Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Bear HD, Tang G, Rastogi P, et al. Bevacizumab added to neoadjuvant chemotherapy for breast cancer. N Engl J Med 2012;366:310-20.

NSABP PROTOCOL B-40

A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

NATIONAL SURGICAL ADJUVANT BREAST AND BOWEL PROJECT (NSABP)

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All institutions that are not aligned with the NSABP will enroll patients via the NCI Cancer Trials Support Unit (CTSU).

Protocol B-40 IND# 73,913 (gemcitabine, capecitabine, and bevacizumab), sponsored by the NSABP

STUDY DRUGS:	NSC#	SUPPLIED BY:
Bevacizumab	704865	Genentech, Inc.
Capecitabine	712807	Roche Laboratories, Inc.
Cyclophosphamide	26271	Commercially Available
Docetaxel	628503	Commercially Available
Doxorubicin	123127	Commercially Available
Gemcitabine	613327	Eli Lilly and Company

Version Date: April 1, 2011 (Replaces all other versions)

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NSABP PROTOCOL B-40

A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

National Surgical Adjuvant Breast and Bowel Project (NSABP)

Trial Activated:	November 20, 2006
Protocol Revision Record	
Original Version:	August 18, 2006
Amendment #1:	May 30, 2007 Sections Changed: Cover Page, Table of Contents, Information Resources, Sections 8.5.2, 8.6.2, 8.7.2, 14.0, Appendix F
Amendment #2:	February 15, 2008 Sections Changed: Cover Page, Table of Contents, Information Resources, Sections 1.0, 2.2.2, 5.1, 5.2.6, 5.3.3, 5.3.8, 6.4, 7.0 (Tables 4 and 5), 8.1 (text and Table 6), 8.3, 8.4, 8.5, 8.7.1, 9.0, 10.4.3 (Table 22), 11.1, 11.3, 12.1.1, 12.1.4, 13.1, 15.3.5, 15.4.1, 15.4.2, 15.4.4, 16.2, 17.1, 18.6, 18.8, 20.0, Appendices A, E, F, and G
Amendment #3:	April 1, 2011 Sections Changed: Cover Page, Table of Contents, 2.4, 2.5, 20.0, Appendix G

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05/30/07, 02/15/08, 04/01/11

INFORMATION RESOURCES

NSABP http://www.nsabp.pitt.edu		
NSABP Operations Center	East Commons Professional Building Four Allegheny Center – 5th Floor Pittsburgh, PA 15212-5234	Phone: 412-330-4600 Fax: 412-330-4660
NSABP Biostatistical Center	One Sterling Plaza 201 North Craig Street, Suite 500 Pittsburgh, PA 15213	Phone: 412-624-2666 Fax: 412-624-1082 (General office fax)
Questions/problems regarding IRB review & informed consent	NSABP Operations Center Division of Regulatory Affairs	Phone: 412-330-4600 Fax: 412-330-4661
Submission of IRB approval	CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103	Phone: 1-888-823-5923 Fax: 215-569-0206
Questions concerning eligibility and clinical aspects of the trial	NSABP Operations Center Clinical Coordinating Division	Phone: 1-800-477-7227 E-mail: ccd@nsabp.org
Patient entry information (see Section 16.0)	NSABP Biostatistical Center Patient Entry Coordinator	Phone: 412-383-4900 Consult the Patient Entry Guidelines section in the Members' Area of the NSABP Web site.
Information concerning drug orders, shipments, transfers, and returns (see Section 15.0)	NSABP Biostatistical Center	***See above***
Submission of all pre-entry core biopsy samples, questions concerning specimen collection and shipment, and requests for kits should be directed to Precision Therapeutics, Inc. (see Section 8.6)	Precision Therapeutics, Inc. 2516 Jane Street Pittsburgh, PA 15203	Phone: 1-888-557-7834 Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site.
Submission of blood/serum specimens and kit requests (see Section 8.8)	Baylor College of Medicine Breast Center NSABP Serum Bank Room N1220 One Baylor Plaza Houston, TX 77030	Phone: 713-798-1647 Fax: 713-798-1642 Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site.
Submission of tumor blocks and unstained sections; when sending blocks or other materials, please indicate on the package "Pathology Specimens Enclosed" (see Section 8.7)	NSABP Biostatistical Center One Sterling Plaza 201 North Craig Street, Suite 500 Pittsburgh, PA 15213	Phone: 412-624-2666 Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site.
Arrangement for return of blocks that are not to be stored or to request kits for 2 mm core sampling of existing blocks	NSABP Division of Pathology	Phone: 412-359-3312
Submission of expedited adverse event reports/questions concerning expedited adverse event reporting (see Section 14.0)	NSABP Biostatistical Center B-40 AE Reporting Nurse	Phone: 412-383-2557 Fax: 412-622-2113
Submission of data forms/questions concerning data management (see Section 17.0)	NSABP Biostatistical Center B-40 Data Manager	Phone: 412-624-2666 Data Fax: 412-622-2111 Refer to the B-40 Data Forms page in the Members' Area of the NSABP Web site.

Cancer Trials Support Unit (CTSU) Information Resources

This study is supported by the NCI CTSU.

Institutions not aligned with the NSABP will participate through the CTSU mechanism as outlined below and detailed in the CTSU logistical appendix (Appendix F).

To submit site registration documents:	**For patient enrollments:**	**Submit study data directly to the NSABP unless otherwise specified in the protocol:**
CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 Phone: 1-888-823-5923 Fax: 1-215-569-0206	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) [For CTSU patient enrollments that must be completed within approximately one hour, or other extenuating circumstances, call 301-704-2376. Please use the 1-888-462-3009 number for ALL other CTSU patient enrollments.]	Preferred method: Fax: 412-622-2111 **NSABP Biostatistical Center** One Sterling Plaza 201 North Craig Street, Suite 500 Pittsburgh, PA 15213 Do not submit study data or forms to CTSU Data Operations. Do not copy the CTSU on data submissions.

For patient eligibility and treatment-related questions, contact the Clinical Coordinating Division at the NSABP Operations Center at 1-800-477-7227.

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 https://members.ctsu.org

- 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://members.ctsu.org.
- Send completed **site registration documents** to the CTSU Regulatory Office. Refer to Appendix F for specific instructions and documents to be submitted.
- **Patient enrollments** will be conducted by the CTSU. Refer to Appendix F for specific instructions and forms to be submitted.
- Data management will be performed by the NSABP. **Case report forms** (with the exception of patient enrollment forms), **clinical reports, and other documents** must be sent to the NSABP Biostatistical Center 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 the NSABP Biostatistical Center. Please send query responses and delinquent data to the NSABP Biostatistical Center and do not copy the CTSU Data Operations. If the query is sent with a fax transmittal form, return the data to the fax number on the transmittal form, otherwise fax to 412-624-1082.
- Each site should have a designated CTSU Administrator and Data Administrator and must keep their CTEP AMS account contact information current. This will ensure timely communication between the clinical site and the NSABP Biostatistical Center.

02/15/08 1.0 **SUMMARY OF THE STUDY**

The primary aims of this Phase III study are to determine whether adding capecitabine or gemcitabine to docetaxel followed by AC will increase the pathologic complete response (pCR) rates in patients with palpable and operable HER2-negative breast cancer and to determine whether the addition of bevacizumab to three docetaxel-based regimens followed by AC will increase pCR rates. Secondary aims include determination of whether pCR in the breast and axillary nodes, clinical overall response rates (cOR), and clinical complete response rates (cCR) can be increased by the additional therapies summarized above. Other secondary aims include determination of whether the addition of bevacizumab to the chemotherapy regimens will improve disease-free survival and/or increase surgical complication rates, toxicity, and adverse effects on cardiac function.

Patients will initially receive one of the following docetaxel-based regimens:

- docetaxel (100 mg/m^2) ***with or without*** bevacizumab 15 mg/kg every 3 weeks for 4 cycles
- docetaxel (75 mg/m^2 Day 1) + capecitabine (825 mg/m^2 po BID Days 1-14) ***with or without*** bevacizumab 15 mg/kg every 3 weeks for 4 cycles
- docetaxel (75 mg/m^2 Day 1) + gemcitabine (1000 mg/m^2 IV Days 1 and 8) ***with or without*** bevacizumab 15 mg/kg every 3 weeks for 4 cycles

The docetaxel-based regimens will be followed by doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (AC) every 3 weeks for 4 cycles with or without bevacizumab 15 mg/kg given only with the initial 2 cycles of AC. Treatment groups randomized to receive bevacizumab will resume bevacizumab postoperatively at 15 mg/kg every 3 weeks for an additional 10 doses.

Following completion of neoadjuvant therapy, patients will undergo lumpectomy or mastectomy. Post-neoadjuvant chemotherapy axillary staging will be required, but the choice of the procedure will be at the physician's discretion. Postoperative radiation therapy will be given at the physician's discretion except for prohibiting the use of partial breast irradiation techniques utilizing brachytherapy. The choice of hormonal therapy for patients with hormone receptor-positive tumors will also be at the physician's discretion.

Pathology specimens will be collected and used to identify gene expression profiles that can predict pCR and to test a chemoresponse assay as a predictor of pCR. Attempted collection of 4 core needle biopsy specimens, submitted in RNAlater® (a solution that preserves RNA), formalin, and shipping medium for Precision Therapeutics, Inc. is required for participation in B-40. A tumor block from any gross residual disease ≥ 1 cm at time of surgery is also required for all patients. For patients who have agreed, blood and serum specimens will be collected at baseline. Serum will also be collected before the 3rd cycle of the docetaxel-based regimen and at least 3 weeks after completion of chemotherapy, but prior to surgery. In addition, histopathology findings must be provided for all patients enrolled after local IRB approval of Amendment #2 for use by the NSABP Biostatistical Center in calculating Residual Cancer Burden index.

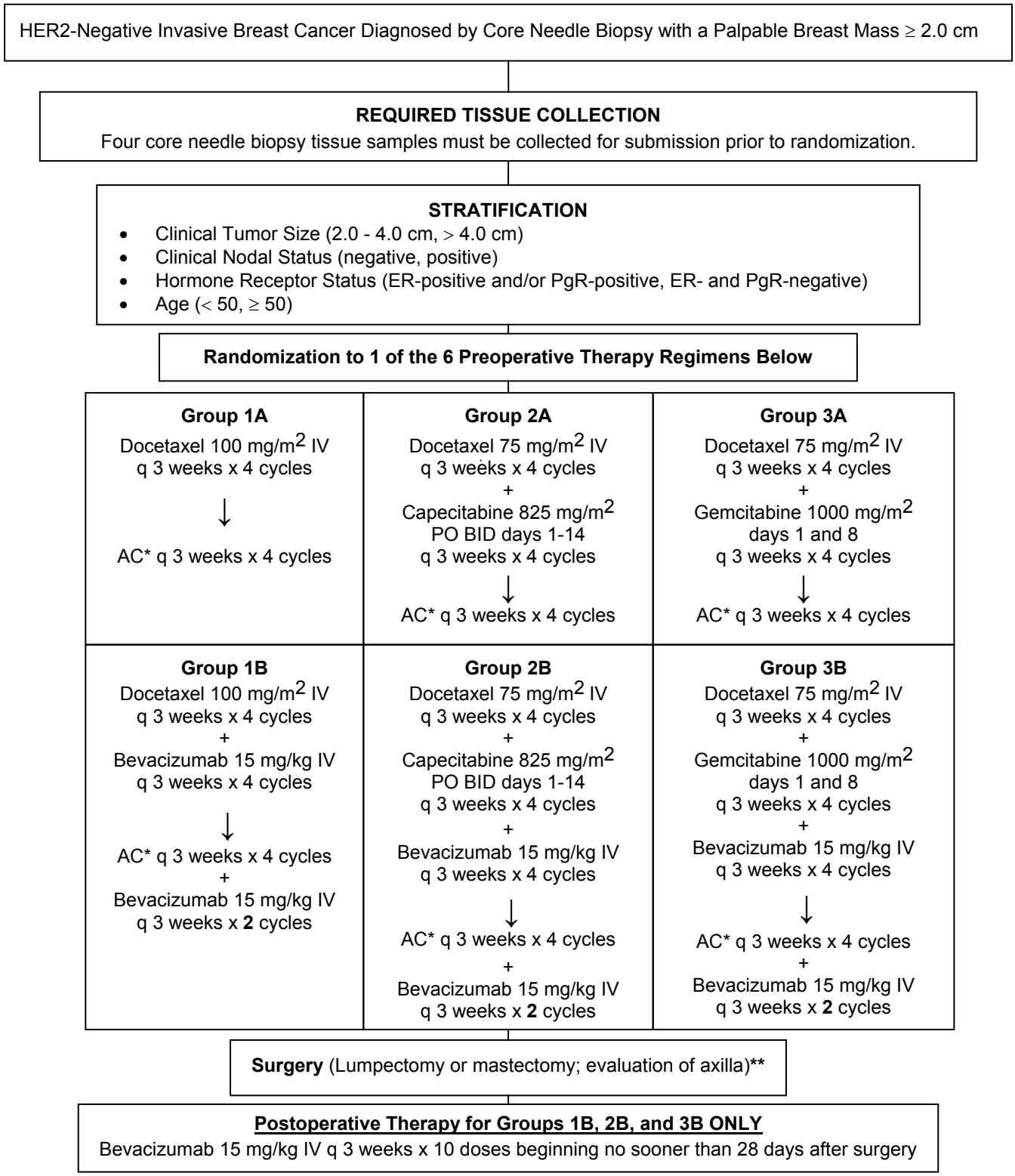
Tumor assessments by palpation to determine clinical response will be reported between chemotherapy regimens and will be used to test the accuracy of a chemoresponse assay as a predictor of cOR with each of the docetaxel-based regimens administered without bevacizumab. Following the last chemotherapy cycle prior to surgery, cOR will be assessed for the sequential regimens (with and without bevacizumab).

To determine the impact of bevacizumab on cardiac function, all patients will have evaluation of left ventricular ejection fraction (LVEF) by MUGA scans or echocardiograms performed at baseline and following preoperative chemotherapy (before surgery). A third LVEF assessment at 18 months after study entry is required for patients randomized to receive bevacizumab.

The sample size for B-40 will be 1200 patients accrued over a period of 2 years. Definitive analysis of the primary endpoints is expected at year 3.

Figure 1

B-40 SCHEMA



* 60/600 mg/m^2 IV of doxorubicin/cyclophosphamide (AC)

** See Section 9.9 for surgery-related instructions and Section 9.10 for radiation therapy instructions. Hormonal therapy is at the discretion of the investigator. Patients with ER-positive and/or PgR-positive tumors should receive a ***minimum*** of 5 years of hormonal therapy.

2.0 BACKGROUND

2.1 Introduction

The American Cancer Society projects 211,000 new cases of cancer of the female breast will be diagnosed in 2005, with the disease confined to the breast in 63% and regional lymph nodes in 29%.[1] With early detection and routine use of appropriate systemic therapies, 75 to 80% of these women will enjoy long-term freedom from recurrence. While these improved outcomes are welcome, they have come at a cost of treating many women with therapy that is in reality, either unnecessary or ineffective. This is in part due to the fact that accurate prognostic factors for outcome and predictive factors for response to treatment of individuals are lacking. Neoadjuvant chemotherapy is an established approach for managing patients with larger early stage breast cancers, which provides an opportunity for collection of specimens for correlative science studies to identify predictive markers for response to specific agents. Gene expression profiling has shown promise in neoadjuvant trials as a tool for defining patterns predictive of pathologic complete response to specific agent(s).[2-4] Similarly, in vitro chemoresponse assays have the potential to predict response to therapy,[5-7] but need validation in large, well-controlled trials.[8] A primary goal of B-40 will be to expand this promising avenue of research into a large scale multi-institutional setting.

2.2 Rationale for conduct of the study

The rationale for evaluating preoperative chemotherapy in patients with operable breast cancer was provided by hypotheses formulated from findings obtained in laboratory investigations,[9-12] from results of preoperative chemotherapy for locally advanced cancers,[13-21] and by the establishment of lumpectomy as the surgical treatment of choice in patients with operable breast cancer.[22,23]

2.2.1 *Neoadjuvant chemotherapy in locally advanced breast cancer*

Initial trials of neoadjuvant chemotherapy administered for locally advanced tumors, including those in breast cancer, demonstrated that chemotherapy could induce sufficient tumor regression to allow for the resection of otherwise unresectable tumors.[13-18] Subsequent demonstration of the equivalence of lumpectomy to mastectomy in patients with operable breast cancer,[22,23] stimulated interest in the concept of using preoperative chemotherapy to reduce large, but operable primary tumors, to allow for lumpectomy in these women who would otherwise require a mastectomy.[19-21] Findings from a number of non-randomized trials demonstrated that chemotherapy administered preoperatively resulted in high rates of clinical response (47% to 85%) but low rates of pathologic complete response (4% to 7%).[19,21,24,25] Some of these studies confirmed that reduction in tumor size allowed for more breast-conserving procedures. These results provided justification for Phase III trials comparing neoadjuvant chemotherapy with postoperative chemotherapy in women with operable breast cancer.

02/15/08

2.2.2 *Phase III trials of neoadjuvant chemotherapy in women with operable breast cancer*

NSABP B-18 was a landmark Phase III trial that compared preoperative with postoperative chemotherapy in women with operable breast cancer. In this study, 1523 patients were treated with 4 cycles of doxorubicin/cyclophosphamide (AC) either before or after surgery. In the group randomized to neoadjuvant chemotherapy, the overall clinical response rate was 79%, with a clinical complete response (cCR) of 36%. Pathologic evaluation of surgical specimens demonstrated 13% of patients had a pathologic complete response (pCR) in the breast. The incidence of pathologically positive axillary nodes in the preoperative group was 41% compared to 57% in the postoperative group, representing a 37% relative reduction in the rate of positive nodes with chemotherapy.[26] In addition, significantly more women in the neoadjuvant group underwent lumpectomies compared to those in the adjuvant group (68% vs. 60%, p=.002).[27] When the two groups were compared in terms of outcome, there were no significant differences in disease-free survival (p=.99), distant disease-free survival (p=.70) or overall survival (p=.83). However, a significant correlation between complete pathologic tumor response following neoadjuvant chemotherapy and patient outcome was noted. Patients achieving a pCR had superior disease-free survival (DFS) (p=.0001) and a strong trend for overall survival (OS) (p=.06) when compared to the other patient cohorts (cCR but with residual invasive carcinoma in the breast specimen [pINV], clinical partial response [cPR] or a clinical non-response [cNR]). When the prognostic effect of pCR was examined after adjusting for known clinical prognostic factors such as clinical nodal status, clinical tumor size and age, pCR remained a significant independent predictor of disease-free survival (p=.0005).[26] Through 9 years of follow-up, results continue to demonstrate equivalence in outcome between neoadjuvant and adjuvant chemotherapy, and the significant correlation between pCR and outcome remains. At 9 years, the disease-free survival rate for patients achieving a pCR was 75% compared with 58% for patients with pINV. For overall survival, the respective rates were 85% for patients with a pCR and 73% for patients with pINV. Overall primary tumor response classified as pCR, pINV, cPR, or cNR remained strongly associated with outcome measures (DFS: p=.00005; OS: p=.0008).[28]

EORTC 10902 was similar in design to the NSABP B-18 trial, but employed 4 cycles of FEC (fluorouracil, epirubicin, cyclophosphamide) rather than 4 cycles of AC. A total of 698 patients with $T1_c$ - $T4_b$, N0-1, M0 breast cancers were entered. The overall clinical response was 49% in the patients randomized to the neoadjuvant chemotherapy regimen (7% complete and 42% partial), with a pCR rate of only 2%. There was evidence of axillary nodal down-staging with neoadjuvant chemotherapy, as 38% of these patients were found to have negative nodes compared to 32% in the postoperative chemotherapy group. With a median follow up of 56 months, there was no significant difference between the two groups in terms of overall survival, progression-free survival or time to loco-regional recurrence.[29]

2.2.3 *Potential advantages of neoadjuvant chemotherapy*

These results demonstrate that equivalent disease-free and overall survival in patients with operable breast cancer can be achieved with the two approaches. However, neoadjuvant therapy has several advantages as well as some minor limitations. From the clinical perspective, a major advantage of neoadjuvant chemotherapy is the ability to increase the rate of breast conserving surgery. Additionally, by reducing the size of primary tumors, better cosmetic results with lumpectomies may be obtained in patients with larger tumors who were technically lumpectomy candidates before neoadjuvant chemotherapy. Recently, a body of evidence supporting the feasibility and accuracy of sentinel node biopsy following neoadjuvant chemotherapy has evolved.[30-37] Since the accuracy of negative sentinel nodes in predicting pathologic status of axillary nodes is similar whether the procedure is performed before or after systemic chemotherapy, neoadjuvant treatment should reduce the rates of axillary dissection by clearing involved axillary nodes.

The presence of clinically and pathologically assessable disease allows for observation of the response to neoadjuvant therapy, and provides opportunity to evaluate pretherapy specimens for predictive biomarkers. The importance of tumor sensitivity to systemic therapy is supported by the results from B-18 which showed disease-free survival and survival correlate with primary tumor complete response assessed either clinically or pathologically.[26] Early identification of tumor resistance makes it possible to avoid further administration of ineffective therapy with the attendent unnecessary adverse events, and to allow change to a more potentially effective regimen. Furthermore, it is possible that, by assessing changes in tumor biomarkers as the tumor undergoes neoadjuvant chemotherapy, patients could be categorized into more refined prognostic and predictive groups than is possible with clinical or histologic evaluation.

Potential disadvantages to preoperative chemotherapy include the lack of pre-chemotherapy, pathologic axillary node assessment and the relatively small amount of tumor tissue obtained by biopsy techniques such as fine needle aspiration. The latter problem has been addressed by the availability of core needle biopsy for diagnosis and assessment of biomarkers. Additionally, lack of knowledge of pathologic nodal status before neoadjuvant chemotherapy is not of major concern, since clinical decisions regarding systemic therapy, as well as adjuvant radiotherapy can be based on the initial clinical stage, and the pathologic information at the time of surgery. Furthermore, nodal status following chemotherapy, which is reflective of both initial node status and chemoresponse, has at least equal, if not greater, prognostic power than pre-treatment nodal pathology.

2.2.4 *Phase III trials evaluating the addition of taxanes to anthracycline-based neoadjuvant chemotherapy regimens*

Results from initial randomized trials of neoadjuvant chemotherapy strengthened the biologic and clinical rationale for continuing to evaluate its role in patients with operable breast cancer. Since response to neoadjuvant chemotherapy correlated with patient outcome, it was hypothesized that more active neoadjuvant chemotherapy regimens should increase the pCR rates and improve

disease-free and overall survival. More active chemotherapy regimens could also further reduce the extent of surgery in the breast and the axilla. If higher pCR rates achieved with more effective regimens continued to predict for improved outcome, then pCR could be used as an intermediate endpoint in testing new chemotherapy regimens as well as newer targeted therapies.[38]

The demonstration of significant antitumor activity of taxanes in advanced breast cancer provided the opportunity to test these hypotheses in the neoadjuvant setting. Several randomized trials were designed to evaluate neoadjuvant chemotherapy regimens that employed anthracyclines and taxanes.[36,39-45] As shown in Table 1, rates of pCR (22%-31%) have essentially been doubled with the addition of a sequential taxane to the anthracycline/cyclophosphamide combinations.

TABLE 1. Randomized trials comparing anthracycline→taxane vs. anthracycline-based neoadjuvant chemotherapy regimens in patients with operable breast cancer

Clinical Trial	# of Patients	Tumor Stage/Size	Chemotherapy Regimen	pCR %	DFS Benefit	OS Benefit
Smith IC, et al[43] Hutcheon AW, et al[44]	145	Operable T_{1c}-T_3 N_0 T_{1-3} N_1	CVAP x 4 vs. CVAP →TXT x 4	15 31	CVAP-TXT > CVAP	CVAP-TXT > CVAP
Bear H, et al[36,45,46] (NSABP)	2411	Operable T_{1c}-T_3N_0 T_{1-3} N_1	AC x 4 vs. AC x 4 → TXT x 4	13.7 25.6	No	No
Green MC, et al[39]	258	Operable	Q 3 W TXL x 4 → FAC x 4 vs. Q W TXL x 12 → FAC x 4	13.6 28.8	NR	NR
Untch M, et al[40]	631	Primary BC T > 3 cm or inflammatory	Q 2 W E x 3 → TXL x 3 (dose-dense) vs. ETXL x 4	18 10	NR	NR
Von Minckwitz G, et al[41]	913	Operable	AC x 4 → TXT x 4 vs. ATXT x 4 q 2 W (dose-dense)	22.4 11.5	NR	NR
Evans T, et al[42]	362	Locally advanced, operable	AC x 6 vs. ATXT x 6	12 8	NR	NR

CVAP: cyclophosphamide, vincristine, doxorubicin, prednisone; TXT: docetaxel; AC: doxorubicin, cyclophosphamide; TXL: paclitaxel; FAC: 5-fluorouracil, doxorubicin, cyclophosphamide; E: epirubicin
PCR: pathologic complete response; DFS: disease-free survival; OS: overall survival; NR: not reported

The largest of these trials, NSABP B-27, was a three-arm study to determine if 4 cycles of post-AC docetaxel administered before surgery would improve pCR rates, and if post-AC docetaxel administered before or after surgery would improve OS and DFS when compared to preoperative AC alone. Comparisons of the clinical and pathologic response rates in patients receiving 4 cycles of AC (n = 1534) to those receiving 4 cycles of AC followed by 4 cycles of docetaxel (n = 752) are available. A significant benefit in favor of the docetaxel group was seen both for cCR (63.6% vs. 40.1%, $p < .001$), and pCR (26.1% vs. 13.7%, $p < .001$).[36] In addition, there was significant nodal down-staging for the group randomized to AC followed by docetaxel when compared to the group randomized to AC alone (negative nodes 58.2% vs. 50.8%, $p < .001$).

However, the addition of docetaxel, either preoperatively or postoperatively, did not significantly increase OS. Preoperative docetaxel increased DFS by 5%, with a hazard ratio of 0.86, but this was not statistically significant. The impact on DFS was decreased by an unexpected increase in second primaries of the contralateral breast and other sites, as well as the inclusion of low-risk patients which decreased the rate of events. Examining relapse-free survival (RFS), which was similar to DFS as defined in the CALGB's 9344 trial, preoperative docetaxel improved outcomes significantly, with a HR of 0.81 ($p=0.03$). Most of the effect of adding docetaxel so far has resulted from significant decreases (by nearly half) in local recurrences. At the time of this analysis, with a median follow-up of 68.8 months, the magnitude of effects and hazard ratios were comparable to the effects of adding taxanes to the adjuvant therapy of node-positive patients in NSABP B-28 and CALGB 9344.[46] Patients whose tumors had a clinical partial response at the end of 4 cycles of AC derived the greatest benefit from the addition of preoperative docetaxel, with a significant increase in DFS (HR=0.68, $p=0.003$). Interestingly, postoperative docetaxel did not produce a significant benefit in this subset of patients. Furthermore, as in B-18, complete pathologic response in the breast remained a powerful predictor of DFS and OS in all treatment groups.

In contrast, the Aberdeen trial demonstrated significant improvement in DFS ($p=0.004$) and OS ($p=0.04$) with crossover to docetaxel in patients who had an objective clinical response to CVAP.[44] Both CALGB 9344 and NSABP B-28 compared 4 cycles of AC followed by 4 cycles of paclitaxel with 4 cycles of AC alone, and have shown significant improvements in DFS in the groups receiving paclitaxel (9344: $p=0.0023$; B-28: $p=0.008$).[47,48] One of the studies, CALGB 9344, has also shown a statistically significant improvement in OS ($p=0.0064$).[47] An additional adjuvant trial, PACS 01, reported at San Antonio in 2004, also demonstrated significant improvement in DFS and OS for node-positive patients who received 3 cycles of anthracycline-based therapy followed by 3 cycles of docetaxel compared to 6 cycles of anthracycline-based therapy alone.[49]

Reversal of the sequence of the regimens does not appear to reduce the activity. A neoadjuvant trial reported by MD Anderson evaluated 2 schedules of paclitaxel followed by 4 cycles of fluorouracil/doxorubicin/cyclophosphamide (FAC) in standard doses every three weeks. A total of 258 patients were randomized to receive paclitaxel either weekly (for a total of 12 doses) or every three weeks (4 cycles), followed by FAC. Patients receiving weekly paclitaxel

followed by FAC had a pCR rate of 28.2%[50] which is similar to the pCR rate reported in NSABP B-27.

Final results of a neoadjuvant Phase III trial of docetaxel and capecitabine (TX) versus doxorubicin and cyclophosphamide (AC) as primary therapy in early stage breast cancer was reported at San Antonio in 2005. A total of 209 patients were randomized to receive docetaxel and capecitabine (four cycles) or doxorubicin and cyclophosphamide (four cycles) in the preoperative setting. Patients then proceeded to surgery. Patients who received TX in the preoperative setting, received AC in the postoperative setting. Patients who received AC in the preoperative setting, received TX in the postoperative setting. Patients receiving TX had an increased clinical response rate (84% vs 67%; p=0.0047) and an increased pCR rate (23% versus 10%) compared to patients receiving AC.[51]

With the above results, it is appropriate to continue development of sequential chemotherapy regimens to improve on clinical and pathologic response rates. We propose to use preoperative sequential docetaxel followed by AC as the control arm for B-40 and to compare the docetaxel/AC regimen with 2 different docetaxel doublets, which have shown significant activity in metastatic and locally advanced breast cancer, followed by AC.

2.2.5 *Justification for use of pCR as primary endpoint*

Since achievement of pCR with neoadjuvant therapy has been shown to predict favorable long-term outcome, we will use pCR rate in the breast as the primary endpoint for this trial. Use of traditional adjuvant endpoints such as DFS and OS require a large number of patients which limits the choice of investigational arms, and require lengthy follow-up for determination of outcome. Additionally, use of these endpoints in neoadjuvant trials requires that post surgical therapy be applied uniformly in women receiving therapy on these trials to avoid introducing bias. Since pCR identifies a small group with a favorable outcome and a larger group with a poorer outcome, it is difficult to justify a uniform approach to clinical management or clinical investigation in these dichotomous patient groups. Current standard therapies following neoadjuvant chemotherapy and surgery include hormonal therapy, if the tumor was hormone receptor-positive, and consideration for radiation therapy. There is no evidence that additional chemotherapy provides benefit to either group, though consideration for additional chemotherapy is sometimes given for patients with significant residual disease. Patients with residual disease following taxane/anthracycline-based sequential neoadjuvant chemotherapy regimens have sufficient risk to warrant investigation of additional systemic treatments such as novel targeted agents. Availability of residual tumor for gene expression profiling and identification of putative targets for new investigational agents provides an important opportunity for evaluation of these therapies. Exclusion of patients with residual disease following treatment with chemotherapy regimens on neoadjuvant trials from participating in trials evaluating novel agents simply to obtain long-term endpoints on the initial study is not appropriate based on the prognostic information already provided by the failure to achieve pCR status.

The efficient use of precious patient resources, which can be achieved by using pCR as the primary endpoint in neoadjuvant trials, has become critical with the

large number of promising therapies entering clinical development. Thorough, but efficient evaluation of new agents in the neoadjuvant setting can reduce the risks of further development of ineffective therapies or incorrectly terminating a potentially effective therapy. Importantly, the development time of effective therapies can also be substantially reduced.

Finally, recent findings from small single institution trials have demonstrated the potential of using molecular markers, particularly gene expression profiles, for predicting pCR of breast cancers to neoadjuvant chemotherapy. A report from MD Anderson demonstrated the potential power of genomic signatures to predict pCR of primary breast cancers to 4 cycles of paclitaxel followed by 4 cycles of FAC.[2] Similarly, a group from Baylor reported gene expression profiles could be constructed to identify those breast cancers that were most likely to respond to single agent docetaxel therapy.[3] These reports, along with others that have demonstrated the predictive power of similar techniques, suggest the neoadjuvant setting could be most valuable for validating genomic profiles in large patient populations.[4] Therefore, this trial will evaluate baseline gene expression profiles and other molecular markers as potential predictors for pCR to the chemotherapy regimens evaluated in this study. Following completion of the first part of the regimen, activity of the first portion of the sequential regimen will be assessed by clinical evaluation. Following completion of the second part of the regimen, activity will be assessed by clinical evaluation and pathologic evaluation of the surgical specimens.

2.3 Rationale for docetaxel doublets to be compared to docetaxel alone

2.3.1 *Capecitabine + docetaxel*

The oral fluoropyrimidine capecitabine (Xeloda®, Roche Laboratories, Inc.) was rationally designed to provide prolonged exposure to 5-FU and to generate 5-FU preferentially in tumor tissue. Capecitabine[52-58] is a rapidly absorbed oral compound, which is converted to 5-FU by a three-enzyme pathway. A carboxylesterase cleaves the drug to make 5'-deoxy-5-fluorocytidine, which is then further metabolized to 5'-deoxy-5-fluorouridine (5'DFUR) by cytidine deaminase. The final enzyme, thymidine phosphorylase (TP), converts 5'DFUR to 5-FU. Capecitabine[59-61] is rapidly absorbed with a t_{max} of 1-2 hours and 70% of it can be recovered as parent compound or metabolites in urine. Approximately 40% is detected as 5'DFUR in urine. It has a $t_{½}$ of 0.85 hours. Capecitabine C_{max} and AUC increase in a dose proportional manner over the dose range of 666 – 1255 mg/m^2. The enzyme TP is present in tumor tissue in much higher concentrations than in normal tissues. Exploitation of this higher level of thymidine phosphorylase (TP) in many human tumor tissues relative to healthy tissue provides a degree of tumor selectivity.[62,63]

Preclinical studies in human cancer xenograft models demonstrated that administration of docetaxel or paclitaxel results in further upregulation of TP in tumor tissue.[64] This has been confirmed in women with primary breast cancer who were treated with pre-operative docetaxel.[65] Co-administration of capecitabine and either docetaxel or paclitaxel in xenograft models resulted in synergistic antitumor activity, whereas, taxanes in combination with either 5-FU or UFT (uracil plus tegafur) demonstrated only additive efficacy.[64]

Capecitabine has been approved in the U.S. for patients with previously treated metastatic breast cancer and is being evaluated in the adjuvant setting. The Phase II pivotal trial which resulted in expedited approval of capecitabine showed an overall response rate of 20% in a population of patients who had previously received treatment with anthracyclines and taxanes.[66] These results were later confirmed in a second multicenter trial.[67] Capecitabine has also been shown to produce a higher response rate and a better median survival than CMF in women over 55 years of age.[68]

A Phase III comparison of capecitabine plus docetaxel vs. docetaxel alone in patients with advanced or metastatic disease who either progressed while receiving anthracycline-containing therapy or relapsed after the completion of treatment showed an increase in objective response (32% vs. 23%, p = .025), as well as significant prolongation in median time to progression (6.1 months vs. 4.2 months, p = .0001) and median survival (14.5 months vs. 11.5 months, p = .0126) for patients assigned to the combination. At the initial doses used (docetaxel 75 mg/m^2/D1/q3w and capecitabine 1250 mg/m^2/D1-14/q3w), Grade 3 hand foot syndrome (HFS) was 24%, Grade 3/4 diarrhea was 14.4%, and Grade 3/4 stomatitis was 17.4% The overall incidence of HFS, diarrhea, and stomatitis were each in excess of 60%. Patient withdrawal due to treatment-related adverse events occurred in 26% of patients.[69] Because of the high rate of HFS in the combination arm, dose modifications were frequently necessary.

The U.S. Oncology Research Network (USON) is conducting an adjuvant trial of sequential AC/docetaxel vs. AC/docetaxel with capecitabine. The trial was initiated employing the doses of docetaxel and capecitabine used in the Phase III metastatic trial. However, the protocol has been modified to reduce the starting dose of capecitabine due to unacceptable rates and severity of toxicity which initially occurred. With reduction in the starting dose, the tolerability of the regimen has substantially improved.[70] Based on discussions with Dr. O'Shaughnessy and reports from the Data Monitoring Committee for the study, the manufacturer of capecitabine now recommends use of the modified starting dose of capecitabine when using the regimen either in research or routine clinical practice.[71] We will therefore use this schedule in B-40.

Dr. O'Shaughnessy has also shared the current dose modification program for the regimen being employed in the USON adjuvant trial. A key aspect of the schema is use of filgrastim as secondary prophylaxis following neutropenia related toxicities. When filgrastim has been administered concurrently with capecitabine, it has been effective without apparent increase in side effects.[70] Based on this experience, we will also administer filgrastim concurrently with capecitabine when needed for secondary prophylaxis.

2.3.2 *Rationale for gemcitabine + docetaxel doublet*

Gemcitabine (Gemzar®) is a nucleoside analog antimetabolite, which exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S phase) and also blocking the progression of cells through the G1/S phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which lead to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleotide causes reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA, which allows only one additional nucleotide to be added to the growing DNA strands. This results in inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal fragmentation, one of the characteristics of programmed cell death.

Gemcitabine possesses a broad range of antitumor activity against solid tumors and leukemias *in vitro* and *in vivo*.[72,73] It is active in several tumor cell lines and human tumor xenografts. Gemcitabine has undergone considerable testing for various malignancies and exhibited activity in non-small cell lung cancer (NSCLC), pancreatic cancer, bladder cancer, advanced breast carcinoma, and cisplatin-refractory ovarian carcinoma.[74-78]

Multiple studies have established the activity of single agent gemcitabine in patients with metastatic breast cancer. Response rates of 37% to 42% have been seen in less heavily pretreated patients.[79-82] Activity of gemcitabine has also been shown in patients previously treated with up to three prior regimens in the metastatic setting, as well as in patients resistant to both an anthracycline and a taxane (see Table 2).[83-85] The weekly dose of gemcitabine has ranged from 800-1250 mg/m^2 administered every week for 3 out of 4 weeks. The toxicities seen have typically been mild and included neutropenia, thrombocytopenia, fatigue, flu-like syndrome and anemia.[79-85]

TABLE 2. Activity of gemcitabine in advanced breast cancer

Author	Weekly Dosing (mg/m^2)	Prior Chemo Regimens MBC	# Pts	Percent CR/PR/OR	Major Toxicities (> 5% grade 3 or 4)
Blackstein[79]	1200	0	35	11/26/37	WBC, nausea
Carmichael[80]	800	0-1	40	8/17/25	WBC, nausea
Possinger[81]	1000	0-1	42	0/14/14	WBC, LFTs
Gerson[82]	1250	NA	19	11/31/42	Anemia, plts
Valerio[83]	1000	Resistant A and T	22	5/18/23	WBC, fatigue, flu-like syndrome
Brodowicz[84]	1250	2nd	9	0/22/22	WBC
		3rd	16	6/6/12	
Spielmann[85]	1200	0-1 prior A	43	9/19/28	WBC, anemia, asthenia

MBC: Metastatic breast cancer; CR: complete response; PR: partial response; OR: overall response; LFTs: liver function tests; A: anthracycline; T: taxane

Gemcitabine in combination with taxanes has been evaluated in both pre-clinical and clinical studies. Additive, but not synergistic, effects have been observed in vitro and in vivo when paclitaxel and gemcitabine have been combined.[86,87] When paclitaxel was administered prior to gemcitabine, increased accumulation of the active metabolite dFdCTP was observed, contributing to the additive effect.[88]

Recently, results from a Phase III randomized trial comparing paclitaxel alone to paclitaxel with gemcitabine in patients with metastatic breast cancer have been reported.[88] All patients (529 subjects in both arms) included in the study had received anthracycline in the adjuvant setting and no prior treatment for metastatic disease. This study demonstrated significant improvement in response rates (40.8% vs. 22.1%, $p<0.0001$), median time to progression (5.2 vs. 2.9 mos., $p<0.0001$) and median overall survival (18.5 vs. 15.8 months, $p=0.018$) with the addition of gemcitabine to paclitaxel.

While these results are impressive, we propose to evaluate docetaxel/gemcitabine rather than paclitaxel/gemcitabine for several reasons. First of all, the superiority of the combination of gemcitabine with a taxane relative to the taxane alone justifies evaluation of a gemcitabine/taxane combination in the neoadjuvant setting. Second, when docetaxel has been administered prior to gemcitabine in multiple adenocarcinoma cell lines, synergistic effects were observed. Additionally, the combination of gemcitabine and docetaxel has been evaluated in ten Phase II trials conducted in a total of 408 evaluable patients with metastatic breast cancer.[90-99] Studies have shown activity in patients with multiple lines of previous therapy, including patients treated with prior taxanes. Overall responses have ranged from 36% to 79%. The major side effects of the combination have been neutropenia, anemia, asthenia, neuropathy, nausea, mucositis, and neutropenic fevers (see Table 3).

TABLE 3. Data for docetaxel and gemcitabine combinations in Phase II studies

Author	Weekly *Gemcitabine* Dosing (mg/m^2)	*Docetaxel* Dosing (mg/m^2)	Prior Chemo Regimens MBC	# Pts Eval	Percent CR/PR/ORR	Major Toxicities ≥ 5% grade 3 or 4
Fountzilas[90]	1000 D 1, 8	75 D 1 q 3 wks	Prior adj (19 pts) Prior A (36 pts)	40	8/28/36	Neutropenia, anemia, fatigue, N/V, asthenia, infection
Mavroudis[91]	900 D 1, 8	100 D 8 q 3 wks	Prior T (25 pts) A resistant (all pts)	52	14/40/54	Infection with neutropenia, anemia, platelets
	G-CSF 150 mcg/m^2 D 9-16					
Laufman[92]	800 D 1, 8, 15	100 D 1 q 4 wks	Prior A (33 pts) Prior C (37 pts)	39	5/74/79	Neutropenia, F/N, anemia, nausea, asthenia, infection, edema
Brandi[93]	1000 D 1, 8	80 D 8 q 3 wks	≥ 2 prior regimens (17 pts) 1 prior regimen (13 pts) Prior A (26 pts) Prior T (4 pts)	30	7/53/60	Neutropenia, anemia, nausea, LFTs, mucositis
Kornek[94]	1500 D 1, 15	50 D 1, 15 q 4 wks	1 prior regimen (14 pts) Refractory to A (10 pts)	51	11/50/61	Neutropenia, N/V, infection, neuropathy, use of G-CSF based on counts
Alexopoulos[95]	900 D 1, 8 If pt had SD or PD while on docetaxel	100 D 8 q 3 wks	Prior A 1st line and prior T 2nd line (all pts) 30 SD on Doc and 20 PD on Doc	50	6/40/46	Neutropenia, platelets, asthenia
Mavroudis[96]	1500 D 1	65 D 1 q 2 wks	Prior adj chemo (24 pts)	37	14/62/76	Neutropenia, F/N, anemia, platelets
Slee[97]	1000 D 1, 8	75 D 8 q 3 wks	Prior adj A therapy (10 pts) Prior therapy for MBC (16 pts)	26	8/54/62	Not reported
Garle[98]	2500 D 1	65 D 1 q 2 wks	Prior adj chemo (45%) Prior A therapy (33%)	48	17/54/71	Neutropenia, nausea
Lenz[99]	800 D 1, 8, 15	35 D 1, 8, 15 q 4wks	Prior A adj or neoadj therapy (100%)	35	ORR - 54	Neutropenia, platelets

MBC: metastatic breast cancer; CR: complete response; PR: partial response; ORR: overall response rate; adj: adjuvant; A: anthracycline; N/V: nausea/vomiting; T: taxane; F/N: febrile neutropenia; C: cyclophosphamide; LFT: liver function tests; SD: stable disease; PD: progressive disease; DOC: docetaxel

Finally, a Phase III trial in 305 patients with anthracycline pre-treated metastatic breast cancer has shown docetaxel/gemcitabine to have similar efficacy to docetaxel/capecitabine. The study is comparing the regimen of docetaxel 75 mg/m^2 Day 1 and gemcitabine 1000 mg/m^2 Days 1 and 8 every 21 days with docetaxel 75 mg/m^2 Day 1 and capecitabine 1250 mg/m^2 bid Days 1-14. Both arms demonstrated an overall response rate of 32% and a median progression-

free survival of 35 weeks. Overall the gemcitabine combination was associated with less grade 3/4 diarrhea (7 vs. 18%), less grade 3/4 mucositis (4 vs. 17%) and fewer drug-related discontinuations due to toxicity (13 vs. 28%). Grade 3 hand-foot syndrome was not seen in the gemcitabine cohort. Hematologic toxicity was greater with gemcitabine but only one patient developed grade 4 thrombocytopenia, and the incidence of febrile neutropenia was actually less in the gemcitabine arm (12 vs. 19%).[100]

04/01/11 2.4 **Rationale for evaluating the addition of bevacizumab to neoadjuvant chemotherapy**

See Section 2.5 for additional bevacizumab information provided at the time of Amendment #3.

