumab should be reconstituted with sterile water for injection.) The reconstituted solution should be further diluted in 250 ml of 0.9% sodium chloride for IV infusion. Dextrose solutions should not be used for dilution of trastuzumab.

Administration

The loading dose of trastuzumab, 4 mg/kg, is administered by IV infusion over 90 minutes. Subsequent weekly doses of 2 mg/kg are administered by IV infusion over 30 minutes, assuming the first dose was well tolerated. If the initial or subsequent doses are not well tolerated (e.g., the patient experiences fever or chills), subsequent infusions may be shortened only after a dose is well tolerated.

Toxicity

The most common adverse event associated with trastuzumab are infusion reactions. Although they have been reported to occur in up to 40% of patients, infusion reactions, consisting primarily of fever or chills, are generally of mild to moderate severity. Infusion reactions most often occur with the first infusion.

The most serious adverse reactions associated with trastuzumab include cardiomyopathy, pulmonary events, including adult respiratory distress syndrome and death, and febrile neutropenia/exacerbation of chemotherapy-induced neutropenia.

Left ventricular dysfunction is characterized by signs and symptoms of CHF and decrease in LVEF. Asymptomatic decreases in LVEF can been seen. See Section 9.5 for cardiac function monitoring and dose modifications.

Serious infusion reactions including bronchospasm, hypoxia and hypotension have been described in a small percentage of patients. Fatalities following infusion reactions have been reported from hours to days after the reaction.

Severe pulmonary events include bronchospasm, hypoxia, dyspnea, pulmonary infiltrates and pleural effusions, pulmonary edema (non-cardiogenic) and ARDS. Severe pulmonary toxicity may be part of serious infusion reactions.

When used as a single agent, trastuzumab causes myelosuppression in < 1% of patients. However the incidences of neutropenia and febrile neutropenia were higher in patients receiving trastuzumab plus chemotherapy than in patients receiving chemotherapy alone in randomized trials.

12.8 Collaborative agreement provisions

The lapatinib supplied by CTEP, DCTD, NCI used in this protocol is provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between GlaxoSmithKline (hereinafter referred to as the "Collaborator") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the Intellectual Property Option to Collaborator (http://ctep.cancer.gov/industry/ipo.html) contained within the terms of award, apply to the use of the lapatinib in this study:

1. Lapatinib may not be used for any purpose outside the scope of this protocol, nor can it be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for lapatinib are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected , used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Regulatory Affairs Branch, CTEP, DCTD, NCI
   Executive Plaza North, Suite 7111
   Bethesda, Maryland 20892
   FAX 301-402-1584
   Email: anshers@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13.0 ANCILLARY THERAPY

13.1 Supportive care

Patients should receive full supportive care, including transfusions of blood and blood products, epoetin, antibiotics, antiemetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on Form C-1712.

13.2 Other therapy

Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure; hormones administered for non-disease-related conditions (e.g., insulin for diabetes); and intermittent use of dexamethasone as an antiemetic in solid tumor protocols.

13.3 CALGB policy concerning the use of growth factors

G-CSF/GM-CSF may be used, according to ASCO guidelines, at the discretion of the treating physician. The use of Peg-filgrastim (Neulasta®) is not permitted.

The use of growth factors will be documented and reported on Forms C-1712 and 1714.

13.4 Management of lapatinib associated diarrhea

Loperamide should be routinely used with any grade of diarrhea, and may be considered with grade 1. It should be administered with an initial dose of 4 mg followed by 2 mg every 2 hours

(or 4 mg every 4 hours during the night), up to a maximum of 16 mg/day, until diarrhea free for 12 hours.

In addition, for grade 3 or 4 diarrhea or for < grade 3 with complicating features (e.g., grade 3 or 4 neutropenia, fever, dehydration), hydrate aggressively with IV fluids as appropriate and consider octreotide therapy. Begin antibiotic therapy with a fluoroquinolone for diarrhea persisting longer than 24 hours or accompanied by fever or neutropenia.

In addition, maintain adequate hydration and begin dietary modifications according to institutional preferences for diarrhea management. Consider counseling patients regarding dietary modifications with avoidance of greasy, fatty, or spicy foods, cruciferous vegetables such as cabbage, Brussels sprouts, broccoli and cauliflower. Hydration is important, with the goal of replacing lost volume with a combination of plain water and water containing some salt and sugar. The BRAT (bananas, rice, apple sauce, toast) diet may be used until the diarrhea begins to resolve.

13.5 Suggested management strategies for lapatinib associated rash

Skin rash (usually grade 1-2) has been observed during the first several days of treatment with EGFR inhibitors and has been observed to diminish in severity after 4 weeks of treatment in many patients. It is recommended that patients who develop dermatological reactions receive evaluations for management on the specific side effect. Treating physicians may want to consult a dermatologist as appropriate. Patients should be encouraged to avoid exposure to sunlight. Broad spectrum sunscreens (containing titanium dioxide or zinc oxide) with an SPF of at least 15 can be recommended. See Section 4.8 for lapatinib dose modifications.

In some patients, this rash appeared to be treatable with standard acne therapies, including topical and oral antibiotics used to treat acne. Anecdotal reports of improvement have occurred with any of the following: minocycline, topical tetracycline, topical clindamycin, mild-to-moderate strength steroid creams (fluticasone prioprionate 0.5%), topical or systemic antihistamines and immunomodulatos and hypoallergenic moisturizers and emollients for dry skin (5-10% urea in cetomacrogel cream or soft paraffin). Oral retinoids are not recommended because of theoretical concerns about negatively affecting the lapatinib mechanism of action. Additionally, for paronychia, antiseptic bath and local potent corticosteroids in addition to tetracycline therapy is recommended and if no improvement in these known to be resistant to treatment lesions, a dermatology or surgery consultation may be necessary. For infected lesions, appropriate, culture driven, systemic or topical antibiotics are indicated.

13.6 Lapatinib drug interactions

Patients should avoid taking medications thought to interact with lapatinib, see Section 12.5.

13.7 Adjuvant Therapy

Radiation therapy will be administered according to the treating radiation oncologist's practice. See Section 8.6.2 for radiation therapy guidelines.

14.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE

A baseline and presurgical radiographic study of the breast is required; MRI is recommended. The same radiographic modality should be used consistently. The baseline scan must be obtained within 28 days of beginning therapy. The pre-surgical scan should occur after the last chemotherapy administration. If the patient clinically progresses, repeat imaging is required. If there is discordance (clinical progression, but radiographic stable disease or response), contact the study chair. For the purposes of this study, patients should be clinically re-evaluated every 4 weeks.

14.1 Target lesions

14.1.1 Pathologic assessment

In the event of multifocal or multicentric disease in the breast, the investigator must determine which will represent the target lesion. This should remain consistent throughout the study. The target lesion should be selected on the basis of its size (lesion with the longest diameter) and suitability for accurate repetitive measurements (either by imaging techniques or clinically).

14.1.2 Pathologic complete response

Complete pathological response is defined as the absence of residual invasive carcinoma in the breast at the time of definitive surgical removal. Pathologic complete response in the lymph nodes is defined as no detectable invasive tumor by H&E. The sentinel/axillary lymph node status should be noted on the CALGB 40601 Post Surgery Histopathology Review (C-1717).

14.2 Radiographic assessment

Each patient will have a pre-therapy baseline radiographic tumor measurement, preferably by MRI, however if logistic or practical issues preclude MRI use, mammogram or ultrasound may be substituted. The longest diameter (LD) of the target lesion at the time of study initiation will be reported as the baseline LD. The baseline LD of the target lesion will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

14.2.1 Radiographic complete response: Disappearance of the target lesion.

14.2.2 Radiographic partial response (PR): At least a 30% decrease in the longest diameter (LD) of the target lesion taking as reference the baseline LD.

14.2.3 Radiographic progression (PD): At least a 20% increase in the LD of target lesion taking as references the baseline LD or the appearance of one or more new lesions.

14.2.4 Radiographic stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as reference the baseline LD.

14.3 Clinical assessments

Both target and, in the event of multifocal or multicentric invasive breast cancer, nontarget lesions should be followed clinically and their clinical size recorded at baseline. Measurements thereafter are required, however these lesions should be categorized at subsequent visits regarding whether there is evidence of progression. If "yes", the study chair should be notified in order to determine whether the patient should come off protocol treatment. In-situ carcinoma does not represent a non-target lesion and should not be recorded or followed.

15.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

15.1 Duration of treatment

15.1.1 Protocol neoadjuvant chemotherapy continues until surgery. Post-surgical therapy is at the discretion of the treating physician (recommended therapy is dose-dense AC x 4 cycles followed by trastuzumab see Section 8.6).

15.1.2 Disease Progression: If progression occurs during protocol neoadjuvant chemotherapy, consider surgery if possible. After surgery treatment will be at the discretion of the treating

physician. Continue to follow patient per protocol documenting details, including tumor measurements, on Forms C-817 and C-1715.

15.2 Extraordinary medical circumstances

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Notify the Study Chair.
- Document the reason(s) for discontinuation of therapy on Form C-1715.
- Follow the patient for first local and first distant recurrence, survival, DCIS and new primary of invasive cancer in any site for a maximum of 10 years following registration (see Section 6.1.1).

16.0 STATISTICAL CONSIDERATIONS

16.1 Study design

Update #5
6/15/11

This is a Phase III trial in which patients will be randomized with equal probability to one of three treatment arms:

- Paclitaxel, trastuzumab and lapatinib (THL),
- Paclitaxel plus trastuzumab (TH),
- Paclitaxel plus lapatinib (TL).

The THL and TL arms are the experimental arms, while TH is the control arm. The primary objective of the study is to compare pathologic complete response (pCR) in the breast between the control arm and each experimental arm.

Update #5 Closure of the paclitaxel plus lapatinib (TL) treatment arm

With Update #5, treatment Arm III, paclitaxel and lapatinib, was closed to accrual. Patients will continue to be randomized to the other two arms in a 1:1 fashion. The primary objective will be restricted to the comparison of THL vs TH on an equal basis.

16.2 Endpoints

Primary

The primary study endpoint is pCR in the breast. It will be determined post neoadjuvant therapy at time of surgery.

Secondary

- Pathologic stage in the breast and in the breast plus axilla, as defined by the AJCC TNM (v6).
- Clinical response, measured at the end of neoadjuvant therapy.
- Radiographic response, measured at the end of neoadjuvant therapy.
- Overall survival (OS), measured from study entry to death due to any cause. Survivors will be censored at the date last known to be alive.
- Relapse-free survival (RFS), measured from definitive surgery to ipsilateral invasive breast tumor recurrence, regional invasive breast cancer recurrence, distant recurrence, or death from any cause, whichever occurs first. Patients who have not experienced any of these events will be censored at the date of last clinical assessment. Patients who do not undergo definitive surgery will not be assessable for RFS [55].
- Time to first failure (TFF), measured from study entry to ipsilateral invasive breast tumor recurrence, regional invasive breast cancer recurrence, distant recurrence or death from

any cause. Patients who have not experienced any of these events will be censored at the date of last clinical assessment.

- Toxicity defined by the NCI Common Terminology Criteria for Adverse Events version 3.

16.3 Stratification

Prior to randomization patients will be stratified by:

(1) pretreatment clinical stage:

- II
- III

(2) hormone receptor status:

- ER and/or PgR positive
- ER and PgR negative

16.4 Sample size and power calculations

Update #5
6/15/11

Statistical power is based upon a single comparison of pCR between THL and TH. We will use a 2-sided alpha of 0.05.

We assume that the incidence of pCR on the control regimen, TH, is 30%. A 20% increase in pCR incidence on the experimental regimens would be considered clinically meaningful. The null hypothesis is then H_0: $\pi_{THL} = \pi_{TH}$ where π_{THL} and π_{TH} represent the expected proportions of patients with pCR on each respective treatment regimen. Analyses are powered for the alternative hypothesis H_1: $\pi_{THL} = 0.5$, $\pi_{TH} = 0.3$

A total of 230 patients in the THL and TH arms will give at least 85% power. Power calculations take into account the interim analyses described in Section 16.6. The total sample size will be 230 plus whatever the number of patients assigned to the L arm at the time Update #5 is approved and applied to the study.

16.5 Accrual rate

We estimate a monthly accrual of 12 patients. This estimate derives from CALGB's previous experience and anticipated participation, as follows. CALGB 150007, the current neoadjuvant correlative sciences protocol, accrued approximately 12 patients per month in a limited access program. Because eligibility to the present study is restricted to HER2-positive disease, it will be appropriate for only 20% of breast cancer patients. Unlike CALGB 150007, it will be open to all CALGB institutions. In addition, this trial will be proposed to the Breast Intergroup, whose participation would further augment accrual. Given a monthly accrual of 12 patients, it will take 34 months to reach the target of 400 patients.

16.6 Interim analysis

Update #5
6/15/11

Interim analyses on pCR will be conducted for comparison THL versus TH. Efficacy analyses will use a chi-square test with 1 degree of freedom for the pairwise differences in proportions of patients with pCR. Nine five percent (95%) confidence intervals around pCR will be calculated according to exact binomial methods.

Two interim analyses and one final analysis are planned. They are scheduled to occur at 50%, 75% and 100% information. With a target of 230 patients in the two arms, 50% of the information corresponds to the first 115 patients in the two arms combined, 75% corresponds to 173 patients and 100% corresponds to all 230 patients on the two arms. Interim analyses will allow the trial to be stopped early for either significance (superiority/inferiority) or for futility. Interim boundaries for a significant difference in pCR were calculated using the O'Brien-Fleming adjustment. Futility boundaries were defined from Bayesian predictive probabilities using a non-informative prior distribution for the true treatment effect. If the

observed test statistic crosses a futility boundary at either the first or second interim analysis, we will conclude that the predictive probability to show a statistically significant difference in pCR after all patients are accrued is less than 5%.

The table below shows the z-score superiority/inferiority and futility boundaries and their corresponding predictive probabilities.

Interim Analysis	% Information	Number Patients	Normalized Z-value Boundary		Predictive Probability	
			Significant Difference	Futility	Significant Difference	Futility
1	50%	115	+/- 3.226	+/- 0.404	0.991	0.050
2	75%	173	+/- 2.641	+/- 1.101	0.925	0.050
3	100%	230	+/- 2.290	----------	0.500	--------

If the observed test statistic crosses a superiority/inferiority boundary we will reject the null hypothesis and conclude significant difference in pCR between the control and experimental arm. Crossing a lower boundary will lead us to conclude that the experimental arm is inferior to the control arm, while crossing an upper boundary will cause us to conclude that the experimental arm is significantly superior to the control arm. If the boundary is crossed at the first interim analysis, then the predictive probability of showing a statistically significant difference if the total target accrual were reached is 99.1%. If the boundary is crossed at the second interim analysis, then the predictive probability is 92.5%

Results of efficacy analyses will be presented to the Data and Safety Monitoring Board (DSMB) at the meeting following each interim analysis.

Analyses of toxicity will be presented to the DSMB twice yearly at each DSMB meeting, beginning as soon as adverse event data are available.

16.7 Data analysis

Update #5
6/15/11

The primary analysis will use a chi-square test for the difference in proportions of patients on the THL arm versus the TH arm who achieve a pCR. We will use exact binomial methods to construct 95% confidence intervals around the pCR incidence for each arm.

All analyses of secondary time-to-event endpoints will test for a possible difference among arms while controlling for other clinical variables using proportional hazards regression. Treatment arm effects will be assessed using 1 degree-of-freedom tests. Analyses on overall survival (OS) and time to first failure (TFF), both measured from study registration, will include all patients. Analyses on relapse-free survival (RFS) will be limited to patients who undergo surgery. Clinical, radiographic and pathologic response will also be included in RFS models to test an association between response and RFS. Each of the three response variables will be dichotomized into response versus not. Time-to-event distributions will be shown graphically using the Kaplan-Meier product-limit technique. The difference between two or more distributions will be assessed using the logrank test.

We will dichotomize clinical, radiographic and pathologic response variables into response versus not, and assess the correlation among the three using 3x2 contingency table analysis. For each arm, we will tabulate the percentage of patients by pathologic stage in the breast and in the breast plus axilla.

Patients who do not undergo surgery will be considered failures in pCR analyses. Reasons for not undergoing surgery will be documented. Patients who do not have any repeat radiographic assessments will be considered failures in radiographic response analyses.

The type and grade of treatment-related toxicity will be tabulated by treatment arm.

Results of the surgical substudy will document current practices on two surgical issues, first lymphatic mapping/sentinel lymphadenectomy (LM/SL) and second breast conservation.

To address the first issue, we will calculate the proportion (and 95% confidence interval) of patients who undergo LM/SL prior to and after neoadjuvant therapy, and the results of that procedure. For all patients who undergo LM/SL, we will calculate the proportion of patients who subsequently undergo axillary dissection, and for those patients calculate the median number of nodes removed and the number determined to be positive.

Regarding the second issue, we will test for changes in the pre- and post-therapy determination of a patient's candidacy for breast conservation using the McNemar's test. Of those women who are actually offered breast conservation, we will calculate the proportion who accept it. Finally, among those women who undergo breast conservation, we will tabulate the number of patients with clear margins at surgery.

Results of the radiotherapy substudy will document current practices on two RT issues, namely, the use of adjuvant regional RT and post-mastectomy RT (1) by degree of nodal burden after neoadjuvant chemotherapy, and (2) by results of sentinel node biopsy.

To address the first issue, we will assess the relation of post-chemo nodal burden (yes/no) and irradiation or not using 2x2 contingency table analysis. From this contingency table we will also calculate the percent and 95% confidence interval of patients who do and do not receive RT by nodal status.

Regarding the second issue, we will assess the relation of sentinel node result (positive/negative) and irradiation or not using 2x2 contingency table analysis. As above, we will calculate the percent and 95% confidence interval of patients who do and do not receive RT by sentinel node results.

Finally, the highest grade of RT-related toxicity will be tabulated by treatment arm.

Because the current trial is powered for the primary treatment study, and not for the surgery or radiation substudies, analyses on the substudies are intended to document practices and serve as hypothesis generating rather than hypothesis confirming.

While we do not expect any differences in clinical outcome by patient race, and we will not have sufficient power to detect small or moderate differences, we will describe the relation of race on clinical outcomes in the primary treatment study and in the two substudies.

16.8 Correlative science statistical plan

Update #5
6/15/11

16.8.1 Biomarkers of response to trastuzumab and lapatinib (150702)

We anticipate that tissue will be available from core biopsies from about 90% of the 230 patients. Therefore, we expect about 208 patients in this correlative study, approximately 104 in each of the THL and TH treatment arms.

A primary goal is to relate marker expression with pCR regardless of treatment arm. A secondary goal is to address this relationship as it depends on treatment group.

Consider first the primary goal. The power for assessing this relationship will depend on the marker. We will treat markers as dichotomous. For some markers, the expression cut-off has been defined (see Table A APPENDIX I, Part A). For these markers this cut-off will define the primary analysis. A secondary analysis for these markers and the primary (but exploratory) analysis or all other markers will be to consider various cut-offs. The entire range of cut-offs will be considered, with the focus on two, namely the median and the third quartile of the distribution (e.g. cut-off for which 50% are "overexpressors" and cut-off for which 25% are overexpressors). A marker will be flagged as being a

statistically significant predictor of pCR if either it is significant for its predetermined cut-off (if known) or for both the 50% and 25% cut-offs (for other markers).

Assuming the median cut-off, approximately 104 patients will be in the two marker groups (above versus below the median). We will have over 80% power to discriminate an increase in pCR rate from 30% to 50% (assuming two-sided significance level of 0.05). Assuming 25% overexpressors, the breakdown will be approximately 52 overexpressors and 156 non-overexpressors, with over 70% power.

The secondary goal of assessing the relationship between marker expression and pCR with treatment arm is more complicated. In two independent analyses we will repeat the above analyses of marker expression with pCR separately within the THL arm and TH arm, respectively. Assuming 104 patients in each treatment arm, each separate analysis has about 55% power using the median cut-off and slightly over 40% power using the upper quartile cut-off. Moreover, concluding that a relationship does or does not exist for one treatment arm should not affect the corresponding conclusion in the other treatment arm. Since these issues are exploratory, we will not carry out a formal hypothesis testing analysis of interaction between marker expression and treatment arm.

Another primary goal will be to build a model that predicts pCR. We will proceed as follows. We will divide the full set of patients into two equal subsets, with approximately 104 patients in each subset (having the same representation of the two treatment arms in each subset). In the first half, the "training set," we will build a multivariate logistic model for predicting whether the patient experiences a pCR. First, each marker will be considered in turn, univariately, with its cut-off chosen to maximize its statistical significance (restricting to the training set). Those markers achieving statistical significance at a two-sided level of 0.05 will be included in the multivariate logistic model. The coefficients of each marker will be determined by this multivariate model. This model thereby establishes a single number for each patient, her "probability" of achieving a pCR. Call this her "index." This index will be determined for each patient in the second ("confirmatory") subset. A univariate logistic model in which this index is the only independent variable will be carried out in the confirmatory set. Statistical significance of this model will be claimed if this index is a statistically significant predictor.

As a secondary extension of this model, we will include clinical variables along with the patient's index. These will include age, ER-status, tumor size, grade, and treatment group [31].

Recognizing that there may be limitations to the number of markers which can be tested in core biopsy specimens, we will prioritize the markers below to those which are most likely to be associated with response to trastuzumab, or to lapatinib based upon the existence of preliminary data supporting their evaluation. The markers indicated by an asterisk (*) are deemed to have the highest likelihood of association with response and will be used for initial analysis using the sample size calculations above. Of note, DNA based assays require very little material and are not likely to be limiting. Secondary markers will be tested if tissue resource allows. If fewer specimens are available for secondary markers, exploratory analyses will be conducted but their validation will await testing in a larger cohort.

16.8.2 Serum HER2 extracellular domain (ECD) and circulating tumor cells (CTC) (150702) Update #5 6/15/11

We anticipate that about 70% of the patients accrued to this trial will contribute laboratory data; thus the total sample size for the correlative studies is expected to be as large as 160

with equal allocation to the two treatment arms if no early stopping rules are applied during analysis.

The presence of HER2 ECD is 20-30% at baseline in nonmetastatic disease [56], [57], although it may be higher in the high risk, HER2-overexpressing tumors in this population. We hypothesize that the presence of baseline HER2 ECD correlates with a lower pathological response rate. Assuming that 30% of patients will be ECD positive (48 patients) and 70% negative (112 patients), we will have 71% power to compare a 20% pathological complete response (pCR) rate in marker positive patients to a 34% in negative patients (30% pCR rate overall), using a one-sided alpha of 0.10.

In an exploratory analysis, with little power, we will compare the association of pCR rates to ECD status by treatment arm. We anticipate having approximately 80 patients on each of the two arms. In addition, we will compare the changes in baseline ECD marker to time of surgery marker in the pCR group versus the residual invasive disease group.

The presence of CTCs is assumed to be 20-30% at baseline in nonmetastatic disease [51, 52], although it may be higher in the high risk population. CTC counts will be assessed as one of 6 ordinal categories (0,1,2,3,4 and 5+) and we will use descriptive statistics and confidence intervals to report the distributions within and across treatments. Combining the data from the two arms, we will use logistic regression models to examine the prognostic and predictive value of CTC counts measured at baseline. We will address independent prognostic effects by including known prognostic factors in the model: arm, patient age, estrogen-receptor status, and number of lymph nodes. Specifically, various functional relationships between the ordinal variable and the outcome of pCR will be evaluated in an exploratory fashion (e.g., linear, quadratic, ordinal). LOESS will also be used in an exploratory manner to determine the best functional form.

In addition to the general exploratory analyses of cell counts that ignore arm, the following three specific exploratory analyses will be performed. (A) We will examine, both descriptively and inferentially, the interaction between baseline presence of CTCs and arm in the prediction of pCR. Specifically, zero and non-zero CTC counts will be used to estimate the observed treatment arm effect with odds ratios and their 90% confidence intervals. (B) Within each arm separately, we will examine whether CTC counts measured at day 15 of study therapy and before the final week (week 16) infusion of paclitaxel has been started predict pCR. For this analysis, CTC counts will be dichotomized as 0-4 versus 5+. CTC odds ratios will be calculated with 90% confidence intervals and will be tested within the logistic model controlling for the covariates listed in the previous paragraph. (C) We will examine whether two a priori defined CTC cutpoints predict pCR using the logistic model. These cutpoints are ≤ 1 versus ≥ 2 and ≤ 4 versus ≥ 5. Specifically, CTC cutpoint adjusted odds ratios will be calculated with 90% confidence intervals controlling for the covariates listed in the previous paragraph.

Power calculations for this aim are made for a comparison of pCR rates in patient subgroups identified by cell counts. Based upon the hypothesized pCR rate in each of the two arms of the clinical trial, the overall pCR rate is 40%. We assume that a binary classification exists from the ordinal measures of CTC to identify subgroups of patients of equal size. Under these assumptions, there is 74% power to detect a 50% pCR rate in the subgroup with low CTC levels and a 30% pCR rate in the subgroup with high CTC levels (using a two-sided alpha of 0.05).

The feasibility of detecting CTCs will be investigated by an interim analysis after 90 patients are accrued with evaluable samples. To confirm the incidence of CTCs at baseline is at least 30%, a one-sided 90% Binomomial confidence interval will be used. Thus, if fewer than 27 of 90 samples contain CTCs, a decision may be made to stop the CTC study

or to modify the protocol to increase the numbers of CTCs collected (e.g., increase the number of blood samples drawn at each time point.

For CTCs collected at baseline, HER2 and EGFR status will be determined by FISH analysis. To characterize quantitative assessments of HER2-positive and EGFR-positive CTCs, as both cell counts and also as percentages of enumerated CTCs, descriptive statistics will summarize observations within and across the two arms of neoadjuvant therapy. To conduct an exploratory analysis of the association of baseline HER2-positive and EGFR-positive CTCs to pCR, logistic regression models will examine the prognostic and predictive value using main effects and interactions terms respectively for the two treatment arms. We will address independent prognostic effects of the two markers by including known prognostic factors in the model: arm, patient age, estrogen-receptor status, and number of lymph nodes.

For serially collected CTCs over the course of neoadjuvant therapy, descriptive statistics will be used to summarize the distributions of phosphorylated and total HER2 and EGFR levels that are observed. Subject-specific trends in biomarker levels will be assessed using longitudinal mixed effects regression models. Residual analysis and Box-Cox power transformations will be considered to ensure Gaussian errors prior to conducting statistical inferences. A variety of covariance structures will be considered for the repeated measurements per subject including independence, compound symmetric and time-dependent Markov structures. Optimal models will be selected using Bayesian Information Criteria (BIC) under REML estimations. Statistical inferences on the association with treatment arms and patient response to therapy will be made using likelihood ration tests under ML estimation. All exploratory analysis will be conducted using two-sided alpha = 0.05, and reported with 95% confidence intervals.

16.8.3 Pharmacogenomic determinants of response and toxicity in CALGB 40601 (60701)

16.8.3.1 Candidate gene approach

The primary endpoint for this pharmacogenomic study is response as defined by pCR. More specifically, we hypothesize that patients with the CYP2C8*3 allele (group A) to have superior response compared to those patients who do not have this allele (group B). Let π_A and π_B denote the probability of response for arms A and B respectively. The hypothesis of interest can be canonically presented as testing H0: $\pi_A=\pi_B$ versus H1: $\pi_A>\pi_B$.