Bevacizumab (rhuMAb VEGF), developed by Genentech, Inc., is a recombinant humanized monoclonal antibody against human VEGF. Results of ECOG E2100 have demonstrated substantial activity of bevacizumab added to paclitaxel in the front-line treatment of metastatic breast cancer. Similar benefits have been demonstrated with the addition of bevacizumab to combination chemotherapy in front-line treatment of metastatic colorectal cancer and lung cancer. These results suggest bevacizumab may be the most broadly effective anti-cancer therapy to enter clinical trials to date. It is imperative that the clinical research community evaluate this promising agent as quickly and efficiently as possible in adjuvant trials of these common malignancies since delays in demonstration of efficacy of a highly active agent in common malignancies would allow for a substantial number of potentially avoidable deaths to occur. However toxicities and costs of adjuvant therapies require that correlative science studies be conducted to understand mechanisms of action and resistance, and ultimately to define predictive factors for benefit from specific agents. Neoadjuvant trials provide a unique opportunity for early correlation between potential molecular predictive factors and outcomes such as overall clinical response and complete pathologic response. Given the promise of bevacizumab it is important to evaluate the agent in a large Phase III neoadjuvant trial incorporating correlative science studies on tumor specimens.

Recently, Miller et al reported results of ECOG E2100, a randomized Phase III trial comparing weekly paclitaxel with weekly paclitaxel and bevacizumab 10 mg/kg every 2 weeks in the front-line treatment of metastatic breast cancer. The large majority of the patients had HER-2 negative primary breast cancer. Results demonstrated an improvement in progression-free survival (primary endpoint) from 6.11 months to 11.4 months (HR=.51; 0.43-0.62, $p<0.0001$) an improvement in response rate from 13.8% to 29.9% ($p<0.0001$) with the addition of bevacizumab.[101]

Based on these results, we will evaluate the benefit of adding bevacizumab to the sequential docetaxel-based regimens being evaluated in NSABP B-40 by incorporating a 3 X 2 factorial design. Patients will be randomized to one of the 3 chemotherapy regimens with a secondary randomization to receive bevacizumab or no bevacizumab. Bevacizumab 15 mg/kg every 3 weeks will be administered with the first 6 cycles of chemotherapy in the neoadjuvant setting and for 10 doses in the postoperative setting.

Since the definitive adjuvant trial of bevacizumab being developed by ECOG is evaluating 6 months versus 12 months of therapy, it is important to employ a treatment schedule of bevacizumab in B-40 that will be interpretable regardless of the duration outcome in the ECOG study. If longer durations of bevacizumab appear to be more

effective than shorter durations, applicability of results from B-40 could be problematic if bevacizumab therapy was ended after 4 months, since safety data regarding post-operative resumption of bevacizumab following neoadjuvant chemotherapy combined with bevacizumab would not be available. Even if the ECOG study does not demonstrate a difference between 6 and 12 months of bevacizumab, uncertainty would remain about the efficacy of only 4 months of neoadjuvant bevacizumab on disease-free survival if bevacizumab is limited to the preoperative period in B-40. By administering 10 doses of bevacizumab post-operatively, B-40 patients randomized to bevacizumab will receive a total exposure of 12 months, which will be comparable to the longest treatment duration being evaluated in the ECOG trial.

Since E2100 evaluated bevacizumab with a taxane and bevacizumab will only be administered for the first half of the second regimen in the sequential program, reversal of the chemotherapy sequences will maximize the concurrent administration of bevacizumab with the docetaxel regimens in B-40. Additionally, since the cardiac safety of prolonged co-administration of bevacizumab and anthracyclines is still being defined, reversing the sequence of chemotherapy regimens will limit exposure of concurrent bevacizumab to the AC combination.

2.4.1 *Mechanism of action of bevacizumab*

Vascular endothelial growth factor (VEGF) is a critical regulator of both normal and pathologic angiogenesis.[102] It is a highly conserved homodimeric glycoprotein whose dominant isoform has a molecular weight of 45kD. The biologic activity of VEGF is mediated by binding to two receptors on the surface of endothelial cells, namely Flt-1 and KDR. Increased levels have been demonstrated in colon cancer and presumably represent an important factor needed to support the cancer through the growth of tumor vasculature.[103] Folkman and coworkers provide convincing evidence linking tumor growth and metastases with angiogenesis, and several investigators have demonstrated a correlation between vascular density in colorectal cancer and metastases, recurrence, and survival.[104-111] Inhibition of VEGF, either alone or in combination with chemotherapy, results in growth inhibition of a number of human tumor xenografts in the nude mouse model, including colorectal cancer cell lines, LS174T, HM7, and LSLiM6.[102,112]

2.4.2 *Phase I clinical experience with bevacizumab*

The dose and toxicities associated with bevacizumab were investigated in two phase I trials.[113,114] Study AVF0737g was an open-label dose-escalation study of single agent bevacizumab in patients with advanced cancers. Three patients in this trial experienced tumor-related hemorrhagic events (2 cases were considered serious). Linear pharmacokinetics were observed for doses of drug in excess of 1 mg/kg. The half-life of bevacizumab was found to be approximately 15 days.

Study AVF0761g evaluated bevacizumab in combination with 3 cytotoxic regimens including 5-FU + LV, carboplatin + paclitaxel, and doxorubicin in patients with advanced cancers. No patients in this study experienced serious hemorrhagic events, and the co-administration of bevacizumab with chemotherapy did not alter the pharmacokinetics of the cytotoxic agents. Neither study detected antibodies to bevacizumab.

2.4.3 *Bevacizumab improves tumor response rates in patients with breast cancer*

A Phase I/II study of bevacizumab monotherapy conducted in patients with previously treated metastatic breast cancer demonstrated an overall response rate of 9.3% with 16 % of the patients having stable disease or an ongoing response at 22 weeks. The data from this trial supported the initiation of trials combining bevacizumab with chemotherapy in metastatic breast cancer.[115]

The preliminary results of a Phase II study comparing neoadjuvant docetaxel with or without bevacizumab in patients with locally advanced breast cancer has been reported. Patients received neoadjuvant therapy with bevacizumab (10 mg/kg qo wk) and docetaxel (two 8-week cycles of 35 mg/m^2 x 6 with a two-week break) or docetaxel alone. Patients with disease response underwent definitive surgery and radiation therapy, followed by 4 cycles of adjuvant conventional doxorubicin and cyclophosphamide and tamoxifen (if ER/PR+). Treatment converted 84% of tumors to being surgically resectable. Statistically significant reduction in tumor volume and perfusion associated with the combination bevacizumab and docetaxel compared to docetaxel alone were also noted. The combination of docetaxel and bevacizumab was safe and well tolerated. At the time of the report, there had been no episodes of uncontrolled hypertension, proteinuria, thrombosis, or change in left ventricular function associated with bevacizumab or subsequent doxorubicin administration.[116]

In a second trial for locally advanced or inflammatory breast cancer, patients were treated with bevacizumab, doxorubicin, and docetaxel. The treatment program consisted of bevacizumab (15 mg/kg) alone for cycle one followed by 6 cycles of bevacizumab (15 mg/kg) with doxorubicin (50 mg/m^2) and docetaxel (75 mg/m^2) every 3 weeks. Following surgery and radiation therapy, bevacizumab was given for an additional 8 cycles with hormonal therapy in estrogen-positive patients. In this trial, the overall response rate was 67% (95% CI; 43%-85.4%). Grade 2 asymptomatic decrease in left ventricular ejection fraction (LVEF) was observed in 2 patients. The LVEF dysfunction occurred in both after 7 cycles of neoadjuvant therapy (total 300 mg/m^2 doxorubicin and 450 mg/m^2 docetaxel), surgery, and radiation therapy prior to starting cycle 8. One patient was taken off study therapy. Her LVEF normalized 6 months after discontinuing therapy and receiving an ACE inhibitor. The second patient had normalization of her LVEF within 3 weeks and remained on study. Other than these 2 patients, no patient had >15% absolute decrease in LVEF or a decrease in LVEF below the lower limits of normal, and no segmental abnormalities were noted on echocardiogram. Left ventricular dysfunction was asymptomatic and recovered in both cases.[117]

2.4.4 *Phase III trials with chemotherapy and bevacizumab in breast cancer*

A Phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with metastatic breast cancer previously treated with anthracyclines and taxanes has been reported. This study revealed significantly higher response rates with the combination therapy (19.8% vs. 9.1%; p=0.001) but did not meet its primary endpoint (time to tumor progression). Bevacizumab was well tolerated in this treatment population. No significant differences were found in

the incidence of diarrhea, hand-foot syndrome, thromboembolic events, or serious bleeding episodes between treatment groups.[118]

A subsequent Phase III trial led by the Eastern Cooperative Oncology Group (E2100) compared paclitaxel with or without bevacizumab as first line therapy for locally recurrent and metastatic breast cancer. The large majority of patients enrolled had HER2-negative tumors since administration of trastuzumab was not allowed in the trial. Women received paclitaxel (90 mg/m^2) weekly for 3 out of 4 weeks with or without bevacizumab at 10 mg/kg every 2 weeks. The primary endpoint was progression-free survival which was improved from 6.11 months to 11.4 months ($p<0.0001$). The overall response rate was also improved with bevacizumab and paclitaxel versus paclitaxel alone (29.9 % versus 13.8 %: $p<0.0001$.[101]

04/01/11 2.5 **Updated bevacizumab information provided at the time of Amendment #3**

A meta-analysis of 16 published, randomized controlled trials was performed to determine the overall risk of fatal adverse events associated with bevacizumab and chemotherapy compared to chemotherapy alone. A total of 10,217 patients with a variety of advanced solid tumors including colorectal, non-small cell lung, pancreatic, prostate, breast, and renal cell cancers were included in this analysis. The addition of bevacizumab was associated with an increased risk of fatal adverse events compared with chemotherapy alone (2.5% vs. 1.7% with a RR=1.46; p=0.01). The highest relative risk was observed in patients with prostate cancer (RR 3.85; 95% CI 1.58–9.37; incidence 4.4% vs. 1.1%), and lung cancer (RR 2.12; 95% CI 0.78–5.78; incidence 5.3% vs. 2.5%). The lowest relative risk was in renal cell carcinoma (RR 1.11; 95% CI 0.29–4.20; incidence 0.9% vs. 0.8%) and breast cancer. The incidence of fatal adverse events in the breast cancer trials was 0.9 in the chemotherapy plus bevacizumab cohorts and 1.3 in the chemotherapy control cohorts (RR=0.69) suggesting that different patient populations may be at different degrees of risk for fatal adverse events when bevacizumab is added to chemotherapy.[119]

On December 16, 2010, the United States Food and Drug Administration (FDA) announced that the agency was initiating a process to remove the indication for bevacizumab in metastatic breast cancer from the approved label. This decision was made following an updated review of the results of four randomized trials of bevacizumab (E2100, AVADO, RIBBON-1, RIBBON-2) in patients with metastatic breast cancer. The FDA concluded that "the drug does not prolong overall survival in breast cancer patients or provide a sufficient benefit in slowing disease progression to outweigh the significant risks to patients". The key efficacy data from the four trials in metastatic breast cancer are summarized in Table 4.

TABLE 4. Efficacy data from four trials using bevacizumab therapy to treat metastatic breast cancer

	Median PFS (months)	Hazard Ratio (bevacizumab vs. nil)	P-Value	Response Rate (%)	P-Value	Median OS (months)
E2100 (first-line)[120]						
Weekly paclitaxel	5.9	--	--	21.2	--	25.2
Weekly paclitaxel with bevacizumab	11.8	0.60	< 0.001	36.9	< 0.001	26.7
AVADO (first-line)[121]						
Docetaxel with placebo	8.2	--	--	46.4	--	31.9
Docetaxel with bevacizumab 7.5 mg/kg	9.0	0.86	0.12	55.2	0.07	30.8
Docetaxel with bevacizumab 15 mg/kg	10.1	0.77	0.006	64.1	< 0.001	30.2
RIBBON-1 (first-line)[122]						
Capecitabine with placebo	5.7	--	--	23.6	--	22.8
Capecitabine with bevacizumab	8.6	0.69	0.0002	35.4	0.0097	25.7
Anthra or taxane with placebo	8.0	--	--	37.9	--	NR
Anthra or taxane with bevacizumab	9.2	0.64	< 0.001	51.3	0.0054	27.5
RIBBON-2 (second-line)[123]						
Chemotherapy with placebo	5.1	--	--	29.6	--	16.4
Chemotherapy with bevacizumab	7.2	0.78	0.0072	39.5	0.019	18.0

The first studies to evaluate the potential of bevacizumab to improve the efficacy of adjuvant chemotherapy, NSABP C-08 and AVANT, were conducted in patients with colon cancer. NSABP C-08 was designed to evaluate the addition of bevacizumab to modified FOLFOX6 (infusional/bolus fluorouracil, leucovorin, and oxaliplatin) for the treatment of patients with stages II and III colon cancer.[124] The addition of bevacizumab to mFOLFOX6 did not result in an overall significant increase in DFS (HR 0.89; 95% CI 0.76–1.04; p=0.15). The point estimates for 3-year DFS were 74.4% for bevacizumab plus mFOLFOX6 and 75.5% for chemotherapy alone. The AVANT study evaluated bevacizumab in combination with either capecitabine plus oxaliplatin (XELOX) or fluorouracil/leucovorin with oxaliplatin (FOLFOX4) versus FOLFOX4 alone in patients with high-risk stage II or III colon cancer.[125] With a median follow-up of 48 months, bevacizumab did not prolong DFS or OS when added to FOLFOX or XELOX. The 3-year DFS rates were 73% for FOLFOX plus bevacizumab, 75% for XELOX plus bevacizumab, and 76% for chemotherapy alone. FOLFOX with bevacizumab was associated with a hazard ratio of 1.17 compared to FOLFOX alone (95% CI 0.98–1.39), and XELOX with bevacizumab was associated with a hazard ratio of 1.07 (95% CI 0.90–1.28) compared to FOLFOX alone, neither of which was statistically different from chemotherapy alone. The efficacy results numerically favored the chemotherapy-alone control arm. No benefit was apparent from the addition of bevacizumab to oxaliplatin/fluoropyrimidine-based chemotherapy in either trial. On the basis of these results, accrual to other adjuvant trials in colon cancer has been stopped.

The AVANT results have renewed concerns about possible accelerated tumor growth and distant metastases following discontinuation of prolonged antiangiogenic agents. Updated survival results of C-08 with a median follow-up of 4 years continue to show no evidence to support this hypothesis. Additionally, a pooled analysis of 5 placebo-controlled trials of bevacizumab conducted in patients with metastatic breast, renal, colon, and pancreatic cancers did not show evidence of a decreased time to

progression, increased mortality, or altered disease progression patterns after discontinuation of bevacizumab.[126] The median time from discontinuation as a result of adverse events to progression or death was 4 months (95% CI 3.4 to 4.6 months) in the bevacizumab group and 3.0 months (95% CI 2.6 to 3.8 months) in the placebo group (HR 0.93; 95% CI 0.79 to 1.10).

The results of the 3 large trials in the first-line therapy of metastatic breast cancer, and the RIBBON-2 study in the second-line setting, justify the completion of this trial.

2.6 Importance of this trial

This trial will further develop the role of primary chemotherapy for patients with operable breast cancer. Use of the neoadjuvant setting to test and compare new drug combinations correlating pCR with gene expression profiles and in vitro chemoresponse profiles represents a paradigm shift in the study of primary breast cancer. At the present time, hormone-receptor status and HER-2 status are the only available predictive markers on which breast cancer treatments can be based. By using genomics technology on samples of malignant tissue collected before treatment and assessing the in vitro response to chemotherapy on tumor biopsy material before treatment on neoadjuvant clinical trials, we hope to define genetic and in vitro chemoresponse profiles that ultimately can be used to tailor specific therapies for individual patients.

Bevacizumab has been shown to have activity in combination with anthracyclines, taxanes and capecitabine. It would be of clinical value to investigate the addition of bevacizumab to taxanes/anthracycline based regimens in patients with locally advanced breast cancer. These regimens have the potential to provide anti-tumor activity with an increase in pCR rate.

The NSABP has a long history of conducting clinical trials evaluating pre-operative or primary chemotherapy in the treatment of patients with operable breast cancer. The proposed trial represents the next logical step in the evolution of this treatment approach and builds on the results of clinical trials and correlative science studies performed by the NSABP as well as by other investigators.

3.0 STUDY AIMS

3.1 Primary aims

- To determine whether the combination of docetaxel/capecitabine→AC or docetaxel/gemcitabine→AC, with or without bevacizumab, will increase the rate of pathologic complete response in the breast (pCR breast) relative to docetaxel→AC with or without bevacizumab.

- To determine whether the addition of bevacizumab to the docetaxel/anthracycline-based regimens (docetaxel→AC, docetaxel/capecitabine→AC, and docetaxel/gemcitabine→AC) will increase the rate of pCR breast relative to the same docetaxel/anthracycline-based regimens without bevacizumab.

3.2 Secondary aims

- To determine whether the combination of docetaxel/capecitabine→AC or docetaxel/gemcitabine→AC, with or without bevacizumab, will increase the rate of pathologic complete response in both the breast and all post-therapy lymph nodes evaluated histologically (pCR breast and nodes) relative to docetaxel→AC with or without bevacizumab.

- To determine whether the addition of bevacizumab to the docetaxel/anthracycline-based regimens (docetaxel→AC, docetaxel/capecitabine→AC, and docetaxel/gemcitabine→AC) will increase the rate of pCR breast and nodes relative to the same docetaxel/anthracycline-based regimens without bevacizumab.

- To determine whether the addition of capecitabine or gemcitabine to docetaxel, with or without bevacizumab, will increase the rate of clinical overall response (cOR) of primary breast cancers relative to docetaxel alone with or without bevacizumab.

- To determine whether the addition of bevacizumab to the docetaxel/anthracycline-based regimens (docetaxel→AC, docetaxel/capecitabine→AC, and docetaxel/gemcitabine→AC) will increase the rate of cOR of primary breast cancers relative to the same docetaxel/anthracycline-based regimens without bevacizumab.

- To determine whether the addition of capecitabine or gemcitabine to docetaxel, with or without bevacizumab, will increase the rate of clinical complete response (cCR) of primary breast cancers relative to docetaxel alone with or without bevacizumab.

- To determine whether the addition of bevacizumab to the docetaxel/anthracycline-based regimens (docetaxel→AC, docetaxel/capecitabine→AC, and docetaxel/gemcitabine→AC) will increase the rate of cCR of primary breast cancers relative to the same docetaxel/anthracycline-based regimens without bevacizumab.

- To identify gene expression profiles that can predict pCR with each of the sequential docetaxel/anthracycline-based regimens with or without bevacizumab.

- To identify gene expression profiles that can predict cOR with docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine with or without bevacizumab.

- To test the accuracy of an in vitro chemoresponse assay (ChemoFx®, Precision Therapeutics, Inc.) as a predictor of pCR with each of the sequential docetaxel/anthracycline-based regimens when administered without bevacizumab.

- To test the accuracy of an in vitro chemoresponse assay (ChemoFx®, Precision Therapeutics, Inc.) as a predictor of cOR with docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine when administered without bevacizumab.

- To determine the impact of preoperative bevacizumab and sequential chemotherapy regimens and postoperative bevacizumab therapy on cardiac function.

- To determine the impact of bevacizumab on surgical complications.

- To determine the toxicity of the preoperative regimens and the toxicity of postoperative bevacizumab employed in this trial.

- To determine whether the addition of bevacizumab to the docetaxel/anthracycline-based regimens (docetaxel→AC, docetaxel/capecitabine→AC and docetaxel/gemcitabine→AC) will increase disease-free survival (DFS) relative to the same docetaxel/anthracycline-based regimens without bevacizumab.

4.0 STUDY ENDPOINTS

4.1 Primary endpoint

The primary endpoint is pathologic complete response of the primary tumor in the breast (pCR breast). This is defined as no histologic evidence of invasive tumor cells in the breast specimen removed at surgery.

4.2 Secondary endpoints

- pCR breast and nodes defined as no histologic evidence of invasive tumor cells in the breast specimen or the regional lymph nodes removed at surgery. Regional lymph nodes include axillary nodes (both sentinel and non-sentinel) and non-axillary sentinel nodes.

- cOR following docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine, with or without bevacizumab, as assessed by physical exam at the completion of the docetaxel-based portion of the chemotherapy program. If either cCR or clinical partial response (cPR) is identified at that time, the patient would be considered to have cOR following the docetaxel-based therapy, with or without bevacizumab (see Section 12.1.4 for definitions of cCR and cPR).
- cOR following the entire sequential chemotherapy program as assessed by physical exam at the completion of the sequential chemotherapy regimens. If either cCR or cPR is identified at that time, the patient would be considered to have a cOR following docetaxel-based therapy followed by AC, with or without bevacizumab.

- cCR following docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine, with or without bevacizumab, as assessed by physical exam at the completion of the sequential chemotherapy regimens.

- cCR following completion of the sequential chemotherapy program as assessed by physical exam at the completion of the sequential chemotherapy regimens.
- The percentage of cardiac events defined as NYHA Class III/IV congestive heart failure.

- The percentage of surgical complications (from mastectomy, lumpectomy, and axillary staging procedures) defined as:
  - wound dehiscence
  - infection
  - seroma
  - hematoma
- Toxicities, including cardiac events other than congestive heart failure, of chemotherapy alone, bevacizumab with chemotherapy, and bevacizumab alone.
- Events for the DFS endpoint include local recurrence following mastectomy, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, contralateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, colon carcinoma in situ, or lobular carcinoma in situ of the breast), and death from any cause prior to recurrence or second primary cancer.

5.0 PATIENT ELIGIBILITY AND INELIGIBILITY

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5.1 Patient selection guidelines

The guidelines in Section 5.1 will not be considered exclusion criteria; however, in addition to the formal eligibility/ineligibility criteria in Sections 5.2 and 5.3, investigators should consider each of these factors when selecting patients for B-40. Investigators should also consider all other relevant factors (medical and non-medical), as well as the risks and benefits of the study therapy when deciding if a patient is appropriate for B-40. These considerations should be weighed carefully, as they may make a patient an unsuitable candidate for B-40 and may increase risk to the patient.

- Patients with a life expectancy less than 10 years, excluding her diagnosis of breast cancer. (Comorbid conditions should be taken into consideration, but not the diagnosis of breast cancer.)

- Any patient who intends to have a mastectomy with immediate breast reconstruction using tissue expanders, regardless of the clinical response to neoadjuvant therapy, must be in agreement with not having expansion performed for 2 weeks prior to the first postoperative bevacizumab dose, during bevacizumab, and for at least 6 weeks following the last dose of bevacizumab. (See Section 9.9.2 for further details on breast reconstruction.)

- Women of reproductive potential must agree to use an effective non-hormonal method of contraception during therapy ***and for at least 3 months after completion of bevacizumab.***

- Psychiatric or addictive disorders or other conditions that, in the opinion of the investigator, would preclude the patient from meeting the study requirements.

5.2 Conditions for patient eligibility

Patients who satisfy all of the following conditions are the only patients who will be considered eligible for the study:

5.2.1 The patient must have consented to participate and must have signed and dated an appropriate IRB-approved consent form that conforms to federal and institutional guidelines *for the study treatment and submission of pre-entry core biopsy material* for correlative studies (see Section 16.1).

5.2.2 Patients must be female.

5.2.3 Patients must be 18 years of age or older.

5.2.4 Patients must have an ECOG performance status of 0 or 1 (see Appendix A).

5.2.5 The diagnosis of invasive adenocarcinoma of the breast ***must have been made by core needle biopsy***.

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5.2.6 The primary breast tumor must be palpable and measure ≥ 2.0 cm on physical exam.

5.2.7 All patients must have their left ventricular ejection fraction (LVEF) assessed by MUGA scan or echocardiogram within 3 months prior to study entry. The LVEF must be ≥ the lower limit of normal (LLN) for the cardiac imaging facility performing the study. *Note: If the cardiac imaging facility cannot provide a LLN, use 50% as the LLN value.*

Note: Since the pre-entry LVEF serves as the baseline for comparing subsequent LVEF assessments to determine if bevacizumab therapy can be continued, it is critical that this baseline study be an accurate assessment of the patient's LVEF. *If the baseline LVEF is > 75%, the investigator should have the study reviewed for accuracy prior to study entry.* Following study entry, the LVEF determination may be reviewed up until the time of the post-chemotherapy (preoperative) evaluation. Please note that if a more accurate value is obtained from the review of the baseline MUGA or echocardiogram, the correct value must be submitted to the NSABP before the post-chemotherapy (preoperative) MUGA or echocardiogram is performed or it cannot be used for managing postoperative bevacizumab.

5.2.8 All patients must have an EKG within 3 months prior to study entry.

5.2.9 At the time of randomization:

- Absolute neutrophil count (ANC) must be ≥ 1200/mm^3.
- Platelet count must be ≥ 100,000/mm^3.
- Hemoglobin must be ≥ 10 g/dL.
- There must be evidence of adequate hepatic function by these criteria:
  - Total bilirubin must be ≤ the ULN for the lab unless the patient has a grade 1 bilirubin elevation (> ULN to 1.5 x ULN) resulting from Gilbert's disease or similar syndrome due to slow conjugation of bilirubin; *and*
  - Alkaline phosphatase must be ≤ 2.5 x ULN for the lab (refer to Sections 5.2.10 and 5.2.11); *and*
  - AST must be ≤ 1.5 x ULN for the lab (refer to Section 5.2.11).
  - *Alkaline phosphatase and AST may not both be > the ULN.* For example, if the alkaline phosphatase is > the ULN but ≤ 2.5 x ULN, then the AST must be ≤ the ULN. If the AST is > the ULN but ≤ 1.5 x ULN, then the alkaline phosphatase must be ≤ ULN.

5.2.10 Patients with either skeletal pain or alkaline phosphatase that is > ULN but ≤ 2.5 x ULN are eligible for inclusion in the study if bone scans do not demonstrate metastatic disease. Suspicious findings on bone scan must be confirmed as benign by x-ray, MRI, or biopsy.

5.2.11 Patients with AST or alkaline phosphatase > ULN are eligible for inclusion in the study if liver imaging does not demonstrate metastatic disease and the requirements in criterion 5.2.9 are met.

5.2.12 The following criteria for evidence of adequate renal function must be met:

- Serum creatinine ≤ ULN for the lab.
- Calculated creatinine clearance must be > 50 mL/min (see Appendix C).

5.2.13 Urine protein/urine creatinine (UPC) ratio must be < 1.0 (see Appendix B).

5.2.14 Patient must be able to swallow oral medications.

5.3 **Conditions for patient ineligibility**

Patients with one or more of the following conditions will be ineligible for this study:

5.3.1 Tumor determined to be strongly HER2-positive by immunohistochemistry (3+) or by fluorescent in situ hybridization (positive for gene amplification).

5.3.2 Excisional or incisional biopsy for this primary breast tumor.

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5.3.3 Surgical axillary staging procedure prior to study entry. Exceptions: 1) FNA or core biopsy of an axillary node is permitted for any patient, and 2) although not recommended, a pre-neoadjuvant therapy sentinel lymph node biopsy for patients with *clinically negative axillary nodes is permitted*. (See Section 9.9.3 for post-neoadjuvant therapy axillary staging requirements.)

5.3.4 Tumors clinically staged as T_4.

5.3.5 Ipsilateral cN_{2b} or cN_3 disease. (Patients with cN_1 or cN_{2a} disease are eligible.)

5.3.6 Definitive clinical or radiologic evidence of metastatic disease.

5.3.7 Synchronous bilateral breast cancer (invasive or DCIS).

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5.3.8 Treatment including radiation therapy, chemotherapy, biotherapy, and/or hormonal therapy for the currently diagnosed breast cancer prior to study entry (see Section 9.11).

5.3.9 Any sex hormonal therapy, e.g., birth control pills, ovarian hormonal replacement therapy, etc. (These patients are eligible if this therapy is discontinued prior to randomization. See Section 9.12.)

5.3.10 Therapy with any hormonal agent such as raloxifene, tamoxifen, or other selective estrogen receptor modulator (SERM), either for osteoporosis or breast cancer prevention. (Patients are eligible only if these medications are discontinued prior to randomization.)

5.3.11 Prior history of breast cancer, including DCIS. (Patients with a history of LCIS are eligible.)

5.3.12 Prior therapy with anthracyclines, taxanes, capecitabine, 5-FU, gemcitabine, or bevacizumab for any malignancy.

5.3.13 Other malignancies unless the patient is considered to be disease-free for 5 or more years prior to randomization and is deemed by her physician to be at low risk for recurrence. Patients with the following cancers are eligible if diagnosed and treated within the past 5 years: carcinoma in situ of the cervix, carcinoma in

situ of the colon, melanoma in situ, and basal cell and squamous cell carcinoma of the skin.

5.3.14 Cardiac disease that would preclude the use of anthracyclines. This includes:
- angina pectoris that requires the use of anti-anginal medication;
- history of documented congestive heart failure;
- serious cardiac arrhythmia requiring medication;
- severe conduction abnormality;
- valvular disease with documented cardiac function compromise; and
- uncontrolled hypertension defined as BP > 150/90 on antihypertensive therapy. (Patients with hypertension that is well controlled on medication are eligible.)

5.3.15 History of myocardial infarction documented by elevated cardiac enzymes or persistent regional wall abnormalities on assessment of LV function.

5.3.16 History of TIA or CVA.

5.3.17 History of other arterial thrombotic event within 12 months before study entry.

5.3.18 Symptomatic peripheral vascular disease.

5.3.19 Any significant non-traumatic bleeding within 6 months before study entry.

5.3.20 Serious or non-healing wound, skin ulcers, or incompletely healed bone fracture.

5.3.21 Gastroduodenal ulcer(s) determined by endoscopy to be active.

5.3.22 Invasive procedures defined as follows:
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to planned start of study therapy. (Note: Placement of a vascular access device is not considered a major surgical procedure. See Sections 9.2, 9.4, and 9.6 for instructions regarding initiation of therapy after device placement.)
- Anticipation of need for major surgical procedures (other than the required breast surgery) during the course of the study.

5.3.23 Known bleeding diathesis or coagulopathy. (Patients on warfarin with an in-range INR [usually between 2 and 3] are eligible.)

5.3.24 Sensory/motor neuropathy ≥ grade 2, as defined by the NCI's Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE v3.0).

5.3.25 Other non-malignant systemic disease (cardiovascular, renal, hepatic, etc.) that would preclude treatment with any of the treatment regimens or would prevent required follow-up.

5.3.26 Conditions that would prohibit administration of corticosteroids.

5.3.27 History of severe hypersensitivity reaction to drugs formulated with polysorbate 80.

5.3.28 Administration of any investigational agents within 30 days before study entry.

5.3.29 Pregnancy or lactation at the time of proposed randomization.

6.0 CARDIAC SAFETY MONITORING

6.1 Timing of LVEF assessments

LVEF assessments using the same method (MUGA scan or echocardiogram) are required as follows:

- For **all patients**:
  - At baseline (prior to study entry)
  - 3-4 weeks after the last chemotherapy cycle (before surgery)
- For **patients in Groups 1B, 2B, and 3B**:
  - If the LVEF assessment performed following chemotherapy (before surgery) did not meet criteria to administer postoperative bevacizumab (see Section 11.2 [Table 28]), the LVEF assessment must be repeated 3-5 weeks following surgery. If the postoperative LVEF assessment met criteria to initiate bevacizumab, an additional LVEF assessment must be performed after 4 postoperative doses of bevacizumab.
  - At 18 months following study entry for patients who received any bevacizumab (before or after surgery).

6.2 Important LVEF assessment guidelines

- **All LVEF assessments must be performed by the same method**, either MUGA scan or echocardiogram, that was performed at baseline (before study entry).

- Investigators are strongly urged to schedule the LVEF assessment at the same cardiac imaging facility where the patient's baseline LVEF assessment was done. *Note: If the cardiac imaging facility cannot provide a LLN, use 50% as the LLN value.*

- **Submission of the LVEF report form (Form LVA) with the LVEF assessment (MUGA scan or echocardiogram) report attached is required**. Please submit all LVEF evaluation forms within 4 weeks after the LVEF assessment to the NSABP Biostatistical Center (see the B-40 section of the NSABP Web site for submission instructions). For all patients, regardless of treatment assignment, Form LVA must be submitted for every MUGA scan or echocardiogram done for any reason until 2 years following study entry.

- LVEF assessment and submission of Form LVA with the MUGA scan or echocardiogram report continue to be required even if the patient has had any of the following:
  - discontinuation of study therapy
  - congestive heart failure (CHF)
  - breast cancer recurrence
  - second primary cancer

6.3 Reporting cardiac events

- Cardiac events will be graded utilizing CTCAE v3.0.
- Left ventricular systolic dysfunction and left ventricular diastolic dysfunction ≥ grade 3 requires expedited adverse event reporting via AdEERS (see Section 14.2.2). Also submit Form LVA and the MUGA scan or echocardiogram report, if applicable. (See Section 14.0 for additional adverse event reporting requirements.)
- Other cardiac events will be reported according to routine and expedited reporting instructions in Section 14.0.

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6.4 Reporting cardiac history

Cardiac history and risk factors will be collected for all patients at baseline.

7.0 REQUIRED ENTRY AND FOLLOW-UP STUDIES

TABLE 5. Studies required for study entry and during preoperative therapy

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Required studies[a]	**Prior to study entry** (within 4 wks unless indicated otherwise)	**Within 72 hrs of Day 1 of Each Pre-op Therapy Cycle** (beginning with Cycle 2)	**Between chemotherapy regimens**	**Prior to surgery** 3-4 wks after last chemotherapy dose
History & physical exam[b]	X (within 3 months)	X		X
Height (baseline only) and weight	X	X		
BP & anti-HTN meds assessment	X	X		
Cardiac history	X			
Tumor assessment by physical exam[c]	X	X (recommended)	X	X
Measurement of target lesion(s)	X		X[d]	X[d,e]
Adverse event assessment		X		X[f]
CBC/differential/platelet count	X	X		X
Serum creatinine	X	X (before cycles 3, 5, and 7)		X
Calculated creatinine clearance	X	X (only Groups 2A and 2B)[g]		
Urine protein/creatinine (UPC) ratio[h]	X	X (before cycles 3 and 5; only Groups 1B, 2B, and 3B)[i]		
Bilirubin/AST/Alkaline phosphatase	X	X (before cycles 3, 5, and 7)		X
Serum βhCG (women of childbearing potential)	X (within 2 weeks)			
Chest CT or chest x-ray (PA and lateral)[j]	X (within 3 months)			
Liver imaging[j]	X[k]			
Bone scan[j]	X[l]			
MUGA or echocardiogram[m]	X (within 3 months)[n]			X
EKG	X (within 3 months)			
Bilateral breast imaging	X[o,p]			X (ipsilateral)[o]
Ultrasound of ipsilateral axilla	X[q] (before or after entry but before therapy begins)			
Marking of primary tumor site	X[r] (before or after entry but before therapy begins)			
Core biopsy tissue for submission	X (primary tumor)[s]			
Blood/serum collection[t]	X (after entry - before therapy begins)	X (serum only; before cycle 3 of docetaxel-based regimen)		X (serum only)

a H&P, bloodwork, x-rays, scans, and other testing may be performed more frequently at the discretion of the investigator.
b Complete H&P including performance status (see Appendix A) prior to study entry; targeted assessment at remaining timepoints (physician or other healthcare professional).
c Includes assessment of the primary breast tumor and palpable regional lymph nodes.
d Required tumor measurement for evaluation of clinical response.
e Surgery should be performed after recovery from chemotherapy, clinical tumor assessment, and preop LVEF assessment. (See Section 9.9.2 regarding timing of surgery for Groups 1B, 2B, & 3B.)
f Final AE assessment for ***preoperative therapy*** should be performed 3-4 weeks after completion of preoperative therapy (before surgery). See Table 31 (footnote c) and Table 32 (footnote b) for expedited reporting requirements for AEs that occur > 30 days.
g Only if serum creatinine is ≥ grade 2 at any time during treatment with capecitabine.
h See Appendix B for calculation of UPC ratio.
i For patients who permanently discontinue bevacizumab prior to surgery: If UPC ratio was ≥ 1.0 at last assessment during bevacizumab, repeat UPC ratio every 3 months for 12 months after the last dose of bevacizumab. If the UPC ratio improves to < 1.0 before 12 months, no additional UPC ratios are required.
j PET scan is permitted as an alternative to CT scans of the chest and abdomen and bone scan to rule out metastatic disease. Canadian investigators: Refer to Section 16.2 for regulatory requirements related to PET scan.
k Liver imaging is required if alkaline phosphatase or AST is > ULN.
l Bone scan is required if alkaline phosphatase is > ULN or if the patient has unexplained bone pain.
m ***The same method of LVEF assessment (MUGA scan or echocardiogram) must be performed at each time point.*** If possible, all LVEF assessments should be performed at the same facility.
n If LVEF is > 75%, the investigator should consider having the LVEF assessment result reviewed prior to study entry (see Section 5.2.7).
o MRI is permitted as a substitute for mammogram (ultrasound is not) at baseline and before surgery; all other assessments must be performed by mammogram.
p Ipsilateral within 6 months; contralateral within 12 months.
q Strongly recommended to assist in planning the axillary staging procedure. If abnormal, FNA is recommended (see Section 9.9.3).
r Clip placement, tattoo, or other method of marking the primary tumor site should be done before therapy begins (see Section 9.9.1).
s ***Study entry requirement for all patients***; includes cores in formalin, RNAlater, and shipping medium for PTI; ship to Precision Therapeutics, Inc. (see Sections 8.1 and 8.6.)
t For patients who have consented to blood/serum submission (see Section 8.8).

TABLE 6. Studies required after surgery and during follow-up (all patients); during postoperative bevacizumab (Groups 1B, 2B, and 3B) *02/15/08*

Required studies[a]	**All patients**			**Patients in Groups 1B, 2B, and 3B**			**All patients**	
				If postop bevacizumab is anticipated	If postop bevacizumab is re-initiated[b]			
	3–5 weeks postop	**At 9 months** following study entry	**At 12 months** following study entry	**Before re-initiating bevacizumab**	**During Postop Bevacizumab** Every 6 weeks	**At 18 months** following study entry	**Years 2 through 5** Following study entry	**Years 6 through 10** Following study entry
Updated history & physical exam[c]	X	X[d]	X	X[e]	X		X (every 6 months)	X (every 12 months)
Weight				X	X			
BP & anti-HTN meds assessment				X	X[f]			
Adverse event assessment	X[g]	X[d,g]	X[g]		X[h]			
CBC/differential/platelet count				X				
Urine protein/creatinine (UPC) ratio[i]				X	X[j]		X[j]	
Serum creatinine				X				
Bilirubin/AST/Alkaline phosphatase				X				
MUGA or echocardiogram[k]				X (3-5 weeks postop)[l]	X[m]	X[n]		
Mammogram[o]			X[p]				X (every 12 months)	X (every 12 months)
Tumor block collection	X[q]							

a H&P, bloodwork, x-rays, scans, and other testing may be performed more frequently at the discretion of the investigator.

b For patients in Groups 1B, 2B, and 3B who ***will NOT re-initiate bevacizumab postoperatively,*** bevacizumab-related studies are not required.

c Targeted/updated assessment by physician or other healthcare professional.

d At this time point, it is preferred that patients have an exam that includes assessment of adverse events related to surgery. However, if an exam is not possible, the AE assessment at this time point can be based on notes and other source documentation from other physicians (for example, surgeon, radiation oncologist, or primary care physician) and by telephone contact with the patient.

e If initiation of postoperative bevacizumab is delayed > 6 weeks after the most recent postoperative exam, a repeat assessment should be performed within 10 days of initiation of postoperative bevacizumab.

f BP required ***every 3 weeks*** prior to each postoperative bevacizumab dose.

g Assessment of adverse events related to surgery.

h Final AE assessment 30 days after last bevacizumab dose; assessment may be based on office notes from other physician visits or telephone contact with the patient.

i See Appendix B for UPC ratio instructions.

j ***For Groups 1B, 2B, and 3B patients who had a UPC ratio ≥ 1.0 at the last assessment during bevacizumab***, repeat every 3 months for 12 months after the last dose of bevacizumab. If the UPC ratio improves to < 1.0 before 12 months, no additional UPC ratios are required.

k ***The same method of LVEF assessment (MUGA scan or echocardiogram) must be performed at each time point.*** If possible, all LVEF assessments should be performed at the same facility.

l LVEF assessment must be performed at this time point **ONLY** if the LVEF obtained after chemotherapy (before surgery) did not meet requirements for initiating postoperative bevacizumab (see Section 11.2 [Table 28]).

m LVEF assessment must be performed ***after 4 postoperative doses*** of bevacizumab **ONLY** if an LVEF assessment was required at 3-5 weeks after surgery (because the assessment performed following chemotherapy [before surgery] did not meet criteria to administer postoperative bevacizumab) *and* the postop assessment met LVEF criteria to initiate postoperative bevacizumab (see Section 11.2 [Table 28]).

n Required for all Group 1B, 2B, and 3B patients who have received any bevacizumab, even if the patient has not received any postoperative bevacizumab. For patients whose last dose of bevacizumab was administered < 4 weeks prior to 18 months following study entry, the 18-month LVEF assessment should be delayed until 4 weeks after the last dose.

o MRI is not permitted as a substitute for mammogram.

p Ipsilateral or contralateral mammogram is required every 12 months; specific timing of the first postoperative mammogram depends on the timing of the pre-entry and pre-op mammograms.

q Blocks from tumor and positive node (if gross residual disease ≥ 1 cm) are ***required*** (see Section 8.7). ***Reminder: Histopathology findings must be submitted on Form RCB for ALL B-40 patients enrolled after local IRB approval of Amendment #2 for use by the NSABP Biostatistical Center in calculating the RCB index (see Section 8.4).***

8.0 PATHOLOGY AND CORRELATIVE STUDIES

02/15/08

8.1 Summary of pathology-related requirements (also see Sections 8.4 and 8.6-8.8)

NSABP B-40 requires the collection and submission of tumor and blood/serum samples and histopathology findings.

- A total of 4 core biopsy samples will be collected from the primary tumor prior to randomization. ***These tumor samples are required for B-40 study entry.***
  - One core biopsy sample will be collected in RNAlater
  - One core biopsy sample will be collected in formalin
  - Two core biopsy samples will be collected in tissue culture transport medium for Precision Therapeutics, Inc. (referred to as shipping medium for PTI throughout the protocol)
- Representative block(s) of gross residual disease ≥ 1 cm must be submitted to the NSABP following definitive surgery. See Section 8.7 for specific requirements. ***These samples are required.***
- Histopathology findings to calculate residual cancer burden (RCB) must be provided by the pathologist who reviewed the case. See Section 8.4.
- Three serial blood/serum collections are required elements of the study, ***unless a patient refuses to have these specimens collected and submitted to the NSABP***. Such patients may still participate in the trial. Specimens are to be collected at the following times:
  - At baseline before chemotherapy (blood and serum)
  - Before the 3rd cycle of T (docetaxel), TCape (docetaxel/capecitabine), or TG (docetaxel/gemcitabine) (serum)
  - At the completion of chemotherapy but before surgery (serum)

Non-submission of required tissue or blood/serum samples will be a protocol violation (unless, for the blood/serum collections, a patient has not consented to collection, use, and storage of her samples).

NOTE: The tissue and blood/serum samples collected in this study will be used for B-40 studies as described in Section 8.2. DNA extracted from blood specimens will be used specifically to examine the role of gene polymorphism on response to chemotherapy and toxicity. ***The specimens procured, including DNA samples, will not be used for hereditary genetic studies involving genes conferring susceptibility to cancer or other diseases.*** Central review of a patient's tumor pathology and markers examined will not be reported to the patient or her physician and will not have any bearing on her treatment.

All pretreatment core biopsy samples are to be submitted in a single shipping kit to Precision Therapeutics, Inc. (PTI) (refer to Information Resources, page v). Tissue samples in RNAlater and formalin are subsequently forwarded to the NSABP Division of Pathology. All biopsy samples will be identified by the PTI Control Number (a unique coded number provided by the NSABP Biostatistical Center).

Submitted slides and blocks from the definitive surgery are initially shipped to and logged into the database at the NSABP Biostatistical Center (refer to Information Resources, page v). These samples are then stripped of patient identifiers except NSABP Patient ID numbers and forwarded to the NSABP Division of Pathology where they are assigned a code number for further processing and study. Tissue microarrays of 1 mm cores will be constructed from collected blocks from residual tumors.

Blood/serum samples are to be submitted to the NSABP Serum Bank at Baylor where they are logged into the database and assigned a Serum Bank number. Blood/serum samples are stripped of patient identifiers, except the NSABP Patient ID numbers, and are processed and stored.