It is not expected that there are any interactions between this genotype and the therapies of the study. Assuming that the study is not closed early, the study plans to evaluate a minimum of N=300 patients. Assuming that 85% will consent and provide usable samples, it is expected that a minimum of n=255 will be available for the proposed analysis. The putative prevalence for groups A and B are 0.13 and 0.87 respectively. That corresponds to having 33 patients from group A and 221 patients from group B. The overall response rate over the study arms is π=0.4 (=0.5*0.3+0.5*0.5).

The power of Fisher's test, at the two-sided level of 0.05, is illustrated in the table below for various choices of (B. Note that for each example, $0.4=\pi=0.13\pi_A+0.87\pi_B$. In this table, Ω denotes the odds-ratio (the canonical effect size for comparing two probabilities).

π_A	π_B	Ω	Power
0.65	0.362	3.27	0.85
0.61	0.3686	2.68	0.81
0.59	0.3716	2.43	0.73

The above analysis will be carried out for other candidate genes such as (but not limited to) CYP3A4, CYP3A5, ABCB1, ABCC2 and CYP2B6 as well as polymorphisms in HER2. Other endpoints such as PFS and OS will be considered as well. For the time-to-event endpoint, the log-rank test will be employed to assess the discrepancy in the time-to-event profiles.

We have assumed that there are no genotype/treatment interactions in predicting clinical response. We will explore potential multiplicative effects in the framework of two-way multiplicative logistic (for binary endpoints) and log-linear Cox (for time-to-event endpoint) models. We will also consider the effect of having locally advanced disease on the results of these analyses.

16.8.3.2 Genome wide approach

The primary clinical endpoint for this study is tumor response. The primary statistical objective is to identify specific SNPs and/or copy number variations that are associated with the prevalence of this event.

Other endpoints of interest include adverse events and recurrence-free survival. In addition to association analyses, we will also explore potential SNP by drug interactions with respect to outcome.

We will also seek to validate results found in other CALGB studies (e.g., 80303, 40101, 90401) and perform a risk analysis by comparing the 40601 SNP data to SNP data from controls (patients thought not to have cancer). The latter will be obtained from public databases.

Pre-processing

For pre-processing (QC and genotype calls) the Illumina chips, we will use the commercial program Bead Studio developed by Illumina. Although, Illumina does not provide a Linux port of Bead Studio, one can run the software on VMWARE running on a Linux host. A two CPU dual core (four cores) AMD Opteron Socket F workstation with 16GB of RAM will be available for this purpose (the statistical analyses will be carried out on a Linux server with 8 dual core Opteron Socket F CPUs (16 cores) with 64GB of RAM [expandable to 128GB if needed]).

Analyses to Assess Genotyping Quality and Population Stratification

Initial quality studies will be conducted to identify SNPs that have generated sufficiently poor quality genotype data that they should be removed from analyses. Call rate, patterns of missing data, and departures from Hardy-Weinberg equilibrium (HWE) assessed using an exact test will all be scrutinized to identify markers that will not be used in analysis. In general, SNPs with call rates <95% and those with highly significant departures from HWE ($p<10^{-7}$) will not be included in analyses. Non-random patterns of missing data are sometimes encountered in data generated on high-throughput genotyping platforms; the most common non-random missing data problem is that heterozygous genotypes are more likely to be assigned as missing than either homozygous genotype. We will perform analyses using blind duplicates as well as analyses assessing the relationship between heterozygous call rates and missing data to identify any SNPs in which data are clearly not missing at random. Depending on the number and degree of difficulty observed, we will either remove problematic SNPs from analysis, or assign quality scores to reflect the extent of the non-random missing data.

Additional preliminary quality control analyses will be conducted to insure that the sample does not include duplicated samples or closely related individuals. These analyses can be

rapidly conducted using PLINK [58]. Duplicated samples (or unrecognized identical twins) will be reduced to a single sample for further analyses. Although we do not expect to have closely related individuals included in this sample, only one member of any set of first-degree relatives will be included in subsequent analysis. For each sample, we will also generate a gender call based on the SNPs on the X chromosome and study the missingness patterns for the SNP on the Y and XY chromosomes in order to convincingly determine that all samples are from male patients.

Population structure that is not appropriately recognized and accommodated can lead to both false positive and false negative results in association studies. We will conduct studies using structure [59] to estimate ancestry proportions using 10,000 SNPs chosen for having no pairwise LD with unrelated individuals from the HapMap CEU, YRI and CHB+JPT samples used to model the ancestral populations. Substantial previous research has shown this to be a rapid and effective approach to defining historical geographic ancestry. Although self-identified race/ethnicity is usually highly correlated with estimated historical geographic ancestry, there are often a few individuals who appear to be misclassified with self-defined labels, and it is the genetically defined ancestry that is critical to correctly accommodate to insure robust results from association studies. Each individual will then have estimates of European, African and Asian ancestry. For individuals with high ancestry proportion for a single group (>98%), we will conduct further analyses with EIGENSTRAT [60] using all SNPs to determine whether there are additional important sources of variation among individuals leading to detectable stratification by allele frequencies (reflecting, for example, differences in ethnic make-up within individuals of European descent from different U.S. cities from which subjects for the trial were obtained). Primary analyses, described below, will be conducted within groups defined by historical geographic ancestry. Secondary analyses will be conducted using logistic regression with ancestry proportions (and any additional stratification identified using EIGENSTRAT) as covariates.

Feature Discovery

The association between the genotype call (say AA, AB or BB) for each autosomal SNP and the clinical outcome will be investigated within the framework of 2 by 3 contingency table stratified by ancestry. The analyses will be powered to detect additive genetic models (to be defined shortly). The Cochran-Armitage [61] will be used for carrying out inference on these tables. A feature (SNP) will be considered significant if the corresponding nominal unadjusted two-sided P-value is less than 0.05/K, where K is number of features which pass the pre-processing step. Needless to say, this approach may be slightly conservative. It does however guarantee strict type I error control.

For the sake of discussion, let B denote the risk allele with an assumed relative allelic frequency of q. Under the Hardy-Weinberg equilibrium assumption, the genotypes AA, AB or BB will have relative genotypic frequencies of $(1-q)^2$, $2q(1-q)$ and q^2, respectively. Let D denote the binary clinical outcome (D=1 if patient responds or =0 otherwise) and define the probability of a response given the copies of the risk allele on the genotype, to be denoted by G, as $p_g=P[D=1|G=g]$, for g=0,1 or 2. The relationship between the event probability $p=P[D=1]$ in the general population is then expressible as the following mixture $p=(1-q)^2p_0+2*q*(1-q)p_1+q^2p_2$. The effect size in the context of genome-wide association studies is typically quantified using the genotype relative risk (GRR) whose definition depends on the disease model. Under the recessive disease model, $p_0=p_1$ and $p_2=GRRp_0$ while under the dominant disease model $p_1=p_2=GRRp_0$. Finally, under the log-additive model, $GRR=p_1/p_0=p_2/p_1$. Under these disease models, the event probability in the population, p, can then be reformulated as the mixture $p=(1-q)^2p_0w_0+2*q*(1-$

$q)p_0w_1+q^2p_0w_2$, where $w=(w_0,w_1,w_2)=(1,1,GRR)$, for the recessive model, $=(1,GRR,GRR)$ for the dominant model and $=(1,GRR,GRR^2)$ for the additive model.

The probability of response is expected to be 0.4. The Illumina Huma610-Quad chip, which types about 620,000 SNPs, is expected to be used for this analysis. For a given q, the GRR detectable with a power of 0.8, at the two sided 0.05/600,000 level (i.e., assume K=600,000 autosomal SNP markers pass through the pre-processing step), is illustrated in Table 1.

	q	0.4	0.5	0.6	0.7	0.8
recessive						
	GRR	**2.845**	**2.725**	**2.78**	**3.125**	**4.45**
dominant	q	0.1	0.2	0.3	0.4	0.5
	GRR	**2.78**	**2.8**	**3.25**	**4.4**	**10.5**
Additive	q	0.22	0.36	0.51	0.65	0.8
	GRR	**2.325**	**2.025**	**2.125**	**2.45**	**3.9**

Table 1. Power illustration: The minimum Genotype Relative Risk (GRR) detectable with a power of 0.8, at the two-sided level of 0.05/600,000 for a range of relative allele frequencies (q) assuming the probability of response is P[D=1]=0.4, under recessive, dominant and additive models assuming HWE. The sample size used in the illustration is based on N0=102 (non-responders) and N1=153 (responders) for a total of N=255 patients.

Submission of Molecular Data

The laboratory of Dr. Yusuke Nakamura will submit the Illumina *.idat image files using secure means to the CALGB Statistical Center. The lab will also submit a table along with this transmission, which at the minimum will provide the following information for each sample received from the repository.

- The lab ID number provided by the repository.
- The experimental ID, a concatenation of the plate, well and replicate information, generated by the lab.
- The idat file names (the file string name will contain the Lab ID).
- The md5sum signature of the idat files to ensure data integrity.
- The date the specimen was received from the repository.
- The date the sample was analyzed by the RIKEN laboratory.

Additionally, the lab will also provide the complete results from any quality control measures carried out. If a sample had to be redone (e.g., defective or poor quality array), the lab will provide all replicate idat files and add an appropriate column to the supplementary table. The molecular data generated for this aim may not be shared with other investigators or used for any analysis not specified in the protocol until a formal approval from the CALGB Statistical Center is obtained.

Secondary Objectives

Logistic regression models and conditional inference trees (or more generally conditional random forests) will be used to construct multi-variable models based on the SNPs identified as interesting. These models also allow for inclusion of other potentially relevant clinical and demographic variables.

The Illumina Human610-Quad chip contains approximately 60,000 additional markers, developed in collaboration with deCODE Genetics, specifically target regions known or likely to contain CNVs, including segmental duplications and regions in the unSNPable genome. Given the complex structure of CNVs, it is not always clear how to define the genotype of a CNV. Instead of categorizing copy numbers into genotypes, we will estimate relative genomic abundance probe intensities. This approach allows for the consideration of other CNVs beyond deletions, including duplications and combinations of both. For notational brevity, we shall refer to these as CNV markers.

For each objective, the association between each CNV marker and the response endpoint, will be assessed using the Wilcoxon two-sample test. The family-wise error rate will be controlled at the 0.05 level using permutation resampling (based on B=10,000 replicates).

Regression methods, as in the case of the SNP markers, will be employed to construct multivariable models based on the CNV markers.

Secondary relevant clinical endpoints include adverse events (e.g., proteinuria and hypertension), recurrence-free and overall survival. For censored time-to-event outcomes, the stratified log-rank test will be primarily used for assessment of significance.

A risk analysis will be carried out by comparing the genotypic distributions of the SNPs from the CALGB 40601 data to those from controls (thought to not to have cancer). The SNP data from the controls will be obtained from public databases.

In addition to conducting analyses on all features directly assessed on the high-throughput platform used in these studies, we will also interrogate all additional HapMap SNPs that are not in strong pairwise LD with any genotyped SNP but for which there is sufficient multi-locus LD to SNPs on the high-throughput platform. TUNA (Testing UNtyped Alleles) is a robust approach for conducting such analyses that provides inexpensive in silico follow up to the initial analysis and allows us to more efficiently design any follow up genotyping studies [62, 63]. For example, use of Illumina HumanHap300 enables direct testing of 270K-450K SNPs, and indirect testing of 750K-1.5M additional SNPs (i.e. these SNPs are so highly correlated with SNPs that are directly tested for association that testing them would provide little additional information). The ranges given above bracket the expectations for different human populations, with European populations at the high end of the range, and populations of recent African descent at the lower end. Use of TUNA enables interrogation of an additional 100K-250K SNPs that are neither on the platform nor highly correlated with any individual SNP on the platform. Note that use of TUNA will facilitate comparisons to genome wide association studies on potentially related phenotypes (e.g. clinical trials of the same or related drugs) conducted using other high-throughput platforms or candidate gene studies utilizing SNPs not directly genotyped on the high-throughput platform chosen for our studies.

Finally, we note that the methodology field for the analysis of genome-wide SNP data is in its infancy. We will consider the employment of “newer” methods if they are deemed to be statistically sound and enable us to better interrogate, and more importantly, understand the data.

Statistical Software

The R statistical environment [64] and Bioconductor [65] packages will be used for all of the primary statistical analyses relating features to phenotypes. Specialized statistical genetics software, including PLINK [58], structure [59], EIGENSTRAT [60], and TUNA [62, 63] will be used for some of the quality or secondary analyses, and R will be used for logistic regression analyses allowing for ancestry covariates.

16.8.4 Somatic mutations (60701)

The immediate objective of the gene resequencing aim is to create a predictive model for pathological complete response based on binary information on gene mutation status (amplicon present yes/no and gene mutant yes/no). Several state-of-art classification methods can incorporate predictors of different variable types in the analysis. Random forest (RF) algorithm and support vector machine (SVM) are two well-established classification methods. The random forest [66] is one of the “ensemble learning” algorithms which builds many classification trees-a forest, for each bootstrap sample of a dataset. Consequently this methodology usually yields a robust misclassification error rate estimate and more critically "importance measurements" of predictors (Gini index). This algorithm can provide clues for researchers to narrow down their search for important biomarkers in cancer research. Another method we will utilize is the "LASSO penalized logistic" [67] particularly if we are faced with a larger number of variables. This approach is an application of a relatively new shrinkage technique “least absolute shrinkage and selection operator” (LASSO) to ordinary logistic regression. Attractive features include simultaneous parameter estimation and variable selection, which leads to a parsimonious model for high dimensional datasets. We will compare the prediction performance of these methods across the study.

17.0 ADVERSE EVENT REPORTING (AER)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Investigators are required to notify the Investigational Drug Branch (IDB), the CALGB Central Office, the Study Chair, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting beginning October 1, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov). All reactions determined to be "reportable" in an expedited manner must be reported using the NCI Adverse Event Expedited Reporting System (AdEERS).

CALGB requires investigators to route all AdEERS reports through the CALGB Central Office for CALGB-coordinated studies.

17.1 CALGB 40601 reporting requirements

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: AdEERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the treatment

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Unexpected without Hospitalization	Expected with Hospitalization	Expected without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hrs; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment including an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:
Grade 4 and Grade 5 unexpected events
AdEERS 10 calendar day report:
Grade 3 unexpected events with hospitalization or prolongation of hospitalization
Grade 5 expected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.
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Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:

- "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
- "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific exclusions (see below).
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

17.2 Additional instructions or exclusions from AdEERS expedited reporting requirements for CALGB 40601:

- CALGB 40601 uses a drug under a CTEP IND. The reporting requirements for investigational agents under a CTEP IND should be followed for all arms in this trial.
- Deaths occurring greater than 30 days after the last dose of treatment, that are due to disease progression, do not require AdEERS.
- Grade 3/4 myelosuppression and hospitalization resulting from such do not require AdEERS, but should be submitted on the routine Adverse Event Forms for this study.
- Febrile neutropenia and hospitalization resulting from such do not require AdEERS, but should be submitted on the routine Adverse Event forms for this study.
- Grade 3/4 paclitaxel-induced neuropathy or arthralgia/myalgia and hospitalization resulting from such do not require AdEERS, but should be submitted on the routine Adverse Event Forms for this study.
- Grade 3/4 paclitaxel-induced hypersensitivity reactions and hospitlization resulting from such do not require AdEERS, but should be submitted on the routine Adverse Event Forms for this study.
- For the purposes of expedited adverse event reporting, the CAEPRS (which include expected adverse events) for lapatinib and trastuzumab may be found below in Sections 17.3 and 17.4 respectively. Note: The ASAEL column of the trastuzumab and lapatinib CAEPRs have been replaced with the Specific Protocol Exceptions to Expedited Reporting (SPEER) list. This now includes "expected" severity grades in addition to event terms. Expected adverse events for paclitaxel may be found in the package insert or in Section 12.6.
- AdEERS reports are to be submitted electronically using AdEERS http://ctep.cancer.gov/reporting/adeers.html to the CALGB Central Office (calgb@uchicago.edu). In the rare occurrence when Internet connectivity is lost, an AE report may be submitted using CTEP's Adverse Event Expedited Report-Single Agent or Multiple Agent paper template (available at http://ctep.cancer.gov) and faxed to CTEP at 301-230-0159 and to CALGB at 312-345-0117. A 24-hour notification is to be made to CTEP by telephone at 301-897-7497, only when Internet connectivity is disrupted. Once Internet connectivity is restored, an AE report submitted on a paper template or a 24-hour notification phoned in must be entered electronically into AdEERS by the original submitter at the site.

- New primary malignancies including AML/MDS should be reported using study form C-1001, and via AdEERS.
- The reporting of adverse events described above is in addition to and does not supplant the reporting of adverse events as part of the report of the results of the clinical trial, e.g., study summary forms or cooperative group data reporting forms (see Section 6.0 for required CALGB forms).
- All adverse events reported via AdEERS (i.e., serious adverse events) should also be forwarded to your local IRB.

17.3 Comprehensive adverse events and potential risks list (CAEPR) for lapatinib (GW572016, NSC 727989)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1890 patients.* Below is the CAEPR for Lapatinib (GW572016).

NOTE: Report AEs on the SPEER ONLY IF they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.5, January 13, 2012[1]

Adverse Events with Possible Relationship to Lapatinib (GW572016) (CTCAE 4.0 Term) [n= 1890]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
CARDIAC DISORDERS			
		Left ventricular systolic dysfunction	***Left ventricular systolic dysfunction (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal distension		***Abdominal distension (Gr 2)***
	Abdominal pain		***Abdominal pain (Gr 2)***
	Anal mucositis		***Anal mucositis (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Dyspepsia		***Dyspepsia (Gr 2)***
	Flatulence		***Flatulence (Gr 2)***
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
	Rectal mucositis		***Rectal mucositis (Gr 2)***
	Small intestinal mucositis		***Small intestinal mucositis (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatic failure	***Hepatic failure (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	
INVESTIGATIONS			

	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
		Electrocardiogram QT corrected interval prolonged	***Electrocardiogram QT corrected interval prolonged (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Laryngeal mucositis		***Laryngeal mucositis (Gr 2)***
	Pharyngeal mucositis		***Pharyngeal mucositis (Gr 2)***
		Pneumonitis	
	Tracheal mucositis		***Tracheal mucositis (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Nail loss		
	Pruritus		***Pruritus (Gr 2)***
	Rash acneiform		***Rash acneiform (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***
VASCULAR DISORDERS			
	Flushing		***Flushing (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Gastrointestinal obstruction includes Colonic obstruction, Duodenal obstruction, Esophageal obstruction, Ileal obstruction, Jejunal obstruction, Obstruction gastric, Rectal obstruction, and Small intestinal obstruction under the GASTROINTESTINAL DISORDERS SOC.

[4]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on Lapatinib (GW572016) trials but with the relationship to Lapatinib (GW572016) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Anemia; Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Restrictive cardiomyopathy
EYE DISORDERS - Blurred vision
GASTROINTESTINAL DISORDERS - Constipation; Dysphagia; Gastritis; Gastrointestinal hemorrhage[2]; Gastrointestinal obstruction[3]
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs; Fever; Pain
INFECTIONS AND INFESTATIONS – Infection[4]
INVESTIGATIONS - Alkaline phosphatase increased; Creatinine increased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; Weight loss; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Hyperglycemia; Hypoalbuminemia; Hypoglycemia; Hypokalemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Myalgia
NERVOUS SYSTEM DISORDERS - Cerebrospinal fluid leakage; Depressed level of consciousness; Dizziness; Intracranial hemorrhage
PSYCHIATRIC DISORDERS - Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Epistaxis; Pleural effusion
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Palmar-plantar erythrodysesthesia syndrome; Skin and subcutaneous tissue disorders - Other (seborrheic dermatitis); Urticaria
VASCULAR DISORDERS - Hypotension; Thromboembolic event; Vascular disorders - Other (hypovolemia)

Note: Lapatinib (GW572016) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

17.4 Comprehensive adverse events and potential risks list (CAEPR) for trastuzumab (NSC #688097)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with *bold* and *italicized* text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via AdEERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

NOTE: Report AEs on the SPEER ONLY IF they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.2, September 29, 2011[1]

Adverse Events with Possible Relationship to Trastuzumab (CTCAE 4.0 Term)			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr. 3)***
	Febrile neutropenia[2]		
CARDIAC DISORDERS			
	Cardiac disorders - Other (cardiomyopathy)		
	Left ventricular systolic dysfunction		***Left ventricular systolic dysfunction (Gr. 2)***
	Pericardial effusion		
	Pericarditis		
	Sinus tachycardia		***Sinus tachycardia (Gr. 3)***
	Supraventricular tachycardia		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr. 3)***
	Diarrhea		***Diarrhea (Gr. 3)***
	Mucositis oral		***Mucositis oral (Gr. 3)***
	Nausea		***Nausea (Gr. 3)***
	Vomiting		***Vomiting (Gr. 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr. 3)***
	Fatigue		***Fatigue (Gr. 3)***
	Fever		***Fever (Gr. 3)***
	Flu like symptoms		***Flu like symptoms (Gr. 3)***
	Infusion related reaction		***Infusion related reaction (Gr. 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr. 3)***
	Pain		***Pain (Gr. 3)***

IMMUNE SYSTEM DISORDERS			
		Allergic reaction[3]	
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[4]		***Infection[4] (Gr. 3)***
INVESTIGATIONS			
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr. 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr. 3)***
	GGT increased		***GGT increased (Gr. 3)***
	Neutrophil count decreased[2]		***Neutrophil count decreased[2] (Gr. 3)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr. 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr. 2)***
	Back pain		***Back pain (Gr. 3)***
	Bone pain		***Bone pain (Gr. 3)***
	Myalgia		***Myalgia (Gr. 3)***
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Tumor pain		***Tumor pain (Gr. 3)***
NERVOUS SYSTEM DISORDERS			
	Headache		***Headache (Gr. 3)***
	Peripheral sensory neuropathy		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome[3]	
	Allergic rhinitis		***Allergic rhinitis (Gr. 3)***
		Bronchospasm[3]	
	Cough		***Cough (Gr. 3)***
	Dyspnea[3]		***Dyspnea[3] (Gr. 2)***
	Hypoxia[3]		***Hypoxia[3] (Gr. 2)***
		Pneumonitis[3]	
		Pulmonary edema	
		Pulmonary fibrosis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash acneiform		***Rash acneiform (Gr. 3)***
	Rash maculo-papular		***Rash maculo-papular (Gr. 3)***
	Urticaria		***Urticaria (Gr. 3)***
VASCULAR DISORDERS			
	Hypertension[5]		
	Hypotension[5]		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting **mailto: PIO@CTEP.NCI.NIH.GOV**. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Fatal event when given in combination with Xeloda® (capecitabine) and Taxotere® (docetaxel).

[3]Severe hypersensitivity reactions including angioedema and pulmonary adverse events (e.g., hypoxia, dyspnea, pulmonary infiltrates, pleural effusion, and acute respiratory distress syndrome) have been reported.

[4]Infection may include any of the 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[5]Associated with infusion reactions.

Also reported on trastuzumab trials but with the relationship to trastuzumab still undetermined:

CARDIAC DISORDERS - Acute coronary syndrome; Atrial fibrillation; Cardiac arrest; Myocardial infarction; Ventricular arrhythmia; Ventricular fibrillation; Ventricular tachycardia
EAR AND LABYRINTH DISORDERS - Hearing impaired
ENDOCRINE DISORDERS - Hypothyroidism
EYE DISORDERS - Blurred vision; Extraocular muscle paresis
GASTROINTESTINAL DISORDERS - Colitis; Dyspepsia; Enterocolitis; Esophageal ulcer; Gastritis; Pancreatitis; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Sudden death NOS
IMMUNE SYSTEM DISORDERS - Immune system disorders - Other (autoimmune thyroiditis)
INVESTIGATIONS - Alanine aminotransferase increased; Blood bilirubin increased; Cardiac troponin I increased; Creatinine increased; Platelet count decreased
METABOLISM AND NUTRITION DISORDERS - Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Avascular necrosis; Generalized muscle weakness; Muscle weakness left-sided; Muscle weakness lower limb; Muscle weakness right-sided; Muscle weakness trunk; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (myopathy)
NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Depressed level of consciousness; Dizziness; Hydrocephalus; Ischemia cerebrovascular; Neuralgia; Seizure; Syncope
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Depression; Psychosis
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria; Proteinuria; Urinary tract obstruction
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Fallopian tube obstruction; Prostatic obstruction; Spermatic cord obstruction; Uterine obstruction; Vaginal obstruction
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Apnea; Laryngeal edema; Pharyngolaryngeal pain; Pleural effusion; Pneumothorax; Pulmonary hypertension; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Nail loss; Pruritus; Skin ulceration
VASCULAR DISORDERS - Thromboembolic event

Note: Trastuzumab in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

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APPENDIX I 150702: CORRELATIVE SCIENCE STUDIES FOR CALGB 40601

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PART A: BIOMARKERS OF RESPONSE TO TRASTUZUMAB AND LAPATINIB

1.0 BACKGROUND

The introduction of trastuzumab has been a significant advance in breast cancer therapy but current regimens for HER2-positive disease are only partially effective, and alternative HER2-targeting agents, including lapatinib, are under investigation. The optimal use of trastuzumab, like other anti-cancer agents, requires a detailed molecular explanation for the marked variation in clinical outcomes. A rational approach is to consider HER2 in the context of a receptor tyrosine kinase signaling pathway. Trastuzumab and/or lapatinib resistance may reflect incomplete inhibition of HER2 signaling and/or the presence of parallel or "down-steam" molecular anomalies that allow critical mediators of cell cycle progression and cell survival to remain active despite the presence of HER2 inhibitory molecules. While this hypothesis does not address the aspect of trastuzumab's action that depends on antibody-dependent cytotoxicity [1], a signal transduction focus does present a series of testable possibilities regarding the action of trastuzumab and lapatinib that can be addressed in the setting of this neoadjuvant trial. Measurement of downstream signaling molecules also provide useful biomarkers to investigate the hypothesis that when these signal transduction pathways are dominant, the combination HER1 and HER2 blockade produced by lapatinib and trastuzumab will produce higher rates of pCR than lapatinib or trastuzumab alone. The markers of interest include pHER2, pHER1, Akt, pAkt, S6, pS6, MAPK, pMAPK, as well as downstream - Ki67, p27, cyclin D1, CD34, Tdt, TUNEL.

HER family members. Most breast tumors express more than one HER family receptor receptor tyrosine kinase (RTK) which may engender trastuzumab-insensitive signaling [2] [3]. Modulation of downstream signaling is clearly dependent on expression of coreceptors in HER2-overexpressing cells, however, measurement of other HER family members in human tissue has been challenging with widely varying frequencies in published studies [4]. This is partly due to variation in populations studied but more likely due to the fact that these coreceptors are expressed at relatively low receptor density, compared with HER2, and their detection is at the limit of sensitivity of traditional, immunohistochemical techniques. We will evaluate the frequency of expression of the HER family members using quantitative RT-PCR for RNA expression and a more sensitive immunohistochemical method that takes advantage of the improved sensitivity of immunofluorescence along with subcellular localization of expressed proteins [5]. The strength of this analysis is illustrated in a series of recent papers by Rimm and colleagues showing results not discernable by conventional pathologist-based analysis. For example, quantitation of HER2 protein with this method not only influences prognosis [6], but may be a better predictor of trastuzumab response than either Herceptest(r) or FISH (D. Rimm, unpublished data). Furthermore, the sensitivity of this method allows detection of lower abundance proteins otherwise difficult to study. Where feasible we will also evaluate the phosphorylation status of these receptors as it relates to responsiveness to trastuzumab- and lapatinib-based therapy. Finally, we will further explore the relationship of Insulin-like growth factor 1 receptor (IGF1R) with response to HER2 inhibitors as activation of PI3K by IGF1R is thought to be a mechanism of resistance to trastuzumab [7] [8].