TABLE 7. Summary of B-40 pathology-related requirements *02/15/08*

	Pre-entry	After study entry before therapy begins	Prior to cycle 3 of T, TCape, or TG regimen	At least 3 weeks following last chemotherapy dose (before surgery)	At the time of definitive surgery	
					No gross residual disease or gross residual disease < 1 cm	Gross residual disease ≥ 1 cm
Core biopsy tissue in RNAlater	1 core[a]	No	No	No	No	No
Core biopsy tissue in formalin	1 core[a]	No	No	No	No	No
Core biopsy tissue in shipping medium for PTI	2 cores[a]	No	No	No	No	No
Paraffin blocks	No	No	No	No	No	Representative blocks of residual tumor and the node with the largest focus of metastasis[b]
Pathology information needed for RCB index	No	No	No	No	Yes[c]	Yes[c]
Diagnostic H & E slides	No	No	No	No	No	No
Blood/serum	No	Yes[d]	Yes[d,e]	Yes[d,e]	No	No

a Biopsy tissue must be collected before study entry. All cores must be from the primary tumor and will be submitted in the same shipping kit. (For core biopsy specimen procurement, follow instructions provided with the Precision Therapeutics, Inc. specimen collection kit or the B-40 Pathology Instructions.*)

b Alternative submission – 2 mm core sampling of an existing tumor block plus 20-30 unstained sections (mounted on charged slides) from both tumor and positive node.

c Histopathology findings must be submitted for ALL B-40 patients enrolled after local IRB approval of Amendment #2 for use by the NSABP Biostatistical Center in calculating RCB index. See Section 8.4.

d For patients who have consented to blood/serum collection.

e Only serum.

**** PLEASE NOTE: Refer to the Members' Area of the NSABP Web site for the B-40 Pathology Instructions. (CTSU investigators should refer to Section 3.0 in Appendix F and to the B-40 Web page located on the CTSU Registered Member Web site for the B-40 Pathology Instructions.)***

8.2 Scientific and clinical rationale for pathology and correlative science studies

8.2.1 *Predictive markers – needs and barriers to development*

In an ideal situation, physicians would base the treatment of patients on individual risk prediction using precise prognostic factors, and select specific treatments based on accurate predictive markers for therapeutic response. Results from NSABP Protocol B-18 and other published studies clearly suggest that only a subset of patients with early breast cancer derive benefit from a specific chemotherapy regimen.

Data from NSABP B-11, B-15, and B-20 suggest that the response to chemotherapy may be determined by molecular changes in the tumor cells.[128-130] Development of a panel of molecular markers that would predict complete response to specific chemotherapeutic agents would improve the overall effectiveness of adjuvant therapy by tailoring treatments for individuals based on responsiveness of their disease to specific regimens.

While there have been advances in development of prognostic markers, little progress has been made in defining predictive markers for response to specific chemotherapy regimens, or response to chemotherapy in general. There are two main reasons for this lack of progress in developing these markers. First, discovery tools that could be applied to clinical specimens initially were limited or too expensive. This problem has been largely resolved with the development of gene expression microarrays that allow interrogation of the entire human transcriptome at once.[131] However, a more serious problem remains unresolved, which is the limitation in statistical power inherent in utilizing materials from adjuvant trials to study predictive markers. For studies addressing predictive markers (marker-by-treatment interaction), as many as four times the event rate is required to have the same statistical power as is needed in addressing treatment effect.[132] For example, in a hypothetical situation in which 1300 patients are randomized equally to two adjuvant treatment arms with the expected total number of events being 425, the power to detect a hazard ratio of 1.5 between the two treatments would be 99%. With a hypothetical marker prevalence of 23% (similar to HER2), and assuming all tumor blocks would be available for assay, the power to detect a hazard ratio of 1.5 between marker-negative versus marker-positive cohorts (thus, this marker being a prognostic factor) would be reduced to 73%. For detection of marker-by treatment interaction, the power to detect a hazard ratio of 1.5 would be decreased to 47%. Therefore, studies utilizing specimens procured through Phase III adjuvant treatment trials are generally seriously underpowered to address marker-by-treatment interactions.

8.2.2 *Neoadjuvant setting provides solutions*

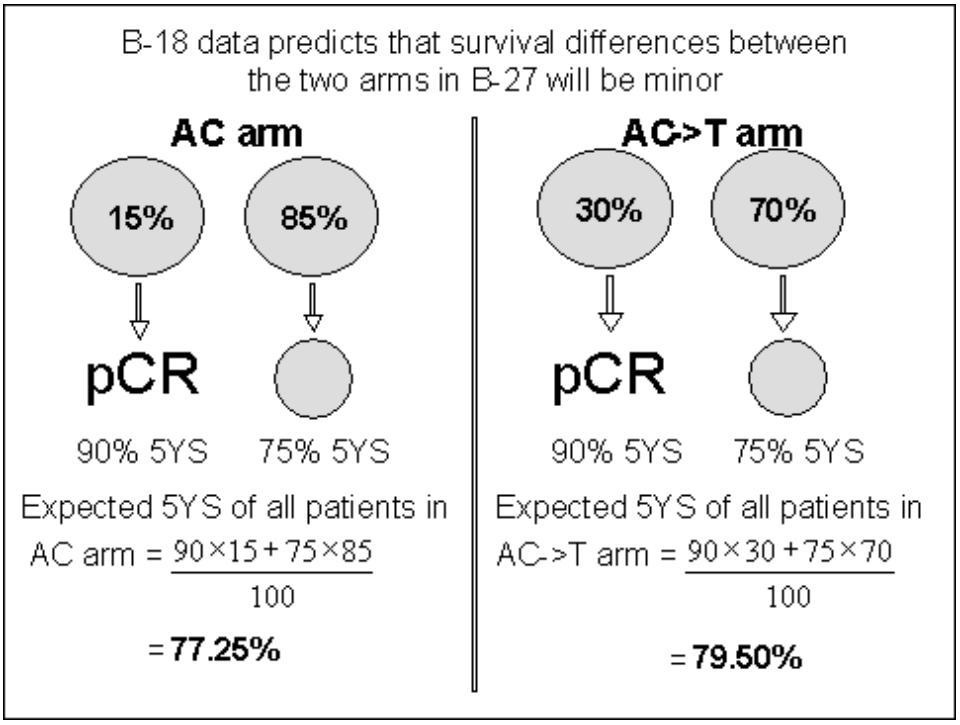
Neoadjuvant studies provide a unique setting in which molecular measurement in the tumor tissue before therapy can be correlated with in-situ response of tumor to chemotherapy. The most objective measurement of tumor response in neoadjuvant trials is pathologic complete response (pCR). Previous studies including NSABP Protocols B-18 and B-27 have demonstrated the value of pCR as a surrogate marker for clinical outcome measures.[36,133]

There are two major advantages in using pCR as a surrogate endpoint for marker analysis. First, long-term clinical follow-up is not required. The second reason, which is not widely recognized, is statistical. When using materials collected from an adjuvant trial, marker-by-treatment analysis is usually severely underpowered as described above. In the neoadjuvant setting, direct correlation with pCR rather than serial follow-up data, provides much greater statistical power for marker studies.

8.2.3 ***Justification for not using a survival endpoint as primary endpoint***

In NSABP B-27, the doxorubicin/cyclophosphamide (AC) followed by docetaxel (T) arm had twice the rate of pCR compared to the AC arm.[36] While this result is exciting, it does not appear to correlate well with the small absolute benefit demonstrated in adjuvant trials such as NSABP B-28 for similar sequential taxane regimens. However, a simple mathematical calculation using survival estimates from cohorts in NSABP B-18 illustrates that doubling of the pCR rate may not translate into a large increase in the survival rate.

Figure 2. B-18 data prediction for survival



This dichotomy between adjuvant and neoadjuvant clinical trial data is most likely due to the fact that the average survival of non-pCR patients is still fairly good due to the contamination of this group with inherently good prognosis patients such as those with ER-positive tumors with a low proliferation rate which are known to have a low pCR rate. More recently, in the retrospective analysis of NSABP B-20 cases, we found that low risk groups as defined by the OncotypeDx assay gain minimum benefit from chemotherapy in support of this notion.[134] Ideally, patients with such a favorable prognosis who do not tend to

completely respond to chemotherapy should be left out of neoadjuvant studies. However, at present they do not confound our aim of evaluating predictive markers in this study even if they are included.

In summary, continued pursuit of survival endpoints in neoadjuvant trials obviates the inherent advantages of this neoadjuvant clinical trial model now that correlation of pCR with favorable long-term outcome has been established. For this reason, coupled with the clinical justifications outlined in Section 2.2.5, we will use pCR as the primary endpoint.

8.2.4 *Hypotheses to be tested*

- We hypothesize that we can use gene expression profiling techniques such as the Affymetrix GeneChip or RT-PCR assays to identify a set of genes that correlate with pCR to each treatment regimen used in this study.

- We hypothesize that an in vitro chemoresponse assay (ChemoFx® assay) can be used to predict tumor response to each regimen. The dose-response curves produced by the ChemoFx® assay from core biopsies before treatment will enable the identification of patients who will achieve a clinical response after treatment with T, TCape, TG (without bevacizumab). Further, for each arm of the trial, pCR can be predicted utilizing the dose-response curves; one for T, TCape, or TG treatment, and a second for AC (all without bevacizumab) depending on the patient's regimen. Further exploratory analysis will be conducted for subgroups with bevacizumab, for example, T and TBev, TCape and TCapeBev, TG and TGBev, and AC and ACBev to quantify how the prediction is or is not affected by addition of bevacizumab.

- We hypothesize that there are serum proteins that can be used to predict pCR at earlier cycles of treatment. A proteomics approach will be used to identify such markers.

- We hypothesize that polymorphisms in specific genes may influence tumor response and toxicity. We will perform a focused survey of candidate genes as well as a general survey of all genes in order to identify such polymorphic markers.

8.2.5 *Rationale for each marker, technical feasibility and preliminary data*

Response to chemotherapy is thought to be predicated on a combination of complex factors such as the anticancer effectiveness of the drug (drug factors), tumor cell sensitivity or resistance, which includes the tumor's expression of drug targets and other proteins such as p-glycoprotein drug (tumor factors), physical delivery of the drug to the tumor as well as the body's ability to effectively metabolize and clear the drug (host factors).[135] Very few tools are available to the clinician to incorporate tumor factors or host factors into the choice of chemotherapy and dosage regimen, even though it is hypothesized that these factors dictate a major part of a patient's response to chemotherapy, future health, and survival.

In this study, we will examine tumor molecular characteristics, cellular response to chemotherapy in vitro, and host characteristics that may influence chemotherapy response. The rationale for each study component and currently available data in support of such studies are described in this section. Since there is rapid progress in the field of genomics, especially in methodological aspects, the actual conduct of many of the listed molecular studies will be based upon funding and available technologies at the conclusion of accrual. Any unused materials will be stored permanently for future studies related to the study aims of the B-40 protocol – this will allow us to further develop/enhance predictive tests using improved technologies that will be developed in the future.

- Gene expression analysis – available data from other published microarray studies in support of the proposed gene expression profiling study

  There are two studies that have used microarray-based gene expression analysis to identify predictive markers for pCR with pre-operative chemotherapy. Both are small exploratory studies, but they suggest that the approach we are proposing may provide valuable information.

  The first study was by Chang et al from the Baylor College of Medicine Breast Center.[136] Using the Affymetrix GeneChip, the investigators examined a handful of cases treated preoperatively with docetaxel. Due to the small number of cases, they attempted to identify genes that correlated with the degree of pathologic response rather than pCR. Differential patterns of expression of 92 genes correlated with docetaxel response (p=0.001). In leave-one-out cross validation analysis, 10 of 11 sensitive tumors (90% specificity) and 11 of 13 resistant tumors (85% sensitivity) were correctly classified, with an accuracy of 88%. This 92-gene predictor had positive and negative predictive values of 92% and 83%, respectively.

  Ayers et al from MD Anderson recently reported their analysis of 45 cases treated with pre-operative paclitaxel (+/- trastuzumab) plus the FAC regimen.[2] Using FNA specimens procured in RNAlater, they were able to identify a set of genes that correlated with pCR in the initial training set of 24 cases and were able to validate them using an additional 21 cases. The predictive value of the gene expression pattern was 75% for all 21 patients and 100% when only those not getting trastuzumab were considered. From this study, the investigators identified Tau protein as a candidate predictive marker for response to the paclitaxel-based regimen. An immunohistochemistry assay was developed for tissue in paraffin blocks.[137] We will utilize specimens collected in the B-40 trial to validate Tau as a predictor of response to our taxane regimen. These two studies, although having a small sample size, provide solid support for the proposed correlative science studies for this trial.

- NSABP experiences from the NSABP Foundation Research Program GET Trial (FB-GE-001)

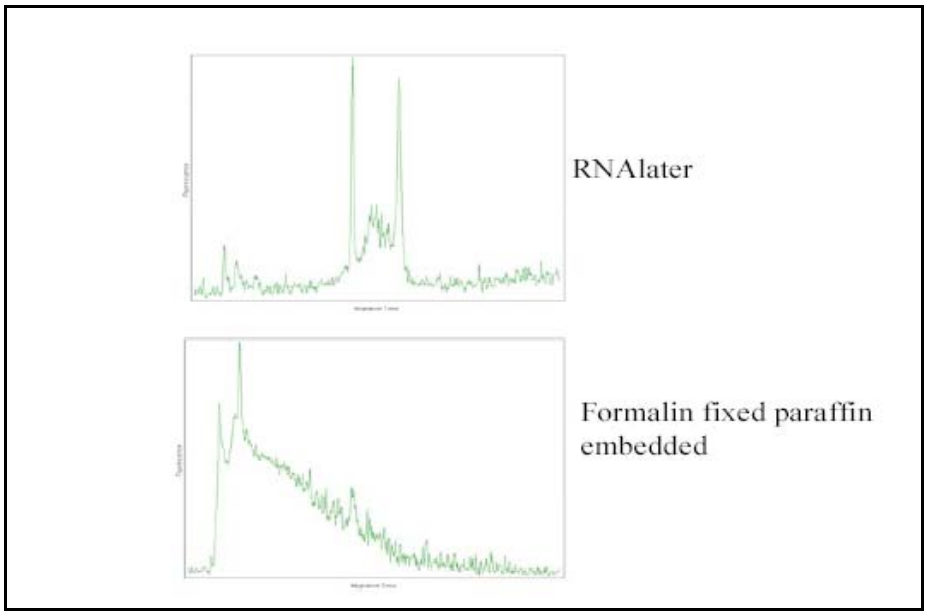
  Two critical aspects need to be satisfied before launching a large scale clinical study such as this one. First, a specimen procurement method to allow easy procurement at the multiple sites needs to be established.

  The ideal method of tissue procurement and long-term storage is snap-freezing in liquid nitrogen. However, the logistical limitations of a multi-center trial require a more pragmatic approach for tissue procurement. We have developed and tested a simple tissue procurement kit using RNAlater (Ambion). RNAlater is a non-toxic, non-flammable, high-salt solution that allows procurement of tissue at room temperature. It has been shown that RNA is stable without degradation for a week in RNAlater, even when tissue is stored at room temperature.[138,139] Two studies have demonstrated the value of RNAlater in preserving good quality RNA in core biopsy and FNA specimens of breast cancer.[140,141] The median yield of total RNA from RNAlater-fixed single 14-gauge core biopsy specimens was 1.34 and 2.0 micrograms in studies performed at the Lombardi Cancer Center and MD Anderson Cancer Center, respectively.[140,141] The success rate for getting a sufficient amount of good quality RNA was 92% and 75%, respectively, in these two studies.

  We have finished a pilot study of procuring core biopsy specimens in RNAlater from multiple institutions as part of a Phase II trial (GET) in locally advanced breast cancer conducted by the NSABP Foundation Research Program. The trial had a sample size of 76 with a 100% procurement rate, using a shipping kit developed in the NSABP Division of Pathology. In the GET trial, we were able to extract high quality total RNA from most of the core biopsies procured in RNAlater that were received from participating member sites using the in-house developed kits.

  The case illustrated in Figure 3 is from the NSABP GET trial. Note clearly visible 18S and 28S ribosomal bands in the RNAlater sample as compared to highly degraded RNA extracted from the paraffin block.

Figure 3. Quality of RNA extracted from tissue procured in RNAlater versus same tissue fixed in formalin and embedded in paraffin



The second important aspect is the demonstration of the ability to amplify the RNA without losing the original representation of relative mRNA quantity in the samples. In the GET pilot study, we tested the use of the Ovation RNA amplification and labeling kit (Nugen Technologies, San Carlos, CA) for Affymetrix GeneChip assays. Using 9 nanograms of starting RNA extracted from core biopsies collected in RNAlater, we were able to identify 2698 genes that are differentially expressed between ER-positive and ER-negative cases (defined by member reported IHC assays). The top gene was the estrogen-receptor gene with geometric means of 1650 vs 146 in ER-positive and -negative cases, respectively. As expected, other genes that were also found to be highly significant were GATA3 (335 vs 72), and HER4 (534 vs 82) as expected. Using the Biometrics Research Branch (BRB) Array Tools and Prediction Analysis of Microarray (PAM), we tested the accuracy of a predictive model for estrogen receptor status based on gene expression levels with leave-one-out cross validation.[141] Accuracy of 82% to 86% was obtained. One example based on the Support Vector Machine is shown in Table 8.[142] Considering the known misclassification rate of clinical ER immunohistochemistry assays performed by the NSABP member institutions (20% false-negative rate when compared to central ER assay), this degree of prediction accuracy, together with the identification of known genes including estrogen-receptor and GATA3, confirm our ability to conduct a microarray-based study using the Ovation RNA amplification protocol with total RNA extracted from core biopsies collected in RNAlater.[143]

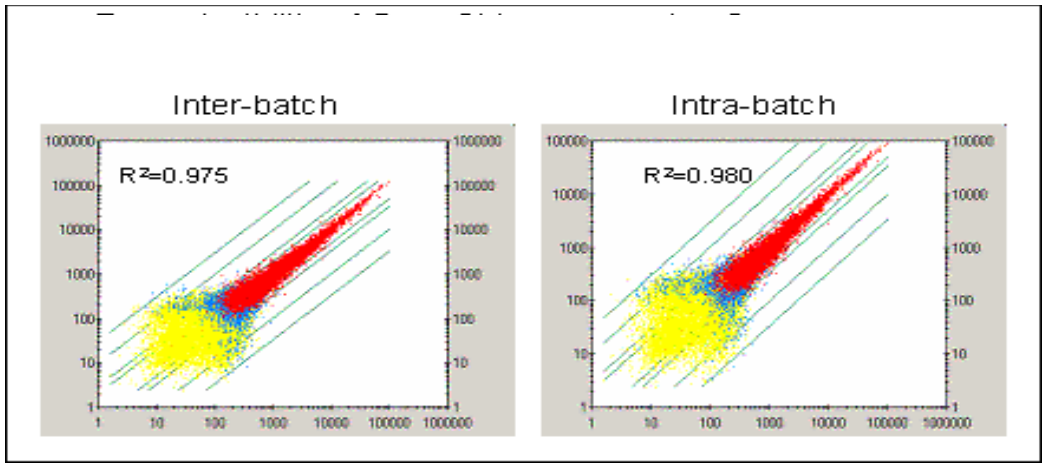
TABLE 8. Performance of the Support Vector Machine Classifier for ER

Class	Sensitivity	Specificity	PPV	NPV
N	0.833	0.829	0.806	0.853
P	0.829	0.833	0.853	0.806

The Significance Analysis of Microarray (SAM) also identified 278 genes differentially expressed between those cases with pCR vs non-pCR to the GET regimen with a false discovery rate of 0.0995.[144] Among these genes, 144 were also identified as significant by PAM.[141] Similar to what has been described by Gianni et al using the RT-PCR method, our list includes, surprisingly, many immune-related genes.[4] It is of interest that Damaria et al have observed that pretreatment lymphocytic infiltrate in the tumor was minimal in the majority of patients in their neoadjuvant study and showed no relationship with clinical response.[145] In the patients without tumor infiltrating lymphocytes (TILs) before treatment, development of TILs after treatment was noted in 0/3 (0%) patients with stable disease, 3/12 (25%) patients with clinical partial response, and 4/6 (67%) patients with clinical complete response and pathological residual disease. Therefore, immunological processes may influence the response of breast cancer patients to neoadjuvant treatment. However, performance of the gene expression as a predictor in our leave-one-out cross validation exercise was disappointing with accuracy of 62% to 76% with low-positive predictive and high-negative predictive value for predicting pCR. This could be due to the fact that pCR rate in the GET study was 25% with a small sample size. It is also possible that limited sampling of the tumor (single core) may not provide representative information for the entire tumor. Regardless, these data clearly argue for exploration of complementary approaches for discovery of predictive markers for pCR including proteomics and cell-based approaches as included in this protocol.

We have also tested the interbatch and intrabatch reproducibility of the Affymetrix GeneChip assay based on the Ovation kit and found it to be highly reproducible as shown in Figure 4. This data is also supported by a recent study by Dobbin et al who also found high within-laboratory and between-laboratory correlations using frozen tumor tissue.[146]

Figure 4. Reproducibility of GeneChip assay using 9 nanograms of starting RNA



We will be using the RNAlater in this trial to procure pre-treatment core biopsy tissue. While it is not yet clear whether RNAlater-procured tissue specimens are a suitable substrate for proteomics platforms, it does provide a method for investigators participating in NSABP trials to procure materials ideal for planned genomics studies.

Use of core-biopsy specimens instead of the entire index tumor for gene expression profiling requires the potential impact of tumor heterogeneity to be addressed. According to the data from Perou et al samples obtained from the same patients (index tumors vs metastasis or pre-chemotherapy vs post-chemotherapy) always cluster together when compared to samples obtained from different patients.[131] Two independent studies using either FNA or core biopsies in breast cancer have succeeded in demonstrating the feasibility of obtaining gene expression profiles potentially predictive of pCR to pre-operative therapy.[2,136]

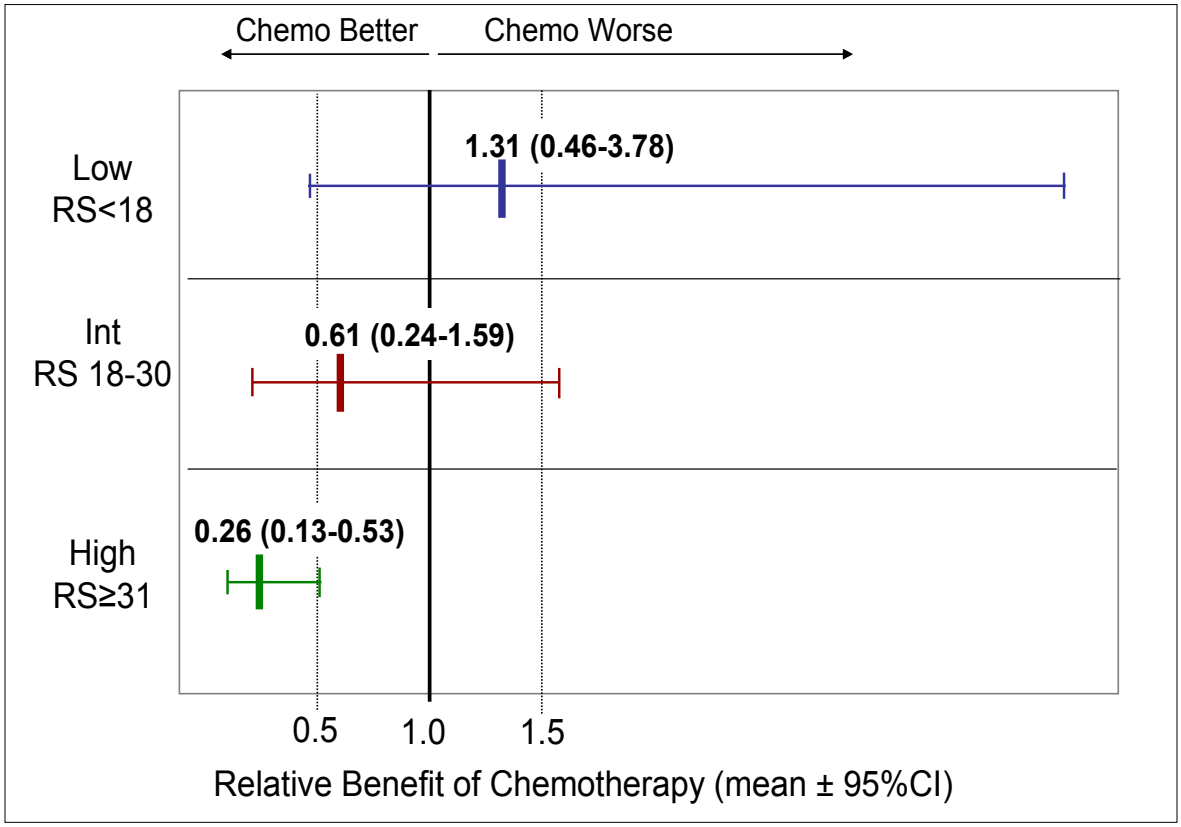
- Alternative approaches for gene expression profiling

  While the primary correlative science aim of B-40 is to profile gene expression using RNA extracted from tissue procured in RNAlater, ideal clinical tests would be the ones that can utilize routinely processed formalin-fixed paraffin-embedded tissue blocks. Several methods that allow gene expression profiling of many genes using fragmented RNA extracted from paraffin blocks have recently been developed. These include quantitative real-time reverse transcription-polymerase chain reaction (QRT-PCR),[147] ASL (cDNA-mediated annealing, selection, extension and ligation),[148] and microarray-based assays. Once an RNAlater based study yields a meaningful result, we will attempt to develop a predictive assay that works with paraffin blocks. The core biopsy procured in formalin will be used for this purpose as well as for validation of gene expression profiling data with immunohistochemical assays for the estrogen and progesterone receptors, and HER2 by fluorescence in situ hybridization (FISH) assay.

The NSABP has been involved in the development of one such assay (OncotypeDx™ developed by Genomic Health, Inc, Redwood City, CA).[129] Using the method developed by scientists at Genomic Health, Inc., we were able to develop a multi-gene prognostic index for patients diagnosed with node-negative estrogen receptor-positive breast cancer treated with tamoxifen.

In a subsequent study using blocks available from NSABP Protocol B-20, we have observed a statistically significant interaction (p=0.03 for DDFS and DFS) between the OncotypeDx assay results and a clinical benefit from CMF/MF regimen as shown in Figure 5.[134] Patients in the low risk group had minimal benefit from chemotherapy whereas those in the high risk group had a large benefit. We do not know whether these data will also be applicable to ER-negative or node positive patients or those receiving chemotherapy regimens different from those used in B-20. Validation of this result will be important and the B-40 trial, despite the differences in regimens used, may provide insight as to the generalizability of this finding from the retrospective analysis of B-20.

Figure 5. Relative risk of patients receiving chemotherapy in addition to tamoxifen in ER-positive and node negative patients enrolled in the B-20 trial



Using the same RT-PCR method, Dr. Luca Gianni's group at Instituto Nazionale Tumori Milan has examined the expression of 384 genes in 89 pre-treatment core biopsy specimens procured in their neoadjuvant study of doxorubicin/paclitaxel x 3 cycles → weekly paclitaxel x 12 cycles.[4] They found expression of 83 genes that highly correlated with pCR. The pCR correlated with higher expression of **proliferation genes** (CDC20, E2F1, MYBL2, TOPO2A, FBXO5, MCM2, MCM6, CDC25B), higher expression of **immune-related genes** (MCP1, CD68, CTSB, CD18, ILT-2, CD3z, FasL, HLA.DPB1), and lower expression of **estrogen-related genes** (PR, SCUBE2 (CEGP1), ER, NPD009, GATA3, IGF1R, IRS1). Protocol B-40 will provide a validation set for the latter 83 gene RT-PCR profile using higher quality RNA extracted from RNAlater-procured core biopsy tissue as well as paired paraffin blocks.

Some methods (such as the Paradise kit™ from Arcturus Microgenomics or the DASL assay from Illumina) have been developed that allow more comprehensive gene expression profiling of degraded RNA extracted from paraffin blocks using microarray or bead array format.[147,148] We are currently evaluating these methods using 600 core biopsy paraffin blocks collected in NSABP Protocol B-27.2. If successful, we will have candidate predictive markers for AC and AC→Taxotere before B-40 finishes accrual. B-40 will then provide a validation platform for a B-27.2 gene expression study. With the current methods in NSABP Pathology Laboratory, we can generate Affymetrix GeneChip data using a single 5 micron thick section from paraffin embedded core biopsy from B-27.2. Using RNA amplification method (Transplex Whole Transcriptome Amplification method from Rubicon Genomics), we are able to get gene expression profiles with over 85% accuracy in predicting ER status defined by immumohistochemistry. Top in the list of genes identified as predictive genes by GeneChip are ER, GATA3, Keratin 7 suggesting that this method does work despite the fact that the number of genes we find is less than when using RNA extracted from RNAlater procured tissue. However, since we are still at an early stage of evaluating the feasibility of these methods, we will develop a separate protocol with a committed predictive model to validate the findings from B-27.2 using specimens collected in the B-40 protocol.

At this point in time in the evolution of methodology, we have to make sure that we procure the most pristine material as logistically possible for gene expression studies. Therefore, procurement of tissue in RNAlater still is necessary. Once we complete the studies described in the B-40 protocol, we will be able to determine whether we can reliably predict response to chemotherapy using routinely available tissue or whether we will need to require RNAlater-procured tissue.

- ChemoFx® chemoresponse assay

  While chemoresponse assays have shown promise in predicting clinical response to chemotherapy, they need to be studied within the context of large, prospective clinical trials for more widespread clinical utility and adoption.[8]

Viable explant cultures for testing in the ChemoFx® assay have been successfully grown in monolayers from a variety of solid tumors, including breast and ovarian tumors. The average assessability rate for breast tumors in the ChemoFx® assay is 80%. In a study of 148 primary, recurrent, or metastatic breast cancer specimens, in vitro patterns of response were shown to be similar to reported clinical population response rates. In vitro, 69% of the specimens were responsive to the anticancer effects of cyclophosphamide, 57% to fluorouracil, 45% to doxorubicin, 39% to docetaxel, 27% to paclitaxel, and 36% to gemcitabine.[149] Of note, the higher response rate noted in vitro in this study for doxorubicin, cyclophosphamide and fluorouracil, as compared to docetaxel, paclitaxel and gemcitabine, mimics clinical experience with these agents. In addition, when two of the agents (doxorubicin and cyclophosphamide) were tested in combination, the anticancer effect was additive (88% response rate) compared to the effect of either agent when tested alone (48% and 61%, respectively).[150] In comparison, in the NSABP Protocol B-27, the overall clinical response rate after doxorubicin and cyclophosphamide combination treatment was 86%.[36]

Similar studies were also performed using ovarian tumors. In a study of the ChemoFx® assay, in vitro response rates to standard therapies (carboplatin, cisplatin, paclitaxel, docetaxel) for 268 primary and 15 recurrent ovarian tumors were determined and compared to clinical population response rates reported in the literature. In primary ovarian tumors, nearly all in vitro results were highly correlative to clinical patterns of response.[151] In an additional study, more than half of 51 primary ovarian cancer cases evaluated responded in vitro to treatment with nonstandard chemotherapy, such as cyclophosphamide, doxorubicin, gemcitabine and etoposide.[152] In vitro, individual tumors demonstrate heterogeneity in responsiveness to both standard and nonstandard chemotherapeutic agents for a given tumor type. In some instances, in vitro response even differed among agents of the same mechanistic drug class for the same patient tumor.[153] In light of the observed heterogeneity of responsiveness, it is suggested that in vitro chemoresponse testing is likely to increase the efficacy of the current chemotherapy decision-making process for each individual patient.

In a retrospective review of 18 patients with late-stage, papillary, serous ovarian cancer undergoing 21 episodes of chemotherapy, the ChemoFx® assay appeared to predict clinical responsiveness. The ChemoFx® assay had a positive predictive value (PPV) of 63.6% and a negative predictive value (NPV) of 100%. In addition, a survival advantage was noted in patients whose tumor cells were ChemoFx® sensitive compared with patients whose tumor cells were ChemoFx® resistant.[5] More recently, a large, retrospective clinical study in ovarian cancer has evaluated the ability of the ChemoFx® assay to predict progression-free interval (PFI) in patients with ovarian cancer. Data were collected on 317 ovarian and peritoneal cancer specimens tested with the ChemoFx® assay between April 1997 and April 2002. Of those, 256 cases had a partial or exact match between drug(s) assayed and those prescribed clinically, and displayed a statistically significant correlation between assay prediction of response (sensitive, intermediate, resistant) and clinical PFI. For the 135 cases with an exact match between assay and clinical treatment, the hazard ratio (HR) for progression of the assay-resistant

group was 2.9 (CI: 1.4 to 6.3; P<0.01) compared to the assay-sensitive group; and the HR for the assay-intermediate group compared to the assay-sensitive group was 1.7 (CI: 1.2 to 2.5). The PFI for assay-resistant patients was 9 months, compared to 14 months for assay-intermediate patients, and "not achieved" for assay-sensitive patients.[6] In a subsequent analysis of 84 cases of primary ovarian cancer treated clinically with platinum-based chemotherapy and evaluated in vitro with the ChemoFx® assay with a single agent platinum compound, the HR for progression in patients with assay-predicted platinum resistance was 2.9 times that of patients with non-resistant tumors (p=0.007). In addition, when prediction of response was graded (sensitive, intermediate, resistant), the HR of assay-resistant patient tumors was 3.6 times that of assay-sensitive patient tumors (p=0.005). On multivariate analysis, the ChemoFx® assay was the strongest independent predictor of response when compared to known clinical, prognostic factors such as FIGO stage and debulking status (p=0.0006).[7]

The reproducibility of the ChemoFx® assay has been evaluated with the use of immortalized human cell lines (HTB-77, ovarian adenocarcinoma; HTB-19, breast carcinoma; or HTB-26, breast adenocarcinoma; ATCC, Manassas, VA, USA). In brief, each immortalized cell line was subjected to a non-overlapping subset of the twenty-one drugs commercially available on the ChemoFx® assay. Percentage cell kill was recorded at each dose for a period of 7 months resulting in a minimum of 52 and a maximum of 76 unique data points for each drug. The assay was performed by multiple clinical laboratory personnel over the course of the reproducibility study. The average coefficient of variation of percentage cell kill across drug-dose combinations was found to be 18% for drug-dose combinations in which the percent reduction in cell survival was greater than 50%.[7]

- Proteomics studies

  Susanne Ragg, MD, PhD, Assistant Professor of Pediatrics and Director of the Center for Computational Diagnostics, Indiana University, will be the coordinator for the proteomics studies.

  Serum protein markers that change early during the course of chemotherapy and are predictive of tumor response or toxicity will be extremely useful. Although there have been numerous studies demonstrating the potential of proteomics approaches, proteomics is still in its infancy, and we may have to wait for the field to mature to be able to effectively interrogate samples collected in this study to identify desired markers. Most likely, the sera collected in this trial are not going to be the ideal materials for most of the demanding discovery oriented proteomics studies. However, the sera may provide samples for validation studies for markers discovered from other studies due to expected variations in sample collection and processing in the multi-center trial setting. We will make an effort, however, to collect samples at time points that may be most informative. Picking the right point of sampling is tricky, however. Pusztai et al have examined protein changes on day 3 of neoadjuvant chemotherapy using Surface-enhanced laser desorption/ionization mass spectrometry (SELDI-MS) and found one peak that was induced by paclitaxel treatment, and this peak did not correlate with

response.[154] This could be due to the fact that serum was collected too early on day 3 of treatment. According to studies by Kiang et al and Vogelzang et al some serum/plasma markers may rise initially due to acute tumor cytolysis and then decline.[155,156] Leukemia is probably the best example for utilizing risk-adapted therapy. While it is usually very easy to find the very fast responders by following the blast count in the bone marrow, it is much harder to predict early who needs more aggressive therapy, and a later time point (day 14) has been more informative in this regard. Since we are not sure when is the best timing of sampling to identify the markers, we will collect at three time points – before chemotherapy, before the 3rd cycle of T, TC, or TG, as well as at the end of chemotherapy.

- Pharmacogenomics study

  Variation in drug disposition and response among patients is a major concern associated with many pharmaceuticals, particularly anticancer agents.[135,157] The clinical relevance of variability is most evident with drugs that have narrow therapeutic indices (i.e., the dose used is close to the dose that is likely to produce toxicity in most individuals). Typically, anticancer drugs have low therapeutic indices because of their nonspecific cytotoxicity and because the doses necessary for optimal eradication of malignant cells are often close to those that damage normal cells.

  Every gene contains some level of polymorphism, with single nucleotide polymorphisms (SNPs) occurring every 1000–3000 base pairs throughout the human genome. Genetic variations in both normal host tissue and tumor tissue can influence the net effects observed following administration of an anticancer drug.[135,157] Polymorphisms in both host and tumor genes regulate drug availability, retention and efflux, determining whether or not anticancer drugs can access the tumor. Polymorphisms occur in enzymes that metabolically activate or inactivate drugs both in the systemic circulation and within cells. Variations in genes that control drug transport can alter the amount of drug absorbed into the systemic circulation following oral administration and across tumor cell membranes. The tumor genome possesses most of the polymorphisms that affect tumor invasiveness and drug sensitivity. The potential mechanistic targets of anticancer drugs are subject to polymorphisms and/or mutations that can alter or determine bioactivity. Furthermore, genes that control the cellular repair process following insult with an anticancer drug can have important variants. On the other hand, host polymorphisms determine the risk of host toxicity (genes such as those that encode thiopurine methyltransferase or dihydropyrimidine dehydrogenase), with little contribution by the tumor genome.

  The net effect of the genetic variants in normal tissues is regulation of the amount of anticancer drug in the bloodstream over time (systemic exposure) resulting in altered pharmacological effects and/or toxicity for an individual patient. The net effect of genetic variants in the tumor can have a profound influence on efficacy because of their control of both anticancer drug tumor concentration and pharmacological events such as receptor binding and cellular repair.

Numerous studies have demonstrated relationships between the systemic pharmacokinetics of anticancer drugs and their effects (primarily toxicity). This indicates that polymorphisms in genes that encode crucial drug-metabolizing enzymes can be relevant clinically. Table 9 summarizes some of the better-characterized polymorphic enzymes that are involved in the metabolism of anticancer drugs.

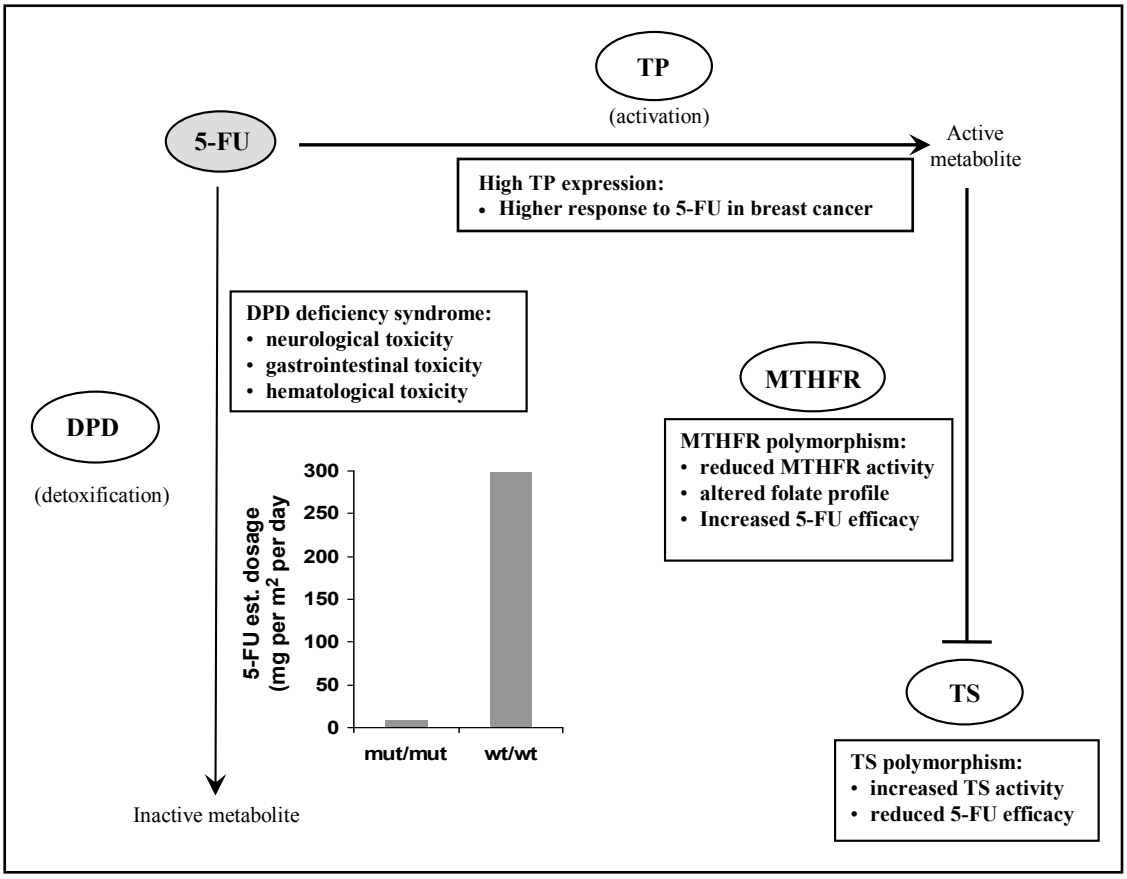
TABLE 9. Metabolic enzymes with polymorphisms that might affect the disposition of anticancer drugs[135,157]

Metabolic enzyme	Potential anticancer drug substrate
CYP1B1*	Estrogen metabolites
CYP2B6*	Cyclophosphamide
CYP2C8*	Paclitaxel
CYP2C9*	Cyclophosphamide
CYP3A4,5,7*	Cyclophosphamide, etoposide, teniposide, tamoxifen, docetaxel, steroids, ifosfamide, paclitaxel, irinotecan, anthracyclines and vinca alkaloids
Aldehyde dehydrogenase (ALDH2)	Cyclophosphamide
Dihydropyrimidine dehydrogenase	5-Fluorouracil and capecitabine
NAD(P)H:quinone oxidoreductase 1	Mitomycin C
Thiopurine methyltransferase	6-Mercaptopurin and 6-thioguanine
UDP-glucuronosyltransferase 1A1	Irinotecan
Glutathione-*S*-transferases	Carmustine and platinum analogs
Sulfotransferases	Estrogen and tamoxifen
* Abbreviation: CYP, cytochrome P450.	

Genome variability can also influence the targets of anticancer agents. For example, genetic variability in enzymes that detoxify, activate and are involved in the response to 5-FU can influence the toxicity and efficacy of 5-FU (and capecitabine). Individuals who have a polymorphism in the gene encoding dihydropyrimidine dehydrogenase (DPD), the 5-FU-detoxification enzyme, might have a severe toxicity syndrome because of delayed elimination (i.e., high blood concentrations of 5-FU). As shown in Figure 6, the dose of 5-FU tolerated in patients who are homozygous for the mutant allele (mut/mut) is substantially less than in those who are homozygous for the wild-type allele (wt/wt). Thymidine phosphorylase (TP) is thought to increase the activation of 5-FU and be an angiogenic factor.[158] Thus, 5-FU-based therapy is more efficacious in patients with breast cancer who possess high concentrations of TP, compared with similar patients who have lower levels of TP. Folates are important co-factors required for binding the active metabolite of 5-FU to its target enzyme. Methylenetetrahydrofolate reductase (MTHFR) regulates the metabolism of folates and, thus, a polymorphism in the gene encoding MTHFR can influence the efficacy of 5-FU. An important mechanism that leads to 5-FU cytotoxicity is thought to be its inhibition of thymidylate synthase (TS). The expression of TS is also influenced by a polymorphism that is associated with altered efficacy of 5-FU.[159] The human thymidylate synthase gene promoter is polymorphic, having either double or triple repeats of a 28-bp sequence. Park et al have examined the correlation between TS gene polymorphism and response to capecitabine in advanced colorectal cancer.[160] They found that 75% (3/4) of

individuals with the S/S variant responded to capecitabine, compared to 8% (1/12) and 25% (2/8) of those with the S/L and L/L variants, respectively.

Figure 6. Effect of genetic variability on the toxicity and efficacy of 5-FU



Although advances in molecular technology hold promise for reducing the cost and effort involved in genotyping individuals, the techniques to accurately genotype individuals for specific pharmacogenetic polymorphisms remain expensive and specialized, hindering their widespread use. We will collect adequate specimens (blood and tumor samples) for future analysis and conduct the study when technology becomes more affordable.