1.1 Proliferation and apoptosis

We hypothesize that response will be higher in more proliferative tumors, and that the benefit in terms of improved response to combination of trastuzumab and lapatinib over monotherapy will be greater among more proliferative tumors than those with a low proliferative index. The Ki67 proliferation biomarker will be investigated in pretreatment or archival tissue. Ki67 will be measured by IHC (expressed as percent positive) and mitotic index on H&E specimens at baseline. Trastuzumab functions in part by inducing apoptosis in tumor cells [9] [10] [11], and improved chemotherapy efficacy has been postulated to result from reversal of the anti-apoptotic effect of HER2 [12]. Higher response may occur in those tumors with lower

pretreatment apoptotic indices, particularly in those treated with combined therapy compared to monotherapy. Induction of apoptosis will be measured by TUNEL scores and compared on each arm.

1.2 Topoisomerase II (Topo II) and c-myc co-amplification

Recent data from adjuvant trials has implicated both Topo II and c-myc amplification in response to trastuzumab-containing adjuvant regimens for HER2+ disease. In a comparison between an anthracycline-containing regimen and a non-anthracycline containing regimen, the presence of Topo II co-amplification with HER2 appeared to identify a group in which the anthracycline was particularly effective. For HER2-positive, Topo II-negative tumors the two regimens appeared equivalent [12]. Controversy exists, however, as Topo II studies in CALGB 8541 did not support the hypothesis that benefit of doxorubicin was due to Topo II amplification [13]. In addition, a European study which compared AC versus CMF showed benefit from the addition of the anthracycline in both Topo II-amplified and Topo II-deleted cases [14]. Although an interaction with anthracycline benefit cannot be examined in this study, we will examine whether Topo II abnormalities relate to pCR in a nonanthracycline chemotherapy/HER2 targeted regimen. In NSABP B-28, a study which randomized HER2-positive patients to chemotherapy with or without trastuzumab, amplification of c-myc was associated with improved disease-free and overall survival only in the trastuzumab-treated cohort [15]. Based on these data, myc-amplified tumors will be compared by arm to confirm these findings and examine whether there is an interaction between trastuzumab and/or lapatinib benefit and myc status. Finally, related chromosomal regions found by microarray (9.1.3) to be predictors of pCR will be further characterized by FISH using custom probes.

Genome-wide studies. RNA microarrays permit the study of multiple gene expression changes in a single biologic sample. These types of studies have refined the taxonomy of breast cancer by recognizing complex gene expression patterns characteristic of particular subtypes [16]. Recent studies suggest that subsets of genes identified by profiling can be used to predict response to therapy [17] [18] [19]. Within HER2-positive breast cancers, it is likely that there are patterns of gene expression that are predictive of outcome with trastuzumab-containing treatment. In addition, profiling may identify novel genes associated with resistance to therapy and suggest new treatment strategies. Several studies, using different approaches, have assessed transcriptional profiles in HER2-overexpressing breast cell lines and/or tumors. Expression profiling using cDNA microarays of immortalized human mammary luminal epithelial cells and variants expressing moderate or high levels of HER2 identified 61 differentially expressed genes [20]. Bertucci et al. analyzed 213 breast tumors and 16 breast cancer cell lines and identified 61 differentially expressed genes and ESTs associated with the HER2 gene signature [21]. Five of the overexpressed genes were located on the HER2 amplicon. Expression profiles have also been compared before and after trastuzumab treatment of HER2-positive breast cancer cell lines [22]. Most of the differentially expressed genes encode proteins involved in cellular processes like transcription, signal transduction, protein processing, cell metabolism, and transport. Trastuzumab treatment induced the expression of several genes involved in RNA processing and DNA repair, while some cell adhesion mediators, several genes involved in fatty acid synthesis, and some proto-oncogenes were down-regulated. These studies show that HER2-amplified and non-amplified tumors express different sets of genes and trastuzumab alters gene expression patterns in a predictable fashion, in vitro. However, whether these gene expression patterns are consistently observed in breast cancer in vivo and whether the pattern of changes seen in human breast tumors is modeled adequately by HER2-amplified cell lines remains unclear. In an attempt to identify surrogate markers of response to trastuzumab and chemotherapy, investigators from Dana Farber Cancer Institute profiled tumor RNAs from HER2+ breast cancer patients enrolled in a preoperative study of trastuzumab and vinorelbine [23]. We found that extremes of response fell into

separate classes on unsupervised hierarchical clustering, suggesting that response to trastuzumab and vinorelbine may be determined by different gene expression patterns. Attempts to identify genes associated with pathologic complete response were not fruitful, suggesting that this group of patients did not represent a discrete molecular group. This observation is supported by a recent study, which evaluated 82 breast tumors treated with preoperative 5 fluorouracil, doxorubicin and cyclophosphamide [24]. In this study, the authors were able to identify a gene signature for basal-like tumors which achieved pCR (10/22) but were unable to identify such a signature in HER2-amplified tumors with pCR (9/20). Furthermore, they note that HER2-amplified tumors that achieve pCR do not share any of the differentially expressed genes noted in the basal-like group. A collaboration between the study co-chair, Dr. Harris, with Jenny Chang (Baylor University) combining microarray data sets from patients treated with preoperative trastuzumab and chemotherapy (vinorelbine or docetaxel) revealed evidence of a complex signature for pathologic complete response. Differentially expressed genes were not reproducibly identified for tumors which achieved a pathologic complete response, however higher level analysis showed that regions of contiguous gene expression (functional amplicons on Chr2p23, Chr6q24, Chr7q33, Chr2p2, Chr12q21.31, Chr14q32.2, Chr1p34.2, Chr8q21.3) were associated with pathologic complete response (preliminary data). In addition, analysis of DNA copy number changes showed corresponding regions of genetic amplification, suggesting a potential mechanism for contiguous aberrant expression. In contrast to the lack of a robust signature in pCR tumors, non-responding tumors seemed to represent a particular tumor phenotype in this study. T4 tumors were more frequent in the non-responding cluster and these tumors were more likely to express genes associated with the 'basal-like' phenotype first described by Perou and Sorlie [16] [25]. This observation was highly significant and transferable across platforms and datasets (p=0.00001 for both). In addition, non-responding tumors expressed higher levels of growth factors (IGF-1, HGF, PDGF, pleotropin), growth factor receptors (IGF1-R, Leptin receptor, c-met), anti-apoptosis genes (alpha B crystallin, clusterin, NAV2) and the PI3K p85 subunit, suggesting potential mechanisms of resistance in this subgroup.

In addition to gene expression array, the technology for array CGH is rapidly evolving. Array CGH allows the detection of multiple chromosomal abnormalities simultaneously and can be considered complementary to gene expression profiling in identifying regions of pathogenic amplifications that may be targetable [26]. The Nimblegen-tiled oligoarray platform is proposed for this analysis because it offered ultra-high resolution (http://www.nimblegen.com). For this study, 2 micrograms of peripheral blood DNA and the same quantity of tumor DNA will be required. The sample will be analyzed by Nimblegen and the data returned for analysis.

2.0 OBJECTIVES

2.1 To examine gene copy number and/or expression of the HER family members and related proteins, proliferation and apoptosis, topoisomerase II alpha, c-myc and gene expression patterns in a cohort of HER2-positive tumors.

2.2 To evaluate the effect of variations of these markers on response to HER2-targeted therapy in order to identify putative predictive factors for testing in large adjuvant datasets.

3.0 METHODS

All patients will have 2 frozen and 2 fixed pre-therapy tumor samples taken prior to the initiation of protocol therapy. Samples obtained at the time of diagnosis for other studies, prior to enrollment on CALGB 40601, may be submitted for this correlative analysis if they meet the protocol requirements. We anticipate that tissue will be available from core biopsies from about 90% of the

400 patients. Therefore, we expect about 360 patients in this correlative study, approximately 1/3 in each of the trastuzumab, lapatinib, and the combination subgroups.

A primary goal is to relate marker expression with pCR. A secondary goal is to address this relationship as it depends on treatment group. Consider first the primary goal. The power for assessing this relationship will depend on the marker. We will treat markers as dichotomous. For some markers, the expression cut-off has been defined (see Table A). For these markers this cut-off will define the primary analysis. A secondary analysis for these markers and the primary (but exploratory) analysis for all other markers will be to consider various cut-offs. The entire range of cut-offs will be considered, with the focus on two, the median and the third quartile of the distribution (e.g., cut-off for which 50% are "overexpressors" and cut-off for which 25% are overexpressors). A marker will be flagged as being a statistically significant predictor of pCR if either it is significant for its predetermined cut-off (if known) or for both the 50% and 25% cut-offs (for other markers). Assuming the median cut-off, approximately 180 patients will be in the two marker groups. We will have over 90% power to discriminate an increase in pCR rate from 30% to 50% (assuming two-sided significance level of 0.05). Assuming 25% overexpressors, the breakdown will be approximately 270:90 with over 90% power.

The secondary goal of assessing relationship with treatment group is more complicated. Repeating the above restricting to 120 patients in each treatment group gives only about 60% power for the median cut-off and 50% for the upper quartile cut-off. Moreover, concluding that a relationship does or does not exist for one treatment group should affect the corresponding conclusion in the other treatment groups. Since these issues are exploratory, we will not carry out a formal hypothesis testing analysis of interaction across the three HER2-targeted treatment groups.

Another primary goal will be to build a model that predicts pCR in these patients. We will proceed as follows. We will divide the full set of patients into two equal subsets, with approximately 180 patients in each subset (having the same representation of the three treatment groups in each subset). In the first half, the "training set," we will build a multivariate logistic model for predicting whether the patient experiences a pCR. Each marker will be considered in turn, with its cut-off chosen to maximize its statistical significance (restricting to the training set). Those markers achieving statistical significance at two-sided level 0.05 will be included in the multivariate logistic model. The coefficients of each marker will be determined by this multivariate model. This model thereby establishes a single number for each patient, her "probability" of achieving a pCR. Call this her "index." This index will be determined for each patient in the second ("confirmatory") subset. A univariate logistic model in which this index is the only dependent variable will be carried out in the confirmatory set. Statistical significance of this model will be claimed if this index is a statistically significant predictor.

As a secondary extension of this model, we will include clinical variables along with the patient's index. These will include age, ER-status, tumor size, grade, and treatment group [24].

Recognizing that there may be limitations to the number of markers which can be tested in core biopsy specimens, we will prioritize the markers below to those which are most likely to be associated with response to trastuzumab, or to lapatinib based upon the existence of preliminary data supporting their evaluation. The markers indicated by an asterisk (*) are deemed to have the highest likelihood of association with response and will be used for initial analysis using the sample size calculations above. Of note, DNA based assays require very little material and are not likely to be limiting. Secondary markers will be tested if tissue resource allows. If fewer specimens are available for secondary markers, exploratory analyses will be conducted but their validation will await testing in a larger cohort.

Table A

Biomarker	Reagent	Cut-off Defined	Cut-off value	*Frequency*	*Hypothesis*
HER2 AQUA*	Dako	Y	>18	30%	increases pCR from 30 to 50%
IGF1R*	Clone 24-31	Y	3+ IHC membrane staining	22%	decreases pCR from 50 to 30%
Ki67 (MIB-1)	Dako	Y	continuous variable	No cut off	Positive interaction
Apoptosis*	Cell Signaling	Y	continuous variable	No cut off	exploratory
PIK3CA mut'n*	*NM_006218.2* Ensembl Gene ID:ENSG00000121879	Y	Mutation in 'hot spot'	30%	decreases pCR from 50 to 30%
PTEN loss*	*NM_000314.3* Ensembl Gene ID: ENSG00000171862	Y	LOH	30%	decreases pCR from 50 to 30%
Topo-II*	Vysis LSI TOP2A Spectrum Orange/CEP 17 Spectrum Green	Y	Amplified – >2 TII/cep 17 Deleted – <.67 TII/cep 17	Amp-30% Del-25%	increases pCR from 30 to 50%
c-myc*	Vysis LSI C-MYC (8q24.12-q24.13) SpectrumOrange Probe	Y	Amplified >2 cmyc/cep 8	Amp-44%	increases pCR from 30 to 50%
EGFR*	Dako (PharmDx)	N	Not defined	Not defined	exploratory
HER3	SantaCruz (RTJ.2)	N	Not defined	Not defined	exploratory
HER4	SantaCruz (C-18)	N	Not defined	Not defined	exploratory
2C4	Genetech (2C4.E12)	N	Not defined	Not defined	exploratory
Erk1/2	Transduction Labs (M37520-050)	N	Not defined	Not defined	exploratory
PTEN*	Zymed (2F4C9)	N	Not defined	Not defined	exploratory
Akt1	BD Biosciences (#610860)	N	Not defined	Not defined	exploratory
Akt2	Upstate (#06-606)	N	Not defined	Not defined	exploratory
Akt3	Santa Cruz (C-14)	N	Not defined	Not defined	exploratory
*pAKT**	Cell Signaling	N	Not defined	Not defined	exploratory
pS6K	Cell Signaling	N	Not defined	Not defined	exploratory
pERK1/2	Cell Signaling	N	Not defined	Not defined	exploratory
pIGF1R	Cell Signaling	N	Not defined	Not defined	exploratory
*pHER2**	M. Digiovanna	Y	Any membrane staining	40%	pCR 20% higher in lapatinib treated pts

Gene expression studies. Gene expression array-identified predictors of response will be developed using the data from the baseline frozen sample. To minimize variation generated during the hybridization process, all the microarrays should be hybridized under the same conditions. Once the microarray data have been generated, files with the unadjusted expression data will be sent to the CALGB Statistical Center for analysis. We anticipate a sample size of 100 per arm (T, L, T+L)

with available frozen tissue. The overall response rate is estimated at 30% combining all arms leaving 90 patients with pCR.