02/15/08 8.3 **Recommended processing of tissue at the time of definitive surgery to evaluate pCR**

While we are interested only in pre-treatment tumor and patient characteristics that can be used to predict response before we initiate treatment, since the primary endpoint of this trial is pCR rate rather than clinical outcome, accurate and reproducible assessment of pCR will be important.[161] Rates of pCR may fluctuate between trials due to variability of the extent of tissue sampling for histopathologic examination at the time of definitive surgery. Less extensive sampling will result in falsely high pCR rates. *Therefore, a minimum of 10 to 15 paraffin blocks from the initial primary tumor site should be examined before assigning a response of pCR (see Appendix E).*

A description of the procedure for tissue processing of breast specimens at MD Anderson Cancer Center (MDACC) is provided in Appendix E of this protocol. Participating

institutions will be encouraged to develop procedures which incorporate as many of the MDACC elements as is possible within their institution.

02/15/08 8.4 **Residual cancer burden**

Although pCR is a dichotomous variable, in reality, tumor response to chemotherapy is a continuous variable with non-response ranging from very small residual tumor burden to frankly resistant tumors with progressive disease. Therefore, continuous measures of residual cancer burden (RCB) would be expected to be more predictive of clinical outcome than simple dichotomous classification as currently practiced.

RCB determined from routine pathologic materials may be a significant predictor of distant relapse-free survival.[162] Pathologic slides and reports were reviewed for 382 patients who had received neoadjuvant chemotherapy at MD Anderson Cancer Center. Residual cancer burden was calculated as a continuous index combining pathologic measurements of primary tumor (size and cellularity) and nodal metastases (number and size). RCB was independently prognostic in a multivariate model that included age, pre-treatment clinical stage, hormone receptor status and hormonal therapy, and pathologic response (pCR versus RD) (HR, 2.50, CI, 1.70-3.69, $P < 0.001$). Although evaluated in the independent validation cohort of the MD Anderson study, RCB has not been validated in a prospectively designed large multi-institution study.

Pathologists examining the specimen for pathologic response are required to provide information to generate RCB. This information (listed below) will be collected on Form RCB ***for ALL B-40 patients*** enrolled after local IRB approval of Amendment #2 and will be used by the NSABP Biostatistical Center to calculate the RCB index.

- size of the tumor bed
- cellularity of residual primary tumor
- percentage of DCIS component
- number of positive nodes
- size of macrometastasis

For more information on RCB, refer to the publication by WF Symmans et al[162] or www.mdanderson.org/breastcancer_RCB. Appendix 1 of the WF Symmans et al article provides detailed pathology methods; note that this appendix is only accessible through the online version of the article, published at www.jco.org.

02/15/08 8.5 **Correlative studies aims**

Correlative science aims are as follows:

- Discover predictive gene expression profiles for pCR to study regimens (T→AC, TCape→AC, TG→AC) using the Affymetrix GeneChip Assay (or other gene expression profiling platform that is identified as the best at the time of initiation of the marker study).
- Discover and validate: (1) predictive ChemoFx® dose-response curves for clinical overall response (cOR) after treatment with T, TCape, or TG; (2) predictive ChemoFx® dose-response curves for pCR including dose-response curves for AC and either T, TCape, or TG depending on treatment arm.
- Validate Genomic Health 21 gene Recurrence Score (OncotypeDx) and Milan 83 gene predictive expression profile as predictors of pCR to chemotherapy with a

hypothesis that there is an interaction between Recurrence Score and response to chemotherapy (higher pCR rate in higher RS category).

- Validate Tau expression as a marker of pCR (by immunohistochemical assay as well as RT-PCR assay).
- Identify serum markers that may predict pCR when measured early in the course of chemotherapy (This will be conducted in collaboration with Dr. Susan Ragg, Indiana University. A separate protocol will be submitted to CTEP/NCI once the proper technical platform is decided; however, informed consent for future proteomics analyses will be obtained through this protocol.)
- Identify genetic polymorphisms that correlate with pCR and severe toxicity (blood samples are collected for this purpose but a separate protocol will be developed for this aim once suitable technology is identified to achieve this aim).
- Validate residual cancer burden index as a predictor of disease-free survival outcome.

8.5.1 ***Discover predictive gene expression profiles for pCR to study regimens (T→AC, TCape→AC, TG→AC +/-bevacizumab)***

We will examine the gene expression profiles of pre-treatment core biopsy specimens procured in RNAlater using the Affymetrix GeneChip or another array platform determined to be best suited for our purpose to build a predictive model for pCR to combination regimens used in this protocol, i.e., T→AC, TCape→AC and TG→AC +/- bevacizumab. Validation of these models will require additional trials. We will also look for the presence of a common set of genes that are correlated with response to all regimens in this study to build a general predictor of response to chemotherapy.

Method for the Affymetrix GeneChip

Core biopsy specimens procured in RNAlater will be stored in a -70°C deep freezer until the time of batch assay. The specimen is frozen-sectioned to assess tumor cellularity, and a grossly tumor-enriched area is dissected while the tissue is still in an OCT cold embedding medium. We are currently evaluating whether microdissection is required by comparing microdissected vs macrodissected samples in the GeneChip analyses of the B-27.2 cases. If found to be necessary, we will use a PixCell LCM® or PALM device to microdissect tumor cells before extracting RNA. If microdissection is not to be used, macrodissected tissue will be kept frozen in RNAlaterICE solution (Ambion) at -70°C for at least 14 hours and then RNA will be extracted using the Qiagen RNAeasy kit. One microliter of extracted RNA will be run on a Bioanalyzer (Agilent) to check the quality of RNA. Only those samples with distinct ribosomal peaks will be used for the GeneChip assay. Nine nanograms of total RNA will be linearly amplified and labeled with biotin using the Ovation™ kit (Nugen Technologies) and hybridized to the Affymetrix GeneChip. Hybridization signals will be scanned using the Affymetrix scanner.

Alternative method

Depending on development in the field, we may choose to use other gene expression profiling methods such as DASL (Ilumina) if they are more robust and reproducible than the Affymetrix GeneChip based approach.[148]

8.5.2 ***Validate (1) predictive dose-response curves for cOR after treatment with T, TCape, and TG; (2) predictive dose-response curves for pCR including dose-response curves for AC and either T, TCape, or TG depending on treatment arm.***

We will analyze the dose-response curves generated by the ChemoFx® assay to build a predictive algorithm for determining cOR after treatment with T, TCape, or TG. For each arm of the trial, we will extend the algorithm to include prediction of pCR by incorporating additional information on the dose-response curves generated by the secondary treatment.

Method:

ChemoFx® Assay

Two core biopsies will be obtained from each neoadjuvant breast cancer patient prior to the initiation of chemotherapy for use in the research version of the ChemoFx® assay to be performed by PTI. PTI's research version of the ChemoFx® assay involves in vitro isolation, short-term growth, and drug treatment of epithelial cells derived from solid tumors. At the time of biopsy of the breast tumor site, two of the obtained core biopsies will be placed in the supplied bottle of the sterile shipping medium for PTI for overnight shipment to the laboratories of PTI in Pittsburgh, PA. Upon arrival in the laboratory, the core biopsies are minced into small pieces and placed in culture media in three small flasks for cell outgrowth. Over time, cells move out of the tumor pieces and form a monolayer on the bottom of the flask. Once enough cells have migrated out of the in vitro explant pieces, they are then trypsinized and reseeded into microtiter plates for either the research version of the ChemoFx® assay or for immunohistochemsitry (IHC) analysis for confirmation of epithelial cell growth.[163]

For the research version of the ChemoFx® assay, a cell suspension is prepared from the cell monolayer which is delivered to a large basin situated on the stage of the Oasis LM liquid handling machine (Dynamic Devices, Inc.). The liquid handler then seeds a set number of cells into the wells of a 384-well microtiter plate in replicates of four per drug treatment. Cells are then allowed to adhere to the plate and grow for 24 hours at 37°C. Following this incubation, the liquid handler prepares ten doses of each drug or drug combination in the appropriate growth medium via serial dilutions in a 96-well deep-well microplate. The appropriate volume of each dose of each drug or drug combination is then added to the appropriate well of cells in the 384-well plate via the liquid handler; each drug treatment will also contain a control well to which medium alone is added. The system ensures that all cells are treated with the correct drug or drug combination at the correct dosage. The cells are incubated with drugs for 72 hours, thus necessitating their preparation in complete growth medium. At the end of the 72 hour drug incubation period, the liquid handler removes media and any nonadherent, dead cells from each well. The remaining, live cells are fixed in 95% ethanol containing the DNA-intercalating blue fluorescent dye, 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Molecular Probes). As a result of the fixing and staining process, these cells become non-viable at the completion of the assay testing.

Following fixation and staining, an automated microscope captures UV images of the stained cells in each well. Afterwards, the number of cells per well, in both visible and UV light, is quantified. For each dose of each drug treatment tested, a cytotoxic index (CI) is calculated which represents the percentage of cells killed as a result of that treatment. CI is determined using the following formula.

> CI (Cytotoxic Index) for each drug dose concentration = # cells counted in treated wells / # cells counted in untreated-control wells

Using calculated CI values, a complete dose-response curve is generated for each drug or drug combination evaluated.

We will take the data from the first 100 patients randomized to the T followed by AC control arm (Group 1A patients) to investigate various methods for using the ChemoFx® assay to predict cOR and pCR, in order to facilitate the final analysis on the ChemoFx® assay data.

To this aim, we will discover and validate in the control arm (T followed by AC, no bevacizumab): (1) predictive ChemoFx® dose-response curves for clinical overall response (cOR) after treatment with T (without bevacizumab); predictive ChemoFx® dose-response curves for pCR including dose-response curves for T and AC (without bevacizumab); (3) prediction of pCR using ChemoFx® dose-response curves for T and for AC and clinical response to T (without bevacizumab).

8.5.3 *Validate Genomic Health 21 gene Recurrence Score (OncotypeDx assay) and 83 gene predictive gene expression profile as predictors of pCR to chemotherapy*

Gianni et al have examined gene expression levels of 380 genes in paraffin-embedded core biopsy tissue procured from patients enrolled in a pre-operative chemotherapy study.[4] Eighty-three genes were identified whose expression levels correlated with pCR. In addition, Recurrence Scores (RS) based on 21 genes were also correlated with pCR, with higher RS correlating with higher rate pCR (p=0.005). In a recent analysis of NSABP B-20, statistically significant interaction between RS and chemotherapy was observed in ER+ N- patients who received tamoxifen.[134] We will examine this set of 83 genes and use the OncotypeDx assay to validate their association with pCR.

Method

Two 5 micron thick sections from formalin fixed paraffin-embedded tumor blocks will be mounted on glass slides, and a tumor rich area will be macrodissected and sent to Genomic Health, Inc. QRT-PCR analysis will be performed as described previously.[129,147]

8.5.4 *Validate predictive gene expression profiles for pCR to AC and AC→Taxotere developed from B-27.2*

Soonmyung Paik, MD, Director of the NSABP Division of Pathology, is currently analyzing core biopsy specimens collected in NSABP B-27.2 using multiple gene expression profiling platforms including the Affymetrix GeneChip X3P (with Arcturus Paradise kit) and DASL assay.[148] The gene expression profiles that correlate with pCR to AC and AC→Taxotere may become available from this study during accrual of B-40. Therefore, formalin-fixed cores collected from this trial embedded in paraffin will provide a validation set for such gene expression profiles. Once a model is built from B-27.2, a separate validation study protocol will be written with a predefined model to use the samples from the B-40 protocol.

8.5.5 *Validate Tau expression as a marker of pCR*

Pusztai et al previously used DNA microarrays to identify gene expression patterns associated with pCR to preoperative taxane-containing chemotherapy in 60 patients with breast cancer.[164] Low mRNA expression of microtubule-associated protein Tau in cancers at the time of diagnosis was significantly associated with pCR. To validate this finding, Rouzier et al performed immunohistochemistry (IHC) on a tissue array containing 122 breast cancer specimens. All patients received 24 weeks of preoperative paclitaxel followed by 4 courses of anthracycline-containing chemotherapy. None of these patients was included in the microarray study. Thirty-eight patients experienced pathologic CR (31%). Cytoplasmic expression of Tau protein was seen in normal breast epithelium and blood vessels. Sixty-four tumors (52%) were considered Tau-negative, including 14 with complete absence of Tau by immunohistochemistry (IHC score 0) and 50 tumors with less Tau expression than normal controls (IHC score 1+). Fifty-eight tumors (48%) were positive for Tau protein expression, defined as IHC score 2+ that had uniform staining of similar or slightly greater intensity than normal controls or IHC score 3+ that had uniform high intensity staining. This dichotomization of staining results was determined after inspection of the distribution of results and without knowledge of the clinical outcome data. There were more pathologic CRs among the Tau-negative tumors (28/64, 44%) than among the Tau-positive tumors (10/58, 17%). Most tumors that achieved pathologic CR were Tau-negative (28/38, 74%). The odds ratio for pathologic CR in Tau-negative tumors was 3.7 (95% confidence interval 1.6, 8.6, $p = 0.0013$). A multiple logistic regression model with pCR as the outcome, and age, tumor size, nodal status and histology, nuclear grade, estrogen receptor (ER), progesterone receptor (PR), and HER2 expression as covariates identified high nuclear grade ($p = 0.0018$), ER-negative ($p = 0.08$) and Tau-negative status ($p = 0.04$) as independent predictors of pCR. A similar multiple logistic regression model with Tau as the outcome and including the same clinicopathologic parameters as covariates, identified low or intermediate nuclear grade ($p = 0.05$), ER ($p = 0.06$) and PgR ($p = 0.005$) as independent predictors of Tau status. ER-negative and high-grade tumors tended to be Tau-negative. The Tau-pCR odds ratio when adjusted for age, tumor size, nodal status, nuclear grade and ER, PgR, and HER2 status was 2.7 (0.9, 7.9) with $p = 0.059$. These results confirm the microarray data that low Tau expression is associated with higher probability of achieving pCR.[137]

Method

Unstained sections cut from core biopsy paraffin blocks at the NSABP Division of Pathology will be sent to Dr. Pusztai's lab at MDACC for staining. Sections will be deparaffinized as per standard procedure. Endogenous peroxidase activity will be blocked and antigen retrieval will be performed by heating the slides in citrate buffer (pH 6.0) x 10 minutes in a high temperature microwave oven. The slides will be incubated with anti-Tau antibody (1:50 dilution, clone T1029, US Biological) overnight at 4°C. Bound antibody will be detected by using an antimouse horseradish peroxidase-labeled polymer secondary antibody (DAKO Envision® TM+ System, DAKO, Carpentia, CA) with DAB substrate. Normal breast epithelium will serve as an internal positive control. Negative control will include omission of the primary antibody in a control slide.

Cytoplasmic staining intensity will be graded as either negative (0/1+) or positive (2+/3+) based on our previously established cut-off system. Slides will be scored independently by two pathologists (WF Symmans and S Paik) without knowledge of the clinical outcome. As an exploratory analysis, we will utilize the TMAx image analysis program (Beecher Instruments) based on Cellenger Engine (Definiens) that has capability to automatically distinguish tumor cells vs host cells and to quantify staining results as a continuous variable based on intensity of staining and percent positive cells. The Aperio ScanScope will be used to scan stained slides at 200x magnification to be analyzed by TMAx.

8.5.6 ***Identify serum markers that may predict pCR when measured early in the course of chemotherapy***

A serum marker that changes early during the course of chemotherapy and whose change correlates with eventual pCR at the completion of the chemotherapy would be a useful surrogate for pCR to allow us to stop non-efficacious treatment earlier. We will collect serum before the initiation of therapy, just prior to the third cycle of the first chemotherapy regimen, and at the completion of chemotherapy. The samples will undergo proteomics analysis to identify such markers and also will be used to validate predictive serum markers developed by other investigators.

Method:

Proteomics is a rapidly evolving field, and we will store the collected serum and wait until the end of the B-40 accrual to determine which method will be used.

8.6 **Pre-entry core biopsy specimens**

8.6.1 ***Requirements***

Procurement of core biopsies before study entry is an eligibility requirement for all patients. Baseline biopsy specimens must be procured in RNAlater, formalin, and shipping medium for PTI using the kit supplied from Precision Therapeutics, Inc.

Provide one core to be immersed in RNAlater solution. The core has to be immersed in RNAlater solution (kept at room temperature) immediately after the tissue is removed from the patient. Any delay at this step will result in degradation of RNA and will negate the purpose of the procurement in RNAlater. Please note that it is critical that the specimen is rinsed in saline if it is contaminated with blood since it seems to result in suboptimal RNA protection either by poor penetration of RNAlater or dilution of the RNAlater solution. The next two cores should be placed in the supplied bottle of shipping medium for PTI. The final core sample should be placed in the formalin vial.

05/30/07

8.6.2 ***Submission instructions***

Submit all 4 collected core biopsies directly to Precision Therapeutics, Inc. Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site for submission instructions. (CTSU investigators should refer to the CTSU Web site for the B-40 Pathology Instructions.)

Place all three containers with the core samples in the specimen kit and seal. Kits should be shipped immediately with cold packs. If sent on Friday, mark the shipping envelope for Saturday delivery. Keep the specimen kit in the refrigerator until pickup for shipping the same day.

8.7 Paraffin blocks

02/15/08

8.7.1 ***Requirements***

The submission of pathology materials from definitive surgery is required for all patients with gross residual disease ≥ 1 cm.

- **If grossly evident residual disease ≥ 1 cm:**

  Tumor and lymph node blocks from definitive surgery

  At least one representative block of residual primary tumor and a positive lymph node containing the maximum amount of tumor are required. We will use these blocks to evaluate pathologic response and also evaluate markers discovered in the future that may relate to drug resistance. While tissue microarray is useful for mass screening of markers, we found in NSABP B-18 that tumor cell density tends to be low in post-chemotherapy specimens so that it is difficult to construct tissue microarrays with 0.6 mm cores. We will be using 1 mm cores for these specimens. However, to prevent destruction of diagnostic blocks, we will be sampling only one 1 mm core per block.

  Alternative submission

  While it is desirable that tissue blocks are submitted, for institutions that do not allow submission of the tissue blocks, we recommend submitting the following two items as a substitute:

  – 20-30 unstained sections of 4-5 micron thickness mounted on charged slides (do not oven bake slides); and

– a 2 mm diameter core sampling from a tumor-cell-rich area or a similar size fragment cut from the original block (Kits can be obtained from the NSABP Division of Pathology [see Information Resources on page v]).

- **If grossly evident residual disease < 1 cm or if no grossly evident residual disease, tissue submission is *not* required.**

8.7.2 ***Submission***

Submit paraffin blocks (or alternative specimens as described in 8.7.1) to the NSABP Biostatistical Center at the address listed under "Information Resources" (see page v). Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site for submission instructions. (CTSU investigators should refer to the CTSU Web site for the B-40 Pathology Instructions.)

8.8 **Blood/serum specimens**

8.8.1 ***Requirements/timing of blood collections***

Specimens will be collected at the following 3 timepoints for patients who have consented to specimen collection:

– At baseline before chemotherapy *(blood and serum)*

– Before the 3rd cycle of T, TCape, or TG *(serum)*

– At least 3 weeks after completion of chemotherapy but before surgery *(serum)*

05/30/07

8.8.2 ***Submission instructions***

Refer to the B-40 Pathology Instructions in the Members' Area of the NSABP Web site for submission instructions. (CTSU investigators should refer to additional information regarding supplies for serum collection in Section 3.0 in Appendix F and to the CTSU Web site for the B-40 Pathology Instructions.)

9.0 TREATMENT REGIMEN

02/15/08

9.1 Group 1A (docetaxel→AC)

- Chemotherapy should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.***

TABLE 10. Treatment regimen for Group 1A

Drug	Dose	Dosing Interval	Planned Duration
Docetaxel	100 mg/m^2 IV over 60 minutes *(See footnote **a** for docetaxel pre-medication regimen and footnote **b** for **REQUIRED** cytokine support)*	Day 1 q 21 days	4 cycles
***21 days after Day 1 of the last cycle of docetaxel*[c]**			
Doxorubicin	60 mg/m^2 IV over 15 minutes	Day 1 q 21 days	4 cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[d]			

a ***Pre-medication for docetaxel:*** All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the discretion of the investigator, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

b ***Cytokine support****:* Primary prophylaxis with pegfilgrastim or filgrastim is **REQUIRED for Group 1A docetaxel cycles**. Use of pegfilgrastim is preferred.
- Pegfilgrastim should be administered on Day 2 at a fixed dose of 6 mg SQ.
- Filgrastim, if used, should be administered according to the package insert.

c Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

d After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed.

02/15/08 9.2 **Group 1B (docetaxel + bevacizumab→AC + bevacizumab)**

- Chemotherapy and bevacizumab should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.*** A delay in initiation of chemotherapy and bevacizumab of 1 week following vascular access device placement ***is recommended but not required.***

TABLE 11. Treatment regimen for Group 1B

Drug	Dose	Dosing Interval	Planned Duration
Bevacizumab	15 mg/kg IV over: 90 minutes – 1st dose**a** 60 minutes – 2nd dose**a** 30 minutes – all subsequent doses**a** Flush infusion line**b**	Day 1 q 21 days	**4** cycles
Docetaxel	100 mg/m^2 IV over 60 minutes *(See footnote* **c** *for docetaxel pre-medication regimen and footnote* **d** *for* ***REQUIRED*** *cytokine support)*		
21 days after Day 1 of the last cycle of docetaxel[e]			
Bevacizumab	15 mg/kg IV over 30 minutes**a** Flush infusion line**b**	Day 1 q 21 days	**2** cycles *(only with AC cycles 1 & 2)*
Doxorubicin	60 mg/m^2 IV over 15 minutes		**4** cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[f]			
At least 4-6 weeks after surgery, initiate postoperative bevacizumab[g]			
Bevacizumab	15 mg/kg IV over 30 minutes**h**	Every 21 days	**10** cycles**i**

a See Table 29 in Section 11.3 for instructions regarding infusion-related or allergic reactions with bevacizumab.

b Add an additional 50 mL of 0.9% NaCl for injection to the bevacizumab infusion bag (or use a new 50 mL bag of 0.9% NaCl) and infuse a volume equal to the volume contained in the tubing.

c ***Pre-medication for docetaxel:*** All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the investigator's discretion, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

d ***Cytokine support****:* Primary prophylaxis with pegfilgrastim or filgrastim is **REQUIRED for Group 1B docetaxel cycles**. Use of pegfilgrastim is preferred.
- Pegfilgrastim should be administered on Day 2 at a fixed dose of 6 mg SQ.
- Filgrastim, if used, should be administered according to the package insert.

e Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

f After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed (see Section 9.9.2 for important instructions regarding the timing of surgery).

g See Section 11.2 (Table 28) for the LVEF requirements for initiating postoperative bevacizumab. ***If the LVEF meets requirements,*** bevacizumab should resume no sooner than 28 days after breast surgery. The surgical incision must be healed before resuming therapy. If bevacizumab cannot begin ≤ 84 days after surgery, postoperative bevacizumab may not be given.

h Infusion time for the postoperative bevacizumab should be at the rate that was tolerated preoperatively. If premedications were required for preoperative bevacizumab, premedications should continue to be administered with postoperative bevacizumab. See Table 29 in Section 11.3 for instructions regarding infusion-related or allergic reactions with bevacizumab.

i ***Bevacizumab must end by 18 months following study entry*** whether or not 10 postoperative cycles have been administered.

02/15/08 9.3 **Group 2A (docetaxel + capecitabine → AC)**

- Chemotherapy should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.***

TABLE 12. Treatment regimen for Group 2A

Drug	Dose	Dosing Interval	Planned Duration
Docetaxel	75 mg/m^2 IV over 60 minutes *(See footnote **a** for docetaxel pre-medication regimen.)*	Day 1 q 21 days	4 cycles
Capecitabine	825 mg/m^2 po BID	Days 1-14 q 21 days	
***21 days after Day 1 of the last cycle of docetaxel + capecitabine*[b]			
Doxorubicin	60 mg/m^2 IV over 15 minutes	Day 1 q 21 days	4 cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[c]			

a ***Pre-medication for docetaxel****:* All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the discretion of the investigator, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

b Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

c After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed.

02/15/08 9.4 **Group 2B (docetaxel + capecitabine + bevacizumab → AC + bevacizumab)**

- Chemotherapy should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.*** A delay in initiation of chemotherapy and bevacizumab of 1 week following vascular access device placement ***is recommended but not required.***

TABLE 13. Treatment regimen for Group 2B

Drug	Dose	Dosing Interval	Planned Duration
Bevacizumab	15 mg/kg IV over: 90 minutes – 1st dose**a** 60 minutes – 2nd dose**a** 30 minutes – all subsequent doses**a** Flush infusion line**b**	Day 1 q 21 days	**4** cycles
Docetaxel	75 mg/m^2 IV over 60 minutes (*See footnote* **c** *for docetaxel pre-medication regimen.)*		
Capecitabine	825 mg/m^2 po BID	Days 1-14 q 21 days	
21 days after Day 1 of the last cycle of docetaxel + capecitabine[d]			
Bevacizumab	15 mg/kg IV over 30 minutes**a** Flush infusion line**b**	Day 1 q 21 days	**2** cycles *(only with AC cycles 1 & 2)*
Doxorubicin	60 mg/m^2 IV over 15 minutes		**4** cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[e]			
At least 4-6 weeks after surgery, initiate postoperative bevacizumab[f]			
Bevacizumab	15 mg/kg IV over 30 minutes**g**	Every 21 days	**10** cycles**h**

a See Table 29 in Section 11.3 for instructions regarding infusion-related or allergic reactions with bevacizumab.

b Add an additional 50 mL of 0.9% NaCl for injection to the bevacizumab infusion bag (or use a new 50 mL bag of 0.9% NaCl) and infuse a volume equal to the volume contained in the tubing.

c ***Pre-medication for docetaxel****:* All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the investigator's discretion, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

d Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

e After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed (see Section 9.9.2 for important instructions regarding the timing of surgery).

f See Section 11.2 (Table 28) for the LVEF requirements for initiating postoperative bevacizumab. ***If the LVEF meets requirements,*** bevacizumab should resume no sooner than 28 days after breast surgery. The surgical incision must be healed before resuming therapy. If bevacizumab cannot begin ≤ 84 days after surgery, postoperative bevacizumab may not be given.

g Infusion time for the postoperative bevacizumab should be at the rate that was tolerated preoperatively. If premedications were required for preoperative bevacizumab, premedications should continue to be administered with postoperative bevacizumab. See Table 29 in Section 11.3 for instructions regarding infusion-related or allergic reactions with bevacizumab.

h ***Bevacizumab must end by 18 months following study entry*** whether or not 10 postoperative cycles have been administered.

02/15/08 9.5 **Group 3A (docetaxel + gemcitabine →AC)**

- Chemotherapy should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.***

TABLE 14. Treatment regimen for Group 3A

Drug	Dose	Dosing Interval	Planned Duration
Docetaxel	75 mg/m^2 IV over 60 minutes *(See footnote **a** for docetaxel pre-medication regimen.)*	Day 1 q 21 days	4 cycles
Gemcitabine	1000 mg/m^2 IV over 30 minutes	Day 1 and Day 8 q 21 days	
***21 days after Day 1 of the last cycle of docetaxel + gemcitabine*[b]**			
Doxorubicin	60 mg/m^2 IV over 15 minutes	Day 1 q 21 days	4 cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[c]			

a ***Pre-medication for docetaxel:*** All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the discretion of the investigator, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

b Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

c After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed.

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9.6 Group 3B (docetaxel + gemcitabine + bevacizumab → AC + bevacizumab)

- Chemotherapy should begin as soon as possible following randomization and collection of baseline blood and serum (if patient has consented).
- ***Central venous access is strongly recommended.*** A delay in initiation of chemotherapy and bevacizumab of 1 week following vascular access device placement ***is recommended but not required.***

TABLE 15. Treatment regimen for Group 3B

Drug	Dose	Dosing Interval	Planned Duration
Bevacizumab	15 mg/kg IV over: 90 minutes – 1st dose**a** 60 minutes – 2nd dose**a** 30 minutes – all subsequent doses**a** Flush infusion line**b**	Day 1 q 21 days	**4** cycles
Docetaxel	75 mg/m^2 IV over 60 min. (*See footnote* **c** *for docetaxel pre-medication regimen.)*		
Gemcitabine	1000 mg/m^2 IV over 30 minutes	Day 1 and Day 8 q 21 days	
21 days after Day 1 of the last cycle of docetaxel + gemcitabine[d]			
Bevacizumab	15 mg/kg IV over 30 minutes**a** Flush infusion line**b**	Day 1 q 21 days	**2** cycles *(only with AC cycles 1 and 2)*
Doxorubicin	60 mg/m^2 IV over 15 minutes		**4** cycles
Cyclophosphamide	600 mg/m^2 IV over 30 minutes		
BREAST SURGERY[e]			
At least 4-6 weeks after surgery, initiate postoperative bevacizumab[f]			
Bevacizumab	15 mg/kg IV over 30 minutes**g**	Every 21 days	**10** cycles**h**

a See Table 29 in Section 11.3 for instructions regarding infusion-related or allergic reactions with bevacizumab.

b Add an additional 50 mL of 0.9% NaCl for injection to the bevacizumab infusion bag (or use a new 50 mL bag of 0.9% NaCl) and infuse a volume equal to the volume contained in the tubing.

c ***Pre-medication for docetaxel:*** All patients should receive the following before each docetaxel dose:
- Dexamethasone 8 mg po BID the day before and the morning of chemotherapy. At the physician's discretion, dexamethasone may be continued after chemotherapy.
- At the investigator's discretion, other non-steroidal pre-medications such as diphenhydramine hydrochloride 50 mg IV and H-2 blocker IV (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) may be given in addition to the dexamethasone.

d Prior to initiation of AC, all adverse events must be resolved to ≤ grade 1, except ANC, which must be ≥ 1200/mm^3, and bilirubin, which must be ≤ the baseline grade.

e After recovery from chemotherapy and after the LVEF assessment before surgery (MUGA or echocardiogram) and final clinical response assessment have been performed (see Section 9.9.2 for important instructions regarding the timing of surgery).

f See Section 11.2 (Table 28) for the LVEF requirements for initiating postoperative bevacizumab. ***If the LVEF meets requirements,*** bevacizumab should resume no sooner than 28 days after breast surgery. The surgical incision must be healed before resuming therapy. If bevacizumab cannot begin ≤ 84 days after surgery, postoperative bevacizumab may not be given.

g Infusion time for the postoperative bevacizumab should be at the rate that was tolerated preoperatively. If premedications were required for preoperative bevacizumab, premedications should continue to be administered with postoperative bevacizumab. See Table 29, Section 11.3 for instructions if infusion-related or allergic reactions occur with bevacizumab.

h ***Bevacizumab must end by 18 months following study entry*** whether or not 10 postoperative cycles have been administered.

9.7 Dose determinations

9.7.1 *Calculation of BSA and chemotherapy doses*

- Based on the patient's weight and BSA, recommended chemotherapy and bevacizumab doses will be provided by the NSABP at study entry. Recalculations of BSA and doses are required if the patient has a ≥ 10 lb. weight change from baseline. Drug doses may be recalculated prior to each chemotherapy and bevacizumab cycle at the physician's discretion.

- The first postoperative bevacizumab dose should be recalculated.

- At the physician's discretion, the dose(s) may be capped using a BSA of 2.2 m^2.

9.7.2 *Rounding drug doses*

- Doxorubicin, cyclophosphamide, docetaxel, gemcitabine, and bevacizumab: rounding of doses is optional. If the treating physician decides to round the dose(s), follow these guidelines. (These also apply to dose modifications.)

  – ***Bevacizumab*** (15 mg/kg IV)

    Doses should be rounded to the nearest 5 mg.

  – ***Cyclophosphamide*** (600 mg/m^2 IV)

    Doses should be rounded to the nearest 25 mg.

  – ***Docetaxel*** (Group 1 – 100 mg/m^2 IV; Groups 2 and 3 – 75 mg/m^2 IV)

    Doses should be rounded to the nearest mg.

  – ***Doxorubicin*** (60 mg/m^2 IV)

    Doses should be rounded to the nearest mg.

  – ***Gemcitabine*** (1000 mg/m^2 IV on Days 1 and 8)

    Doses should be rounded to the nearest 25 mg.

- ***Capecitabine*** (825 mg/m^2 po BID)

  Refer to Appendix D for dose instructions based on BSA.

9.8 Supportive therapy

Patients should receive full supportive care.

9.8.1 *G-CSF*

- G-CSF ***must be used as primary prophylaxis*** during docetaxel cycles for Groups 1A and 1B patients (see Tables 10 and 11).
- G-CSF will be used as secondary prophylaxis during treatment with AC (all groups), docetaxel and capecitabine (Groups 2A and 2B), and docetaxel and gemcitabine (Groups 3A and 3B). Refer to the appropriate tables in Section 10.0. *(Note: Pegfilgrastim is not permitted with capecitabine.)*

9.8.2 *Prophylactic antibiotics*

At the investigator's discretion, primary prophylaxis with fluoroquinilones may be administered, e.g., levofloxacin 500 mg po qd or ciprofloxacin 500 mg po BID, administered. Other antibiotics may be used as an alternative in case of allergy prohibiting levofloxacin and ciprofloxacin.

Note: Prophylaxis with an antibiotic ***may not*** *be used as a substitute for required cytokine support or dose modifications.*

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9.8.3 *Erythropoietin*

Administration of erythropoiesis-stimulating agents should be consistent with the approved indication for cancer patients receiving chemotherapy in current package inserts, and in accordance with dose recommendations and modifications as well as safety warnings. Choice of agent is at the investigator's discretion.

9.8.4 *Mucositis prophylaxis for Groups 2A and 2B patients*

During docetaxel/capecitabine, it is recommended that patients in Groups 2A and 2B use nystatin mouth rinse TID Days 2 through 6 of each regimen. Salt and soda mouth rinse can also be used as needed.

9.8.5 *Antiemetic therapy*

Antiemetic therapy should be administered at the physician's discretion according to National Comprehensive Cancer Network (NCCN) or American Society of Clinical Oncology (ASCO) clinical practice guidelines.

9.9 Surgery

9.9.1 *Marking the primary tumor site prior to initiation of therapy*

Patients who are considered candidates for breast conservation should have the primary tumor site marked prior to initiating chemotherapy. This can be achieved with such methods as insertion of a radiopaque marker or clip, tattoos of the tumor boundary on the skin (especially for smaller breasts), or by making a transparent template with the tumor site marked on it. Other techniques are acceptable, as long as they provide assurance that the primary tumor site can be located and excised. If a clip is used, a specimen radiograph should be performed to confirm its removal.

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9.9.2 ***Breast surgery***

- As soon as possible following recovery from chemotherapy, final clinical tumor assessment, and preoperative LVEF assessment the patient should undergo a lumpectomy or mastectomy.

  For patients randomized to Groups 1B, 2B, and 3B, who complete 4 cycles of AC, breast surgery will occur at least 9 weeks following the last dose of bevacizumab. This schedule was selected to minimize the risk of bevacizumab increasing rates of postoperative wound complications. If for any reason the patient discontinues therapy before completion of the 4 cycles of neoadjuvant AC, surgery should be delayed at least 6 weeks following the last dose of bevacizumab. If the investigator determines that a 6-week delay is unacceptable for management of the breast cancer, surgery may take place sooner than 6 weeks but **MUST be delayed at least 4 weeks after the last dose of bevacizumab**.

- Breast conservation may be selected according to patient and surgeon preferences.
  - Patients who are deemed to be good candidates for breast conservation will undergo segmental excision of the primary tumor bed. If the residual tumor is non-palpable, methods to ensure adequate excision of the primary tumor site should be used to guide the excision. If tumor location was marked with clips, a specimen radiograph should be obtained intraoperatively to document that the lesion has been removed including the clips.
  - The margins of the resected specimen of patients treated with breast conservation must be histologically free of invasive tumor and DCIS. In patients for whom pathologic examination demonstrates tumor at the margin, additional operative procedures should be performed to obtain clear margins.
  - Patients who are not considered candidates for breast conservation or do not desire breast conservation will undergo a total mastectomy.

- Breast reconstruction
  - Important reminder: Surgical incisions must be healed before postoperative bevacizumab is administered.
  - For patients randomized to receive bevacizumab (Groups 1B, 2B, and 3B) who choose to have breast reconstruction using tissue expanders, expansion is prohibited within the 2 weeks prior to the first postoperative bevacizumab dose. Expansion or any surgical procedure (such as exchanging tissue expanders for permanent implants) is prohibited throughout therapy with bevacizumab and for at least 6 weeks after the last dose of bevacizumab. The following instructions must be employed:
    1. Tissue expanders can be placed at the time of mastectomy, with or without acellular dermis placed in the lower pole.
    2. Partial expansion in the operating room is allowed according to individual practitioner preference.

3. If the postoperative course proceeds normally, one or two expansions may be performed within the first 2 weeks BUT no later, since bevacizumab will be resumed as early as 4 weeks following surgery.

4. If prolonged postoperative drainage occurs (greater than 10 days), no additional expansion should be contemplated.

5. No attempt should be made to complete expansion prior to resuming bevacizumab nor to delay resuming bevacizumab for the purpose of expansion.

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9.9.3 *Axillary staging*

- Post-neoadjuvant therapy axillary staging is required for all patients.
- Use of sentinel node (SN) biopsy procedure following completion of neoadjuvant therapy is at the discretion of the investigator. If SN biopsy was not performed, surgical evaluation of the axilla is required.
  - If the post-neoadjuvant therapy SN biopsy is positive, additional surgical evaluation of axilla is required.
  - If the post-neoadjuvant therapy SN biopsy is negative, further surgical nodal staging procedure is not required. However, if the only SN identified by isotope scan is in the internal mammary nodal chain, the axilla should be explored for a blue or suspicious node.
- If axillary nodal staging procedures were performed before study entry (see Section 5.3.3), follow these guidelines.
  - *Even if FNA or core biopsy of an axillary node(s) was performed* ***before*** *initiation of neoadjuvant therapy*, surgical evaluation of the axilla (either SN biopsy and/or axillary dissection as described in the bullet above) must be performed following completion of neoadjuvant therapy, **regardless of whether FNA or core biopsy was positive or negative**.
  - *Performance of SN biopsy* ***prior to*** *initiation of neoadjuvant therapy is discouraged.* However, patients who undergo a SN biopsy prior to initiation of neoadjuvant therapy do not require post-therapy surgical evaluation of the axilla if the pre-neoadjuvant therapy SN biopsy was negative. If the pre-neoadjuvant therapy SN biopsy was positive, surgical evaluation of the axilla should be performed following completion of neoadjuvant therapy.

9.9.4 *Tissue processing and collection*

See Section 8.7 for recommended processing of tissue at the time of definitive surgery and Section 8.1 for tissue collection requirements. Also see Appendix E for suggested procedure for evaluation of postneoadjuvant therapy surgical specimens.

9.10 Postoperative radiation therapy

- Post-lumpectomy breast radiation therapy (RT) must be given.
- Partial breast irradiation techniques utilizing brachytherapy are not permitted for patients in either treatment group.
- Post-mastectomy chest wall RT is at the physician's discretion.
- Regional nodal RT is at the physician's discretion.
- For patients in Groups 1B, 2B, and 3B, RT may be administered concurrently with postoperative bevacizumab therapy.
- All other decisions about the RT method and fractionation are at the physician's discretion.

Refer to the NSABP Guidelines for Breast Cancer Radiation Therapy on the NSABP Web site.

9.11 Postoperative adjuvant hormonal therapy

Patients with ER-positive and/or PgR-positive tumors should receive a minimum of 5 years of hormonal therapy following completion of neoadjuvant chemotherapy and surgery. Selection of agents will be at the physician's discretion.

9.12 Non-protocol therapy guidelines

The following types of treatment, in addition to ***any cancer therapy other than that specified in the protocol***, are prohibited until the time of development of the first tumor progression or breast cancer recurrence or second primary cancer.

9.12.1 *Other postoperative adjuvant systemic therapy*

For patients who have received a total of 6 to 8 cycles of preoperative therapy, postoperative systemic therapy (other than bevacizumab [for patients in Groups 1B, 2B, and 3B] and hormonal therapy as described in Section 9.11) is prohibited. ***For any patient who received fewer than 6 preoperative chemotherapy cycles and who had residual disease at the time of surgery, postoperative chemotherapy is at the physician's discretion. However, postoperative bevacizumab may not be administered if postoperative chemotherapy is used.***

9.12.2 *Radiation therapy*

Partial breast irradiation techniques utilizing brachytherapy ***are not permitted for patients in either treatment group***.

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9.12.3 *Hormonal therapy*

The following types of hormonal therapy ***are prohibited***:

- SERMs other than tamoxifen and toremifene
- Sex hormonal therapy, e.g., estrogen or progesterone-replacement therapy (including low-dose estrogen in the form of vaginal cream) and hormonal contraceptives
- Femring®

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9.12.4 ***Recommendations for management of menopausal vaginal symptoms***

- Non-hormonal therapy interventions (e.g., Astroglide® or Replens®) are strongly encouraged for management of vaginal symptoms.
- *Although the use of vaginal hormonal therapies is not encouraged*, the following are permitted:
  - Conjugated estrogen ring (Estring®).
  - Vagifem® (permitted for patients who are unable to tolerate Estring®).

  For patients receiving an AI for adjuvant hormonal therapy, use of these products is strongly discouraged as they may increase circulating estradiol levels and reduce the effectiveness of the AI.

9.12.5 ***Trastuzumab***

Patients who are candidates for adjuvant trastuzumab (IHC 3+ or FISH positive) are excluded from participation in B-40. If analysis of residual disease following protocol therapy indicates the patient may be a candidate for trastuzumab (IHC 3+ or FISH positive), trastuzumab may be given at the investigator's discretion. However, if trastuzumab is administered, bevacizumab must not be given postoperatively.

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9.12.6 ***Participation in other clinical trials***

- Patients may participate in the Suppression of Ovarian Function Trial (SOFT).

- If a B-40 patient is considering participation in another clinical trial (including supportive therapy trials), contact the NSABP Clinical Coordinating Division (see Information Resources on page v).

10.0 CHEMOTHERAPY DOSE MODIFICATIONS AND DELAYS

Patients should receive 4 cycles of both of their assigned chemotherapy regimens unless there is evidence of progressive disease or excessive toxicity.

10.1 General instructions for dose modifications and delays for all patients

- Dose modifications are detailed in Tables 16 through 27.
- The Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE v3.0) must be used to grade the severity of adverse events (AEs).
- All doses should be based on the AE requiring the greatest modification.
- Doses that have been reduced may not be escalated.
- Chemotherapy should be held for at least 1 week until ***any AE requiring dose modification*** returns to ≤ grade 1. The exceptions are neutrophils, which must be ≥ 1200/mm^3, and bilirubin, which must be at or below baseline grade. If recovery to ≤ grade 1 has not occurred after 3 weeks of delay, protocol therapy must be discontinued. Further treatment is at the investigator's discretion.

10.2 Dose modification for docetaxel alone (Groups 1A and 1B)

- All dose modifications for docetaxel alone are based on the dose level changes in Table 16.
- Instructions for management of toxicities related to docetaxel are listed on Tables 17, 18, 24, and 25.
- The use of G-CSF as primary prophylaxis is **REQUIRED** for all Group 1A and Group 1B patients.
- If docetaxel is discontinued early due to toxicity, proceed to treatment with AC at Dose Level 0 when toxicities from docetaxel are ≤ grade 1. Exceptions are neutrophils, which must be ≥ 1200/mm^3, and bilirubin, which must be at or below the baseline grade.
- If docetaxel is discontinued early due to tumor progression, further treatment is at the discretion of the investigator. *(Note: For patients also receiving bevacizumab, surgery may occur no sooner than 28 days after the last dose of bevacizumab.)*

TABLE 16. Dose levels for docetaxel (Groups 1A and 1B)

	Dose Level 0 ***Starting Dose*** **(mg/m^2)**	**Dose Level -1 (mg/m^2)**	**Dose Level -2 (mg/m^2)**	**Dose Level -3**
Docetaxel	100	75	60	Discontinue

TABLE 17. Dose modifications and delays for docetaxel alone (Groups 1A and 1B) (Also see Tables 20, 24, and 25)

Important table instructions:

- Dose modifications must be based on AEs observed during the cycle (column 2) ***and*** AEs present on the scheduled cycle Day 1 (column 3).
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v3.0 Category/Grade	Modifications for AEs that occurred during a cycle but DID NOT REQUIRE A DELAY IN THE NEXT CYCLE OF TREATMENT[a]	Modifications for AEs that REQUIRED A DELAY IN THE NEXT TREATMENT CYCLE[b,c]
Blood/bone marrow:		
Neutrophils/granulocytes		
Grades 2 (1000-1199/mm^3), 3, 4	Maintain dose	**ANC**: *Hold until ≥ 1200/mm^3. If recovery takes:* 1 week – maintain dose 2 - 3 weeks – ↓ one dose level
Platelets		
Grades 2, 3	Maintain dose	**Platelets**: *Hold until ≥ 75,000/mm^3. If recovery takes:* 1 week – maintain dose 2 to 3 weeks - ↓ one dose level
Grade 4	↓ one dose level	↓ one dose level
GI (if related to chemotherapy):		
Diarrhea		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Mucositis/stomatitis		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Vomiting (*despite antiemetics)*		
Grade 2	↓ one dose level (optional)	↓ one dose level
Grades 3, 4	↓ one dose level or D/C	↓ two dose levels or D/C
Hepatic function:		*Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1.*
Bilirubin or AST or alk phos		
Grade 2	↓ one dose level	↓ one dose level
Grade 3	↓ two dose levels	↓ two dose levels
Grade 4	D/C	D/C
Infection:		
Febrile neutropenia/ Infection with grade 3 or 4 ANC		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Infection with normal ANC		
Grade 3	Maintain dose ***or*** ↓ one dose level	Maintain dose ***or*** ↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Other clinically significant AEs[d]:		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	D/C

a Treatment may not proceed until toxicity is ≤ grade 1, except for ANC/AGC which must be ≥ 1200/mm^3 and bilirubin, which must be ≤ the baseline grade.

b Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1.

c If toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, D/C protocol therapy. Further therapy is at the investigator's discretion.

d Determination of "clinically significant" AEs is at the discretion of the investigator.

10.3 **Dose modifications and delays for docetaxel and capecitabine (Groups 2A and 2B)**

- All dose modifications for docetaxel and capecitabine are based on the dose level changes outlined in Table 18.
- Instructions for management of toxicities related to docetaxel and capecitabine are listed on Table 19.
- Instructions for management of hand-foot skin reaction are listed on Table 20.
- Instructions for management of docetaxel-specific toxicities are listed on Tables 24 and 25.
- ***Please note that use of pegfilgrastim is prohibited with capecitabine. (Refer to footnote d on Table 19.)***
- If both docetaxel and capecitabine are discontinued early due to toxicity, proceed to treatment with AC at Dose Level 0 when toxicities from docetaxel and capecitabine are ≤ grade 1. Exceptions are neutrophils, which must be ≥ 1200/mm^3, and bilirubin, which must be at or below the baseline grade.
- If docetaxel and capecitabine are discontinued early due to tumor progression, further treatment is at the discretion of the investigator. *(Note: For patients also receiving bevacizumab, surgery may occur no sooner than 28 days after the last dose of bevacizumab.)*

TABLE 18. Dose levels for docetaxel and capecitabine (Groups 2A and 2B)

	Dose Level 0 ***Starting Dose*** **(mg/m^2)**	**Dose Level -1 (mg/m^2)**	**Dose Level -2 (mg/m^2)**	**Dose Level -3**
Docetaxel	75	60	50	Discontinue
Capecitabine	825 (BID)	650 (BID)	500 (BID)	Discontinue

TABLE 19. Dose modifications and delays for docetaxel and capecitabine (Groups 2A and 2B) (Also, see Tables 20, 24, and 25 for hand-foot skin reaction, neurosensory and musculoskeletal toxicities.)

Important table instructions:

- Dose modifications must be based on AEs observed during the cycle (column 2) ***and*** AEs present on the scheduled Cycle Day 1 (column 3).
- **UNLESS SPECIFIED OTHERWISE, MODIFICATIONS IN DOSE LEVELS APPLY TO BOTH DRUGS.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v3.0 Category/Grade	Modifications for AEs that occurred during a cycle but DID NOT REQUIRE A DELAY IN THE NEXT CYCLE OF TREATMENT[a]	Modifications for AEs that REQUIRED A DELAY IN THE NEXT TREATMENT CYCLE[b,c]
Blood/bone marrow: **Neutrophils/granulocytes** Grades 2 (1000-1199/mm^3), 3, 4	Maintain dose	**ANC:** *Hold until ≥ 1200/mm^3 If recovery takes:* 1 week - maintain dose and add filgrastim[c] 2-3 weeks - ↓ docetaxel one dose level & add filgrastim[d].
Platelets Grades 2, 3	Maintain dose	**Platelets:** *Hold until ≥ 75,000/mm^3 If recovery takes:* 1 week - maintain dose 2-3 weeks - ↓ docetaxel one dose level.
Grade 4	↓ docetaxel one dose level	↓ docetaxel one dose level
GI (if related to chemotherapy): **Diarrhea or mucositis** Grades 2, 3	Hold and delay treatment until ≤ grade 1. If resolved to ≤ grade 1, may resume capecitabine up to day 14. **Missed doses will not be made up.** 1st occurrence – ↓ capecitabine one dose level 2nd occurrence – ↓ docetaxel one dose level 3rd occurrence – discontinue capecitabine & maintain the docetaxel dose	
Grade 4	1st occurrence – ↓ docetaxel and capecitabine one dose level or D/C 2nd occurrence – discontinue capecitabine & maintain the docetaxel dose or D/C	
Vomiting (*despite antiemetics*) Grade 2	↓ one dose level (optional)	↓ one dose level
Grades 3, 4	↓ one dose level or D/C	↓ two dose levels or D/C
Dysphagia Grade 3	↓ capecitabine one dose level	↓ one dose level
Grade 4	↓ one dose level or D/C	D/C
Hepatic function: **Bilirubin, alk phos, or AST** Grade 2	↓ docetaxel one dose level	*Hold until bilirubin returns to baseline grade and AST and alk phos are ≤ grade 1.* ↓ docetaxel one dose level
Grade 3	↓ one dose level	↓ two dose levels
Grade 4	D/C	D/C
Infection including febrile neutropenia, infection with grade 3 or 4 ANC, and infection with normal ANC: Grade 3 (*First episode)*	Maintain dose; add filgrastim[d]	Maintain dose; add filgrastim[d]
Grade 3 (*Subsequent episodes)*	↓ one dose level	↓ one dose level
Grade 4 (*First episode)*	↓ docetaxel one dose level ; add filgrastim[d]	↓ docetaxel one dose level ; add filgrastim[d]
Grade 4 (*Subsequent episodes)*	D/C	D/C
Renal: **Serum creatinine** Grades 2, 3, 4	*Hold until serum creatinine ≤ grade 1* ***and*** *calculated creatinine clearance is > 50 mL/min. (see Appendix C). Maintain dose unless other AE requires dose reduction.*	
Other clinically significant AEs[e] Grade 3	↓ one dose level[e]	↓ one dose level[e]
Grade 4	↓ two dose levels or D/C[e]	D/C[e]

a Treatment may not proceed until toxicity is ≤ grade 1, except for ANC/AGC, which must be ≥ 1200/mm^3 and bilirubin, which must be ≤ the baseline grade.

b Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1.

c If toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, D/C protocol therapy. Further therapy is at the investigator's discretion.

d Begin filgrastim on Day 2 and continue according to drug package insert. ***Do not administer pegfilgrastim with capecitabine.***

e Determination of "clinically significant" AEs is at the discretion of the investigator. Also at investigator's discretion, the dose levels of capecitabine and docetaxel can be modified independently.

TABLE 20. Dose modifications and delays for hand-foot skin reaction (Groups 2A and 2B)

Hand-Foot Skin Reaction	Treatment Modifications*
Grade 2	1st occurrence – ↓ capecitabine one dose level 2nd occurrence – ↓ docetaxel one dose level 3rd occurrence – discontinue capecitabine & maintain the docetaxel dose
Grade 3	1st occurrence – ↓ docetaxel and capecitabine one dose level 2nd occurrence – ↓ capecitabine one additional dose level 3rd occurrence – discontinue capecitabine & maintain the docetaxel dose
* Hold and delay capecitabine until ≤ grade 1. If resolved to ≤ grade 1, may resume up to Day 14. **(Do not make up missed doses.)**	

10.4 Dose modifications and delays for docetaxel and gemcitabine (Groups 3A and 3B)

10.4.1 *Pulmonary toxicity related to gemcitabine (Groups 3A and 3B)*

- Hold gemcitabine if any of the following occur:
  - Dyspnea ≥ grade 2
  - Hypoxia ≥ grade 2
  - Pneumonitis/pulmonary infiltrates ≥ grade 2
  - Pulmonary fibrosis ≥ grade 2
  - Cough ≥ grade 3

- Rule out interstitial lung disease:
  - If non-infectious interstitial lung disease is confirmed, gemcitabine must be discontinued. Continuation of docetaxel will be at the discretion of the investigator.
  - If non-infectious interstitial disease is ruled out and infection (if any) has resolved, patients may resume treatment with persistent grade 2 dyspnea or hypoxia at the discretion of the investigator.

10.4.2 *Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura (HUS/TTP) possibly related to gemcitabine (Groups 3A and 3B)*

If persistent ≥ grade 2 thrombocytopenia is noted, evaluate for HUS/TTP. If confirmed, discontinue chemotherapy. The diagnosis of HUS should be considered if the patient develops anemia with evidence of microangiopathic hemolysis, elevation of bilirubin or LDH, reticulocytosis, severe thrombocytopenia, or renal insufficiency.

10.4.3 *Dose modifications for docetaxel and gemcitabine (Groups 3A and 3B)*

- All dose modifications for docetaxel and ***Day 1*** gemcitabine are based on the dose level changes outlined in Table 21.
- Instructions for management of toxicities related to docetaxel and ***Day 1*** gemcitabine are listed on Table 22.
- Instructions for dose modifications for ***Day 8*** gemcitabine are listed on Table 23.

- Instructions for management of docetaxel-specific toxicities are listed on Tables 24 and 25.
- If both docetaxel and gemcitabine are discontinued early due to toxicity, proceed to treatment with AC at Dose Level 0 when toxicities from docetaxel and gemcitabine are ≤ grade 1. Exceptions are neutrophils, which must be ≥ 1200/mm^3, and bilirubin, which must be at or below the baseline grade.
- If discontinued early due to tumor progression, further treatment is at the discretion of the investigator. *(Note: For patients receiving bevacizumab, surgery may occur no sooner than 28 days after the last dose of bevacizumab.)*

TABLE 21. Dose levels for docetaxel and Day 1 gemcitabine (Groups 3A and 3B)

	Dose Level 0 ***Starting Dose*** **(mg/m^2)**	**Dose Level -1 (mg/m^2)**	**Dose Level -2 (mg/m^2)**	**Dose Level -3**
Docetaxel	75	60	50	Discontinue
Gemcitabine	1000	750	600	Discontinue

TABLE 22. Dose modifications and delays for docetaxel and Day 1 gemcitabine (Groups 3A and 3B) (Also, see Table 23 for Day 8 gemcitabine instructions and Tables 24 and 25 for docetaxel-specific toxicities.)

Important table instructions:

- Dose modifications must be based on AEs observed during the cycle (column 2) ***and*** AEs present on the scheduled Cycle Day 1 (column 3).
- **UNLESS OTHERWISE SPECIFIED, MODIFICATIONS IN DOSE LEVELS APPLY TO BOTH DRUGS.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v3.0 Category/Grade	Modifications for AEs that occurred during a cycle but DID NOT REQUIRE A DELAY IN THE NEXT CYCLE OF TREATMENT[a]	Modifications for AEs that REQUIRED A DELAY IN THE NEXT TREATMENT CYCLE[b,c]
Blood/bone marrow:		
Neutrophils/granulocytes		
Grades 2 (1000-1199/mm^3), 3, 4	Maintain dose	**ANC**: *Hold until ≥ 1200/mm^3. If recovery takes:* 1 week – maintain dose 2 to 3 weeks – ↓ gemcitabine one dose level and add filgrastim.[d]
Platelets		**Platelets:** *Hold until ≥ 75,000/mm^3. If recovery takes:*
Grades 2, 3	Maintain dose	1 week – maintain dose 2 to 3 weeks - ↓ one dose level
Grade 4	↓ gemcitabine one dose level	↓ gemcitabine one dose level
GI (if related to chemotherapy):		
Diarrhea		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Mucositis/stomatitis		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Vomiting (*despite antiemetics*)		
Grade 2	↓ one dose level (optional)	↓ one dose level
Grades 3,4	↓ one dose level or D/C	↓ two dose levels or D/C
Hepatic function:		*Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1.*
Bilirubin or AST or alk phos		
Grade 2	↓ gemcitabine one dose level	↓ gemcitabine one dose level
Grade 3	↓ one dose level	↓ two dose levels
Grade 4	D/C	D/C
Infection: including febrile neutropenia, infection with grade 3/4 ANC; infection with normal ANC:		
Grade 3 *(First episode)*	Maintain dose; add filgrastim[d]	Maintain dose; add filgrastim[d]
Grade 3 *(Subsequent episodes)*	↓ one dose level	↓ one dose level
Grade 4 *(First episode)*	↓ one dose level; add filgrastim[d]	↓ one dose level; add filgrastim[d]
Grade 4 *(Subsequent episodes)*	D/C	D/C
Dermatology/skin:		
Grade 2	Maintain dose or ↓ gemcitabine one dose level	↓ gemcitabine one dose level
Grades 3, 4	D/C gemcitabine	D/C gemcitabine
Other clinically significant AEs[e]		
Grade 3	↓ one dose level[e]	↓ one dose level[e]
Grade 4	↓ two dose levels or D/C[e]	D/C[e]

a Treatment may not proceed until toxicity is ≤ grade 1, except for ANC/AGC, which must be ≥ 1200/mm^3 and bilirubin, which must be ≤ the baseline grade.

b Hold and check weekly. With exception of ANC/AGC, and bilirubin, resume treatment when toxicity is ≤ grade 1.

c If toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, D/C protocol therapy. Further therapy is at the investigator's discretion.

d Begin filgrastim on Day 2 and continue through Day 7. Management guidelines for treatment beyond Day 7 are provided on Table 23.

e Determination of "clinically significant" AEs is at the discretion of the investigator. Also at the investigator's discretion, the dose levels of gemcitabine and docetaxel can be modified independently.

TABLE 23. Dose modifications for Day 8 gemcitabine administration* (Groups 3A and 3B) *02/15/08*

NCI CTCAE v3.0 Category/Grade	Dose Modification
Blood/bone marrow:	
Neutrophils/granulocytes	
Grades 0-2	Treat at Day 1 gemcitabine dose given for that cycle
Grade 3	Treat at 75% of Day 1 gemcitabine dose given that cycle. • If the patient **was not** on secondary prophylaxis, administer pegfilgrastim 6 mg subcutaneously on Day 9. As an alternative, filgrastim can be initiated on Day 9 and continued through Day 15 and ANC > 5000. Use secondary prophylaxis for subsequent cycles. • If the patient **was** on secondary prophylaxis administer pegfilgrastim 6 mg subcutaneously on Day 9. As an alternative, filgrastim can be resumed on Day 9 and continued through Day 15 and ANC > 5000.
Grade 4	Omit Day 8 gemcitabine • If patient **was not** on secondary prophylaxis, use of prophylactic filgrastim will be required for subsequent cycles. Decision to manage Day 8 Grade 4 neutropenia with filgrastim or pegfilgrastim will be at investigator's discretion. • If patient **was** on secondary prophylaxis, continue daily filgrastim through Day 10 and ANC > 5000.
Platelets	
Grades 0-1	Treat at Day 1 gemcitabine dose given that cycle.
Grade 2	Treat at 75% of Day 1 gemcitabine dose given that cycle.
Grade 3-4	Omit Day 8 gemcitabine.
Non-hematologic toxicities:**	
Grade 1 - regardless of attribution	Treat at Day 1 gemcitabine dose for that cycle.
Grade 2 - related to gemcitabine	Omit Day 8 gemcitabine.
Grade 2 - not related to gemcitabine	Treat at Day 1 gemcitabine dose given for that cycle.
Grades 3, 4 - regardless of attribution	Omit Day 8 gemcitabine

* These dose modifications apply **only to Day 8 gemcitabine** (use the percentages listed on this table, not the dose levels in Table 21). The patient will resume the appropriate Day 1 dose of gemcitabine using the dose levels on Table 21 with the next cycle of chemotherapy.

** Determination of attribution will be at the investigator's discretion.

10.5 **Management of docetaxel-related neurosensory toxicity (all groups)**

TABLE 24. Dose modifications for neurosensory toxicity related to docetaxel

Paresthesias/Dysesthesias	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed**
Grade 1 – Paresthesias/dysesthesias that do not interfere with function	Maintain dose	Maintain dose
Grade 2 – Paresthesias/dysesthesias interfering with function, but not activities of daily living	Maintain dose[a]	Decrease ***docetaxel*** one dose level[b]
Grade 3 – Paresthesias/dysesthesias with pain or with function impairment that also interfere with activities of daily living	**First episode:** Decrease ***docetaxel*** one dose level[a] **Second episode:** Discontinue ***docetaxel***	Discontinue ***docetaxel***
Grade 4 – Persistent paresthesias/dysesthesias that are disabling or life-threatening	Discontinue ***docetaxel***	Discontinue ***docetaxel***

a Must be resolved to ≤ grade 1 on Day 1 of the next cycle.
b Hold chemotherapy for persistent grade 2 neurosensory toxicity. When ≤ grade 1, resume treatment with dose modification. If grade 2 toxicity persists after 3 weeks of delay, discontinue docetaxel and proceed with other chemotherapy (if applicable).

10.6 **Management of docetaxel-related musculoskeletal pain (all groups)**

TABLE 25. Dose modifications for musculoskeletal pain attributed to docetaxel and *not controlled by analgesics**

Musculoskeletal Pain	**1 – 7 Days Duration**	**Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed**
Grade 1	Maintain dose	Maintain dose
Grade 2	Maintain dose	Maintain dose OR Decrease ***docetaxel*** one dose level
Grade 3	**First episode:** Decrease ***docetaxel*** one dose level **Second episode:** Discontinue ***docetaxel***	**First episode:** Decrease ***docetaxel*** one dose level OR Discontinue ***docetaxel*** **Second episode**: Discontinue ***docetaxel***
Grade 4	Discontinue ***docetaxel***	Discontinue ***docetaxel***

* Use of narcotics and NSAIDs is encouraged to maintain dose of docetaxel if possible.

10.7 Dose modifications and delays for AC (all groups)

10.7.1 *Left ventricular dysfunction*

Discontinue AC if the patient develops ≥ grade 2 left ventricular systolic dysfunction or ≥ grade 3 left ventricular diastolic dysfunction. Refer to Section 11.2 for additional instructions.

10.7.2 *Dose modifications for AC*

- All dose modifications for AC are based on the dose level changes in Table 26.
- Instructions for management of toxicities related to AC are listed on Table 27.
- If secondary prophylactic use of G-CSF was required during treatment with the docetaxel/capecitabine or docetaxel/gemcitabine regimens, or dose reduction due to infection was required with docetaxel alone, it should be continued during treatment with AC. If secondary prophylaxis with G-CSF was not required with docetaxel/capecitabine or docetaxel/gemcitabine or infections did not occur with docetaxel supported by primary prophylaxis with G-CSF, primary prophylaxis with G-CSF for AC will be at the investigator's discretion.
- If AC is discontinued early:
  - Due to toxicity, the patient should proceed to surgery.
  - Due to tumor progression, all study therapy must be discontinued and further treatment is at the discretion of the investigator. *Note: See Section 9.9.2 for instructions regarding the timing of surgery for patients who have received bevacizumab. For patients who receive fewer than a total of 6 cycles of preoperative chemotherapy and have residual disease at the time of surgery, refer to Section 9.12.1 for instructions regarding postoperative therapy.*

TABLE 26. Dose levels for AC (all groups)

	Dose Level 0 ***Starting Dose*** **(mg/m^2)**	**Dose Level -1 (mg/m^2)**	**Dose Level -2 (mg/m^2)**	**Dose Level -3**
Doxorubicin	60	50	40	Discontinue
Cyclophosphamide	600	500	400	Discontinue

TABLE 27. Dose modifications and delays for AC (all groups)

Important table instructions:

- Dose modifications must be based on AEs observed during the cycle (column 2) ***and*** AEs present on the scheduled Cycle Day 1 (column 3).
- **ALL MODIFICATIONS IN DOSE LEVELS APPLY TO BOTH DRUGS.**
- Dose modifications must be based on the AE requiring the greatest modification.

NCI CTCAE v3.0 Category/Grade	Modifications for AEs that occurred during a cycle but DID NOT REQUIRE A DELAY IN THE NEXT CYCLE OF TREATMENT[a]	Modifications for AEs that REQUIRED A DELAY IN THE NEXT TREATMENT CYCLE[b,c]
Blood/bone marrow:		
Neutrophils/granulocytes		
Grades 2 (1000-1199/mm^3), 3, 4	Maintain dose	**ANC**: *Hold until ≥ 1200/mm^3. If recovery takes:* 1 week – maintain dose and add G-CSF[d] 2 - 3 weeks – ↓ one dose level and add G-CSF[d]
Platelets		
Grades 2, 3	Maintain dose	**Platelets**: *Hold until ≥ 75,000/mm^3. If recovery takes:* 1 week – maintain dose 2 to 3 weeks - ↓ one dose level
Grade 4	↓ one dose level	↓ one dose level
GI (if related to chemotherapy):		
Diarrhea		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Mucositis/stomatitis		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Vomiting (*despite antiemetics)*		
Grade 2	↓ one dose level (optional)	↓ one dose level
Grades 3, 4	↓ one dose level or D/C	↓ two dose levels or D/C
Hepatic function:		
Bilirubin or AST or alk phos		*Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1.*
Grade 2	↓ one dose level	↓ one dose level
Grade 3	↓ two dose levels	↓ two dose levels
Grade 4	D/C	D/C
Infection including febrile neutropenia, infection with grade 3 or 4 ANC, and infection with normal ANC:		
Grade 3 *(First episode)*	Maintain dose & add G-CSF[d]	Maintain dose & add G-CSF[d]
Grade 3 *(Subsequent episodes)*	↓ one dose level	↓ one dose level
Grade 4 *(First episode)*	↓ one dose level; add G-CSF[d]	↓ one dose level; add G-CSF[d]
Grade 4 *(Subsequent episodes)*	D/C	D/C
Other clinically significant AEs[e]:		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	D/C

a Treatment may not proceed until toxicity is ≤ grade 1, except for ANC/AGC which must be ≥ 1200/mm^3 and bilirubin, which must be ≤ the baseline grade.

b Hold and check weekly. With exception of ANC/AGC and bilirubin, resume treatment when toxicity is ≤ grade 1.

c If toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, discontinue AC (and preoperative bevacizumab) and proceed to surgery.

d Add G-CSF *if not initiated previously*. Pegfilgrastim is preferred, at a fixed dose of 6 mg SQ on Day 2. Filgrastim, if used, should be administered according to the drug package insert.

e Determination of "clinically significant" AEs is at the discretion of the investigator.

11.0 BEVACIZUMAB TREATMENT DELAYS

Instructions in Section 11.0 are for Groups 1B, 2B, and 3B patients.

02/15/08

11.1 General instructions

- The CTCAE v3.0 must be used to grade the severity of adverse events (AEs).
- Treatment decisions should be based on the AE requiring the greatest modification.
- There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain 15 mg/kg when treatment resumes.
- Bevacizumab must end 18 months following study entry, regardless of treatment delays due to toxicity or for any other reason (even if all 10 postoperative doses have not been administered).
- If chemotherapy is held due to chemotherapy-related adverse events, bevacizumab must also be held.
- If ***all*** remaining chemotherapy is discontinued for a ***chemotherapy-related toxicity*** before completion of the scheduled 8 cycles of chemotherapy, preoperative bevacizumab may not be continued as a single agent and the patient should proceed to surgery. (Postoperative bevacizumab may be administered.)
- If alternative (non-protocol) therapy is given at any time, bevacizumab must be discontinued.
- If tumor progression occurs, bevacizumab must be discontinued.
- If bevacizumab must be discontinued before completion of chemotherapy, the remaining chemotherapy cycles should be continued as per protocol. *For patients in Groups 1B, 2B, and 3B, bevacizumab may not be re-initiated postoperatively.*
- Instructions regarding cardiac function adverse events are described in Section 11.2 (Table 28). See Section 11.3 (Table 29) for other adverse events requiring delays or permanent discontinuation of bevacizumab.

11.2 Cardiac adverse events

11.2.1 *Asymptomatic decrease in LVEF*

- To determine if the patient has had an asymptomatic decrease in LVEF, all patients will undergo a scheduled LVEF assessment 3-4 weeks after the post-chemotherapy cycle (before surgery) using the *method (MUGA or echocardiogram) that was performed at baseline*. Also, patients in Groups 1B, 2B, and 3B who receive any bevacizumab (preoperatively or postoperatively) will undergo a scheduled LVEF assessment at 18 months after study entry.

  For patients in Groups 1B, 2B, and 3B, the result of the LVEF assessment performed following the last chemotherapy cycle (before surgery) will be used to determine if bevacizumab can be resumed postoperatively as outlined on Table 28. Depending on the LVEF result at this time point, additional LVEF assessments may be required for the initiation and continuation of postoperative bevacizumab (see Table 28 for additional instructions).

- In asymptomatic Groups 1B, 2B, and 3B patients, the decision to continue or stop bevacizumab is based upon two factors:
  - the measured ejection fraction as it relates to the cardiac imaging facility's lower limit of normal. *Note: If the cardiac imaging facility cannot provide a LLN, use 50% as the LLN value.*
  - the change in ejection fraction from baseline.
- Table 28 summarizes the LVEF requirements for bevacizumab therapy in asymptomatic patients. ***Bevacizumab may not be administered to patients who have a symptomatic decrease in LVEF (see Section 11.2.2).***

TABLE 28. Summary of LVEF requirements for continuation of bevacizumab in ***asymptomatic*** patients (Groups 1B, 2B, and 3B)

Absolute change in LVEF between baseline (before study entry) and any assessment performed following study entry	**Instructions for continuation of bevacizumab and for repeat LVEF assessments**
Increase or no change from baseline	Continue bevacizumab
No more than 15 percentage points below baseline LVEF **and** No more than 5 percentage points below the cardiac imaging facility's LLN	Continue bevacizumab
16 or more percentage points below baseline LVEF **or** 6 or more percentage points below the cardiac imaging facility's LLN	**HOLD BEVACIZUMAB** Follow instructions below based on specific LVEF time points*

*INSTRUCTIONS WHEN BEVACIZUMAB IS HELD:

- **Based on the scheduled post-chemotherapy LVEF assessment (prior to surgery):** Repeat the LVEF assessment 3-5 weeks after surgery.
  - If the repeat LVEF does not meet the criteria to continue bevacizumab, ***bevacizumab may NOT be initiated postoperatively***.
  - If the repeat LVEF meets the criteria to continue bevacizumab postoperatively, postoperative bevacizumab may be initiated. *However, for these patients, an additional LVEF assessment will be required after 4 postoperative doses of bevacizumab. If the LVEF obtained after 4 postoperative doses does NOT meet criteria for continuing bevacizumab,* ***postoperative bevacizumab must be permanently discontinued***.
- **Based on a discretionary LVEF assessment performed at any time during bevacizumab therapy:** The investigator should repeat the LVEF assessment in 4 weeks.
  - If the repeat LVEF meets criteria to continue bevacizumab, bevacizumab may be resumed.
  - If the repeat LVEF does NOT meet criteria to continue bevacizumab, ***further bevacizumab therapy may not be given***.

11.2.2 ***Symptomatic decrease in LVEF***

- *Congestive heart failure (grade 3)*: Patients should be monitored for signs and symptoms of CHF (i.e., dyspnea, tachycardia, cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, etc.). ***If the patient develops these signs and symptoms, bevacizumab (and chemotherapy) must be held.***

  The investigator must confirm the diagnosis of CHF with an LVEF assessment (MUGA scan or echocardiogram). A chest x-ray is also required.

  If the diagnosis of CHF is confirmed, bevacizumab and AC are prohibited. If CHF occurs during the docetaxel-based regimen and bevacizumab, continuation of the docetaxel-based chemotherapy regimen is at the investigator's discretion.

  Report ≥ grade 3 left ventricular systolic dysfunction and left ventricular diastolic dysfunction in an expedited manner via AdEERS (see Section14.2.2). All reports and supporting documentation must be submitted with Form LVA to the NSABP Biostatistical Center within 4 weeks following the LVEF assessment.

  The protocol-specified schedule for obtaining LVEF assessments should be followed even after the discontinuation of protocol therapy or occurrence of a cardiac event.

- *Severe refractory CHF or requiring intubation (grade 4)*: Discontinue all study therapy and submit Form LVA (if MUGA or echocardiogram was performed), and supporting documentation.

11.2.3 ***Cardiac ischemia/infarction***

If the patient develops grade 3 or 4 cardiac ischemia/infarction, protocol therapy must be discontinued. Further therapy is at the investigator's discretion.

11.2.4 ***Other cardiac symptoms***

- If the patient develops grade 2, 3, or 4 cardiac conduction abnormality, grade 3 or 4 supraventricular arrhythmia, or grade 2, 3, or 4 ventricular arrhythmia during the docetaxel-based regimen and bevacizumab, protocol therapy should be discontinued. When symptoms have resolved to ≤ grade 1, the patient should proceed to surgery. ***Postoperative bevacizumab is prohibited.***
- If these conditions occur while receiving AC and bevacizumab, ***AC and bevacizumab must be discontinued***. When symptoms have resolved to ≤ grade 1, the patient should proceed to surgery. ***Postoperative bevacizumab is prohibited.***

11.3 **Bevacizumab delays and instructions for non-cardiac toxicities**

Refer to Table 29 for a summary of bevacizumab instructions for non-cardiac adverse events.

TABLE 29. Bevacizumab treatment delays and instructions for **non-cardiac** adverse events *(See Section 11.2 for cardiac adverse events.)* *02/15/08*

Adverse Event	Grade CTCAE v3.0	Action to be Taken
Cytokine release syndrome/acute infusion reaction **or** **Allergic reaction/hyper-sensitivity (e.g., fever, rash, urticaria, bronchospasm)**	1, 2 or 3	- *If infusion-related or allergic reactions occur, pre-meds should be given with the next dose*, but the infusion time may not be ↓ for the subsequent infusion. If the next dose is well-tolerated with pre-meds, the subsequent infusion time may be ↓ by 30 ± 10 min. as long as pre-meds continue to be used. If infusion-related AEs occur with the 60-min. infusion, all subsequent doses should be given over 90 ± 15 min. (with pre-meds). If infusion-related AEs occur with the 30-min. infusion, all subsequent doses should be given over 60 ± 10 min. (with pre-meds). - *For patients with grade 3 reactions*, the bevacizumab infusion should be stopped and not re-started on that day. At the physician's discretion, bevacizumab may be permanently discontinued or re-instituted with pre-medications and at a rate of 90 ± 15 minutes. If the reaction occurred at the 90-minute rate, initially challenge at a slower infusion rate and gradually increase to 90 minutes. When bevacizumab is re-instituted, the patient should be monitored, per physician's usual practice, for a duration comparable to duration of reaction.
	4	Permanently discontinue bevacizumab.
Hemorrhage[a]	3 or 4	Permanently discontinue bevacizumab.
Thrombosis/thrombus/ embolism-venous (including vascular access device)	2 or 3	Hold bevacizumab until clinical resolution. • If the planned duration of full-dose anticoagulation is < 2 weeks, hold bevacizumab until anticoagulation is complete. • If the planned duration of full-dose anticoag is ≥ 2 weeks, bevacizumab may be resumed during anticoag if no grade 3 or 4 hemorrhage event occurred while on therapy ***and*** coagulation testing shows results within the therapeutic range. Discontinue bevacizumab if thromboembolic events worsen or recur after resuming therapy.
	4	Permanently discontinue bevacizumab.
Visceral or peripheral arterial ischemia	2**[b,c]**, 3 or 4	Permanently discontinue bevacizumab.
CNS ischemia	3 or 4	Permanently discontinue bevacizumab.
Signs/symptoms of RPLS (see Section 15.4.2)	≥ 1	For clinical features suggestive of RPLS, hold bevacizumab and obtain MRI. If RPLS is diagnosed ***or*** if ANY symptoms were grade 4, permanently D/C bevacizumab. If RPLS is not diagnosed, bevacizumab may be resumed when presenting symptoms are ≤ grade 1 ***and*** blood pressure < 150/90.
GI perforation including GI leak and GI fistula	≥1	Permanently discontinue bevacizumab.
Non-GI fistula	≥ 1	Permanently discontinue bevacizumab.
Intra-abdominal abscess[d]	3	Hold bevacizumab until resolved.
	4	Permanently discontinue bevacizumab.
Complication, non-infectious-wound dehiscence[e]	1	Hold bevacizumab for at least 1 month. If, in the physician's opinion, substantial healing has taken place within 1-3 months, bevacizumab may be resumed. If wound dehiscence recurs, permanently discontinue bevacizumab.
	2, 3, or 4	Permanently discontinue bevacizumab.
Proteinuria[f]	3	Hold bevacizumab until UPC ratio improves to ≤ grade 2. Re-check UPC ratio every 3-6 weeks. If UPC ratio does not improve to ≤ grade 2 within 6 weeks, permanently discontinue bevacizumab.
	4	Permanently discontinue bevacizumab.
Hypertension	3	Bevacizumab may be continued in conjunction with standard anti-hypertensive therapy at physician discretion. Bevacizumab should be held for uncontrolled or symptomatic hypertension present on the day that the bevacizumab dose is to be given. If BP is not controlled with medication within 1 month, permanently discontinue bevacizumab.
	4	Permanently discontinue bevacizumab.
Other clinically significant AEs[g]	3	Hold until AE has resolved to ≤ grade 1.
	4	Permanently discontinue bevacizumab.

a If coagulation disorders develop secondary to other medical conditions, hold bevacizumab until the PT/INR and PTT return to ≤ grade 1.
b New grade 2 events. (Therapy may be continued for grade 2 conditions present at baseline.)
c Pts. who develop brief, reversible, exercise-induced claudication (grade 2) not attributable to arterial thromboembolic events may continue on study.
d Refer to grading criteria listed for the appropriate adverse event in the Infection Section of the CTCAE v 3.0.
e Refer to Dermatology/Skin Section of the CTCAE v 3.0.
f For the purpose of this study, CTCAE v3.0 criteria for grading proteinuria in g/24 hours will be estimated by UPC ratio (see Appendix B).
g Determination of "clinically significant" is at the physician's discretion and applies to those ***adverse events that are not clearly associated with chemotherapy and could be related to bevacizumab.***

12.0 ASSESSMENT OF EFFECT

12.1 Clinical assessment of effect

For the purposes of this study, all clinical measurements will be assessed by physical examination of the breast and the axilla. Measurements, response, and progression will be determined using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee.[127]

02/15/08

12.1.1 *Timing of clinical assessments*

Clinical tumor measurement is obtained at baseline (prior to study entry), at the completion of the assigned docetaxel regimen (before starting AC therapy), and at the conclusion of the sequential regimens (prior to surgery). It is recommended that patients also be examined before each cycle of preoperative therapy to ensure that there is no disease progression. *In the event of progressive disease (defined below), treatment will be at the discretion of the investigator.*

12.1.2 *Definition of target and non-target lesions at baseline*

During the baseline assessment, all lesions detected in the breast and the axilla are classified as either target lesions or non-target lesions before the start of protocol treatment.

- **Target lesions include:**
  - Breast tumors ≥ 2.0 cm on baseline physical examination
  - Axillary nodes ≥ 2.0 cm on baseline physical examination
- **Non-target lesions include:**
  - Breast tumors < 2.0 cm on baseline physical examination
  - Axillary nodes < 2.0 cm on baseline physical examination

12.1.3 *Clinical measurement*

For each target lesion in the breast and the axilla, the longest unidimensional measurement *(longest diameter, LD)* is recorded. **The clinical measurement for this assessment is the sum of the LD measurements of the target lesions.**

02/15/08

12.1.4 *Criteria for evaluation of clinical response*

The following criteria will be employed at the completion of the assigned docetaxel regimen (before starting AC therapy) and at the conclusion of the sequential regimens (before surgery). These criteria are summarized in Table 30.

- *Clinical complete response (cCR)*

  Disappearance of all target and non-target lesions identified at baseline, with no evidence of disease progression.

- *Clinical partial response (cPR)*

  At least a 30% decrease in the sum of the longest diameter (LD) of target lesions (taking as reference the baseline sum of the longest diameter). No definite progression of non-target lesions. No evidence of new lesions.

- *Clinical stable disease (cSD)*

  Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

- *Progressive disease (PD)*

  At least a 20% increase in the sum of the longest diameter (LD) of the target lesions (taking as reference the smallest sum of the LD recorded). Other manifestations of progressive disease would also be classified as disease progression, e.g.: appearance of one or more new lesions in the breast, regional lymph nodes or distant sites; unequivocal progression of existing non-target lesions; appearance of inflammatory carcinoma on clinical exam.

TABLE 30. Criteria for evaluation of clinical response

Target Lesions	**Non-Target Lesions**	**New Lesions or Other Signs of Progression**	**Clinical Response Classification**
CR	CR	No	CR
CR	Incomplete response or SD	No	PR
PR	Non-PD*	No	PR
SD	Non-PD*	No	SD
PD	Any category	Yes or No	PD
Any category	PD*	Yes or No	PD
Any category	Any category	Yes	PD

* For non-target lesions, PD is defined as the unequivocal progression, as determined by the investigator, of existing non-target lesions.

12.2 Pathologic assessment of effect

12.2.1 *Timing of evaluation*

The determination of pCR will be performed by the local pathologist following examination of tissue (breast and nodes) removed at the time of surgery. See Appendix E for the suggested procedures for evaluation of surgical specimens for pCR.

12.2.2 *Criteria for evaluation of pathologic complete response*

- *Pathologic complete response in the breast (pCR breast)*

  No histologic evidence of invasive tumor cells in the surgical breast specimen.

- *Pathologic complete response in breast and axillary lymph nodes as well as non-axillary sentinel nodes (pCR breast & nodes)*

  No histologic evidence of invasive tumor cells in the surgical breast specimen, axillary nodes, and sentinel nodes identified after neoadjuvant chemotherapy.

13.0 DIAGNOSIS OF BREAST CANCER RECURRENCE AND OTHER CANCER EVENTS

- The diagnosis of a first breast cancer recurrence or second primary cancer can be made only when both the clinical and laboratory findings meet "acceptable" criteria as defined below. Suspicious findings do not constitute criteria for breast cancer recurrence, nor are they an indication to alter protocol therapy.
- Please submit a copy of the clinic/office note summarizing the work-up and treatment plan for a recurrence or a second primary cancer.
- Treatment of a breast cancer recurrence or second primary cancer will be at the discretion of the investigator.

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13.1 Local recurrence

Recurrent tumor is defined as evidence of invasive or in situ breast cancer (except LCIS) in the ipsilateral breast or skin of the breast. Patients who develop clinical evidence of tumor recurrence in the remainder of the ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis.

- Acceptable: positive cytology or histologic biopsy

13.1.1 *Ipsilateral breast tumor recurrence (IBTR)*

An IBTR event is defined as recurrent tumor in either the ipsilateral breast parenchyma or skin of the breast occurring after lumpectomy.

13.1.2 *Other local recurrence*

Defined as recurrence in the skin of the chest wall (exclusive of the breast) or chest wall.

13.2 Regional recurrence

Defined as the development of tumor in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral infraclavicular and/or ipsilateral axillary nodes, as well as the soft tissue of the ipsilateral axilla, after operation.

- Acceptable: positive cytology or histologic biopsy

13.3 Distant recurrence

Defined as evidence of tumor in any area of the body, with the exception of those described in Sections 13.1 and 13.2.

13.3.1 *Skin, subcutaneous tissue, and lymph node (other than local or regional) metastases*

- Acceptable: (i) positive cytology, histologic biopsy, or (ii) radiologic evidence of metastatic disease

13.3.2 ***Bone marrow metastasis***

- Acceptable: (i) positive cytology, histologic biopsy, or (ii) MRI scan

13.3.3 ***Lung metastasis***

- Acceptable: (i) positive cytology, histologic biopsy, or (ii) radiologic evidence of multiple pulmonary nodules that are judged to be consistent with pulmonary metastases

NOTE: If a solitary lung lesion is found and no other lesions are present on lung tomograms, CT scan, or MRI scan, further investigations such as biopsy or needle aspiration must be performed. Proof of neoplastic pleural effusion must be established by cytology or pleural biopsy.

13.3.4 ***Skeletal metastasis***

- Acceptable: (i) x-ray, CT, or MRI evidence of lytic or blastic lesions consistent with bone metastasis; or (ii) biopsy proof of bone metastases; or (iii) bone scan that is clearly positive for bone metastases

NOTE: If the diagnosis is equivocal by bone scan or radiologic evaluation, a biopsy is strongly recommended. Any positive bone scan in joints or in a recent area of trauma (surgical or otherwise) cannot be used as a criterion for breast cancer recurrence.

13.3.5 ***Liver metastasis***

- Acceptable: (i) an abdominal CT scan, liver scan, ultrasound, or MRI consistent with liver metastases, or (ii) liver biopsy confirmation of the metastatic disease

NOTE: If the radiologic findings are not definitive (especially with solitary liver nodules), a liver biopsy is recommended; however, if a biopsy is not performed, serial scans must be obtained to document stability or progression.

13.3.6 ***Central nervous system metastasis***

- Acceptable: (i) positive CT scan or MRI scan, usually in a patient with neurological symptoms, or (ii) biopsy or cytology (for a diagnosis of meningeal involvement)

13.4 **Second primary breast cancer**

Defined as evidence of invasive or in situ breast cancer (except LCIS) in the contralateral breast or chest wall. The diagnosis of a second primary breast cancer must be confirmed histologically.

- Acceptable: positive histologic biopsy

13.5 Second primary cancer (non-breast)

Any non-breast second primary cancer other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, or colon carcinoma in situ will be considered an event in the analysis of DFS. The diagnosis of a second primary cancer must be confirmed histologically whenever possible.

13.6 Documentation requested following death

- Autopsy reports should be secured whenever possible and should be submitted to the NSABP Biostatistical Center.
- A copy of the death certificate should be forwarded to the NSABP Biostatistical Center if it is readily available or if it contains important cause-of-death information that is not documented elsewhere.
- Please submit the last clinic/office note made before the death or the physician's note summarizing events resulting in death.

05/30/07 14.0 ADVERSE EVENT REPORTING REQUIREMENTS

Please refer to Coordinator Online in the Members' Area of the NSABP Web site for general information regarding adverse event reporting.

14.1 B-40 definitions for adverse event reporting

14.1.1 *Investigational agent*

The investigational agents in B-40 are ***capecitabine, gemcitabine***, ***and bevacizumab***. Capecitabine and gemcitabine are being tested as neoadjuvant therapy for breast cancer; bevacizumab is being tested as both neoadjuvant and adjuvant therapy.

14.1.2 *Commercial agents*

The commercial agents in B-40 are ***cyclophosphamide, doxorubicin, and docetaxel***.

14.1.3 *Investigational combination therapy*

This study includes both investigational and commercial agents. When an investigational agent (***gemcitabine, capecitabine, or bevacizumab***) is administered concurrently or sequentially with a commercial agent (***cyclophosphamide, doxorubicin, or docetaxel),*** and an adverse event occurs that is expected for the commercial agent(s), but is not listed for the investigational agent, the adverse event should be considered expected for the combination. However, ***if based on clinical judgment, the investigator believes the adverse event is possibly, probably, or definitely related to the investigational agent*** (gemcitabine, capecitabine, or bevacizumab) ***rather than the commercial agent(s)*** (cyclophosphamide, doxorubicin, or docetaxel), the adverse event should then be considered unexpected for the combination.

14.1.4 *Adverse event assessment*

The NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0 must be used to identify the type and to grade the severity of adverse events in B-40.

14.2 Expedited reporting of adverse events

The NSABP follows procedures for centralized reporting of adverse events. Adverse events must be reported to the NSABP Biostatistical Center. The NSABP forwards reports to the appropriate regulatory agencies and pharmaceutical companies involved in the trial. B-40 utilizes the NCI's Adverse Event Expedited Reporting System (AdEERS) for all expedited reporting of adverse events. NCI's guidelines for creating an AdEERS report can be found at http://ctep.cancer.gov.

The NSABP Biostatistical Center is identified in AdEERS as the NSABP Lead Group for NSABP protocols which require AdEERS reporting. An AdEERS report must be submitted to the NSABP Lead Group using the electronic web-based application located at http://ctep.cancer.gov. If Web access is not available, the NCI Adverse Event Expedited Report-Multiple Agents template (located at http://ctep.cancer.gov) must be completed and faxed to the NSABP Biostatistical Center at **(412) 622-2113**. When initiating an AdEERS report, the reporter will be directed to refer to the protocol for expedited reporting requirements.