We will take two general approaches to analysis in this proposal. First, for those candidate regions of gene expression that have been identified in previous studies, each candidate region will be expressed as a dichotomous outcome. We will assess the frequency of tumors with these 8 candidate regions in the optimal response group (defined clinically as pCR) compared with the frequency of these regions in the tumors with a less poor response (SD+PD). We will divide the data into two sets, one for model building and one for validation. Patients will be randomly selected for one of the two groups with balance based on treatment study and response rates. Logistic regression modeling will be performed for the model building set to determine if the total number out of 8 chromosomal regions predict response. The logistic model will be tested using the validation set. To estimate the power of the logistic model in the building set we will reverse the factor-response structure and use the power calculations from a t-test. If the average number of genes expressed in the pCR group is 6.0 and the average number of genes expressed in the less favorable response group is 5.2, each group having a standard deviation of 2.0 genes, then the power to detect such differences is 89% assuming a two-sided alpha of 0.05 with 90/300 pCR patients. In addition, we will consider the subset of patients treated with trastuzumab, lapatinib and the combination to determine if pCR is predicted in each arm. If the average number of genes expressed in the pCR subset is 6.0 and the average number of genes expressed in the less favorable response subset is 4.6, each subset having a standard deviation of 2.0 genes, with 30/100 patients estimated to achieve a pCR, our power will be 89%.

The second approach will allow an exploratory study of the available microarray data in order to determine sets of genes that are predictive of response or resistance to trastuzumab, lapatinib and the combination. In this case, we will employ different machine learning methodologies. The clinical outcome used to define optimal response (pCR) or resistant tumors (SD+PD) will be used in a supervised analysis to define genes associated with response. We will use a number of distinct classification methods including a k-nearest neighbor (KNN) classifiers, PAM and other Centroid based methods that utilize SVM, random forests and linear discriminant analysis; for the KNN and Centroid methods, we typically use Euclidian and Spearman correlation to determine the distance of a sample to its nearest sample neighbors [27], and then assign a sample based upon either its 3 nearest neighbors, or that Centroid it is closest to. Before any classifier can be applied, a basic "gene filtering" step will be included to first identify the subset of genes that give a signal intensity of 1-1.5 standard deviations above background in at least 70% of the samples, which will then be used as the input data set. Some of these methods have built in gene/feature selection methods (PAM), while others typically use all genes (SVM); for those that do not have a gene selection method, we will use our own version of a gene selection method that was first described by Dudoit et al. [27]; for example, the KNN and Centroid genes are identified in the training set according to the ratio of between-group to within-group sums of squares. The top n ranked genes were used during each round of cross-validation. The size of the gene subset is increased for subsequent rounds of CV. The set of n top ranked genes that gave the highest average prediction accuracy during CV will also be determined and reported.

For most predictors, we will split the data into a training set and a test set to perform a 10-fold cross-validation (CV) analysis to iteratively optimize the list of genes and to determine prediction accuracies. Each round of CV would begin by splitting the samples into a training set (90% of the samples) and a test set (10% left-out samples), with gene selection and training being performed on the 90%, and then used to predict the status of the withheld 10%. This will be repeated 10 times, each time using a different 10% subset and a different gene set by collaborating investigators (Charles Perou, correlative co-chair of the CALGB HER2-negative preoperative trial).

In protocol Update # 9 the investigators have substituted two more modern genomic assays for the three older assays, for correlative science endpoints to this trial. The three relevant assays to be discussed here are 1) gene expression profiling by DNA microarrays, 2) Somatic mutations detection using Sanger sequencing, and 3) DNA copy number assessments (also called aCGH) by DNA microarray. The investigators will complete these endpoints, but do so using the most modern genomic assays available as opposed to the specific platform types listed in the original protocol text (which was based upon the standards of 2009). Since 2009, tremendous the technological advancements have been made, and thus we simply propose to use these more modern genomic tools instead of the older technologies. No preplanned endpoint has changed, nor will any additional tumor or normal materials be required. To be specific, we propose that 1) The gene expression profiling be changed from DNA microarray-based to mRNA sequencing-based 2) the somatic mutation detection be changed from Sanger sequencing-based to exome capture followed by DNA-sequencing on an Illumina platform, and 3) the DNA copy number assessment be changed from DNA microarray based to DNA-sequencing based (i.e. coming from the exome analysis). The rationale for each change in the relevant correlative section of the protocol is included below, with detailed protocols available upon request.

mRNA-sequencing is a recently developed method for gene expression profiling where mRNA is converted to cDNA, and then all cDNA fragments are sequenced using a NexGen Sequencing technology like the Illumina HiSeq2000 machine [28, 29]. This approach is rapidly replacing conventional DNA microarrays for gene expression profiling and provides multiple advantages including increased sensitivity, increased specificity, improved reproducibility, an ability to identify and quantitate splice variants, and an ability to identify sequence variants in expressed genes including oncogenic mutations. Thus, the gene expression platform has been changed from DNA microarray-based to mRNA-sequencing based. The precise protocol to be used is the one used by the Perou lab to profile >4500 tumor for The Cancer Genome Atlas Project; the first of these mRNA-sequencing results for TCGA are now part of two papers that were published in Nature for Colon/Rectal and Lung Squamous Cell Carcinomas [30, 31]. Please note that all the previously described expression signatures discussed below can also be precisely obtained using mRNA-sequencing data including tumor intrinsic subtype, thus all gene expression pre-planned endpoints can and still will be obtained.

Exome-sequencing is another newly developed gene sequencing approach that is revolutionizing somatic mutation and mendelian trait discovery [32-37]. The main advantage of exome sequencing that using the same amount of DNA, and about the same amount of work, it is as easy to sequence all 25,000 genes today as it was to sequence 100 genes back in 2009. Therefore, the investigators will perform whole exome sequencing using the now standard exome capture approach followed by Illumina HiSeq2000 sequencing, instead of the original proposal that was based upon Sanger sequencing. Similar to the mRNA-sequencing, this will be done using identical protocols as to what is being used in the TCGA, and has been published soon [30, 31].

DNA copy number analysis is the last new approach to be discussed here. The investigators originally proposed that this would be accomplished using DNA microarrays, which could be Agilent aCGH arrays, Illumina SNP arrays, or Affymetrix SNP arrays. It has now been shown that as a by-product of the exome sequencing approach, quantitative DNA copy number estimates for each of the 25,000 genes captured is also obtained [38, 39], thus providing 25,000 values for the scanning of the cancer genome. This information may not be as dense as a million-feature SNP array, however, it is obviously gene focused (and thus interpretations are simplified), and is able to detect all major and recurrent DNA losses and gain in the breast cancer genome. Thus if we perform exome capture, there is no immediate need to perform additional genomic assays for DNA copy number changes as this data will be provided from the exome sequencing.

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PART B: SERUM HER2 EXTRACELLULAR DOMAIN (ECD) AND CIRCULATING TUMOR CELLS (CTC)

1.0 BACKGROUND

Serum HER2 ECD levels have been proposed as a method of monitoring patients on trastuzumab-based treatments and predicting response to treatment [1]. Consistent with these findings, in two studies of preoperative trastuzumab and vinorelbine or paclitaxel, serum HER2 levels >15 ng/ml at any time point were only seen in patients with T3-T4 tumors [2,3]. In a recent study by Fornier and colleagues, serum HER2 declined by a greater percentage in responding patients [4]. In patients treated with preoperative trastuzumab and vinorelbine, patients with stable disease appear to have a slower decline in serum HER2. Furthermore, several patients develop elevated levels of HER2 ECD in followup blood draws, one of whom developed metastatic disease at that time point [2]. Longer follow-up and larger trials are necessary to further evaluate the role of HER2 ECD in monitoring early stage patients with HER2-positive breast cancer. Circulating tumor cells have also been used to measure microscopic tumor burden. Using immunohistochemical and PCR-based strategies, it is possible to detect rare (1 in one million to 1 in 100 million) circulating tumor cells in women with advanced breast cancer. Such assays as detection of circulating tumor cells or ECD have the potential of being sensitive measures for tumor response or progression, and have the virtue of being studied on samples of blood that are easily obtained from patients. For these reasons, there is widespread clinical interest in examining the utility of these methods in patients to see if more sensitive correlates of tumor response can be identified. Recent studies have shown that CTCs are present in over 50% of metastatic patients and 30% of early stage patients, using detection of CTCs by RT-PCR and by fluorescence-based immunohistochemistry using image analysis and flow cytometry [5,6]. These data further suggest that CTCs respond to therapy in a similar fashion to the metastatic sites, and may be a useful marker of response in the early stage setting. In addition, detection of CTCs as much as 6 months prior to the clinical detection of disease in early breast cancer has recently been reported, suggesting their potential application to detection of early relapse [7]. CTCs will be obtained and correlated with response to therapy in this trial.

2.0 OBJECTIVES

2.1 To determine if circulating biomarkers, including malignant epithelial cells and shed extracellular domain, predict response to neoadjuvant therapy that includes HER2-directed therapy.

2.2 To determine if the number of HER2-positive and/or EGFR-positive circulating tumor cells, as determined by FISH assay, predicts response to neoadjuvant therapy that includes HER2-directed therapy.

2.3 To evaluate changes in phosphorylated and total EGFR, and HER2 in circulating tumor cells from serial blood samples obtained from patients receiving neoadjuvant HER2 directed systemic therapy.

3.0 METHODS

Serial blood samples will be obtained at the following time points:

- Prior to the initiation of study therapy
- On day 15 of study therapy (just prior to the second chemotherapy infusion)
- Before the final (week 16) infusion of paclitaxel has been started

At all time points, 3 CellSave tubes (10 mL each), one lavender top (EDTA) tube (10 mL) and one red/grey top serum separator (8.5 mL) should be drawn. Please note that it is critical that each tube be completely filled with the required volume.

Blood will be evaluated for alterations in serum HER2 ECD and CTCs as follows.

3.1 Measurement of HER2 extracellular domain

HER2 ECD in serum will be measured by standard sandwich immunoassay criteria [8] using the blood collected in the 8.5 mL red/grey top tube. To perform the test, an appropriate volume of specimen is incubated in the wells to allow binding of the antigen by the capture antibody. The immobilized antigen is then exposed to the alkaline phosphatase labeled detector antibody. Addition of substrate to the wells allows the catalysis of chromogen, para-nitrophenylphosphate into a colored product, the intensity of which is proportional to the amount of ECD, which has been bound the plate.

3.2 Analysis of circulating tumor cells

Circulating tumor cell analysis will be performed using a variety of methods. All analyses will be performed in the Liu laboratory.

3.2.1 CTE Enumeration

Enumeration will be accomplished using the CellSearch System as previously described [6]. This analysis will be performed on two of the - 10 mL of blood samples collected in CellSave tubes. Briefly, blood is diluted with buffer, enrichment is performed using the AutoPrep machine using antiEpCAM coated magnetic beads. Cells are then stained for CD45 and cytokeratin, and analyzed on an automated fluorescence microscope. The total number of cytokeratin positive, DC45 negative and DAPI positive (a nuclear stain) is reported per sample.

3.2.2 Rare Cell FISH analysis

CTCs enumerated with the CellSearch System, as described in Section 3.2.1, are retained in a cartridge placed within a magnetic cell presentation device (one cartridge per CellSave tube). Following enumeration, each of the cartridges will be subjected to an automated process that allows for in situ cell fixation. FISH analysis for HER2 and EGFR will be performed on the collected CTCs.

3.2.3 Protein profiling for pathway activation

CTCs will be isolated using the CellSearch™ platform in conjunction with the commercially available Profile Kit on the 10 mL blood samples collected in the EDTA tube. These cells will be analyzed using a multiplex proximity enzyme assay that allows for enhanced specificity for phosphorylated proteins through use of concurrent binding by three antibodies. Single cell detection of phosphorylated EGFR and HER2 *in vitro* and in limited clinical samples has been demonstrated.

3.2.4 Future CTC studies

The blood sample collected in the third CellSave tube will be processed using the CellSearch System to collect CTCs for in situ cell fixation and storage at 20°C. These cells will be used for future correlative analyses.

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APPENDIX II 60701: GENOMIC STUDIES IN CALGB 40601

Part A: Pharmacogenomic Determinants of Response and Toxicity in CALGB 40601

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PART A: PHARMACOGENOMIC DETERMINANTS OF RESPONSE AND TOXICITY IN CALGB 40601

1.0 BACKGROUND

Chemotherapy-associated toxicity is highly variable. This may be largely due to host factors such as differences in absorption, distribution, and particularly metabolism and clearance. These sources of variability may be compounded by the presence of vomiting and diarrhea, poor nutritional status, concomitant medications, prior therapies, and liver metastases or effusions [1]. Response to chemotherapy is also highly variable and may be largely due to tumor specific factors, several of which are the subject of other correlative substudies described herein. However, variability in hepatic metabolism and membrane transport may be a key reason for human variability in response to medications, particularly chemotherapy.

1.1 Candidate gene approach

The cytochrome P450 (CYP) system is responsible for the vast majority of oxidative drug metabolism in the liver. In particular the enzyme families CYP1, CYP2, and CYP3 are responsible for the metabolism of many anticancer drugs, including paclitaxel, cyclophosphamide, and lapatinib, which will be used in this study. There are known CYP gene polymorphisms, and the expression of these enzymes shows marked interindividual variation [2],[3]. More recently, functionally significant genetic polymorphisms in drug transporters have been identified and characterized, most notably in the multidrug resistance transporter P-glycoprotein [4],[5].We hypothesize that patients carrying functional variants of drug metabolism and transport genes will have altered response and/or toxicity to paclitaxel. In particular, patients carrying the CYP2C8*2 and CYP2C8*3 alleles will have favorable response and/or increased toxicity with paclitaxel therapy.