14.2.1 ***Expedited reporting methods***

- **AdEERS-24 Hour Notification:** requires that an AdEERS 24-hour notification is electronically submitted to the NSABP Lead Group **within 24 hours** of learning of the adverse event. Each AdEERS 24-hour notification must be followed by a complete AdEERS report within 5 calendar days of learning of the event.

- **AdEERS 5 Calendar Day Report:** requires that an AdEERS report is electronically submitted to the NSABP Lead Group **within 5 calendar days** of the investigator learning of the adverse event.

- Supporting documentation is required for all expedited (AdEERS) reports. Include the protocol number, patient's study number, and AdEERS ticket number on each page, and fax supporting documentation to the NSABP Biostatistical Center at **(412) 622-2113**.

14.2.2 ***Expedited reporting requirements – AdEERS-24, AdEERS and other protocol requirements***

Expedited reporting requirements begin with the administration of the first dose of B-40 study therapy. Expedited reporting requirements including protocol-specific requirements and exceptions for expedited reporting for all B-40 patients who receive study therapy are provided in Table 31 and Table 32.

TABLE 31. AdEERS expedited reporting requirements for adverse events that occur within 30 days of the last dose of an **investigational agent** (gemcitabine, capecitabine, or bevacizumab)[a]

Attribution	Grade 2	Grade 3		Grade 4[c]		Grade 5[b,c]		Protocol-Specific Requirements/ Exceptions
	Unexpected	*Unexpected*	*Expected*	*Unexpected*	*Expected*	*Unexpected*	*Expected*	-See footnote **(d)** for other ***requirements*** -See footnote **(e)** for special ***requirements*** -See footnote **(f)** for special ***exceptions***
Unrelated or Unlikely				AdEERS	AdEERS	AdEERS	AdEERS	
Possible, Probable, Definite		AdEERS if hospitalized		AdEERS-24 and AdEERS		AdEERS-24 and AdEERS		

AdEERS-24: Indicates an AdEERS 24-hour notification must be electronically submitted to the NSABP Lead Group *within 24 hours* of learning of the event.

AdEERS: Indicates a complete expedited report must be electronically submitted to the NSABP Lead Group *within 5 calendar days* of learning of the event.

Hospitalization: Hospitalization associated with an adverse event is defined as any hospitalization lasting ≥ 24 hours (or a prolongation of an existing hospitalization).

All Reports: On all reports, use the NCI protocol number, AdEERS ticket number, and the protocol-specific patient ID provided during trial registration. ***Fax supporting documentation to the NSABP Biostatistical Center.***

a Refer to Section 14.1.3 for instructions regarding assignment of attribution and expectedness for **Investigational Combination Therapy**.

b All deaths within 30 days after the last dose of the investigational agent require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided. **Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a complete AdEERS report is required as outlined in the table.**

c Adverse events that occur greater than 30 days after the last dose of the investigational agent with attribution of possible, probable or definite to the investigational agent require reporting as follows:

- AdEERS 24-hour notification followed by a complete AdEERS report within 5 calendar days of learning of the event for:
  - grade 4 unexpected events
  - grade 5 unexpected events
- AdEERS 5-calendar day report for:
  - grade 5 expected events

d 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.

e **Protocol-specific expedited reporting requirements:** For this study, the adverse events listed below, regardless of attribution, require expedited reporting via AdEERS to the NSABP Lead Group within 5 calendar days of learning of the event.

- Hypertension ≥ grade 4
- Thrombosis/thrombus/embolism ≥ grade 4
- Hemorrhage ≥ grade 3
- Perforation, GI ≥ grade 3
- Proteinuria ≥ grade 3 (if based on UPC ratio – see Appendix B)
- Intra-abdominal abscess/infection ≥ grade 3
- Wound complication, non-infectious ≥ grade 3
- Cardiac ischemia ≥ grade 4
- CNS ischemia ≥ grade 4
- Peripheral arterial ischemia ≥ grade 4
- Visceral arterial ischemia ≥ grade 4
- Left ventricular systolic dysfunction ≥ grade 3
- Left ventricular diastolic dysfunction ≥ grade 3
- Reversible Posterior Leukoencephalopathy Syndrome (RPLS) ≥ grade 1 reported as Neurology – other (leukoencephalopathy syndrome) (see Section 15.4.2)

f **Protocol-specific expedited reporting exceptions:** For this study, the adverse events listed below which occur, including hospitalizations for these events, do **not** require expedited reporting via AdEERS:

- Blood/Bone Marrow: grades 3 and 4 leukocytes (total WBC), neutrophils/granulocytes, lymphopenia, and platelets (Note: These events are exclusions from expedited reporting only during chemotherapy. Beginning 30 days following the last dose of chemotherapy and when a patient is receiving bevacizumab alone, these grade 3 and 4 events should be reported in an expedited manner.
- Constitutional: grades 3 and 4 fatigue
- Endocrine: grade 3 hot flashes/flushes
- Gastrointestinal: grade 3 constipation and heartburn/dyspepsia
- Infection: grades 3 and 4 febrile neutropenia (Beginning 30 days following the last dose of chemotherapy and when a patient is receiving bevacizumab alone, these grade 3 and 4 events should be reported in an expedited manner.)
- Metabolic: glucose, serum – high (grade 3)
- Pain: perineum (grade 3); Pain: uterus (grade 3)
- Renal/Genitourinary: grade 3 urinary frequency/urgency
- Secondary Malignancy: grade 3 and 4
- Sexual/Reproductive Function: Irregular menses (change from baseline) (grade 3)

TABLE 32. AdEERS expedited reporting requirements for adverse events that occur within 30 days of the last dose of study therapy with a **commercial agent** (cyclophosphamide, doxorubicin, and docetaxel)

Attribution	**Grade 2**	**Grade 3**		**Grade 4[b]**		**Grade 5[a,b]**		**Protocol-Specific Requirements/ Exceptions**
	Unexpected	*Unexpected*	*Expected*	*Unexpected*	*Expected*	*Unexpected*	*Expected*	-See footnote **(c)** for other requirements -See footnote **(d)** for special ***requirements*** -See footnote **(e)** for special ***exceptions***
Unrelated or Unlikely				AdEERS		AdEERS	AdEERS	
Possible, Probable, Definite		AdEERS if hospitalized		AdEERS-24 and AdEERS		AdEERS-24 and AdEERS		

AdEERS-24: Indicates an AdEERS 24-hour notification must be electronically submitted to the NSABP Lead Group *within 24 hours* of learning of the event.

AdEERS: Indicates a complete expedited report must be electronically submitted to the NSABP Lead Group *within 5 calendar days* of learning of the event.

Hospitalization: Hospitalization associated with an adverse event is defined as any hospitalization lasting ≥ 24 hours (or a prolongation of an existing hospitalization).

All Reports: On all reports, use the NCI protocol number, AdEERS ticket number, and the protocol-specific patient ID provided during trial registration. ***Fax supporting documentation to the NSABP Biostatistical Center.***

a All deaths within 30 days of the last dose of study therapy require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided. **Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a complete AdEERS report is required as outlined in the table.**

b Adverse events that occur greater than 30 days after the last dose of study therapy with attribution of possible, probable or definite to study therapy require reporting as follows:
- AdEERS 24-hour notification followed by a complete AdEERS report within 5 calendar days of learning of the event for:
  - grade 4 unexpected events
  - grade 5 unexpected events
- AdEERS 5-calendar day report for:
  - grade 5 expected events

c 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.

d **Protocol-specific expedited reporting requirements:** For this study, the adverse events listed below, regardless of attribution, require expedited reporting via AdEERS to the NSABP Lead Group within 5 calendar days of learning of the event.
- Hypertension ≥ grade 4
- Thrombosis/thrombus/embolism ≥ grade 4
- Hemorrhage ≥ grade 3
- Perforation, GI ≥ grade 3
- Proteinuria ≥ grade 3 (if based on UPC ratio – see Appendix B)
- Intra-abdominal abscess/infection ≥ grade 3
- Wound complication, non-infectious ≥ grade 3
- Cardiac ischemia ≥ grade 4
- CNS ischemia ≥ grade 4
- Peripheral arterial ischemia ≥ grade 4
- Visceral arterial ischemia ≥ grade 4
- Left ventricular systolic dysfunction ≥ grade 3
- Left ventricular diastolic dysfunction ≥ grade 3
- Reversible Posterior Leukoencephalopathy Syndrome (RPLS) ≥ grade 1 reported as Neurology – other (leukoencephalopathy syndrome) (see Section 15.4.2)

e **Protocol-specific expedited reporting exceptions:** For this study, the adverse events listed below which occur, including hospitalizations for these events, do **not** require expedited reporting via AdEERS:
- Blood/Bone Marrow: grades 3 and 4 leukocytes (total WBC), neutrophils/granulocytes, lymphopenia, and platelets
- Constitutional: grades 3 and 4 fatigue
- Endocrine: grade 3 hot flashes/flushes
- Gastrointestinal: grade 3 constipation and heartburn/dyspepsia
- Infection: grades 3 and 4 febrile neutropenia
- Metabolic: glucose, serum – high (grade 3)
- Pain: perineum (grade 3); Pain: uterus (grade 3)
- Renal/Genitourinary: grade 3 urinary frequency/urgency
- Secondary Malignancy: grade 3 and 4
- Sexual/Reproductive Function: Irregular menses (change from baseline) (grade 3)

14.2.3 *Other recipients of adverse event reports*

Adverse events determined to require expedited reporting must also be reported by the investigator to the Institutional Review Board responsible for safety and oversight of the patient according to the local policy and procedures.

14.2.4 *Reporting secondary AML/MDS/ALL*

All cases of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and acute lymphocytic leukemia (ALL) that occur in patients on NCI-sponsored trials following their chemotherapy for cancer must be reported to the Investigational Drug Branch (IDB) of the NCI Cancer Therapy Evaluation Program (CTEP). Submit the following information within 30 days of an AML/MDS/ALL diagnosis occurring after treatment for cancer on NCI-sponsored trials:

- completed NCI/CTEP Secondary AML/MDS Report Form;
- copy of the pathology report confirming the AML/MDS/ALL; and
- copy of the cytogenetics report (if available).

Submit the information to the NSABP Biostatistical Center. The NSABP will submit the form and any accompanying reports to the IDB of the NCI.

Note: If a patient has been enrolled in more than one NCI-sponsored study, the NCI/CTEP Secondary AML/MDS Report Form must be submitted for the most recent trial. The NSABP must also be provided with a copy of the report even if the NSABP study was not the patient's most recent trial.

14.2.5 *Pregnancy occurring while patient is on protocol therapy*

If the patient becomes pregnant while receiving protocol therapy, discontinue study therapy and notify the NSABP Clinical Coordinating Division (see Information Resources on page v) immediately.

14.3 Routine reporting of adverse events

14.3.1 *Reporting on the B-40 Adverse Event Form*

- **All grade 3 adverse events not reported via AdEERS** (see Table 31 and Table 32 for expedited reporting requirements) are to be reported on the Adverse Event Form.

- Select **grade 2** adverse events must be reported on the Adverse Event Form. Refer to the Adverse Event Form for instructions.

- The following adverse events **are not reported** on the Adverse Event Form:
  - Adverse events that require expedited reporting (see Table 31 and Table 32)
  - Adverse events that occur after breast cancer recurrence or secondary primary cancer
  - Secondary cancers
- Adverse events related to surgery ***should be reported on the B-40 Assessment of Surgical Complications Form***. (*These adverse events as specified on Tables 31 and 32 must also be reported via AdEERS.)*
- Supporting documentation for each adverse event reported on the Adverse Event Form must be maintained in the patient's research record. When submission of supporting documentation to the NSABP Biostatistical Center is required, fax to 412-622-2111. Remove patient names and identifiers such as social security number, address, telephone number, etc. from reports and supporting documentation.

14.3.2 *Submission of the B-40 Adverse Event Form*

Submit the B-40 Adverse Event Form according to the following B-40 study group schedule:

- Groups 1A, 2A, and 3A: submit the Adverse Event Form at the end of each chemotherapy cycle.
- Groups 1B, 2B, and 3B: submit the Adverse Event Form at the end of each chemotherapy cycle, after every other dose of bevacizumab when given postoperatively, and 30 days after the last dose of bevacizumab.

Note: Online submission of the B-40 Adverse Event Form is available (but not mandatory) through the Online Data Entry function located in the Study Management Area of Coordinator Online in the Members' Area of the NSABP Web site. If not submitted through Coordinator Online, fax the Adverse Event Form to 412-622-2111.

14.4 Reporting cancer recurrence and second primary cancer

Report breast cancer recurrence and all second primary malignancies (including AML/MDS/ALL) on the NSABP B-40 Follow-up Form. Attach supporting documentation that confirms the breast cancer recurrence or second primary cancer diagnosis.

15.0 DRUG INFORMATION

15.1 Commercial chemotherapy

Doxorubicin, cyclophosphamide, and docetaxel must be obtained from commercial sources. Please refer to the current FDA-approved package inserts provided with the chemotherapy or the *Physicians' Desk Reference* for information about possible side effects and instructions for preparation, handling, and storage of the drugs.

15.2 Capecitabine

Please refer to the current FDA-approved package insert for additional information for capecitabine.

15.2.1 *Description*

Capecitabine (Xeloda®) is a fluoropyrimidine carbamate with antineoplastic activity. It is an orally administered systemic prodrug of 5'-deoxy-5-fluorouridine (5' DFUR) which is converted to 5-fluorouracil.

Capecitabine is a white to off-white crystalline powder with an aqueous solubility of 26 mg/ml at 20° C. It is supplied as biconvex, oblong film-coated tablets for oral administration. Each light peach-colored tablet contains 150 mg capecitabine and each peach-colored tablet contains 500 mg capecitabine.

The inactive ingredients in capecitabine include: anhydrous lactose, croscarmellose sodium, hydroxypropyl methylcellulose, microcrystalline cellulose, magnesium sterate, and purified water. The peach or light peach film coating contains hydroxypropyl methylcellulose, talc, titanium dioxide, and synthetic yellow and red iron oxides.

15.2.2 *Storage*

Store at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F), keep tightly closed.

15.2.3 *Toxicity*

Diarrhea: Capecitabine can induce diarrhea, sometimes severe. Patients with severe diarrhea should be carefully monitored and given fluid and electrolyte replacement if they become dehydrated.

Nausea & vomiting: Can be severe and dose limiting. Initiation of prophylactic treatment is recommended.

Stomatitis: Should be treated symptomatically.

Hand-foot syndrome: Is common and dose-limiting. (Redness, pain, numbness, tingling, blistering, and moist desquamation). Syndrome may recur with a rechallenge.

Other known toxicities include: Abdominal pain, constipation, loss of appetite or decreased appetite, dehydration, tiredness, weakness, dizziness, headache, neutropenia, and fever.

15.2.4 ***Precautions/warnings***

- *Capecitabine/warfarin interaction:* Patients receiving concomitant capecitabine and oral coumarin-derivative anticoagulant therapy should have their anticoagulant response (INR or prothrombin time) monitored frequently in order to adjust the anticoagulant dose accordingly. A clinically important Xeloda-Warfarin drug interaction was demonstrated in a clinical pharmacology trial. Altered coagulation parameters and/or bleeding, including death, have been reported in patients taking capecitabine concomitantly with coumarin-derivative anticoagulants such as warfarin and phenprocoumon. Postmarketing reports have shown clinically significant increases in prothrombin time (PT) and INR in patients who were stabilized on anticoagulants at the time capecitabine was introduced. These events occurred within several days and up to several months after initiating capecitabine therapy and, in a few cases, within 1 month after stopping capecitabine. These events occurred in patients with and without liver metastases. Age greater than 60 and a diagnosis of cancer independently predispose patients to an increased risk of coagulopathy.

- *Hypersensitivity to 5-fluorouracil:* Capecitabine is contraindicated in patients who have a known hypersensitivity to 5-fluorouracil.

- *Renal insufficiency:* Capecitabine is also contraindicated in patients with severe renal impairment (creatinine clearance below 30 ml/min).

15.2.5 ***Procurement***

Capecitabine will be supplied free-of-charge by Roche Laboratories, Inc., and will be distributed by an external vendor. Capecitabine is available as a 500 mg tablet in bottles containing 120 tablets and as a 150 mg tablet in bottles containing 60 tablets.

Capecitabine (NSC #712807) must be requested by the principal investigator (or his/her authorized designee) at each participating institution. Investigators must request drug through the NSABP Biostatistical Center (see Information Resources page v). Capecitabine will be distributed directly to the treating investigators whose sites are participating in B-40. Capecitabine may not be used outside the scope of this protocol, nor can capecitabine be transferred or licensed to any party not participating in this clinical study. The transfer of capecitabine between institutions is not permitted.

Drug Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all capecitabine received for this study and be accountable for all inventory.

15.3 Gemcitabine

Please refer to the current FDA-approved package insert for additional information on gemcitabine.

15.3.1 *Description*

Gemcitabine HCl (Gemzar®) is a nucleoside analogue that exhibits antitumor activity. Gemcitabine HCl is 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β-isomer). Gemcitabine HCl is a white to off-white solid. It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

The clinical formulation is supplied in a sterile form for intravenous use only. Vials of gemcitabine contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

15.3.2 *Storage*

Gemcitabine must be stored at controlled room temperature [20-25°C (68-77°F)] in a limited access area at the study site. Reconstitute with 0.9% NaCl; diluted solution is stable for 24 hours and should be stored at room temperature. (Do not refrigerate the reconstituted drug as crystallization may occur.) Discard any unused portion. Expiration dates on individual vials must be strictly adhered to.

15.3.3 *Toxicity*

Hematologic: Suppression of bone-marrow function as manifested by leukopenia, neutropenia, thrombocytopenia, and anemia can occur. Myelosuppression is usually the dose-limiting toxicity.

Gastrointestinal: Nausea and vomiting (mild to moderate), diarrhea, and stomatitis can occur.

Dermatologic: Rash may occur in 30% of patients. The rash is typically a macular or finely granular maculopapular pruritic eruption of mild to moderate severity involving the trunk and extremities. More severe events, including desquamation and bullous skin eruptions, had been reported very rarely.

Fever: May occur in the absence of clinical infection. Fever, usually associated with other flu-like symptoms, is usually mild.

Infection: May occur; sepsis has occurred rarely.

Hepatic: Transient elevations of one or both serum transaminases may occur. Serious hepatotoxicity, including liver failure and death, has been reported very rarely.

Neurotoxicity: Mild paresthesias may occur; rare cases of severe paresthesias have been reported.

Renal: Mild proteinuria and hematuria may occur. Hemolytic Uremic Syndrome (HUS) has been reported rarely. The diagnosis of HUS should be considered if the patient develops anemia with evidence of microangiopathic hemolysis, elevation of bilirubin or LDH, reticulocytosis, severe thrombocytopenia, and/or evidence of renal failure (elevation of serum creatinine or BUN). Gemcitabine therapy should be discontinued immediately if HUS occurs. Renal failure may not be reversible even with discontinuation of therapy and dialysis may be required. Renal failure leading to death has been rarely reported.

Pulmonary: Dyspnea, unrelated to underlying disease, has been reported. Dyspnea was occasionally accompanied by bronchospasm. Parenchymal toxicity, including interstitial pneumonitis, pulmonary fibrosis, pulmonary edema, and adult respiratory distress syndrome, has been reported rarely following one or more doses of gemcitabine administered to patients with various malignancies. Some patients experienced the onset of pulmonary symptoms up to 2 weeks after the last gemcitabine dose. Respiratory failure and death, despite discontinuation of therapy, have occurred very rarely.

Anaphylactic reaction: Has been reported rarely.

Cardiovascular: Congestive heart failure and myocardial infarction have rarely occurred in patients receiving gemcitabine. Arrhythmias, predominately supraclavicular in nature, have been reported very rarely.

Vascular: Clinical signs of peripheral vasculitis and gangrene have been reported very rarely.

15.3.4 ***Precautions/warnings***

- Prolongation of infusion time beyond 60 minutes has been shown to increase toxicity.

- Gemcitabine can cause fetal harm when administered to pregnant women. Gemcitabine is embryotoxic causing fetal malformations (cleft palate, incomplete ossification) at doses of 1.5 mg/kg/day in mice (about 1/200 the recommended human dose on a mg/m^2 basis). There are no studies of gemcitabine in pregnant women.

  Pregnant women are not eligible for this study. If a woman becomes pregnant while on the study, discontinue the gemcitabine immediately.

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15.3.5 ***Procurement***

Gemcitabine will be supplied free-of-charge by Eli Lilly and Company and will be distributed by an external vendor.

Gemcitabine (NSC #613327) 200 mg (10 mL vial) and 1000 mg (50 mL vial) injection must be requested by the principal investigator (or his/her authorized

designee) at each participating institution. Investigators must request drug through the NSABP Biostatistical Center (see Information Resources page v). Gemcitabine will be distributed directly to the treating investigators whose sites are participating in B-40. Gemcitabine may not be used outside the scope of this protocol, nor can gemcitabine be transferred or licensed to any party not participating in this clinical study.

Drug Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all gemcitabine received for this study and be accountable for all inventory.

15.4 **Bevacizumab**

02/15/08

15.4.1 ***Chemical information***

- *Classification:* Recombinant humanized monoclonal antibody
- *Molecular weight*: Approximate molecular weight is 149,000 daltons
- *Mode of action:* Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.
- *Description:* Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody, consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-determining regions.
- *How supplied:* Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration in a 400 mg (25 mg/mL – 16 mL fill) glass vial that contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.
- *Preparation:* Withdraw the necessary amount of bevacizumab for a dose of 15 mg/kg and dilute in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP. For heavier patients, the dose of 15 mg/kg may be diluted in a total volume of 200 to 250 mL of 0.9% sodium chloride injection, USP. Discard any unused portion left in a vial, as the product contains no preservatives. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. No incompatibilities between bevacizumab and polyvinylchloride or polyolefin bags have been observed. Bevacizumab should not be administered or mixed with dextrose solutions.
- *Storage and stability*
  - Storage
    Upon receipt, bevacizumab should be refrigerated (2º to 8º C).
    Do not freeze. Do not shake. Protect from light.
  - Stability
    Shelf-life studies of rhuMAb VEGF are ongoing. The sterile single use vials contain no antibacterial preservatives. Therefore, vials should be

discarded 8 hours after initial entry. Once diluted in 0.9% sodium chloride, solutions of bevacizumab must be administered within 8 hours.

- *Route of administration:* Intravenous – Do not administer as an IV push or bolus.

- *Dose specifics:* 15 mg/kg IV as per Sections 9.2, 9.4, and 9.6.

02/15/08 15.4.2 ***Toxicity***

Refer to the Investigator's Brochure for bevacizumab. The package insert was prepared for commercial Avastin® but not the investigational bevacizumab being used in this protocol. The package insert should, therefore, only be used as a reference in combination with the Investigator's Brochure.

Reported adverse events and potential risks utilizing CTCAE v3.0 terminology:

- *Allergy/immunology*: Allergic reaction/hypersensitivity (including drug fever); allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)
- *Blood/bone marrow:* Hemoglobin; leukocytes (total WBC); neutrophils/granulocytes (ANC/AGC); platelets
- *Cardiac arrhythmia:* Supraventricular arrhythmia NOS; ventricular fibrillation
- *Cardiac general:* Cardiac ischemia/infarction; cardiac troponin I (cTnI); hypertension; hypotension; left ventricular diastolic dysfunction; left ventricular systolic dysfunction; pulmonary hypertension
- *Constitutional symptoms:* Fatigue (asthenia, lethargy, malaise); fever (in the absence of neutropenia (where neutropenia is defined as ANC $< 1.0 \times 10^9$/L); rigors/chills; weight loss
- *Dermatology/skin:* Pruritus/itching; rash/desquamation; ulceration; urticaria (hives, welts, wheals); wound complication – non-infectious
- *Gastrointestinal:* Anorexia; colitis; constipation; diarrhea; fistula; heartburn/dyspepsia; Ileus (functional obstruction of bowel, i.e., neuroconstipation); GI leak (including anastomotic): large bowel; mucositis/stomatitis; nausea; GI perforation; vomiting; ulcer, GI - Select; taste alteration
- *Hemorrhage/bleeding:* GI hemorrhage; CNS hemorrhage; GU: vagina hemorrhage; pulmonary/upper respiratory: nose hemorrhage, lung hemorrhage
- *Infection:* Infection with normal ANC or grade 1 or 2 neutrophils; infection with normal ANC or grade 1 or 2 neutrophils – Select (pelvis, peritoneal cavity, rectum, scrotum, skin, wound)
- *Metabolic/laboratory:* Alkaline phosphatase; ALT (SGPT); AST (SGOT); bilirubin; creatinine; proteinuria; hyponatremia; hypokalemia
- *Musculoskeletal/soft tissue:* Gait/walking

- *Neurology:* CNS cerebrovascular ischemia; dizziness; confusion; Neurology – Other: (Leukoencephalopathy syndrome including reversible posterior leukoencephalopathy syndrome [RPLS])

  RPLS or clinical syndromes related to vasogenic edema of the white matter have been rarely reported in association with bevacizumab therapy (< 1%). Clinical presentations are variable and may include headache, altered mental status, seizure, and cortical visual deficit. Hypertension is a common risk factor and was present in most (though not all) patients on bevacizumab who developed RPLS. MRI scans are key to diagnosis and typically demonstrate vasogenic edema (hyperintensity in T2 and FLAIR images and hypointensity in T1 images) predominantly in the white matter of the posterior parietal and occipital lobes; less frequently, the anterior distributions and the gray matter may also be involved. RPLS should be in the differential diagnosis in patients presenting with unexplained mental status change, visual disturbance, seizure, or other CNS findings. RPLS is potentially reversible with early recognition of symptoms and timely correction of the underlying causes, including control of blood pressure and interruption of the offending drug, which are important in order to prevent progression to irreversible tissue damage.

- *Ocular/visual:* Watery eye
- *Pain:* Abdomen NOS; chest/thorax NOS; head/headache; joint; muscle; NOS
- *Pulmonary/upper respiratory:* Bronchospasm/wheezing; cough; dyspnea; fistula, pulmonary/upper respiratory – Select; nasal cavity/paranasal sinus reactions; voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis); pulmonary/upper respiratory – other (nasal – septal perforation); ARDS; pneumonitis/pulmonary infiltrates; pneumothorax
- *Renal/genitourinary:* Fistula, GU – Select; perforation, GU – Select; renal failure; urinary frequency
- *Syndromes:* Cytokine release syndrome/acute infusion reaction
- *Vascular:* Thrombosis/thrombus/embolism; visceral arterial ischemia (non-myocardial)

15.4.3 ***Administration***

Method of Administration

The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur, the second dose should be administered over a minimum of 60 minutes. If no adverse reactions occur after the second dose, all subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated.

To insure complete delivery of bevacizumab, the IV infusion line must be flushed with 0.9% sodium chloride. The following are two recommended methods for flushing the bevacizumab IV infusion line:

Method 1: When the bevacizumab infusion is complete, add an additional 50 mL of 0.9% sodium chloride for injection to the bevacizumab infusion bag. Continue the infusion until a volume equal to that of the volume contained in the tubing has been administered.

Method 2: Replace the empty bevacizumab infusion bag with a 50 mL bag of 0.9% sodium chloride for injection and infuse a volume equal to the volume contained in the tubing.

Note: the flush is not included in the total recommended infusion times.

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15.4.4 ***Procurement***

Bevacizumab will be supplied free-of-charge by Genentech, Inc. and will be distributed by an external vendor.

The bevacizumab to be supplied for this protocol is intended for clinical trial use only and is not the commercially available Avastin.® Investigational bevacizumab and commercially available Avastin® may be produced at separate facilities and some difference may exist between the two products, although both are required to meet similar product testing criteria and are expected to be very similar in safety and activity. For further details and molecule characterization, see the updated bevacizumab Investigator Brochure.

Bevacizumab (NSC# 704865) 400 mg (25 mg/mL – 16 mL fill) vials must be requested by the principal investigator (or his/her authorized designee) at each participating institution. Investigators must request drug through the NSABP Biostatistical Center (see Information Resources on page v). Bevacizumab will be shipped directly to the treating investigator whose sites are participating in B-40.

Bevacizumab may not be used outside the scope of this protocol, nor can bevacizumab be transferred or licensed to any party not participating in this clinical trial.

15.4.5 ***Drug accountability***

Drug Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all bevacizumab received for this study and be accountable for all inventory.

16.0 PATIENT ENTRY AND WITHDRAWAL PROCEDURES

16.1 Patient consent form

Before the patient is enrolled, the consent form (see Appendix G), including any addenda, must be signed and dated by the patient and the person who explains the study to that patient.

As part of this consent, all patients have agreed to have a second biopsy procedure during which four core biopsy tissue samples will be obtained (see Section 8.0). All biopsy samples will be identified by the PTI Control Number (a unique coded number provided by the NSABP Biostatistical Center). Patients eligible for, and consenting to participate in, the B-40 treatment study, must have the PTI Control Number of their biopsy sample recorded on Form A. Biopsy samples from patients who are ineligible for B-40, or who choose not to participate in the B-40 treatment study, will be destroyed.

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16.2 Regulatory requirement for Canadian institutions

Any Canadian institution that will utilize PET scans in the conduct of this protocol *must* indicate the intent to do so to the NSABP Division of Regulatory Affairs at the NSABP Operations Center *prior to initiating patient enrollment at the institution (and any satellites)*. Use of PET scans as part of this protocol may require submission by the NSABP of specific documentation to Health Canada before activation of the trial at the institution.

16.3 Entry

16.3.1 *NSABP Investigators*

Note: NSABP investigators who also are registered with the CTSU must enroll B-40 patients through the NSABP; they are not permitted to enter patients through the CTSU.

Patient entry instructions can be found in the "Patient Entry Guidelines" section of the Members' Area of the NSABP Web site, https://members.nsabp.pitt.edu.

16.3.2 *CTSU Investigators*

CTSU investigators must follow procedures outlined in Appendix F, Section 1.0, *Site Registration and Patient Entry for CTSU Investigators.*

16.4 NSABP Patient ID number

After all of the eligibility criteria have been met, the institution will receive the patient's nine-digit NSABP Patient ID number.

16.5 **Patient-initiated discontinuation of study therapy**

Even after a patient agrees to take part in this study, she may stop study therapy or withdraw from the study at any time. If study therapy is stopped but she still allows the study doctor to submit information, study data and other materials should be submitted according to the study schedule.

16.6 **Withdrawal from the study**

If a patient chooses to have no further interaction regarding the study, the investigator must provide the NSABP Biostatistical Center with written documentation of the patient's decision to fully withdraw from the study.

16.7 **Investigator-initiated discontinuation of study therapy**

In addition to the conditions outlined in the protocol, the investigator may require a patient to discontinue study therapy if one of the following occurs:

- the patient develops a serious side effect that she cannot tolerate or that cannot be controlled with other medications,
- the patient's health gets worse,
- the patient is unable to meet the study requirements, or
- new information about the study drugs or other treatments for breast cancer becomes available.

17.0 REQUIRED FORMS AND MATERIALS

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17.1 Data collection

A table of required forms and materials is provided in the "Forms and Documents" section in Members' Area of the NSABP Website for Protocol B-40 http://www.nsabp.pitt.edu. (CTSU investigators should also refer to Appendix F of the B-40 Protocol and the B-40 Web page located on the CTSU Registered Member Web site.)

Data will be collected on patient characteristics, tumor characteristics from the core needle biopsy performed prior to randomization, protocol therapy, clinical response, surgical management, pathologic tumor response in the breast and other pathologic findings including those needed for calculation of residual cancer burden, adverse events during systemic therapy, surgical complications, assessments of left ventricular ejection fraction, congestive heart failure, and follow-up for disease-free survival.

17.2 Adverse event reporting

Routine and expedited adverse event reporting requirements are addressed in Section 14.0.

17.3 Pathology specimens

See Section 8.0 for tissue, blood, and serum requirements and submission instructions. (CTSU investigators should also refer to Appendix F in the B-40 protocol.)

18.0 STATISTICAL CONSIDERATIONS

18.1 Randomization and treatment assignments

Assignments of treatment to patients will be balanced with respect to clinical nodal status (negative, positive), clinical tumor size (2.0 - 4.0 cm, > 4.0 cm), hormone receptor status (ER-positive and/or PgR-positive, ER-negative and PgR-negative), and age (< 50, ≥ 50 years old). A biased coin minimization procedure will be utilized for the randomization procedure to avoid imbalance by these factors and extreme inequality in treatment assignment within an institution.[165]

18.2 Endpoints

The primary endpoint for the study is pathologic complete response (pCR) of the primary tumor in the breast.

The secondary endpoints are pathologic complete response of the primary tumor in the breast, sentinel lymph nodes removed following protocol chemotherapy, and axillary lymph nodes (pCR breast and nodes); clinical overall response rate (cOR) following docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine (with or without bevacizumab), as assessed by physical exam at the completion of the docetaxel-based portion of the chemotherapy program; clinical complete response rate (cCR) following docetaxel alone, docetaxel/capecitabine, and docetaxel/gemcitabine (with or without bevacizumab), as assessed by physical exam at the completion of the docetaxel-based portion of the chemotherapy program; clinical overall response rate (cOR) following the sequential chemotherapy program assessed by physical exam at the completion of the sequential chemotherapy regimens; clinical complete response (cCR) following the sequential chemotherapy program assessed by physical exam at the completion of the sequential chemotherapy regimens; the percentage of cardiac events defined as NYHA Class III/IV congestive heart failure among the patients with docetaxel-anthracycline regimens only and that among patients with both bevacizumab and docetaxel-anthracycline regimens; the percentage of surgical complications from mastectomy, lumpectomy, and axillary staging procedures, which include wound dehiscence, infection, seroma, and hematoma; and disease-free survival (DFS). Events for DFS are defined as local recurrence following mastectomy, local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, contralateral breast cancer, second primary cancer (other than squamous or basal cell carcinoma of the skin, melanoma in situ, carcinoma in situ of the cervix, colon carcinoma in situ, or lobular carcinoma in situ of the breast), and death from any cause prior to these events.

18.3 Statistical analysis

The intent-to-treat principle will be used for the analyses of all the primary and secondary endpoints. Accordingly, the analyses will be performed on all patients with endpoint status ascertained and will use the treatment assignments made at randomization.

The rate of pathologic complete response in the breast (pCR breast) of the docetaxel followed by AC (T→AC) arm (with or without bevacizumab) will be compared to the pCR (breast) rates of the docetaxel with capecitabine followed by AC (TC→AC) and docetaxel with gemcitabine followed by AC (TG→AC), with or without bevacizumab. The maximum of the standardized pair-wise differences between the T→AC arm and the

other two arms, with or without bevacizumab, will be used as the test statistic in order to adjust for multiple comparisons.[166,167] The significance level is 0.05. After adjusting for interim analyses, the significance level for the final comparison is 0.0498 and the corresponding critical value for the test statistic is 2.213.

Each of the above pair-wise comparisons of pathologic complete response rates between the T→AC arm and the other two arms, with or without bevacizumab, will be tested and will be judged to be significant if the corresponding standardized pCR rate difference exceeds the critical value 2.213. The pCR rates of the (TC→AC) and (TG→AC) arms will also be compared and the significance level for the test statistic is 0.05.

The rate of pathologic complete response in the breast (pCR breast) of bevacizumab additional to the docetaxel-anthracycline based regimens (T→AC, TC→AC, and TG→AC) will be compared to the pCR (breast) rate of the docetaxel-anthracycline based regimens without bevacizumab. The 2-sided significance level is 0.05. After adjusting for interim analyses, the significance level for the final comparison is 0.0498 and the corresponding critical value for the test statistic is 1.962.

The comparison of the pCR rates of breast and nodes and the cOR and cCR rates that are assessed after the docetaxel-based portion of chemotherapy regimens (T, TC, and TG) or at the completion of the sequential chemotherapy regimens will be performed in a similar manner.

The percentage of surgical complications, from mastectomy, lumpectomy and axillary staging procedures, among the patients with bevacizumab will be compared with that among the patients without bevacizumab.

For docetaxel-anthracycline based regimens without bevacizumab and docetaxel-anthracycline based regimens with bevacizumab, distribution of DFS will be estimated by the Kaplan-Meier method.[168] Difference in DFS between these two groups will be assessed by the stratified log-rank test[169], controlling for docetaxel-based treatment, clinical tumor size, clinical nodal status, hormone receptor status, and age. As a diagnostic check, secondary treatment comparisons using Cox modeling will control for additional prognostic variables such as number of positive nodes and tumor grade. This will be done even though the method of balanced randomization used in the assignment of treatments makes it highly unlikely that prognostic factors could confound the treatment comparison. Tests of treatment by covariate interaction will be carried out by adding interaction terms one at a time to the Cox model. All interaction tests will be two-sided and will be controlled at the 0.05 significance level.

18.4 Sample size requirements and accrual rates

In this trial, patients will be randomized to three docetaxel-anthracycline based regimens (T→AC, TC→AC, and TG→AC) with or without bevacizumab. Therefore two primary hypotheses will be evaluated in this trial:

- Whether TC→AC or TG→AC is superior to T→AC, with or without bevacizumab, with respect to pCR in breast?

- Whether docetaxel-anthracycline based regimens with bevacizumab are superior to docetaxel-anthracycline based regimens alone with respect to pCR in breast?

The sample size for the trial is 1200 patients (200 per treatment arm). We project that the patient population in this protocol will be similar to that of NSABP B-27. Among patients who had preoperative AC plus docetaxel in NSABP B-27, about 26% had pathologic complete response.

For the first primary hypothesis, the test statistic is the maximum of two standardized pair-wise differences, the critical value for a 0.05 significance level is 2.21[167] and this critical value is corresponding to a 2-sided significance level 0.0271 for a standardized pair-wise comparison. Hence power justification for comparing two binomial distributions with a 2-sided significance level 0.0271 should approximate the power justification for the original test statistic with a 2-sided significance level 0.05. Assuming that the pCR rate in the control arm (T→AC, with or without bevacizumab) is 26% and a type I error rate of $\alpha = .05$, a sample size of 400 per docetaxel-anthracycline based regimen (with or without bevacizumab) would provide 80% power to detect as significantly different a pCR rate in one of the other regimens of 36%. With the same sample size and type I error rate, the study would have 90% power to detect as significantly different pCR rate of 37.5%.

For the second primary hypothesis, the test statistic is the standardized pair-wise difference. The critical value is 1.96 corresponding to a 2-sided significance level 0.05. Assuming that the pCR rate in the control arm (docetaxel-anthracycline based regimens without bevacizumab) is 29% and a type I error rate of $\alpha = .05$, this sample size will provide 91% power to detect as significantly different a pCR rate of 38% in the docetaxel-anthracycline based regimens with bevacizumab.

If accrual rates on this study are similar to those in NSABP B-27, then we could expect an accrual rate as high as 600 patients per year. A definitive analysis of the primary endpoint will be performed when pathologic response statuses of all patients are available. It is anticipated, that the definitive analysis could be performed at a time that would be 3 years after the initiation of accrual (after 2 years to accrue 1200 patients plus 1.0 additional year of follow-up).

For the comparison on DFS, with 80% power and a 2-sided type one error rate 0.05, 252 events need to be observed in order to detect a 30% reduction in hazard rate from adding bevacizumab to the docetaxel-anthracycline based chemotherapy regimens. Based on that the pre-op AC→T arm in the NSABP B-27 study has a DFS annual hazard rate 0.07, plus the assumption that additional capecitabine or gemcitabine may reduce the hazard rate by 10%, we would expect that the group without bevacizumab has a DFS annual hazard rate 0.0665. The definitive analysis on DFS could be performed at about 6.75 years after the initiation of the accrual.

18.5 **Cardiac safety analysis**

Cardiac toxicities from the patients who receive bevacizumab will be monitored and formal analysis will be conducted based on the first 200 patients who receive bevacizumab, regardless of the docetaxel-anthracycline based regimen. The endpoint for the cardiac safety analysis is the percentage of cardiac events defined as NYHA Class III/IV congestive heart failure among these 200 patients documented prior to surgery. The monitoring and tabulation of cardiac toxicity will be continuous starting with the first patient who receives bevacizumb. Randomization to bevacizumab in this study will be stopped if more than ten cardiac events are observed at any point in the accrual of the

first 200 patients. Otherwise, randomization of patients to bevacizumab will continue as planned. If the rate of cardiac events on the regimens with bevacizumab is 3%, there is a 0.04 chance that the randomization of bevacizumab will be terminated; if the rate of cardiac events on the regimens with bevacizumab is 10%, there is a 0.992 chance that the randomization of bevacizumab will be terminated.

All patients who develop symptomatic left ventricular dysfunction will have source documentation submitted for review by the B-40 Protocol Officer to determine if the patient experienced NYHA Class III or IV CHF. This will be used for the primary endpoint of the cardiac safety analysis. The rates of CTCAE grade 2 and 3 left ventricular dysfunction will also be determined.

The cardiac toxicity rate of the patients with bevacizumab will be compared with that of the patients without bevacizumab, regardless of the docetaxel-anthracycline based regimens.

18.6 Pathology statistical design

Within each docetaxel-anthracycline based regimen (with or without bevacizumab), baseline genomic profiles of pathologic complete responders to preoperative chemotherapy will be compared to those of non-complete responders. The expression intensities of about 33,000 genes will be evaluated. For each gene, the difference in average log-intensities between responders and non-responders, divided by the corresponding standard error, is used as the test statistic. The corresponding p-values based on these test statistics under appropriate T-distribution approximation, will be rank ordered from the smallest to the largest. In order to control the family-wise error rate at 0.05, we will use the Westfall-Young step-down algorithm to compute adjusted p-values ≤ 0.05 to determine genes that are differentially expressed between the responders and non-responders.[170] Since control of the family-wise error rate at 0.05 for about 33,000 genes is extremely stringent, the algorithm of Korn et al will be applied to control the number of false positives at most 2 with confidence = 95%.[171] Using this mechanism, a list of candidate markers for response to each treatment will be obtained.

For each docetaxel-anthracycline based regimen (with or without bevacizumab), statistical models will be explored to predict response based on the corresponding list of candidate genes and some standard and potentially prognostic factors such as age, clinical tumor size, nodal status and ER status. After considering attrition from improper sample handling, RNA degradation and other reasons, we anticipate 80% of these patients, 320 out of 400, will have their gene expression data collected. We plan to build a statistical prediction model for pathologic complete response based on data from 240 of these 320 patients, and data from the other 80 patients will be used for validation of the prediction model. For each treatment arm, the top 100 individual genes from the available rank list will be considered for the model building. The correlation among these genes will then be explored and a subgroup of these 100 genes that are representative but not mutually highly correlated will be considered as candidates for the prediction model. Some standard factors such as age, clinical tumor size and nodal status will also be considered. Then a logistic regression prediction model will be selected based on an automatic hybrid algorithm of a stepwise selection and "best" sub-models with the same number of predictors. The resulted prediction model will be applied to the rest of the 80 patients for validation. A permutation-based procedure, where the response statuses of the patients

for model building are randomly permuted and similar model building and validation are conducted, will be used to assess the statistical significance of the classification.

For each treatment arm, a support vector machine (SVM) classifier will be developed based on the same group of 100 candidate genes and the training sample of about 240 patients, then applied to the validation sample of about 80 patients.[172] The performance of this SVM classifier will be compared to the above logistic regression prediction model. The better method will then be applied to all the patients in that treatment regimen to build a final prediction model or classifier to predict pCR (pCR breast) to that treatment sequence in future trials.

If 26% have a pCR (pCR breast), there will be 104 complete pathologic responders and 296 non-responders within each docetaxel-anthracycline based regimen (with or without bevacizumab). Because of attrition from improper sample handling, RNA degradation and other reasons, we anticipate 80% of all patients will be available to have their gene expression determined. This would provide gene expression data for 83 responders and 237 non-responders. Under the more conservative Bonferoni justification, compared to the algorithm of Westfall and Young, the nominal $\alpha = 0.05/33{,}000 = 1.52 \times 10\text{-}6$ and it will give a conservative bound for power. Under the current sample size, we expect to have power of 0.75 to detect any gene for which the mean log-intensity of responders differs by 0.7 of standard deviation from that of non-responders. The power would be 0.93 for a separation by 0.8 of the standard deviation. If the group separation is as large as 1 standard deviation, identification of such genes is almost certain. Based on experience with cDNA gene expression arrays, we have estimated that the majority of genes have between-patient standard deviations of no more than 1 on the base 2 log scale.[173] Therefore, we expect to have power of 0.75 to detect any gene for which the ratio of average expression level in responders versus that of non-responders is either smaller than $2^{-0.7}=0.62$ or larger than $2^{0.7}=1.62$. We would have power of 0.93 to detect any gene for which the ratio of average expression level in responders versus that of non-responders is either smaller than $2^{-0.8}=0.57$ or larger than $2^{0.8}=1.74$.

Search and validation of baseline genomic profiles to predict pCR in breast for each docetaxel-anthracycline based regimen only and each docetaxel-anthracycline based regimen with bevacizumab will be conducted in similar procedures.

Based on gene lists that are associated with pCR with these treatment arms, we will build a general prediction model for pCR to chemotherapy. Similar gene expression data analysis will be conducted with outcomes such as pCR of breast and nodes and cOR following docetaxel-based treatments. Gianni et al[4] identified genes that are correlated with pCR. Adjusting for multiple comparisons, we will use t-tests to check whether the pCR-related 83 genes and the other 16 genes are differentially expressed among the pCR responders and non-responders in this study, regardless of the assigned treatment arms. We will build a multivariate model based on these genes. Then apply the final model to predict the pCR for all patients and assess the accuracy of the prediction by the percentage of patients being correctly classified as responders or non-responders.

Two core biopsies will be obtained from each patient prior to the initiation of chemotherapy and ChemoFx® assay will be performed on tumor tissues from these biopsies. The ChemoFx® assay results in a set of scores that characterize the sensitivity and resistance of individual patients to chemotherapy. The prognostic utility of these scores on cOR of primary breast cancer patients following 4 cycles of docetaxel-based

treatments (T, TC, and TG), without bevacizumab, will be investigated. We expect at least 80% of patients will have sufficient tissue to perform the assay. For this purpose, 600 patients from each treatment group are split into two groups: 400 patients as the training set and 200 patients as the validation set. Given the 80% success rate, we expect at least 320 will be usable for training and 160 for validation.

We will use the training set to define an optimal algorithm with cutoffs to allow us to classify patients as sensitive or resistant to specific chemotherapy regimens where the chemotherapeutic regimen may be part of the algorithm. Among the methods used to determine the best algorithm (in the training set) will be fitting of a logistic model with response as the dependent variable and one or more ChemoFx scores and treatment as independent variables that minimizes partial least squares. We will also look at ROC curves for different ChemoFx scores and/or linear combinations of the scores allowing for modifications for different treatments. However, once the training is completed, an optimal algorithm that forms a rule to classify every patient for each treatment as sensitive or resistant will be carried forward to the validation set. The validation set will then be stratified into resistant and sensitive groups and the observed cOR rates among these two groups will be compared and tested.

Similar analyses of ChemoFx® assay scores will be performed using the dose-response curves for AC and each docetaxel-based treatment, to predict pCR with the corresponding sequential chemotherapy regimens. Further analyses that control for the clinical response status after the docetaxel-based treatment for the prediction of pCR will be considered. We will explore the prediction of cOR after four cycles of docetaxel, docetaxel/capecitabine, docetaxel/gemcitabine, docetaxel with bevacizumab, docetaxel/capecitabine with bevacizumab, and docetaxel/gemcitabine with bevacizumab and investigate whether the addition of bevacizumab has an impact on the prediction of cOR using the dose-response curves for these docetaxel-based treatments. Other analyses such as using ChemoFx® assay data to predict cOR, pCR and DFS after sequential chemotherapy regimens with bevacizumab will also be explored.

In order to facilitate the definitive analysis of using ChemoFx® assay scores to predict cOR after docetaxel-based treatments and pCR after the sequential chemotherapy regimens, we will take data from the first 100 patients that will be randomized to the T→AC without bevacizumab arm and study various prediction methods.

The B-27.2 study will provide us a prediction model using a set of gene markers to predict pCR to AC plus docetaxel for tissues procured by RNAlater. We will apply this prediction model to patients enrolled in the AC plus docetaxel arm and assess the classification accuracy. For tumor materials obtained from paraffin-embedded blocks, the B-27.2 study will provide another prediction model based on the same set of candidate gene markers to predict pCR to AC plus docetaxel. We will apply this model to patients enrolled in the AC plus docetaxel too and compare the false-positive and false-negative rates of these two models. In the meanwhile, we will compare the expression of these candidate genes and study how different methods of tissue procurement affect expression levels of these candidate genes.

We will test the relationship between pCR and protein Tau statuses: Tau-positive and Tau-negative by Chi-square test. Then we will build a multivariate model, including patient age, tumor size, histological type and grade, estrogen receptor, progesterone

receptor, HER-2 and Tau staining intensity to predict pCR. This prediction model will be applied to classify pCR of patients and the classification rate will be assessed.

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The residual cancer burden (RCB) index was created based on an MD Anderson Cancer Center study to describe response to neoadjuvant chemotherapy as a continuous measurement to better reflect association between response and distant relapse-free survival.[162] It was determined by the pathologic response status, size and cellularity of residual tumor, and nodal metastasis status. In this study, Cox proportional hazards models will be applied to validate the prognostic utility of RCB on DFS after neoadjuvant regimens. Further exploration on the systemic relationship among treatment, baseline characteristics such as patient age, tumor size and estrogen receptor, characteristics of residual tumor at surgery, and DFS will also be conducted.

18.7 Monitoring of adverse events

The occurrence of adverse events, including toxicities and deaths, will be monitored. Summaries of all adverse events will be prepared and discussed at regularly scheduled meetings of the NSABP Medical Affairs Division.

In addition, throughout the periods in which the protocol is open to accrual or patients are receiving therapy, semi-annual progress reports will be made to the NSABP Data Monitoring Committee (DMC). These reports will include an assessment of toxicities and on-study deaths; a comparison of actual and projected accrual; and an assessment of data quality, including data delinquency and rates of eligibility. After accrual is closed, reports of adverse events, together with the results of planned interim analyses of the primary endpoints will be presented to the DMC in accordance with the schedule presented below.

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18.8 Interim analyses of the primary endpoint

For the comparison on pCR in breast, three formal analyses of endpoints will be conducted. Two interim analyses will occur when pathologic response status is available from 400 and 800 patients. We will consider stopping the trial if the docetaxel/capecitabine followed by AC regimen or the docetaxel/gemcitabine followed by AC regimen, with or without bevacizumab, is shown superior to the control arm (docetaxel alone followed by AC with or without bevacizumab), with a two-sided p-value less than 0.00025 at the first look or less than 0.00025 at the second look. After adjusting for these two interim analyses, the significance level for the final analysis is 0.0498.[174] We will consider modifying the trial to assign bevacizumab to all subsequently enrolled patients if the docetaxel-anthracycline based regimen with bevacizumab is shown superior to the docetaxel-anthracycline based regimen without bevacizumab with a two-sided p-value less than 0.00025 at the first look or less than 0.00025 at the second look. After adjusting for these two interim analyses, the significance level for the final analysis is 0.0498 with respect to the second primary hypothesis.

For the comparison of DFS, three interim analyses will be carried out when 126, 166, and 209 events are observed and the final definitive analysis will be conducted when 252 events are observed. Two-sided p-values 0.0005, 0.0005, and 0.001 will be used for the three interim looks, respectively. Based on current assumptions, the first interim analysis is expected at about 4.5 years after the initiation of the trial. After adjusting for these interim analyses, the significance level for the final analysis is about 0.0499.

18.9 Issues relating to racial and ethnic differences

Possible racial and ethnic variation in response to the treatment under consideration is of great concern in African-Americans. Researchers have noted poorer survival rates for black breast cancer patients as compared to Caucasians.[175,176] This difference has been attributed to many factors including more advanced disease at the time of diagnosis,[177] social and economic factors[178] or specific tumor characteristics such as ER positivity.[179,180] Although outcomes tend to be less favorable in blacks, significant race-by-treatment interactions have not been previously reported, suggesting that, where treatment efficacy exists, both groups appear to benefit. Previous NSABP investigations of the relationship between race and prognosis support these conclusions.[181,182]

Potential for the enrollment of minority patients in this protocol is enhanced by the NSABP's recognition of the importance of increasing minority accrual. To this end, we provide opportunities for greater participation by under-represented racial and ethnic groups. In the NSABP protocol used for the projected sample size calculations (B-27), the study populations consisted of 73% white; 12% black, not of Hispanic origin; 8% of Hispanic origin; 3% other; and 4% unreported. Other studies indicate 3% of the population are Hispanic; 2% are of Asian or Pacific Islander descent; < 1% are American Indian or Alaskan native; and < 1% are classified as other. It is anticipated that this distribution will be maintained for the proposed protocol. The prognostic effect of race/ethnicity will be evaluated using statistical models. Unfortunately, because of power limitations, we will not be able to compare effects separately for the different cultural or racial groups.

TABLE 33. Expected racial and ethnic composition of NSABP B-40

Ethnic Category	Total
Hispanic or Latino	99
Not Hispanic or Latino	1,101
Ethnic Category: Total of all subjects	1,200
Racial Category	
American Indian or Alaskan Native	12
Asian	57
Black or African American	181
Native Hawaiian or other Pacific Islander	4
White	946
Racial Category: Total of all subjects	1,200

Ethnic Categories:	**Hispanic or Latino** – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race.
	Not Hispanic or Latino
Racial Categories:	**American Indian or Alaskan Native** – a person having origins in any of the original peoples of North, Central or South America, and who maintains tribal affiliations or community attachment.
	Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
	Black or African American – a person having origins in any of the black racial Groups of Africa.
	Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
	White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa

19.0 PUBLICATION INFORMATION AND ADMINISTRATIVE AGREEMENTS

The publication or citation of study results will be made in accordance with the publication policy of the NSABP that is in effect at the time the information is to be made publicly available.

02/15/08
04/01/11

20.0 REFERENCES

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. CA Cancer J Clin 2005; 55:10-30.

2. Ayers M, Symmans WF, Stec J, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. J Clin Oncol 2004; 22:2284-2293.

3. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. Lancet 2003; 362:362-369.

4. Gianni L, Zambetti M, Clark K, et al. Gene expression profiles of paraffin-embedded core biopsy tissue predict response to chemotherapy in patients with locally advanced breast cancer. Proc Am Soc Clin Oncol 2004; 22:14S. Abstr 501.

5. Ness RB, Wisniewski SR, Eng H, et al. Cell viability for drug testing in ovarian cancer: in vitro cell kill versus clinical response. Anticancer Res 2002; 22:1145-1150.

6. Gallion HH, Christopherson WA, et al (In press). Relationship between ex vivo chemosensitivity assay and progression free interval in ovarian cancer. Int J Gynec Cancer.

7. Coleman R, Herzog T, Hosford S, et al. Ex vivo chemoresponse assay predicts carboplatin-induced progression-free interval in ovarian cancer. J Clin Oncol 2004, ASCO Annual Meeting proceedings (Post-Meeting Edition) 2004; 22(14S). Abstr 5134.

8. Schrag D, Garewal HS, Burstein HJ, et al. American Society of Clinical Oncology Technology Assessment: chemotherapy sensitivity and resistance assays. J Clin Oncol 2004; 22:3631-3638.

9. Skipper HE. Kinetics of mammary tumor cell growth and implications for therapy. Cancer 1971; 28:1479-1499.

10. Goldie JH, Coldman AJ. A mathematical model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 1979; 63:1727-1733.

11. Gunduz N, Fisher B, Saffer EA. Effect of surgical removal on the growth and kinetics of residual tumor. Cancer Res 1979; 39:3861-3865.

12. Fisher B, Gunduz N, Saffer EA. Influence of the interval between primary tumor removal and chemotherapy of kinetics and growth of metastases. Cancer Res 1983; 43:1488-1492.

13. Rosen G. Preoperative chemotherapy for osteogenic sarcoma. Cancer 1982; 49:1221-1230.

14. Schuller DE, Wilson HE, Smith RE, et al. Preoperative reductive chemotherapy for locally advanced carcinoma of the oral cavity, oropharynx and hypopharynx. Cancer 1983; 51:15-19.

15. Leichman L, Steiger Z, Seydel HG, et al. Preoperative chemotherapy and radiation therapy for patients with cancer of the esophagus: A potentially curative approach. J Clin Oncol 1984; 2:75-79.

16. Perloff M, Lesnick GJ. Chemotherapy before and after mastectomy in stage III breast cancer. Arch Surg 1982; 117:879-881.

17. Schick P, Goodstein J, Moor J, et al. Preoperative chemotherapy followed by mastectomy for locally advanced breast cancer. J Surg Oncol 1983; 22:278-282.

18. Sorace RA, Bagley CS, Lichter AS, et al. The management of nonmetastatic locally advanced breast cancer using primary induction chemotherapy with hormonal synchronization followed by radiation therapy with or without debulking surgery. World J Surg 1985; 9:775-785.

19. Jacquillat C, Weil M, Baillet F, et al. Results of neoadjuvant chemotherapy and radiation therapy in breast-conserving treatment of 250 patients with all stages of infiltrative breast cancer. Cancer 1990; 66:119-129.

20. Bonadonna G, Veronesi U, Brambilla C, et al. Primary chemotherapy to avoid mastectomy in tumors with diameters of three centimeters or more. J Natl Cancer Inst 1990; 82:1539-1545.

21. Tubiana-Hulin M, Malek M, Briffod M, et al. Preoperative chemotherapy of operable breast cancer (stage IIIA): Prognostic factors of distant recurrence. Eur J Cancer 1993; 29A:S76. (Abstr 391, suppl 6).

22. Fisher B, Redmond C, Poisson R, et al. Eight-year results of a randomized clinical trial comparing total mastectomy and lumpectomy with or without irradiation in the treatment of breast cancer. N Engl J Med 1989; 320:820-828.

23. Fisher B, Anderson S, Redmond CK, et al. Reanalysis and results after 12 years of follow-up in a randomized clinical trial comparing total mastectomy with lumpectomy with or without irradiation in the treatment of breast cancer. N Engl J Med 1995; 333:1456-1461.

24. Belembaogo E, Feillel V, Chollet P, et al. Neoadjuvant chemotherapy in 126 operable breast cancers. Eur J Cancer 1992; 28A:896-900.

25. Smith IE, Jones AL, O'Brien ME, et al. Primary medical (neo-adjuvant) chemotherapy for operable breast cancer. Eur J Cancer 1993; 29A:1796-1799.

26. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16:2672-2685.

27. Fisher B, Brown A, Mamounas E, et al. Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer. Findings from National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 1997; 15:2483-2493.

28. Wolmark N, Wang J, Mamounas E, et al. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. J Natl Cancer Inst 2001; Monor 30:96-102.

29. Van der Hage JA, van de Velde CJ, Julien JP, et al. Preoperative chemotherapy in primary operable breast cancer: results from the European Organization for Research and Treatment of Cancer trial 10902. J Clin Oncol 2001; 19:4224-4237.

30. Breslin TM, Cohen L, Sahin A, et al. Sentinel lymph node biopsy is accurate after neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2000; 18:3480-3486.

31. Nason KS, Anderson BO, Byrd DR, et al. Increased false negative sentinel node biopsy rates after preoperative chemotherapy for invasive breast carcinoma. Cancer 2000; 89:2187-2194.

32. Julian TB, Patel N, Dusi D, et al. Sentinel lymph node biopsy after neoadjuvant chemotherapy for breast cancer. Am J Surg 2001; 182:407-410.

33. Fernandez A, Cortes M, Benito E, et al. Gamma probe sentinel node localization and biopsy in breast cancer patients treated with a neoadjuvant chemotherapy scheme. Nucl Med Commun 2001; 22:361-366.

34. Stearns V, Ewing CA, Slack R, et al. Sentinel lymphadenectomy after neoadjuvant chemotherapy for breast cancer may reliably represent the axilla except for inflammatory breast cancer. Ann Surg Oncol 2002; 9:235-242.

35. Haid A, Tausch C, Lang A, et al. Is sentinel lymph node biopsy reliable and indicated after preoperative chemotherapy in patients with breast carcinoma? Cancer 2001; 92:1080-1084.

36. Bear HD, Anderson S, Brown A, et al. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2003; 21:4165-4174.

37. Mamounas EP. Sentinel lymph node biopsy after neoadjuvant systemic therapy. Surg Clin North Am 2003; 83:931-942.

38. Fisher B, Mamounas EP. Preoperative chemotherapy: a model for studying the biology and therapy of primary breast cancer. J Clin Oncol 1995; 13:537-540.

39. Green M, Buzdar A, Smith T, et al. Weekly (wkly) paclitaxel (P) followed by FAC as primary systemic chemotherapy (PSC) of operable breast cancer improves pathologic complete remission (pCR) rates when compared to every 3-week (Q3 wk) P therapy (tx) followed by FAC-final results of a prospective phase III randomized trial. Proc Am Soc Clin Oncol 2002; 21:35a, Abstr 135.

40. Untch M, Kochecny G, Ditsch N, et al. Dose-dense sequential epirubicin-paclitaxel as preoperative treatment of breast cancer: results of a randomized AGO study. Proc Am Soc Clin Oncol 2002; 21:34a, Abstr 133.

41. Von Minckwitz G, Raab M, Schuette N, et al. Dose-dense versus sequential adriamycin/docetaxel combination as preoperative chemotherapy (pCHT) in operable breast cancer (T2-3, N0-2, M0) – primary endpoint analysis of the GEPARDUO-study. Proc Am Soc Clin Oncol 2002; 21:43a, Abstr 168.

42. Evans T, Gould A, Foster E, et al. Phase III randomized trial of adriamycin (A) and docetaxel (D) versus A and cyclophosphamide (C) as primary medical therapy (PMT) in women with breast cancer: an ACCOG study. Proc Am Soc Clin Oncol 2002; 21:35a, Abstr 136.

43. Smith IC, Heys SD, Hutcheon AW, et al. Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. J Clin Oncol 2002; 20:1456-1466.

44. Hutcheon AW, Heys SD, Sarkar, TK, et al. Neoadjuvant docetaxel in locally advanced breast cancer. Breast Cancer Res Treat 2003; 79:19-24.

45. Bear H. The effect on primary tumor response of adding sequential Taxotere to Adriamycin and cyclophosphamide: preliminary results from NSABP protocol B-27. Breast Cancer Res Treat 2001; 69:210, Abstr 5.

46. Bear HD, Anderson S, Smith RE, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 2006; 24:2019-2027.

47. Henderson IC, Berry DA, Demetri GD, et al. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003; 21:976-983.

48. Mamounas EP, Bryant J, Lembersky BC, et al. Paclitaxel (T) following doxorubicin/cyclophosphamide (AC) as adjuvant chemotherapy for node-positive breast cancer: Results from NSABP B-28. Proc Am Soc Clin Oncol 2003; 22:4, Abstr 12.

49. Roché H, Fumoleau P, Speilmann M, et al. Five years of analysis of the PACS 01 trial: 6 cycles of FEC 100 vs 3 cycles of FEC 100 followed by 3 cycles of docetaxel (D) for the adjuvant treatment of node positive breast cancer. Ann San An Breast Cancer Symp, 2004. Abstr 27.

50. Green MC, Buzdar AU, Smith T, et al. Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks. J Clin Oncol 2005; 23 (25):5983-5992.

51. Lee KS, Lee ES, Kwon YM, et al. Mature results from a randomized phase III trial of docetaxel/capecitabine (TX) vs. doxorubicin/cyclophosphamide (AC) as primary chemotherapy for patients (pts) with stage II/III breast cancer (BC). Ann San An Breast Cancer Symp 2005 abstr. 5052.

52. Ishitsuka H, Ishikawa T, Fukase Y, et al. Capecitabine and the dThdPase upregulators IFNgamma or Taxol showed synergistic activity in human cancer xenografts [abstract]. Proc Ann Meet Am Assoc Cancer Res 1996; 37:A27-66.

53. Ishitsuka H, Miwa M, Ishikawa T, et al. Capecitabine: an orally available fluoropyrimidine with tumor selective activity [abstract]. Pro Ann Meet Am Assoc Cancer Res 1995; 36:A24-26.

54. Ishikawa T, Sekiguchi F, Fukase Y, et al. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. Cancer Res 1998; 58:685-690.

55. Ishikawa T, Sawada N, Sekiguchi F, et al. Xeloda (capecitabine), A new oral fluoropyrimidine carbamate with an improved efficacy profile over other fluoropyrimidines. Proc Ann Meet Am Soc Clin Oncol 1997; 16:A796.

56. Schuller J, Cassidy J, Reigner BG, et al. Tumor selectivity of Xeloda in colorectal cancer patients. Proc Ann Meet Am Soc Clin Oncol 1997; 16:A797.

57. Cao S, Lu K, Ishitsuka H, et al. Antitumor efficacy of capecitabine against fluorouracil-sensitive and resistant tumors. Proc Ann Meet Am Soc Clin Oncol 1997; 16:A795.

58. Ishikawa T, Utoh M, Sawada N, et al. Xeloda (capecitabine): an orally available tumor-selective fluoropyrimidine carbamate. Proc Ann Meet Am Soc Clin Oncol 1997; 16:A727.

59. Twelves C, Budman DR, Creaven PJ, et al. Pharmacokinetics (PK) and pharmacodynamics (PD) of capecitabine in two phase I studies. Proc Ann Meet Am Soc Clin Oncol 1996; 15:A1509.

60. Reigner B, Verweij J, Dirix L, et al. Effect of food on the pharmacokinetics of capecitabine and its metabolites following oral administration in cancer patients. Clin Cancer Res 1998; 4:941-948.

61. Van Cutsem E, Findlay M, Osterwalder B, et al. Capecitabine, an oral fluoropyrimidine carbamate with substantial activity in advanced colorectal cancer: results of a randomized phase II study. J Clin Oncol 2000; 18:1337-1345.

62. Miwa M, Ura M, Nishida M, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 1998; 34:1274-1281.

63. Schuller J, Cassidy J, Dumont E, et al. Preferential activation of capecitabine in tumor following oral administration in colorectal cancer patients. Cancer Chemother Pharmacol 2000; 34:293-296.

64. Sawada N, Ishikawa T, Fukase Y, et al. Induction of thymidine phosphorylase activity and enhancement of capecitabine efficacy by Taxol/docetaxel in human cancer xenografts. Clin Cancer Res 1998; 4:1013-1019.

65. Kurosumi M, Tabei T, Suemasu K, et al. Enhancement of immunohistochemical reactivity for thymidine phosphorylase in breast carcinoma cells after administration of docetaxel as a neoadjuvant chemotherapy in advanced breast cancer patients. Oncol Rep 2000; 7:945-948.

66. Blum JL, Jones SE, Buzdar AU, et al. Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. J Clin Oncol 1999; 17:485-493.

67. Blum JL, Buzdar AU, Dieras V, et al. A multicenter phase II trial of Xeloda (capecitabine) in taxane-refractory metastatic breast cancer. Proc Am Soc Clin Oncol 1999; 18:A403.

68. O'Shaughnessy J, Moiseyenko V, Bell D, et al. A randomized phase II study of Xeloda (capecitabine) vs CMF as first line chemotherapy of breast cancer in women aged ≥ 55. Proc Am Soc Clin Oncol 1998; 17:A398.

69. O'Shaughnessy J, Vukelja S, Moiseyenko V, et al. Results of a large Phase III trial of Xeloda®/docetaxel® combination therapy versus docetaxel monotherapy in patients with metastatic breast cancer (MBC) [poster]. 23rd Ann San An Breast Cancer Symp, San Antonio, Texas, 2000.

70. O'Shaughnessy J, (personal communication), 2004.

71. O'Shaughnessy J, (personal communication), 2004.

72. Kristjansen PEG, Quistorff B, Spang-Thomsen M, et al. Intratumoral pharmacokinetic analysis by 19F-magnetic resonance spectroscopy and cytostatic in vivo activity of gemcitabine (dFdC) in two small cell lung cancer xenografts. Annals of Oncology 1993; 4:157-160.

73. Sunkara PS, Lippert BJ, Snyder RD, Jarvi ET, Farr RA. Antitumor activity of 2'-deoxy-,2'-diflurocytidine, a novel inhibitor of ribonucleotide reductase. Proc Ann Meet Am Assoc Cancer Res 1995; 29:324.

74. Lund B, Hansen OP, Theilade K, et al. Phase II study of gemcitabine (2',2'-difluorodeoxycytidine) in previously treated ovarian cancer patients. J Natl Cancer Inst 1994; 86:1530-1533.

75. Guchelaar HJ, Richel DJ, van Knapen A. Clinical, toxicological and pharmacological aspects of gemcitabine. Cancer Treat Rev 1996; 22:15-31.

76. Hansen HH, Sorensen JB. Efficacy of single-agent gemcitabine in advance non-small cell lung cancer: a review. Semin Oncol 1997; 24:S7-38-S7-41.

77. Hui YF, Reitz M. Gemcitabine: A cytidine analogue active against solid tumors. Am J Health Syst Pharm 1997; 54:162-170.

78. Abbruzzese JL, Grunewald R, Weeks EA, et al. A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J Clin Oncol 1991; 9:491-498.

79. Blackstein M, Vogel CL, Ambinder R, et al. Gemcitabine as first-line therapy in patients with metastatic breast cancer: a phase II trial. Oncology 62:2-8, 2002.

80. Carmichael J, Possinger K, Phillip P, et al. Advanced breast cancer: a phase II trial with gemcitabine. J Clin Oncol 1995; 13:2731-2736.

81. Possinger K, Kaufmann M, Coleman R, et al. Phase II study of gemcitabine as first-line chemotherapy in patients with advanced or metastatic breast cancer. Anti-Cancer Drugs 1999; 10:155-162.

82. Gerson R, Senaro A, Villabbos A, et al. Gemcitabine response in advance breast cancer in relation to immunohistochemical factors. Proc Am Clin Oncol 2000; 19:Abstr 572.

83. Valerio M, Cicero G, Armata M. Gemcitabine in pretreated breast cancer. Proc Am Clin Oncol 2001; 20:Abstr 1953.

84. Brodowicz T, Kostler WJ, Möslinger R, et al. Single-agent gemcitabine as second- and third-line treatment in metastatic breast cancer. Breast 2000; 9:338-342.

85. Spielmann M, Kalla S, Llombart-Cussac A, et al. Activity of gemcitabine in metastatic breast cancer (MBC) patients previously treated with anthracycline-containing regimens. Europ J Ca 1997; 33:S149.

86. Data on file, Eli Lilly.

87. Theodossiou C, Cook JA, Fisher J, et al. Interaction of gemcitabine with paclitaxel and cisplatin in human tumor cell lines. Int J Oncol 1998; 12:825-832.

88. Kroep JR, Giaccone G, Tolis C, et al. Sequence dependent effect of paclitaxel on gemcitabine metabolism in relation to cell cycle and cytotoxicity in non-small cell lung cancer cell lines. Br J Cancer 2000; 83:1069-1076.

89. Albain, KS, Nag S, Calderillo-Ruiz G, et al. Global phase III study of gemcitabine plus paclitaxel (GT) vs. paclitaxel (T) as frontline therapy for metastatic breast cancer (MBC): First report of overall survival. Proc Am Soc Clin Onc 2004; 22:14S, Abstr 510.

90. Fountzilas G, Nicolaides C, Bafaloukos D, et al. Docetaxel and gemcitabine in anthracycline-resistant advanced breast cancer: a Hellenic cooperative oncology group phase II study. Cancer Invest 2000; 18:503-509.

91. Mavroudis D, Malamos N, Alexopoulos A, et al. Salvage chemotherapy in anthracycline-pretreated metastatic breast cancer patients with docetaxel and gemcitabine: a multicenter phase II trial. Greek Breast Cancer Cooperative Group. Ann Oncol 1999; 10:211-215.

92. Laufman L, Spiridonidis C, Pritchard J, et al. Monthly docetaxel and weekly gemcitabine in metastatic breast cancer: a phase II trial. Ann Oncol 2001; 12:1259-1264.

93. Brandi M, Giotta F, Vici P, et al. Salvage chemotherapy with docetaxel and gemcitabine in metastic breast cancer: preliminary results of a multicenter phase II trial of GOIM. Proc Am Clin Oncol 2001; 20:Abstr 1956.

94. Kornek GV, Haider K, Kwasny W, et al. Treatment of advanced breast cancer with docetaxel and gemcitabine with and without human granulocyte colony-stimulating factor. Clin Cancer Res 2002; 8:1051-1056.

95. Alexopoulos A, Tryfonopoulos D, Karamouzis MV, et al. Evidence for in vivo synergism between docetaxel and gemcitabine in patients with metastic breast cancer. Ann Oncol 2004; 15:95-99.

96. Mavroudis D, Malamos N, Polyzos K, et al. A multicenter phase II study of docetaxel plus gemcitabine biweekly administration as first line treatment in patients with metastatic breast cancer (MBC). Proc Am Soc Clin Oncol 2003; 22: Abstr 259.

97. Slee PH, De Jong RS, Honkoop A, et al. Combination chemotherapy with docetaxel and gemcitabine in anthracycline pretreated patients with metastatic breast cancer (MBC). Proc Am Soc Clin Oncol 2004; 23: Abstr 792.

98. Garle P, Calvo L, Mayordomo J, et al. Gemcitabine and docetaxel given every other week as first-line therapy for metastatic breast cancer (MBC): Final results of a phase II study conducted within the Breast Cancer Investigation Spanish Group (GEICAM). Proc Am Soc Clin Oncol 2003; 22: Abstr 201.

99. Lenz F, Beldermann F, Gerberth M, et al. A phase II study of first-line combination chemotherapy with docetaxel and gemcitabine in anthracycline-pretreated, Her2-negative metastatic breast cancer (MBC). Europ J of Cancer Suppl 2003; 1(5): S139.

100. Chan S, Romieu G, Huober J, et al. Gemcitabine plus docetaxel (GD) versus capecitabine plus docetaxel (CD) for anthracycline-pretreated metastatic breast cancer (MBC) patients (pts): Results of a European Phase III Study. American Society of Clinical Oncology presentation, 2005.

101. Miller, KD, Wang M, Gralow J, et al. A randomized phase III trial of paclitaxel versus paclitaxel plus bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer: A trial coordinated by the Eastern Cooperative Oncology Group (E2100). San Antonio Breast Cancer Symposium presentation, 2005.

102. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997; 18:4-25.

103. Warren RS, Yuan H, Matli MR, et al. Regulation by vascular endothelial growth factor of human colon cancer tumorigencesis in a mouse model of experimental liver metastasis. J Clin Invest 1995; 95:1789-1797.

104. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990; 82:4-6.

105. Frank RE, Saclarides TJ, Leugans S, et al. Tumor angiogenesis as a predictor of recurrence and survival in patients with node-gegative colon cancer. Ann Surg 1995; 222:695-699.

106. Takahashi Y, Tucker SL, Kitadai Y, et al. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. Arch Surg 1997; 132:541-546.

107. Tanigawa N, Amaya H, Matsumura M, et al. Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res 1997; 57:1043-1046.

108. Tomisaki S, Ohno S, Ichiyoshi Y, et al. Microvessel quantification and its possible relation with liver metastasis in colorectal cancer. Cancer 1996; 77 (8 Suppl):1722-1728.

109. Choi HJ, Hyun MS, Jung GJ, et al. Tumor angiogenesis as a prognostic predictor in colorectal carcinoma with special reference to mode of metastasis and recurrence. Oncology 1998; 55: 575-581.

110. Fox SH, Whalen GF, Sanders MM, et al. Angiogenesis in normal tissue adjacent to colon cancer. J Surg Oncol 1998; 69:230-234.

111. Abdalla SA, Behzad F, Bsharah S, et al. Prognostic relevance of microvessel density in colorectal tumours. Oncol Rep 1999; 6:839-842.

112. Borgstrom P, Gold DP, Hillan KJ, et al. Importance of VEGF for breast cancer angiogenesis in vivo: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin. Anticancer Res 1999; 19:4203-4214.

113. Gordon MS, Margolin K, Talpaz M, et al. Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 2001; 19:843-850.

114. Margolin K, Gordon MS, Holmgren E, et al. Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: Pharmacologic and long-term safety data. J Clin Oncol 2001; 19:851-856.

115. Cobleigh MA, Langmuir VK, Sledge GW, et al. A Phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol 2003 Oct;30(5 Suppl 16): 117-24.

116. Overmoyer B, Silverman P, Leeming R, et al. Phase II trial of neoadjuvant docetaxel with or without bevacizumab in patients with locally advance breast cancer. Ann San An Breast Cancer Symp 2004 abstr. 2088.

117. Wedam SB, Low JA, Yang SX, et al. Anti-angiogenic and anti-tumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. J Clin Oncol 2006; 24(5) 769-777.

118. Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol 2005 Feb 1;23(4)792-799.

119. Ranpura V, Hapani S, Wu S, et al. Treatment-related mortality with bevacizumab in cancer patients: a meta-analysis. JAMA 2011; 305(11):487-494.

120. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007; 357(26):2666-2676.

121. Miles DW, Chan A, Dirix LY, et al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2–negative metastatic breast cancer. J Clin Oncol 2010; 28(20):3239-3247.

122. FDA. Slides for the July 20, 2010 Meeting of the Oncologic Drugs Advisory Committee. July 2010. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM219979.pdf

123. Brufsky A, Bondarenko IN, Smirnov V, et al. RIBBON-2: A randomized, double-blind, placebo-controlled, phase III trial evaluating the efficacy and safety of bevacizumab in combination with chemotherapy for second-line treatment of HER2-negative metastatic breast cancer. Ann San An Breast Cancer Symp 2009; Abstr 42.

124. Allegra CJ, Yothers G, O'Connell MJ, et al. Phase III trial assessing bevacizumab in stages II and III carcinoma of the colon: results of NSABP protocol C-08. J Clin Oncol 2011; 29(1):11-16.

125. De Gramont A, Van Custem E, Tabernero J, et al. AVANT: Results from a randomized, three-arm multinational phase III study to investigate bevacizumab with either XELOX or FOLFOX4 versus FOLFOX4 alone as adjuvant treatment for colon cancer. Presented at the Ann San An GI Cancers Symp 2011.

126. Miles D, Harbeck N, Escudier B, et al. Disease course patterns after discontinuation of bevacizumab: pooled analysis of randomized phase III trials. J Clin Oncol 2011; 29(1):83-88.

127. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000; 92:205-216.

128. Paik S, Bryant J, Tan-Chiu E, et al. Her2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-15. J Natl Cancer Inst 2000; 92(24):1991-1998.

129. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351(27):2817-2826.

130. Paik S, Bryant J, Park C, et al. erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. J Natl Cancer Inst 1998;90(18):1361-1370.

131. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000; 406(6797):747-752.

132. Peterson B, George SL. Sample size requirements and length of study for testing interaction in a 2 x k factorial design when time-to-failure is the outcome [corrected]. Control Clin Trials 1993; 14(6):511-522.

133. Fisher B, Bryant J, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16(8):2672-2685.

134. Paik S, Shak S, Tang G, et al. Expression of the 21 genes in the recurrence score assay and prediction of clinical benefit from tamoxifen in NSABP study B-14 and chemotherapy in NSABP study B-20. Ann San An Breast Cancer Symp 2004; Abstr 24.

135. Petros WP, Evans WE. Pharmacogenomics in cancer therapy: is host genome variability important? Trends Pharmacol Sci 2004; 25(9):457-464.

136. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. Lancet 2003; 362(9381): 362-369.

137. Rouzier R, Rajan R, Hess KR, et al. Microtubule associated protein Tau is a predictive marker and modulator of response to paclitaxel-containing preoperative chemotherapy in breast cancer. Nature Medicine submitted.

138. Grotzer MA, Patti R, Geoerger B, et al. Biological stability of RNA isolated from RNAlater-treated brain tumor and neuroblastoma xenografts. Med Pediatr Oncol 2000; 34(6):438-442.

139. Florell SR, Coffin CM, Holden JA, et al. Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. Mod Pathol 2001; 14(2):116-128.

140. Ellis M, Davis N, Coop A, et al. Development and validation of a method for using breast core needle biopsies for gene expression microarray analyses. Clin Cancer Res 2002;8(5):1155-1166.

141. Symmans WF, Ayers M, Clark EA, et al. Total RNA yield and microarray gene expression profiles from fine-needle aspiration biopsy and core-needle biopsy samples of breast carcinoma. Cancer 2003; 97(12):2960-2971.

142. Furey TS, Cristianini N, Duffy N, et al. Support vector machine classification and validation of cancer tissue samples using microarray expression data. Bioinformatics 2000; 16(10): 906-914.

143. Allred D, Bryant J, Land S, et al. Estrogen receptor expression as a predictive marker of the effectiveness of tamoxifen in the treatment of DCIS: Findings from NSABP Protocol B-24. Ann San An Breast Cancer Symp 2002. Abst 30

144. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 2001; 98(9):5116-5121.

145. Demaria S, Volm MD, Shapiro RL, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res 2001; 7(10): 3025-3030.

146. Dobbin KK, Beer DG, Meyerson M, et al. Interlaboratory comparability study of cancer gene expression analysis using oligonucleotide microarrays. Clin Cancer Res 2005; 11(2 Pt 1): 565-572.

147. Cronin M, Pho M, Dutta D, et al. Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay. Am J Pathol 2004; 164(1):35-42.

148. Bibikova M, Talantov D, Chudin E, et al. Quantitative gene expression profiling in formalin-fixed, paraffin-embedded tissues using universal bead arrays. Am J Pathol 2004; 165(5): 1799-1807.

149. Kornblith, P, Wells A, Gabrin MJ, et al. Breast cancer-response rates to chemotherapeutic agents studied in vitro. Anticancer Res 2003; 23: 3405-3412.

150. Kornblith, P, Wells A, Gabrin MJ, et al. In vitro comparison of response of doxorubicin/cyclophosphamide in combination versus use as individual agents in primary breast cancer. J Clin Oncol 2003; ASCO Annual Meeting Proceedings 22:56. Abstr 223.

151. Kornblith P, Wells A, Gabrin MJ, et al. In vitro responses of ovarian cancers to platinums and taxanes. Anticancer Res 2003; 23: 543-548.

152. Kornblith P, Ochs RL, Wells A, et al. Differential in vitro effects of chemotherapeutic agents on primary cultures of human ovarian carcinoma. Int J Gynec Cancer 2004; 14: 607-615.

153. Ochs RL, Piwowar HA, Gabrin MJ, et al. Heterogeneity in chemoresponsiveness of tumor cells ex vivo. Ann Meeting of the Am Assoc for Cancer Research 2004; Abstr 2086.

154. Pusztai L, Gregory BW, Baggerly KA, et al. Pharmacoproteomic analysis of prechemotherapy and postchemotherapy plasma samples from patients receiving neoadjuvant or adjuvant chemotherapy for breast carcinoma. Cancer 2004; 100(9):1814-1822.

155. Kiang DT, Greenberg LJ, Kennedy BJ. Tumor marker kinetics in the monitoring of breast cancer. Cancer 1990; 65(2):193-199.

156. Vogelzang NJ, Lange PH, Goldman A, et al. Acute changes of alpha-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors. Cancer Res 1982; 42(11):4855-4861.

157. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature 2004; 429(6990):464-468.

158. Morita T, Matsuzaki A, Suzuki K, et al. Role of thymidine phosphorylase in biomodulation of fluoropyrimidines. Curr Pharm Biotechnol 2001; 2(3):257-267.

159. Villafranca E, Okruzhnov Y, Dominguez MA, et al. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J Clin Oncol 2001; 19(6):1779-1786.

160. Park DJ, Stoehlmacher J, Zhang W, et al. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis 2002; 17(1):46-49.

161. Rajan R, Esteva FJ, Symmans WF. Pathologic changes in breast cancer following neoadjuvant chemotherapy: implications for the assessment of response. Clin Breast Cancer 2004; 5(3): 235-238.

162. Symmans WF, Peintinger F, Hatzis C, et al. Measurement of residual cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25(28):4414-4422.

163. Ochs RL, Burholt D, et al. The ChemoFx Assay: An Ex Vivo Cell Culture Assay for Predicting Anticancer Drug Responses. Methods in Molecular Medicine, vol. 110: Chemosensitivity: Vol. 1: In Vitro Analysis. R. Blumenthal. Totowa, NJ, Humana Press Inc. 2004, 110: 155-172.

164. Pusztai L, Ayers M, Stec J, et al. Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. Clin Cancer Res 2003; 9:2406-2415.

165. White SJ, Freedman LS. Allocation of patients to treatment groups in a controlled clinical study. Br J Cancer 1978; 37:849-857.

166. Bristol DR. One-Sided Multiple Comparisons of Response Rates with a Control. Multiple Comparisons, Selection, and Applications in Biometry: a Festchrift in Honor of Charles W. Dunnett, Edited by Hoppe FM, New York: Dekker, 1993.

167. Dunnett, CW. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. Jour Amer Statist Assoc 1955; 50:1096-1121.

168. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457-481.

169. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1996; 50:163-170.

170. Westfall PH, Young SS. Resampling-Based Multiple Testing, New York: Wiley, 1993.

171. Korn EL, Troendle JF, McShane LM, et al. Controlling the number of false discoveries: Application to high-dimensional genomic data. J Statist Plann Inference 2004; 124:379-398.

172. Hastie T, Tibshirani R, Friedman J. The elements of statistical learning. New York, Springer-Verlag, 2001.

173. Simon R, Radmacher MD, Dobbin K. Design of studies using DNA microarrays. Genetic Epidemiology 2002; 23:21-36.

174. Turnbull BW, Jennison C. Group sequential methods with applications to clinical trials. Chapman and Hall, 1999.

175. Baquet CR, Ringen K, Pollack ES, et al. Cancer Among Blacks and Other Minorities: Statistical Profiles. Bethesda MD: National Institutes of Health. DHEW publication (NIH), 1986; 86:2785.

176. Young JL Jr, Ries LG, Pollack ES. Cancer patient survival among ethnic groups in the United States. J Natl Cancer Inst 1984; 73:341-352.

177. Satariano WA, Belle SH, Swanson GM. The severity of breast cancer at diagnosis: A comparison of age and extent of disease in black and white women. Am J Public Health 1986; 76:779-782.

178. Bassett MT, Krieger N. Social class and black-white differences in breast cancer survival. Am J Public Health 1986; 76:1400-1403.

179. Crowe JP Jr, Gordon NH, Hubay CA, et al. The interaction of estrogen receptor status and race in predicating prognosis for stage I breast cancer patients. Surgery 1986; 100:599-605.

180. Mohla S, Sampson CC, Khan T, et al. Estrogen and progesterone receptors in breast cancer in black Americans: Correlation of receptor data with tumor differentiation. Cancer 1982; 50: 552-559.

181. Dignam JJ, Redmond CK, Fisher B, et al. Prognosis among African-American women and white women with lymph node-negative breast carcinoma: Findings from two randomized clinical trials of the National Surgical Adjuvant Breast and Bowel Project (NSABP). Cancer 1997; 80:80-90.

182. Costantino JP, Redmond CK, Wickerham D, et al. A comparison of survival between white and black breast cancer patients: Results from NSABP B-09. Proc Am Soc Clin Oncol 1987; 6:230 Abstr 905.

APPENDIX A

DETERMINATION OF PERFORMANCE STATUS

02/15/08 **Performance status key**

ECOG or Zubrod Scale		Karnofsky Score
0	Fully active; able to carry on all pre-disease performance without restriction	90-100%
1	Restricted in physically strenuous activity but ambulatory	70-80%
2	Ambulatory and capable of self-care; but unable to carry out any work activities	50-60%
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours	30-40%
4	Completely disabled	10-20%

APPENDIX B

URINE PROTEIN/URINE CREATININE (UPC) RATIO INSTRUCTIONS

NSABP B-40 requires screening for proteinuria prior to study entry and at scheduled time points during the study. Because collection of a 24-hour urine specimen to screen for protein is inconvenient, urine protein/creatinine (UPC) ratio will be used to check for proteinuria.

A. Procedure for calculating the UPC ratio

1. The patient provides at least 4 ml of a random urine sample *(24-hour urine collection is not required for this study)*.
2. The lab determines urine protein (or urine albumin) concentration (result should be mg/dL).
3. The lab determines urine creatinine concentration (result should be mg/dL).
4. The coordinator (or lab) calculates the UPC ratio by dividing the urine protein (#2 above) by the urine creatinine (#3 above).

Total urine protein (or urine albumin) ÷ urine creatinine = UPC ratio

Example of UPC calculation:

46.5 mg/dL (urine protein) ÷ 1501.1 mg/dL (urine creatinine) = 0.0309 (UPC ratio)

5. The coordinator documents the UPC ratio to the nearest tenth or to one decimal point. Rounding the value is permitted. If the number being rounded has a 5, 6, 7, 8, or 9 in the hundredth position, round the number up. If the number in the hundredth position is 0, 1, 2, 3, or 4, round the number down. *In the above example, the UPC ratio of 0.0309 would be rounded down to 0.0. Other examples of rounding the UPC ratio:*

   0.167 is rounded up to 0.2
   0.132 is rounded down to 0.1
   0.941 is rounded down to 0.9
   0.951 is rounded up to 1.0

Important reminders:

- Do NOT use <u>serum</u> protein or <u>serum</u> creatinine results for this calculation.
- Do NOT use a dipstick to screen for proteinuria in B-40.
- Do NOT use microalbumin values to calculate the UPC ratio.