Paclitaxel is extensively eliminated by hepatic metabolism, primarily to 6β-hydroxypaclitaxel [6],[7]. CYP3A4/CYP3A5 and CYP2C8 are the major enzymes involved in paclitaxel metabolism [7-9] and functionally significant polymorphisms have been described for each of these genes [10-13]. Genetic polymorphisms resulting in decreased metabolism of paclitaxel are expected to reduce the clearance of paclitaxel, therefore affecting the concentration-response profile. CYP2C8 has two major alleles, CYP2C8*2 and CYP2C8*3, both of which show decreased metabolism of paclitaxel in vitro [11]. CYP2C8*2 is an African American-specific allele found at a frequency of 18% while CYP2C8*3 is Caucasian-specific and found at a frequency of 13% [11]. CYP3A5 is highly polymorphic and varies greatly among populations of differing racial ancestry [14]. The CYP3A5*1 (or so-called wild-type) genotype is associated with significant levels of CYP3A5 protein in the liver such that in carriers of CYP3A5*1, CYP3A5 accounts for 50% of hepatic CYP3A content and may therefore be responsible for higher metabolic clearance of many CYP3A substrates in those individuals. CYP3A5*1 is found in 30% of Caucasians and 50% of African-Americans. By contrast CYP3A5*3 is seen in 95% of Caucasians and only 27% of African-Americans, and homozygotes for this allele have significantly reduced 3A5 expression [12]. This polymorphism may have clinical significance although not all studies are in agreement [14]. For example, in one recent study clearance of midazolam was higher in CYP3A5*1/*3 subjects than in homozygotes for CYP3A5*3 [15]. Other studies have not found phenotype-genotype correlations in systemic clearance of midazolam [16] [17] . Additionally, studies of renal and heart transplant patients receiving tacrolimus showed higher trough levels in CYP3A5*3 homozyotes [15]. A number of genetic polymorphisms have also been described for CYP3A4, although variants with a decrease or loss of function are found at only very low frequencies in all ethnic populations studied to date. While paclitaxel metabolism by CYP3A4 and CYP3A5 variants has not been directly tested, it is reasonable to predict that metabolic function would be decreased in individuals carrying reduced function alleles.

Lapatinib is also a substrate for CYP3A4 and its impact on CYP3A4 activity is currently being studied in a pharmacokinetic study at the University of North Carolina Lineberger Comprehensive Cancer Center and at Dartmouth Cancer Center. As noted above, functionally significant variations in CYP3A4 are of low frequency and therefore are unlikely to contribute to population differences in response and toxicity of CYP3A4 substrates.

Two recent retrospective studies of breast cancer patients who received cyclophosphamide containing chemotherapy have shown correlations between drug metabolizing enzyme polymorphisms and long term outcome [18, 19]. In the first study, DeMichele and colleagues evaluated CYP3A4 and CYP3A5 polymorphisms as well as GST deletions in stored stem cell aliquots from patients who had been treated with high dose chemotherapy including cyclophosphamide and thiotepa for node-positive breast cancer. These investigators found that patients who had the variant alleles CYP3A4*1B and CYP3A5*3 and did not carry deletions in GST had poorer disease-free and overall survival than others who do not have this slow metabolizer genotype [18]. Petros et al. evaluated CYP polymorphisms in cryopreserved lymphocytes from 85 patients who had received high dose therapy including cisplatin, cyclophosphamide and carmustine for metastatic breast cancer. This study found that presence of CYP3A4*1B and CYP3A5*1 correlated with higher AUC parent cyclophosphamide (lower active metabolite) and lower survival [19]. Interestingly, and in contrast to the preclinical studies, Hennigsson et al. did not find correlation between CYP2C8 and CYP3A4 polymorphisms and paclitaxel pharmacokinetics in a recent study of 97 cancer patients receiving paclitaxel [20].

Members of the multidrug resistance family of ATP-binding cassette (ABC) transporters interact with paclitaxel and influence its tissue distribution and elimination. Paclitaxel is a substrate for P-glycoprotein (encoded by ABCB1) and chemical inhibition of P-glycoprotein is associated with increased paclitaxel neurotoxicity in mice [21]. Overexpression of ABCB1 is also associated with paclitaxel resistance in human cell lines [22, 23]. More recently, MRP2 (encoded by ABCC2) expression was shown to be associated with paclitaxel resistance [24]. Genetic polymorphisms in ABCB1 and ABCC2 have been identified through large scale resequencing efforts in ethnically diverse populations and this data is publicly available at www.pharmgkb.org. Functional characterization of amino acid altering variants of ABCB1 have yielded conflicting results [5, 25, 26] although numerous groups have reported associations with the common 2677G>T and 3435C>T polymorphisms in ABCB1 and drug response or toxicity [4, 25, 27, 28]. In acute myeloid leukemia, the reference ABCB1 haplotype was associated with increased relapse rate and decreased survival compared to patients homozygous for the common ABCB1 haplotype of 1236T/2677T/3435T. In a small number of ovarian cancer patients (n=23) treated with paclitaxel, the ABCB1 3435T allele was associated with a higher AUC of 3'-hydroxypaclitaxel but there was no relationship between ABCB1 variants and paclitaxel toxicity [29]. Functional polymorphisms in the promoter region of ABCC2 have been identified in the Kroetz laboratory and are associated with exposure to irinotecan and its metabolites (Kroetz and Ratain, unpublished data). It is reasonable to hypothesize that patients carrying reduced function variants of ABCB1 and ABCC2 may have higher rates of drug response due to more favorable intracellular levels of paclitaxel.

In humans, polymorphisms within the HER2 gene may play a role in response to HER2-targeted therapy. Several coding polymorphisms can be identified from single nucleotide polymorphism (SNP) surveys, and one in particular, I655V, is associated with increased risk of breast cancer, particularly in Caucasians [30]. Interestingly, this residue is located within the transmembrane domain, two amino acids away from the residue that is mutated in transforming variants of rodent Neu. Molecular modeling studies suggest that the bulky isoleucine residue found in the more common variant may impede dimerization by interfering

with alpha helix formation in the transmembrane domain [31]. In contrast, homo- and/or heterodimerization may be facilitated by the less bulky variant allele. HER2+ breast cancer is found even in patients who are homozygous for I655. Because HER2 activation (i.e., homo- or heterodimerization) should be possible at lower concentrations of the V655 allele than the I655 allele, greater levels of HER2I655 expression may be needed for transformation. Alternatively, patients over-expressing HER2I655 may require additional, compensatory oncogenic mutations. Either way, HER2 polymorphisms might influence the trastuzumab/lapatinib response and can be examined in this study.

1.2 Genome wide approach

In addition to specific hypothesis testing for the above candidate genes, this study may also provide the framework for hypothesis generation investigations or genotype an/or haplotype in additional candidate genes of putative importance to drug response of the agents being evaluated.

Most pharmacogenetic analyses have taken a candidate gene approach that utilizes biological data to guide the selection of drug response genes in a pathway. This approach is limited by our knowledge of the mechanisms underlying the phenotypes. In the case of drug response phenotypes, most candidate gene studies have focused on drug metabolizing enzymes and transporters, thus limiting the chance of discovering causal SNPs not involved in mediating drug levels [1, 2]. In contrast, a genome-wide approach collects SNP data across the entire human genome and has significant power to detect common variants that confer a modest risk for a complex phenotype [3]. Genome-wide studies capitalize on the large number of SNPs (more that 10 million available in dbSNP) that have been localized and validated across the genome, a majority of which have resulted from the HapMap project [4]. This valuable collection of publicly available, validated SNPs has provided the framework for performing genome-wide association studies. Recent technological advancements in genotyping platforms have also enabled the development of genome-wide associations. Searching the whole genome in an association study requires genotyping of anywhere between10^5 to 10^6 markers across the genome [5-8]. Until recently, this approach was fiscally prohibitive and impractical. However, new gene chip platforms from Affymetrix and Illumina have made large scale genotyping feasible and cost effective. For example the Illumina HumanHap550 chip has the capacity to genotype over 555,000 SNPs simultaneously. In addition, there are 4,300 SNPs in regions of cop number variations (CNVs), thus allowing for the detection of CNVs as well. This new capability represents a paradigm shift in the number of genotypes that can be evaluated in any given individual with one genotyping assay and provides a platform for the identification of novel genes involved in the response to and toxicity associated with paclitaxel, trastuzumab and lapatinib.

An increasing number of reports of significant findings from genome-wide association studies in cancer are being published. To date, these have largely focused on SNPs associated with risk of developing cancer, and include studies in prostate [9-13], colorectal [14-16], lung [17] and breast cancer [18-20]. The success of these studies illustrates the power and validity of this approach for identifying genetic causes of disease. To date, there is a single published report of genome wide association analyses in cancer pharmacogenetics [32].

2.0 OBJECTIVES

2.1 Primary objective

To determine if the pCR rate after paclitaxel-based neoadjuvant chemotherapy is higher among women carrying CYP2C8*2 and/or *3 alleles compared with women who do not have these allele variants.

2.2 Secondary objectives

2.2.1 To perform an exploratory analysis of CYP2C8*2 and *3 carriage and paclitaxel-induced toxicity.

2.2.2 To perform exploratory analyses of allele frequencies for CYP3A4, CYP3A5, CYP2B6, ABCB1 and ABCC2 polymorphisms, response and toxicity profiles.

3.0 METHODS

A 10 ml whole blood sample in an EDTA (purple) tube will be obtained at baseline (within 60 days of registration) for pharmacogenomic analysis. Samples will be shipped to the Alliance Pathology Coordinating Office (PCO) at Ohio State University. Genomic DNA will be extracted using a commercially available kit from Qiagen. The concentration and quality of DNA will be quantified by ultraviolet spectroscopy. All DNA samples will be stored in the DNA bank at the CALGB PCO. Aliquots of DNA will be sent to the Kroetz (University of California, San Francisco), Ratain (University of Chicago) and McLeod (University of North Carolina) laboratories for genotyping of the candidate genes described above, and any additional genes identified for the paclitaxel, trastuzumab and lapatinib drug response pathways. Genotyping will be carried out using validated assays, typically TaqMan allelic discrimination assays. In some cases, panels of drug response SNP soon high density arrays may be genotyped. Haplotypes will be statistically inferred using the program PHASE.

The genotypes/haplotypes for the candidate genes will be correlated with disease-free survival and toxicity data collected in CALGB 40601 as described above.

In addition to the genes specified above, other genes related to the pharmacokinetics of each drug, as well as the antitumor efficacy (tumor response and other endpoints) and side effects associated with these drugs, might be considered for genotyping.

Furthermore, it is likely that by the time the sample set is near completion, specific typing for these SNPs will be performed by a different, high-performing, more up-to-date method of genotyping including analysis on platforms that allow for genome-wide investigation.

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PART B: SOMATIC MUTATIONS

1.0 BACKGROUND

Large scale tumor DNA resequencing projects, focused on a small number of tumors and the analysis of many genes, suggest that by the time breast cancer enters the lethal phase tens and perhaps hundreds of individual somatic mutations may have accumulated (1). Similarly, array comparative hybridization experiments have uncovered multiple gene amplification and deletion events (2). Understanding the clinical and biological significance of these somatic changes represents one of the most important challenges facing breast cancer researchers today because when complete, a functionally annotated breast cancer genome atlas will provide predictive/prognostic biomarkers and therapeutic opportunities that will transform our approach to this common disease. Discussions between the academic community, NHGRI and the NCI have suggested the following criteria represent the ideal specimens for further investigation. a) cancer samples need to be derived from patients on clinical trials (i.e., uniformly selected, uniformly treated and followed up for disease-related outcomes); b) tumor samples frozen in OCT; c) each tumor sample needs to be 200 mg or greater in size; with at least 80% of the sample being comprised of cancer cells; d) each cancer sample must have a matched normal (e.g. blood) from the same patient; e) at least 500 unique cancer samples of a single histological type, each from a unique patient are required; f) all cancer samples are from the primary tumor site (i.e., not metastases); g) all living patients can be re-consented; h) ideally, all samples are from cancers of the same stage of disease. The CALGB neoadjuvant studies C40601 and C40603 therefore represent an important opportunity to help make a breast cancer genome atlas a reality since there studies will provide specimens that can meet almost all of these criteria.

1.1 Specimen processing and quality

The above criteria state that each tumor sample needs to be 200 mg or greater in size; with at least 80% of the sample being comprised of cancer cells. The samples associated with breast cancer preoperative systemic therapy trials are almost always core needle biopsies, typically yielding between 10 to 30 grams of tissue. At most two or three frozen biopsies are available from each case, containing variable amounts of malignant tissue. However multiple laboratories have demonstrated that smaller amounts of tissue are sufficient for comprehensive genomic screens. For example, in the first one hundred specimen sets accrued from a Phase 2 trial of 4 months preoperative letrozole therapy, 74 cases yielded a sample that contained more than 50% malignant cells, RNA was successfully extracted and gene expression micro-array analysis completed. Further specimen processing by laser capture micro-dissection or macro-dissection where necessary achieved 70% to 80% tumor content for DNA extraction in all cases. The yields were sufficient for resequencing projects in all cases because only 50 to 100 ng of DNA is required as a starting point for Phi29 based whole genome amplification.

2.0 GENE SEQUENCING METHODOLOGY

The Genome Sequencing Center (GSC) at Washington University has received funding from the NHGRI and NCI for tumor re-sequencing projects for other disease sites. These funds have helped establish a fully automated re-sequencing pipeline capable of high-throughput, cost-effective sequencing. At the GSC, the most time-intensive aspects of re-sequencing have led to developing automated mutation analysis based on using phred (3, 4) and crossmatch to assemble and align sequence traces with their corresponding reference sequence. Then, two publicly available software tools, PolyPhred and SNPdetector (5-7), produce raw variant identification flags that are further analyzed by a newly developed SNP/indel detection program, PolyScan (manuscript submitted). PolyScan significantly reduces the false positives generated by the initial screening programs while retaining the true positives. More importantly, it provides highly sensitive and accurate de novo indel detection capability. PolyScan takes a sophisticated approach that integrates signal processing,

pattern recognition, and statistical analysis components that are traditionally performed independently; to better capture the signature characteristics of sequence variations missed by analysis programs that rely on phred trace analysis (i.e., PolyPhred and SNPdetector). Initial evaluation of PolyScan, using sequence data from three human genes known to contain indel polymorphisms indicated it is 5 to 6 times more sensitive than PolyPhred for indel detection, and is especially accurate in predicting indel size. We plan to implement other available software downstream of PolyScan, such as SIFT (8, 9) PolyPhen and nsSNPAnalyzer (10) to predict the functional implications of non-synonymous variants. In addition to the automated analysis, we will manually evaluate detected mutations to ensure accurate analysis results. Manual analysis typically is a process of verifying software-predicted variants using the consed interface, to perform independent evaluation of sequence trace data in these important target regions. In the standard manual analysis process, human analysts empirically assess the relevant features of trace data and can usually distinguish true SNPs and indels from false positives.

2.1 Choosing candidate genes for resequencing

To assign potential significance to their findings, Sjoblom et al. developed a cancer mutation prevalence (CaMP) score for each gene analyzed (1). The CaMP score reflects the probability that the number of mutations observed in a gene reflects a mutation frequency that is higher than that expected to be observed by chance, given the background mutation rate. Unfortunately, the number of cases analyzed in the Sjoblom paper was small, consequently the CaMP scores have very wide confidence intervals and so gene choices based on the published CaMP scores is unwise. Compounding this, their assumptions regarding the background mutation rate have been challenged. Gene function is another approach to narrowing down the gene list. In the Sjoblom paper, functional classifications included cell adhesion and motility, signal transduction, transcriptional regulation, transport, cellular metabolism, intracellular trafficking, RNA metabolism and "other" i.e., response to DNA damage status and protein ubiquitination. We could choose the most prevalent mutation from each of these categories. However from the perspective of drug development, mutations that increase the activity of an enzyme targeted by small molecule inhibitors could also be considered a priority. Gene size and type of mutation (gain of function hot spots or dispersed loss of function mutations) have a strong influence on feasibility and these issues also could be taken into account. Thus, rather than provide a final list of genes for resequencing in the write up for this proposal, we plan to make the question of which genes to resequence a focus for collaborative meetings organized by the CALGB breast committee.

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APPENDIX III: CANCER TRIALS SUPPORT UNIT (CTSU) PARTICIPATION PROCEDURES

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION:

To submit site registration documents:
CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-888-823-5923
Fax: 215-569-0206

For patient enrollments:
CTSU Patient Registration
Voice Mail: 1-888-462-3009
Fax: 1-888-691-8039
Hours: 9:00 AM – 5:30 PM Eastern Time, Monday – Friday (excluding holidays)
[Registrations received after 5:00 PM ET will be handled the next business day.
For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376 between 9:00 AM and 5:30 PM.]

Submit study data directly to the Lead Cooperative Group unless otherwise specified in the protocol:

CALGB Statistical Center
Hock Plaza
2424 Erwin Road, Suite 802
Durham, NC 27705
Tel: 919-668-9350
Data Operations Fax: 919-668-9348
Teleform Fax: 919-416-4990

Sites should submit Teleforms via Fax or Mail. See Section 6.0 Data Submission Section for details on forms submission.

Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility or treatment related questions: Contact the CALGB 40601 Study Chair.

For questions unrelated to patient eligibility, treatment, or 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.

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

The CTSU Registered Member Web site is located at http://www.ctsu.org

REGISTRATION/RANDOMIZATION (SECTION 5.0)

Prior to the recruitment of a patient for this study, investigators must be registered members of the CTSU. 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 registered member Web site or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 a.m. and 4:30 p.m. Eastern time.

Each CTSU investigator or group of investigators at a clinical 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 at http://www.ctsu.org

All forms and documents associated with this study can be downloaded from the CALGB-40601 Web page on the CTSU registered member Web site (https://www.ctsu.org). Patients can be registered only

after pre-treatment evaluation is complete, all eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS.

Requirements for CALGB 40601 site registration:

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

Prestudy requirements for patient enrollment on CALGB 40601:

- Patient must meet all inclusion criteria, and no exclusion criteria should apply.
- Patient has signed and dated all applicable consent and authorization forms.
- All baseline laboratory tests and pre-study evaluations performed (including tumor and blood samples) within the time period specified in the protocol.
- Samples for correlatives collected prior to initiation of neoadjuvant chemotherapy (if patient is participating.

CTSU procedures for patient enrollment:

1. Contact the CTSU Patient Registration Office by calling 1-888-462-3009 between 9:00 a.m. and 5:30 p.m. Eastern Time, Mon-Fri. Leave a voicemail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, e.g. within one hour, call the registrar cell phone at 1-301-704-2376.

2. Complete the following forms:

    - CTSU Patient Enrollment Transmittal Form
    - CALGB 40601 Eligibility Checklist
    - CALGB 40601 Registration Worksheet (indicate participation on substudies 60701 and 150702)

    Note: All patients must be registered to 150702 due to the required submission of pre-treatment tissue. In addition, there are four questions in the model consent form for optional studies included in 150702 (see Section 5.3.1.1 of the protocol).

    CALGB 60701 is an optional pharmacogenomic substudy that must be offered to all patients enrolled on CALGB 40601 (although patients may opt to not participate). CALGB strongly encourages the collection of a pre-therapy sample and registration to CALGB 60701 at the same time the patient is registered to the treatment trial (40601) however, patients may be registered to registered to 60701* within 60 days of registration to 40601. Patients who answer "yes" to "My blood specimen may be used for the genetic research described above," question #7 in the model consent, have consented to participate in the substudy described in Appendix II, Par A. If patients who answer "yes: to I agree to provide my tissue for the DNA sequencing project. I understand that my coded genetic information and coded medical information will be placed in databases accessible by the Internet," question #8 in the model consent, they have consented to participate in the substudy described in Appendix II, Part B. No additional sample submission is required. For patients who answer "yes" to this question, the pre-treatment tissue will be used for this purpose.

3. Fax these forms to the CTSU Patient Registrar at 1-888-691-8039 between the hours of 9:00 a.m. and 5:30 p.m., Mon-Fri, Eastern Time (excluding holidays); however, please be aware that registrations received after 5:00 p.m. will be processed the next day. The CTSU registrar will check the investigator

and site information to ensure that all regulatory requirements have been met. The registrar will also check that forms are complete and follow-up with the site to resolve any discrepancies.

4. Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will contact the CALGB, **within the confines of CALGB's registration hours.** The CTSU registrar will access the CALGB's on-line registration system, to obtain assignment of a treatment arm and a unique patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.

   *Procedures for late enrollment to CALGB 60701:

   -Submit CTSU Patient Enrollment transmittal form (with note indicating delayed registration to the pharmacogenomic companion study).

   -Submit revised 40601 Registration Worksheet (indication patient consent for CALGB 60701).

   Protocol treatment is to begin within 14 days of registration/randomization to allow time for the study specific clinical supply of lapatinib to arrive at study sites.

Data submission and reconciliation (Section 6.0)

1. All case report forms (CRFs) associated with this study must be downloaded from the CALGB-40601 Web page located on the CTSU registered member Web site (https://www.ctsu.org). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.
2. Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and transmittals directly to the CALGB Statistical Center, [see contact table] unless an alternate location is specified in the protocol. Do not send study data to the CTSU. A completed CTSU-CALGB coversheet should accompany all data submissions.
3. The CALGB Statistical Center will send (generally via email, but may be sent via postal mail or fax) query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the CALGB Statistical Center and do not copy the CTSU Data Operations. Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP IAM account contact information current**. This will ensure timely communication between the clinical site and the CALGB Statistical Center.

Special materials or substudies (Section 6.2)

There are two substudies within CALGB 40601. These studies are described in Appendices I and II of the 40601 protocol and do not require separate IRB approval.

All patients must be registered to 150702 due to the required submission of pre-treatment tissue for the correlative studies described in Appendix I Part A.

In addition, there are four questions in the model consent form for optional studies included in 150702 (see Section 5.3.1.1 of the protocol), these question that are for 1) for blood specimens, 2) for tissue core biopsies at surgery and 3) for tissue core biopsy after completion of study treatment if surgery is not performed and 4) for DNA sequencing. These questions **must be asked of all patients** enrolled on CALGB 40601 (although patients may opt to not participate).

If a patient answers "yes" to "My blood specimens may be used for the research described above," question #1 in the model consent, they have consented to participate in the substudy described in Appendix I, Part B. **Samples should be submitted per Sections 6.2, and 6.2.4 and 6.2.5.**

If a patient answers "yes" to "Samples of my tissue may be collected at the time of surgery to evaluate the effects of the study treatment," question #2 or question # 3 "I agree that an additional biopsy of my tumor tissue may be collected for research purposes if I do not have surgery after completing study treatment," in the model consent, they have consented to this future research.

If the patient says "yes" to "I agree to donate cancer tissue and normal tissue to be used for the DNA sequencing project. I understand that my coded genetic information and coded medical information will be placed in databases accessible by the Internet," question #8 in the model consent, they have consented to participate in the substudy described in Appendix II, Part B. No additional sample submission is required. For patients who answer "yes" to this question, the pre-treatment tissue will be used for this purpose.

Note: Specialized tissue specimen collection kits will be used for all correlative tissue collected for this study. The CALGB Pathology Coordinating Office will ship several kits to institutions after local IRB approval has been obtained. Additional kits may be ordered by calling the CALGB PCO at the phone number above.

The kits will also contain the CellSave tubes for the circulating tumor cell (CTC) studies (model consent question #1). Detailed instructions for obtaining, preparing and shipping the tissue specimens will accompany each kit. These instructions will also be available on the 40601 study page on the CTSU web site.

Unused kits or CellSave tubes should be returned to the CALGB PCO.

- Collect, prepare, and submit specimens as outlined in the protocol.
- Do not send specimens, supporting clinical reports, or transmittals to the CTSU.

Serious adverse event (AE) reporting (Section 17.0)

1. CTSU sites must comply with the expectations of their local Institutional Review Board (IRB) regarding documentation and submission of adverse events. Local IRBs must be informed of all reportable serious adverse reactions.
2. CTSU sites will assess and report adverse events according to the guidelines and timelines specified in the protocol. You may navigate to the CTEP Adverse Event Expedited Report System (AdEERS) from either the Adverse Events tab of the CTSU members' area (https://www.ctsu.org) or by drilling down to the Adverse Event Reporting Forms link under the documents folder of the 40601 Web page.
3. Do not send adverse event reports to the CTSU.
4. Secondary AML/MDS/ALL reporting: Report occurrence of secondary AML, MDS, or ALL via the NCI/CTEP AML-MDS Report Form in lieu of AdEERS. Submit the completed form and supporting documentation as outlined in the protocol.

Drug procurement (Section 12.0)

Investigational agents: Lapatinib will be provided for treatment Arms 1 and 3 by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

Commercial agents: Paclitaxel and trastuzumab

1. Information on drug formulation, procurement, storage and accountability, administration, and potential toxicities are outlined in Section 12.0 of the protocol.

2. You may navigate to the drug forms by selecting Pharmacy Forms from the document center drop down list on the CALGB 40601 Web page.

Regulatory and monitoring

Study Audit

To assure compliance with Federal regulatory requirements [CFR 21 parts 50, 54, 56, 312, 314 and HHS 45 CFR 46] and National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) Clinical Trials Monitoring Branch (CTMB) guidelines for the conduct of clinical trials and study data validity, all protocols approved by NCI/CTEP that have patient enrollment through the CTSU are subject to audit.

Responsibility for assignment of the audit will be determined by the site's primary affiliation with a Cooperative Group or CTSU. For Group-aligned sites, the audit of a patient registered through CTSU will become the responsibility of the Group receiving credit for the enrollment. For CTSU Independent Clinical Research Sites (CICRS), the CTSU will coordinate the entire audit process.

For patients enrolled through the CTSU, you may request the accrual be credited to any Group for which you have an affiliation provided that Group has an active clinical trials program for the primary disease type being addressed by the protocol. Per capita reimbursement will be issued by the credited Group provided they have endorsed the trial, or by the CTSU if the Group has not endorsed the trial.

Details on audit evaluation components, site selection, patient case selection, materials to be reviewed, site preparation, on-site procedures for review and assessment, and results reporting and follow-up are available for download from the CTEP web page http://ctep.cancer.gov/monitoring/guidelines.html.

Health Insurance Portability and Accountability Act of 1996 (HIPAA)

The HIPAA Privacy Rule establishes the conditions under which protected health information may be used or disclosed by covered entities for research purposes. Research is defined in the Privacy Rule referenced in HHS 45 CFR 164.501. Templated language addressing NCI-U.S. HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU website.

The HIPAA Privacy Rule does not affect participants from outside the United States. Authorization to release Protected Health Information is NOT required from patients enrolled in clinical trials at non-US sites.

Clinical Data Update System - Web (CDS-Web) Monitoring

This study will be monitored by the Clinical Data System (CDS-web). The sponsoring Group fulfills this reporting obligation by transmitting the CDS data collection from the study specific case report forms, via the Web to the NCI Center for Biometrics (NCICB). Cumulative CDS data are submitted quarterly.