B. Using the UPC ratio for bevacizumab dose modifications and AE reporting

The UPC ratio directly correlates with the amount of protein excreted in the urine per 24 hours. A UPC of 1.0 is roughly equivalent to 1 gram of protein in a 24-hour urine collection. If the UPC ratio is < 1, then the amount of protein in a 24-hour urine specimen can be assumed to be < 1 gram per 24 hours.

For the purposes of B-40, proteinuria will be graded based on the UPC ratio applied to the CTCAE v3.0 criteria for grading proteinuria (measured in grams/24 hours).

*Example of using the UPC ratio for AE grading***:**

UPC ratio of 2.5 approximates 2.5 g/24 hrs = grade 2 proteinuria (> 1.0 - 3.5 g/24 hours)

APPENDIX C

FORMULA TO BE USED FOR CALCULATED CREATININE CLEARANCE

For females, creatinine clearance (mL/min) = $\frac{(140 - \text{age}) \times \text{weight (kg)} \times 0.85}{72 \times \text{serum creatinine (mg/dL)}}$

OR $\frac{(140 - \text{age}) \times \text{weight (kg)} \times 0.85}{0.81 \times \text{serum creatinine (mmol/L)}}$

For males, creatinine clearance (mL/min) = $\frac{(140 - \text{age}) \times \text{weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$

OR $\frac{(140 - \text{age}) \times \text{weight (kg)}}{0.81 \times \text{serum creatinine (mmol/L)}}$

Renal function is classified as normal (> 80 mL/min), mildly impaired (50 – 80 mL/min), moderately impaired (30 – 50 mL/min), or severely impaired (< 30 mL/min).

APPENDIX D

CAPECITABINE DOSE ACCORDING TO BODY SURFACE AREA

Dose Level 0 = 825 mg/m^2 twice daily		**Number of tablets administered *in the morning****		**Number of tablets administered *in the evening****	
Body Surface Area (m^2)	*Dose per administration (mg)*	*150 mg*	*500 mg*	*150 mg*	*500 mg*
< 1.31	1000	-	2	-	2
1.31 – 1.48	1150	1	2	1	2
1.49 – 1.67	1300	2	2	2	2
1.68 – 1.90	1500	-	3	-	3
1.91 – 2.09	1650	1	3	1	3
2.10 – 2.27	1800	2	3	2	3
> 2.27	2000	-	4	-	4
Dose Level -1 = 650 mg/m^2 twice daily		**Number of tablets administered *in the morning****		**Number of tablets administered *in the evening****	
Body Surface Area (m^2)	*Dose per administration (mg)*	*150 mg*	*500 mg*	*150 mg*	*500 mg*
< 1.31	800	2	1	2	1
1.31 – 1.48	950	3	1	3	1
1.49 – 1.67	1000	-	2	-	2
1.68 – 1.90	1150	1	2	1	2
1.91 – 2.09	1300	2	2	2	2
2.10 – 2.27	1500	-	3	-	3
> 2.27	1650	1	3	1	3
Dose Level -2 = 500 mg/m^2 twice daily		**Number of tablets administered *in the morning****		**Number of tablets administered *in the evening****	
Body Surface Area (m^2)	*Dose per administration (mg)*	*150 mg*	*500 mg*	*150 mg*	*500 mg*
< 1.31	650	1	1	1	1
1.31 – 1.48	800	2	1	2	1
1.49 – 1.67	800	2	1	2	1
1.68 – 1.90	950	3	1	3	1
1.91 – 2.09	1000	-	2	-	2
2.10 – 2.27	1150	1	2	1	2
> 2.27	1300	2	2	2	2

Please note: It is important to monitor patients' compliance in taking capecitabine. Investigators are strongly encouraged to provide a patient diary or calendar to record capecitabine dosing information. Pill counts can also be used to supplement the diary or calendar.

APPENDIX E

SUGGESTED PROCEDURE FOR EVALUATION OF SURGICAL SPECIMENS FOLLOWING NEOADJUVANT THERAPY ON B-40

The following procedures used for processing breast specimens following neoadjuvant chemotherapy at MD Anderson Cancer Center are provided as guidelines for NSABP institutions and investigators. They are provided as suggestions, and may be modified as needed to make them functional in participating institutions.

Specimens should be oriented with sutures by the surgeon following removal. The surgeon and breast pathologist should confer to ensure optimal evaluation of the primary tumor site for possible pCR.

02/15/08 Note: Refer to Section 8.4 for histopathology findings required for ALL B-40 patients for use by the NSABP Biostatistical Center in calculating the RCB index.

- **In cases showing significant clinical response:**
  - The breast resection specimen is radiographed to identify metallic markers which were placed during or prior to chemotherapy.
  - Each specimen is inked using multiple colors to identify each face of the specimen, and then sectioned into 3-5 mm slices.
  - The sliced specimen is radiographed and a radiologist reviews the films to determine the presence and extent of residual tumor.
  - The pathologist examines the sliced specimen grossly to identify suspicious areas and notes their proximity to margins.
  - The radiographic and pathological evaluation is discussed with the surgeon who decides whether additional margins should be obtained.
  - Permanent paraffin sections of the suspicious areas and margins are obtained. The number of sections taken is based on the gross inspection, radiologic features, and size of the resection specimen.
  - The entire radiographic abnormality as well as firm and suspicious appearing breast tissue is submitted for histologic evaluation.
  - In general, *for non-palpable (clinical complete response) cases, at least 10-15 blocks are examined* to assess the presence of residual microscopic disease.
- **In cases with residual palpable mass (partial clinical response or no response in the breast):**
  - The resection specimen is inked and sectioned into 3-5 mm slices.
  - The pathologist examines the slices and determines the tumor size on gross evaluation and confirms the tumor size by microscopic evaluation.
- **Evaluation of axillary lymph nodes regardless of response:**

  All axillary lymph nodes are also carefully evaluated by serial gross sectioning.
  - One or two representative histologic sections are evaluated for lymph nodes that contain grossly identifiable metastatic carcinoma.
  - The lymph nodes that do not show grossly identifiable tumor are submitted for histologic evaluation in their entirety. One representative histologic section is evaluated per paraffin block. Immunohistochemical staining for cytokeratin is not routinely performed on negative nodes.

APPENDIX F

CANCER TRIALS SUPPORT UNIT (CTSU) INSTRUCTIONS

These instructions supplement the protocol for CTSU participants. The protocol is to be followed in areas not described in this appendix.

05/30/07
02/15/08

1.0 SITE REGISTRATION AND PATIENT ENTRY FOR CTSU INVESTIGATORS

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 (http://members.ctsu.org) or by calling the PMB at 301-496-5725 Monday through Friday between 8:30 am and 4:30 pm 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. All forms and documents associated with this study can be downloaded from the NSABP B-40 Web page on the CTSU Member Web site (http://members.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 NSABP B-40 site registration:

- CTSU IRB Certification
- IRB/Regulatory Approval Transmittal Sheet

Requirements for patient enrollment on NSABP B-40:

- Patient must meet all inclusion criteria and no exclusion criteria should apply
- Patient has signed and dated the consent
- All baseline laboratory tests and pre-study evaluations performed
- Fresh tumor tissue (four cores) has been collected for submission to Precision Therapeutics, Inc.

CTSU Procedures for Patient Enrollment: Contact the CTSU Patient Registration Office by calling 1-888-462-3009 and leave a voice mail to alert the CTSU Patient Registrar that an enrollment is forthcoming. For immediate registration needs, i.e., within one hour, call the Registrar cell phone at 1-301-704-2376. Complete the following forms:

- CTSU Patient Enrollment Transmittal Form
- Form A (Registration Form) with necessary attachments
- Properly signed and dated B-40 consent form

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. Monday-Friday, Eastern Time (excluding holidays). Registrations received after 5:00 p.m. will be processed the next business day. The CTSU registrar will check the investigator and site information provided 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.

Once investigator eligibility is confirmed and enrollment documents are reviewed for compliance, the CTSU registrar will access the NSABP on-line registration system to obtain assignment of a treatment arm and assignment of a unique NSABP Patient ID (to be used on all future forms and correspondence). The CTSU registrar will confirm registration by fax.

2.0 DRUG ORDERS FOR CTSU INVESTIGATORS

Investigational IND agents

- bevacizumab – request drug through the NSABP Biostatistical Center
- capecitabine – request drug through the NSABP Biostatistical Center
- gemcitabine – request drug through the NSABP Biostatistical Center

Other commercial agents

- doxorubicin, cyclophosphamide, and docetaxel – obtain commercially

05/30/07

3.0 SPECIAL MATERIALS

02/15/08

All tissue, blood, serum specimen requirements, and information regarding ordering kits for core biopsies are outlined in Section 8.1 (Table 7) and the B-40 Pathology Instructions located on the B-40 Web page on the CTSU Registered Member Web site.

3.1 Submission of tissue by CTSU investigators

Tumor tissue procured in RNAlater, formalin, and shipping medium for PTI will be collected prior to randomization (for all patients). Tumor blocks are required from gross residual tumor ≥ 1 cm. (If gross residual tumor < 1 cm or no gross tumor, no tissue submission is required.) CTSU investigators should follow protocol directions for tissue procurement and submission to the NSABP Biostatistical Center (blocks) and Precision Therapeutics, Inc. (core biopsy samples - see Section 8.1). Do not submit specimens to the CTSU. A completed NSABP Form PTI must accompany the pre-entry core biopsy specimens. NSABP Form BLK must accompany the paraffin blocks.

3.2 Submission of blood/serum by CTSU investigators

CTSU will ship supplies for blood/serum collection to the CTSU site at the time of patient registration. Subsequent blood/serum collection kits will be sent to the CTSU site by the NSABP Serum Bank. CTSU investigators should follow protocol directions for collection of blood and serum (see Section 8.8). Specimens should be shipped the same day as collected to the **NSABP Serum Bank at the Baylor College of Medicine**. Clinics should schedule specimen shipments to occur on Monday – Thursday so as not to be received on the weekend. A completed NSABP Form BNK transmittal must accompany all shipments.

05/30/07 4.0 **DATA SUBMISSION FOR CTSU INVESTIGATORS**

All case report forms (CRFs) associated with this study must be downloaded from the NSABP B-40 Web page located on the CTSU Registered Member Web site (http://members.ctsu.org). Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.

Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and other documents directly to the NSABP Biostatistical Center. The preferred method of sending data is via fax at 412-622-2111. Do not include a cover sheet for faxed data.

The NSABP Biostatistical Center will send query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the NSABP Biostatistical Center and do not copy the CTSU Data Operations. If the query is sent with a fax transmittal form, return the data to the fax number on the transmittal form, otherwise fax to 412-624-1082.

Each site should have a designated CTSU Administrator and Data Administrator and **must keep their CTEP AMS account contact information current**. This will ensure timely communication between the clinical site and the NSABP Biostatistical Center.

5.0 **ADVERSE EVENT (AE) REPORTING BY CTSU INVESTIGATORS**

Specific reporting requirements for NSABP B-40 are found in protocol Section 14.0. The Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 is required for reporting adverse events for protocol B-40. A link to the CTCAE version 3.0 is available on the CTSU Member Web site. CTSU investigators should employ definitions of adverse events as described in Section 14.0 of the protocol. All reporting must be conducted within the time frames specified in Section 14.0, and all completed forms should be submitted as outlined in Section 14.0.

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

5.1 **Routine reporting**

CTSU institutions: please refer to Section 14.3 for instructions regarding routine adverse event reporting requirements. When indicated, supporting documentation must be included. The B-40 Adverse Event Form is to be completed by the CTSU institution and sent to the NSABP Biostatistical Center.

5.1 **Expedited reporting**

- Refer to Section 14.2 and Tables 31 and 32 for instructions regarding expedited adverse event reporting requirements.

- Contact the NSABP B-40 Research Nurse Specialist at the NSABP Biostatistical Center (refer to Information Resources, page v) for questions regarding completion of reports, the need for supporting documentation, and submission time constraints.

- Follow the instructions in Section 14.0 of the protocol when AdEERS reporting is required. Access the AdEERS electronic Web based application and complete it fully and accurately. AdEERS reports are submitted electronically to the NSABP

Lead Group, and available supporting documentation is faxed to the NSABP Biostatistical Center (412-622-2113) at the time of the AdEERS submission. Include the NSABP Patient ID number and the AdEERS ticket number on all supporting documentation.

5.3 Secondary AML/MDS/ALL reporting

Refer to protocol Section 14.2.4. CTSU investigators will submit the NCI Secondary AML/MDS Report Form and supporting documentation to the NSABP Biostatistical Center. The NSABP Research Nurse Specialist will review and forward the report form and supporting documentation to the NCI.

5.4 Pregnancy occurring while the patient is on protocol therapy

If a patient becomes pregnant while receiving protocol therapy, notify the NSABP Clinical Coordinating Division.

05/30/07 6.0 REGULATORY AND MONITORING

6.1 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, (e.g., NSABP members may only request credit for protocols pertaining to breast or colorectal cancers). Registrations to protocols for other disease sites may still take place through CTSU without receiving credit for your NSABP activities. Per capita reimbursement will be issued directly from CTSU.

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 CTSU Operations Manual located on the CTSU Registered Member Web site.

6.2. 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-US HIPAA guidelines are provided in the HIPAA Authorization Form located on the CTSU Web site.

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.

6.3 Clinical Data Update System (CDUS) monitoring

This study will monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. The sponsoring Group fulfills this reporting obligation by electronically transmitting to CTEP the CDUS data collected from the study-specific case report forms.

APPENDIX G

NSABP B-40 Sample Consent Form

NSABP PROTOCOL B-40

A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

Study Consent Version: February 15, 2008
Consent Addendum #1 Version: February 15, 2008
Consent Addendum #2 Version: April 1, 2011
To be attached to Protocol Version: April 1, 2011

Instructions to Local Institutional Review Boards Regarding
Local IRB Review of Multicenter Clinical Trials

In order to conform to OHRP guidelines (effective November 9, 1992) regarding local IRB review of multicenter clinical trials, and to provide local IRBs with flexibility in conforming to local standards, the NSABP provides the following instructions regarding the IRB approval process of this multicenter clinical trial.

The protocol and sample consent form provided by the NSABP have been reviewed and approved by the Division of Cancer Treatment and Diagnosis, National Cancer Institute. Local IRBs and the investigator are permitted to make changes to the consent form; however, the editorial changes must not alter the overall content or the intent of the information in the sample consent form. Should an investigator or local IRB delete or make a substantive modification of the information contained in the risks or alternative treatments sections of the consent form, this must be justified in writing by the investigator or the IRB and then approved by the IRB. Also, the NSABP Operations Center requires that, similarly, the NSABP also be notified of substantive changes in the consent form section regarding consent to collect and store samples for possible future testing. Of primary concern are text changes that could potentially affect the future usage of the banked samples. The IRB is responsible for reflecting in the IRB minutes the justification for, and approval of, such deletions or modifications. **The investigator is responsible for forwarding copies of substantive IRB-approved changes with their justifications to the NSABP Operations Center Division of Regulatory Affairs immediately.** It is the responsibility of the principal investigator and the IRB to determine what constitutes a substantive change. Any conflict between the two groups concerning this decision would be resolved at the NSABP Operations Center.

Upon receipt of these documents at the NSABP Operations Center staff will review and approve the changes and their justifications with input (as needed) from the Quality Assurance staff and government agencies.

NSABP SAMPLE CONSENT

Version as of 02/15/08
IRB Approved: 00/00/00

Consent Form
For

A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

(Note: Centers outside of the U.S. and Canada must insert the applicable country and government oversight agencies in place of the FDA and Health Canada where appropriate throughout the consent form.)

This is a clinical trial, a type of research study. You are being asked to take part in this study because you have breast cancer and you have not yet had surgery to remove the tumor. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

Who is conducting the study?

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is conducting this study.

(The NSABP institution must supply appropriate information as to who is conducting the trial locally.)

Why is this research study being done?

This study is being done for a number of reasons.

- One of the main purposes of the study is to learn how breast cancer tumors such as yours respond to several different chemotherapy combinations. Three of the chemotherapy drugs used in this study are docetaxel followed by the combination of doxorubicin and cyclophosphamide (AC), a standard treatment for breast cancer. This study will add the drug capecitabine and the drug gemcitabine to docetaxel to see if either drug improves the effectiveness of the standard drugs at killing all of the tumor cells in the breast and nearby lymph nodes.

  Capecitabine and gemcitabine are both used to treat breast cancer that has spread beyond the breast and nearby lymph nodes, so we expect they will also be effective in treating patients with earlier stage cancer like yours. Capecitabine and gemcitabine are considered investigational because they are still being researched and have not yet received approval from the Food and Drug Administration (FDA) for use in treating early stage breast cancer.

(<u>Canadian Sites</u> must insert the following paragraph <u>in place of</u> the last sentence above:

Health Canada has not approved the use of gemcitabine and capecitabine for the treatment of early stage breast cancer. Their use in this study is considered to be investigational.)

- A second main purpose of the study is to learn how breast cancer tumors like yours respond when the drug bevacizumab is added to the combinations of chemotherapy in this study. Bevacizumab is considered investigational because it is still being researched and has not yet received approval from the FDA for use in treating breast cancer.

(<u>Canadian Sites</u> must insert the following paragraph <u>in place of</u> the last sentence above:

Bevacizumab is not commercially available in Canada. Health Canada considers the use of this agent for treating breast cancer to be investigational.)

Bevacizumab is an "angiogenesis inhibitor." Angiogenesis inhibitors and chemotherapy work in different ways. Bevacizumab keeps the tumor from being able to make new blood vessels. Without new blood vessels, the growth of the tumor is slowed. Chemotherapy drugs kill cancer cells more directly.

- Another purpose of the study is to learn more about the side effects of the combinations of drugs used in this study. Included in what we will learn about side effects will be whether or not adding bevacizumab to chemotherapy for breast cancer will affect the heart. We will also learn if receiving bevacizumab has any effect on how patients recover from breast surgery.
- Another reason for doing this study is that tumor tissue collected before treatment starts will make it possible to do special tests, which should provide researchers at the NSABP with information about how tumors like yours respond to chemotherapy. Researchers hope to determine if results of specialized tests on tumor tissue can predict which tumors will be completely killed by the drug combinations used in this study.

How many people will take part in the study?

About 1200 women will take part in the study.

What will happen if I take part in this research study?

Before you begin the study: You will need to have the following exams, tests, and procedures to find out if you can be in the study. These exams, tests, and procedures are part of regular cancer care and may be done even if you do not join this study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- medical history and physical exam
- blood tests, including a pregnancy test for women of childbearing potential
- chest x-ray or chest CT scan
- breast examination to measure the tumor
- mammogram
- MUGA or echocardiogram (to see how well your heart pumps blood)
- EKG (electrocardiogram)

- bone scan, bone x-rays, or bone tests (only if you have bone pain or your blood tests show an increase in a bone-related protein)
- CT scan of the liver (only if your blood tests show abnormal liver function)
- ultrasound of the breast and underarm area, if your doctor thinks you need this

You will also need the following tests and procedure that are not part of regular cancer care and are being done for the purpose of this study.

- additional breast tumor biopsy to collect tissue for research purposes
- urine test (Because bevacizumab may cause some patients to have abnormal amounts of protein in the urine, a urine sample will be collected before you join the study.)

During the study: If the exams, tests and procedures show that you can be in the study and you choose to take part, then you will be "randomized" into one of the six treatment groups. Randomization means that you are put into a group by chance. A computer program will place you in one of the groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in one of the six groups.

After you have joined the study, you will begin your study therapy. You will receive your drugs on a schedule specific to your group. This schedule will be repeated every 21 days. This 21-day period is known as a ***cycle***. The treatment drugs and schedules are as follows:

Study therapy before surgery for patients randomized to Groups 1A, 2A, or 3A:

Group 1A

Part 1: You will receive docetaxel through a vein once every 21 days for 4 cycles. Each treatment will take about 1½ hours. The day before and in the morning on the day you receive docetaxel you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel. Also, starting the day after you receive docetaxel, you will need to receive another drug, either pegfilgrastim (Neulasta®) or filgrastim (Neupogen®). These drugs help to prevent low white blood cell counts that may lead to infection. These drugs are given as a shot under your skin. Your doctor or his/her staff may give you this shot or may teach you to give yourself this shot, or they can teach a friend or relative to give the shot to you. The shot may be given on 1 or more days, depending on which drug your doctor decides is best for you. Some patients experience bone pain with these drugs. If this happens, let your doctor know about it.

Part 2: After you have received docetaxel for 4 cycles, you will then receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. Each treatment will take about 1 hour.

Group 2A

Part 1: You will receive docetaxel through a vein once every 21 days for 4 cycles. Each treatment will take about 1½ hours. The day before and in the morning on the day you receive docetaxel you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel. You will also take capecitabine pills, twice a day, for 14 days starting on the day you receive docetaxel. Your doctor may ask you to write in a diary or on a calendar each dose of capecitabine that you take. You will then have 7 days without capecitabine before starting the next cycle.

Part 2: After you have received docetaxel and capecitabine for 4 cycles, you will then receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. Each treatment will take about 1 hour.

Group 3A

Part 1: On the first day of each cycle, you will receive docetaxel and gemcitabine through a vein. You will also receive gemcitabine a week after the first dose of each cycle. You will receive this therapy once every 21 days for 4 cycles. The day before and in the morning on the day you receive docetaxel, you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel. Each treatment will take about 2 hours on Day 1. Treatment will take about ½ hour when you receive gemcitabine alone.

Part 2: After you have received docetaxel and gemcitabine for 4 cycles, you will then receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. Each treatment will take about 1 hour.

Summary of study therapy for patients in Groups 1A, 2A, and 3A

PART 1		
Group 1A **Docetaxel** by vein every 3 weeks for 4 cycles	**Group 2A** **Docetaxel** by vein every 3 weeks for 4 cycles + **Capecitabine** by mouth Days 1-14 every 3 weeks for 4 cycles	**Group 3A** **Docetaxel** by vein every 3 weeks for 4 cycles + **Gemcitabine** by vein Days 1 and 8 every 3 weeks for 4 cycles
PART 2		
AC by vein every 3 weeks for 4 cycles	**AC** by vein every 3 weeks for 4 cycles	**AC** by vein every 3 weeks for 4 cycles
You will have breast surgery after completion of study therapy.		

Study therapy before and after surgery for patients randomized to Groups 1B, 2B, or 3B:

Group 1B

Part 1: On the first day of each cycle you will receive bevacizumab and the chemotherapy drug docetaxel through a vein. You will receive this therapy for a total of 4 cycles. It will take about 3 hours to receive your first dose of therapy. After that, if you have no problems receiving bevacizumab, it will take about 2 hours to receive your therapy.

The day before and in the morning on the day you receive docetaxel you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel. Also, starting the day after you receive docetaxel, you will also need to receive another drug, either pegfilgrastim (Neulasta®) or filgrastim (Neupogen®). These drugs help to prevent low white blood cell counts that may lead to infection. These drugs are given as a shot under your skin. Your doctor or his/her staff may give you this shot or may teach you to give yourself this shot, or they can teach a friend or relative to give the shot to you. The shot may be given on 1 or more days, depending on which drug your doctor decides is best for you. Some patients experience bone pain with these drugs. If this happens, let your doctor know about it.

Part 2: After you have received bevacizumab and docetaxel for 4 cycles, you will receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. You will continue to receive bevacizumab. However, during Part 2, you will receive bevacizumab every 21 days for only the first 2 cycles. You are given bevacizumab only during the first 2 cycles of AC so that you have at least one month without bevacizumab before your breast surgery. This will reduce the chance of having wound healing problems after your breast surgery. Each treatment will take about 1½ hours.

After surgery: After you have recovered from surgery but no sooner than 28 days after your surgery, you will receive bevacizumab through a vein once every 21 days for 10 doses. Each treatment will take about 1 hour.

Group 2B

Part 1: On the first day of each cycle you will receive bevacizumab and the chemotherapy drug docetaxel through a vein. You will receive this therapy for a total of 4 cycles. It will take about 3 hours to receive your first dose of therapy. After that, if you have no problems receiving bevacizumab, it will take about 2 hours to receive your therapy. The day before and in the morning on the day you receive docetaxel, you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel. You will also take capecitabine pills, twice a day, for 14 days starting on the day you receive docetaxel and bevacizumab. Your doctor may ask you to write in a diary or on a calendar each dose of capecitabine that you take. You will then have 7 days without capecitabine before starting the next cycle.

Part 2: After you have received bevacizumab, docetaxel, and capecitabine for 4 cycles, you will receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. You will continue to receive bevacizumab. However, during Part 2, you will receive bevacizumab every 21 days for only the first 2 cycles. You are given bevacizumab only during the first 2 cycles of AC so that you have at least one month without bevacizumab before your breast surgery. This will reduce the chance of having wound healing problems after your breast surgery. Each treatment will take about 1½ hours.

After surgery: After you have recovered from surgery but no sooner than 28 days after your surgery, you will receive bevacizumab through a vein once every 21 days for 10 doses. Each treatment will take about 1 hour.

Group 3B

Part 1: On the first day of each cycle, you will receive bevacizumab and the chemotherapy drugs docetaxel and gemcitabine through a vein. You will also receive gemcitabine a week after the first dose of each cycle. You will receive this therapy once every 21 days for 4 cycles. It will take about 3½ hours to receive your first dose of study therapy. After that, if you have no problems receiving bevacizumab, it will take about 2½ hours to receive your therapy when you receive all 3 drugs and about ½ hour when you receive gemcitabine alone. The day before and in the morning on the day you receive docetaxel you will take another drug called dexamethasone. Dexamethasone is a drug you take by mouth to help prevent some of the side effects of docetaxel.

Part 2: After you have received bevacizumab, docetaxel, and gemcitabine for 4 cycles, you will receive doxorubicin and cyclophosphamide (AC) through a vein once every 21 days for 4 cycles. You will continue to receive bevacizumab. However, during Part 2, you will receive bevacizumab every 21 days for only the first 2 cycles. You are given bevacizumab only during the first 2 cycles of AC so that you have at least one month without bevacizumab before your breast surgery. This will reduce the chance of having wound healing problems after your breast surgery. Each treatment will take about 1½ hours.

After surgery: After you have recovered from surgery but no sooner than 28 days after your surgery, you will receive bevacizumab through a vein once every 21 days for 10 doses. Each treatment will take about 1 hour.

Summary of study therapy for patients in Groups 1B, 2B, and 3B

PART 1		
Group 1B **Docetaxel** by vein every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for 4 cycles	**Group 2B** **Docetaxel** by vein every 3 weeks for 4 cycles + **Capecitabine** by mouth days 1-14 every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for 4 cycles	**Group 3B** **Docetaxel** by vein every 3 weeks for 4 cycles + **Gemcitabine** by vein Days 1 and 8 every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for 4 cycles
PART 2		
AC by vein every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for **2** cycles	**AC** by vein every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for **2** cycles	**AC** by vein every 3 weeks for 4 cycles + **Bevacizumab** by vein every 3 weeks for **2** cycles
You will have breast surgery after completion of the study therapy summarized above.		
Study therapy after surgery for Groups 1B, 2B, and 3B Bevacizumab by vein every 3 weeks for 10 cycles.		

During study therapy for all groups:

All patients will have the following exams and tests during study therapy. They are part of regular cancer care.

- a physical exam before each cycle of therapy (During some exams, you will have a breast examination to check your tumor.)
- blood tests to check your blood counts before each cycle of therapy
- blood tests to check how well your kidneys and liver are working before every other cycle of study therapy
- breast exam to measure the tumor after completing Part 1 of study therapy, before beginning AC

The following tests are not part of regular cancer care and are being done for the purpose of this study:

- urine test to check for protein in your urine before the 3rd and 5th cycles of study therapy (Groups 1B, 2B, and 3B patients only)
- optional blood collection for research before beginning study therapy and before the 3rd cycle of chemotherapy that includes docetaxel (only if you answered "yes" to the optional blood collection question at the end of this consent)

Before breast surgery for all groups: After you have recovered from preoperative study therapy, you will see your doctor and have the following exams and tests. They are part of regular cancer care.

- routine blood tests
- a physical exam
- breast examination to measure the tumor
- mammogram

The following tests are not part of regular cancer care and are being done for the purpose of this study:

- MUGA scan or echocardiogram
- optional blood collection for research (only if you answered "yes" to the optional blood collection question at the end of this consent)

You will then have either a lumpectomy or mastectomy to remove the remaining tumor and nearby lymph nodes. Surgery usually takes place 3-6 weeks after you finish study therapy. (If you are a patient in Group 1B, 2B, or 3B, your breast cancer surgery may not take place until at least one month after your last dose of bevacizumab before surgery.)

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Note: If you have a mastectomy (removal of the entire breast), you may decide to have breast reconstruction (plastic surgery to restore the shape and appearance of your breast) at the same time as the mastectomy. If you are randomized to Group 1B, 2B, or 3B and your breast reconstruction plan includes the use of a tissue expander, the expander may be placed at the time of the mastectomy and expanded slightly up until 2 weeks before you resume bevacizumab. No further expansion will be performed until after you complete your bevacizumab therapy. This is to avoid possible problems with wound healing related to bevacizumab. About 6 weeks following your last dose of bevacizumab, expansion of the expander will resume and your breast reconstruction will continue. Your doctor will explain all of your breast reconstruction options to you in more detail.

After surgery for all groups: All patients will have a physical exam or contact from the study doctor at 3 to 5 weeks after surgery and at 9 and 12 months from the time you joined the study.

During study therapy after surgery for Groups 1B, 2B, and 3B only:

You will have a history and physical exam before you begin bevacizumab after your surgery. This is part of regular cancer care.

The following tests will also be performed before you begin bevacizumab after surgery. These tests are not part of regular cancer care and are being done for the purpose of this study.

- blood tests to check blood counts and how well your kidneys and liver are working
- urine test to check for protein
- MUGA or echocardiogram (This will be repeated after surgery, before the start of bevacizumab, only if your test result before surgery was below normal.
  - If this repeat MUGA (or echocardiogram) result has improved to within normal range, bevacizumab will resume after surgery. A MUGA or echocardiogram will be repeated after you have received 4 doses of bevacizumab following surgery.
  - If this result has not improved to within normal range, you will not receive bevacizumab after surgery.

Before every other cycle of bevacizumab, you will have a history and physical exam which is part of regular cancer care.

You will also have the following tests which are not part of regular cancer care and are being done for the purpose of this study.

- urine test to check for protein before every other cycle of bevacizumab
- MUGA or echocardiogram at 18 months from when you joined the study, if you have received at least one dose of bevacizumab.

After completion of all study therapy and surgery for all groups:

- a physical exam about every 6 months for years 2 through 5 and about every 12 months years 6 through 10.
- mammogram about every 12 months

Other therapy for all patients:

Hormonal therapy: If your breast cancer is affected by hormones (estrogen or progesterone), your doctor will also give you at least 5 years of hormonal therapy after you complete your study therapy and surgery.

Radiation therapy: After you complete your study therapy, your doctor may advise you to have radiation therapy after your surgery.

How long will I be on the study?

You will be in this study for about 10 years. Your study therapy will last about 6 months if you are randomized to Groups 1A, 2A, or 3A or about 13 months if you are randomized to Groups

1B, 2B, or 3B. You will have your breast cancer surgery about 3-6 weeks after the last dose of study therapy given before surgery. (If you are a patient in Group 1B, 2B, or 3B, your breast cancer surgery may not take place until at least one month after your last dose of bevacizumab before surgery.) We would like to keep track of your health the entire 10 years you are on the study.

Can I stop being in the study?

Yes, you can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell the study doctor if you are thinking about stopping so any risks from the study drugs can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and tests will be most helpful for you.

You can choose to withdraw in one of two ways. In the first, you can stop your study treatment, but still allow the study doctor to report your health status to the NSABP until 10 years after you join the study. In the second, you can stop your study treatment and request that no new information be reported to the NSABP.

Can anyone else stop me from being in the study?

The study doctor may stop you from taking part in this study at any time if he or she believes it is in the best interest for your health, if you do not follow the study rules, or if the study is stopped by the NSABP.

What side effects or risks can I expect from being in the study?

You may have side effects while on this study. Most of these are listed here, but there may be other side effects that we cannot predict. Side effects will vary from person to person. Everyone taking part in the study will be carefully watched for any side effects. However, doctors do not know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medications to help lessen some of the side effects. Many side effects go away soon after you stop taking your study drugs. In some cases, side effects may be very serious, long-lasting, or may never go away. *There is also a risk of death.*

You should talk with your study doctor about any side effects that you may have while taking part in the study.

During the study, we will do blood tests to see if the dose of the drugs you are receiving during your therapy should be changed or delayed. The tests will also help monitor any side effects you may have. You will not need to be hospitalized unless you have serious side effects.

Risks and side effects related to docetaxel (Taxotere) (Groups 1A and 1B):

Likely effects

These side effects occur in **25% or more** of patients receiving docetaxel:

- Hair loss
- Nausea
- Vomiting
- Taste changes
- Weakness/loss of strength
- Fatigue
- Hot flashes (in premenopausal women)
- Irregular or permanent stoppage of menstrual cycles (periods)
- Inability to become pregnant
- Skin and nail changes, including discoloration and peeling
- Lowered white blood cell count (may lead to infection)
- Lowered red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Time away from work

These side effects occur in **10-24%** of patients receiving docetaxel:

- Diarrhea
- Constipation
- Loss of appetite
- Mouth sores
- Infection
- Pain in muscles, bones, or joints
- Headache
- Fluid retention (bloating or swelling)
- Numbness, tingling, prickling, and burning in the hands and feet

Less likely effects

These side effects occur in **3-9%** of patients receiving docetaxel:

- Ulcers in the stomach or bowels
- Darkening of the soles of the feet or palms of the hands
- Peeling of the skin (including hands and feet)
- Lowered number of platelets (which may lead to increased bruising or bleeding)
- Eye irritation
- Blurred vision
- Dizziness
- Changes (high or low) in blood pressure
- Hardening of the walls of the veins used for chemotherapy
- Reversible changes in blood test results that show possible liver injury

Rare but serious effects

These side effects occur in **less than 3%** of patients receiving docetaxel:

- Liver failure
- Gastrointestinal problems (such as bleeding, blockage, or perforation [opening of a hole] in the stomach or bowel)
- Lowered red blood cell count severe enough to require red blood cell transfusion
- Skin and tissue damage in the area surrounding the catheter where the chemotherapy drugs are injected
- Acute leukemia (cancer of the blood cells)
- Blood clots that may be life-threatening
- Heart damage
- Lung damage
- Severe infection
- Inflammation of the pancreas causing abdominal pain
- Allergic reaction including itching, hives, skin rash, flushing, shortness of breath, wheezing, chest tightness, fever, chills, severe shivering, sinus congestion, or swelling of face, especially eyelids
- A group of symptoms which may include a blister-like rash that may be severe; fever; inflamed eyes; redness, swelling, and painful sores on lips and in mouth. (If this occurs, you may need to be hospitalized and have IV fluids and medicines.)

Risks and side effects related to capecitabine (Xeloda) and docetaxel (Taxotere) (Groups 2A and 2B):

Likely effects

These side effects occur in **25% or more** of patients receiving capecitabine and docetaxel:

- Hair loss
- Nausea
- Vomiting
- Diarrhea
- Taste changes
- Weakness/loss of strength
- Fatigue
- Hot flashes (in premenopausal women)
- Irregular or permanent stoppage of menstrual cycles (periods)
- Inability to become pregnant
- Skin and nail changes, including discoloration and peeling
- Redness and tingling of palms and soles of feet
- Lowered white blood cell count (may lead to infection)
- Lowered red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Time away from work

These side effects occur in **10-24%** of patients receiving capecitabine and docetaxel:

- Constipation
- Loss of appetite
- Sores/inflammation in mouth and/or throat
- Abdominal pain
- Numbness, tingling, prickling, and burning in the hands and feet
- Pain in muscles, bones, or joints
- Headache
- Fluid retention (bloating or swelling)
- Infections
- Eye irritation (tearing)

Less likely effects

These side effects occur in **3-9%** of patients receiving capecitabine and docetaxel:

- Ulcers in the stomach or bowels
- Darkening of the soles of the feet or palms of the hands
- Peeling of the skin (including hands and feet)
- Lowered number of platelets (which may lead to increased bruising or bleeding)
- Rash or hives
- Flu-like symptoms
- Blurred vision
- Dizziness
- Changes (high or low) blood pressure
- Hardening of the walls of the veins used for chemotherapy
- Reversible changes in blood test results that show possible liver injury

Rare but serious effects

These side effects occur in **less than 3%** of patients receiving capecitabine and docetaxel:

- Liver failure
- Gastrointestinal problems (such as bleeding, blockage, or perforation [opening of a hole] in the stomach or bowel)
- Lowered red blood cell count severe enough to require red blood cell transfusion
- Skin and tissue damage in the area surrounding the catheter where the chemotherapy drugs are injected
- Acute leukemia (cancer of the blood cells)
- Blood clots that may be life-threatening
- Heart damage
- Lung damage
- Severe infection
- Inflammation of the pancreas causing abdominal pain
- Allergic reaction including itching, hives, skin rash, flushing, shortness of breath, wheezing, chest tightness, fever, chills, severe shivering, sinus congestion, or swelling of face, especially eyelids
- A group of symptoms that may be severe enough to require hospitalization and includes a blister-like rash; fever; inflamed eyes; redness, swelling, and painful sores on lips and in mouth (Stevens-Johnson Syndrome)

Risks and side effects related to gemcitabine (Gemzar) and docetaxel (Taxotere) (Groups 3A and 3B):

Likely effects

These side effects occur in **25% or more** of patients receiving gemcitabine and docetaxel:

- Hair loss
- Nausea
- Vomiting
- Taste changes
- Weakness/loss of strength
- Fatigue
- Irregular or permanent stoppage of menstrual cycle (periods)
- Hot flashes (in premenopausal women)
- Inability to become pregnant
- Skin rash
- Skin and nail changes, including discoloration and peeling
- Lowered white blood cell count (may lead to infection)
- Lowered red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Time away from work

These side effects occur in **10-24%** of patients receiving gemcitabine and docetaxel:

- Constipation
- Pain in muscles, bones, or joints
- Fluid retention (bloating or swelling)
- Loss of appetite
- Weight loss/gain
- Headache
- Fever
- Diarrhea
- Mouth sores
- Blood test results that show changes in liver function (usually mild and temporary)
- Numbness, tingling, prickling, and burning in hands and feet
- Flu-like symptoms (headache, fever, chills, cough, muscle pain, weak feeling, sleeplessness)
- Infection

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Less likely

These side effects occur in **3-9%** of patients receiving gemcitabine and docetaxel:

- Darkening of the soles of the feet or palms of the hands
- Peeling of the skin (including hands and feet)
- Lowered number of platelets (may lead to increased bruising or bleeding)
- Ulcers in stomach or bowels
- Changes in blood pressure (high or low)
- Dizziness
- Hardening of the walls of the veins used for chemotherapy
- Blurred vision
- Eye irritation

02/15/08 ***Rare but serious effects***

These side effects occur in **less than 3%** of patients receiving gemcitabine and docetaxel:

- Lowered red blood cell count severe enough to require red blood cell transfusions
- Lowered number of platelets severe enough to require a transfusion
- Liver failure
- Acute leukemia (cancer of the blood cells)
- Heart damage
- Blood clots that may be life-threatening
- Skin and tissue damage in the area surrounding the catheter where the chemotherapy drugs are injected
- Protein or blood in the urine that could mean kidney damage
- A breakdown of red blood cells and kidney failure known as hemolytic uremic syndrome
- Gastrointestinal problems (such as bleeding, blockage, or perforation [opening of a hole] in the stomach or bowel)
- A group of symptoms including a blister-like rash that may be severe; fever; inflamed eyes; redness, swelling, and painful sores on lips and in mouth.
- Severe infection
- Severe lung problems (including shortness of breath, inflammation, and damage that could be permanent)
- Inflammation of the pancreas causing abdominal pain
- Decreased blood flow to the hands and feet that may cause tissue damage that may result in the loss of tissue.
- Allergic reaction including itching, hives, skin rash, flushing, shortness of breath, wheezing, chest tightness, fever, chills, severe shivering, sinus congestion, or swelling of the face, especially eyelids

Risks and side effects related to doxorubicin (Adriamycin) and cyclophosphamide (All Groups):

Likely effects

These side effects occur in **25% or more** of patients receiving doxorubicin and cyclophosphamide:

- Hair loss
- Nausea
- Vomiting
- Low white blood cell count (may lead to infection)
- Fatigue
- Hot flashes (in premenopausal women)
- Inability to become pregnant
- Irregular or permanent stoppage of menstrual cycles (periods)
- Low red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Temporary red discoloration of urine (not blood)
- Time away from work

These side effects occur in **10-24%** of patients receiving doxorubicin and cyclophosphamide:

- Mouth sores
- Infection
- Taste changes
- Skin and nail changes, including discoloration and peeling

Less likely effects

These side effects may occur in **3-9%** of patients receiving doxorubicin and cyclophosphamide:

- Diarrhea
- Constipation
- Loss of appetite
- Darkening of the soles of the feet or palms of the hands
- Low platelet count (may lead to bleeding)
- Irregular heartbeat
- Eye irritation
- Hardening of the walls of the veins used for chemotherapy
- Temporary changes in liver function blood tests
- Headache
- Pain in muscles, bones, or joints

Rare but serious effects

These side effects occur in **less than 3%** of patients receiving doxorubicin and cyclophosphamide:

- Skin damage (due to leakage of drugs during treatment)
- Acute leukemia (cancer of the blood cells)
- Blood clots that may be life-threatening
- Decreased ability of the heart to pump blood. If severe, you could have shortness of breath and other symptoms of heart failure. (If mild, you may not have any symptoms.)
- Lung damage
- Severe infection
- Severe bladder irritation
- Lowered red blood cell count severe enough to require red blood cell transfusion
- Allergic reaction including itching, hives, rash, flushing, shortness of breath, wheezing, chest tightness, fever, chills, swelling of face, severe breathing problems

Risks and side effects related to bevacizumab (Groups 1B, 2B, and 3B):

Likely

These side effects occur in **25% or more** of patients receiving bevacizumab:

- Nose bleeds
- High blood pressure
- Protein in the urine

These side effects occur in **10-24%** of patients receiving bevacizumab:

- Shortness of breath
- Watery eyes
- Skin and nail changes (including dryness, itching, rash, discoloration, ulcers, or peeling)
- Sores in mouth and/or throat
- Taste changes
- Mild to moderate bleeding in the gastrointestinal tract (serious and life-threatening bleeding events were rare)

02/15/08 ***Less likely***

These side effects occur in **3-9%** of patients receiving bevacizumab:

- Gastrointestinal upset (which may include heartburn, gas, constipation, diarrhea, nausea, vomiting, or loss of appetite)
- Abdominal pain
- Abnormally slow bowel contraction
- Headache
- Pain in the chest area
- Flu-like symptoms, such as fevers, chills, muscle aches, joint pain, and stiffness
- Low red blood cell count (may lead to anemia, tiredness, shortness of breath)
- Low platelet count that might interfere with clotting
- Low white blood cell count (may make you more likely to get infections)
- Allergy type symptoms like stuffy nose and sneezing
- Tiredness/weakness
- Weight loss
- Confusion
- Unsteady walk or loss of balance
- Low sodium and/or potassium levels that might make you feel weak or dizzy
- Cough
- Frequent urination
- Bleeding from the lining of the vagina
- Voice changes (hoarseness)
- Blood clots in a vein
- Changes in blood tests that indicate possible kidney damage
- Blood clots in your arteries which may cause stroke or heart attack or other problems. Several studies comparing chemotherapy with bevacizumab to chemotherapy alone have been done in patients with advanced cancers. Side effects of each study were looked at together. Problems due to blood clots in arteries were seen in about 2% of patients receiving chemotherapy alone and about 4.5% of patients receiving bevacizumab with chemotherapy. Patients who were 65 or older and those with past problems with blood clots in their arteries appeared to be at greatest risk. Problems due to blood clots in the arteries were seen in about 2.9% of patients 65 or older receiving chemotherapy alone and about 8.5% of patients 65 or older receiving bevacizumab with chemotherapy. Patients who were both 65 or older and reported a history of past problems with blood clots in their arteries appeared to be at even higher risk, although further study is required before an estimate of the risk can be provided. These conditions can be life-threatening or fatal.

02/15/08 ***Rare but serious***

These side effects occur in **less than 3%** of patients receiving bevacizumab:

- Bleeding in various parts of the body leading to disability (including stroke) or death (especially in lung cancer patients).
- Allergic reaction including: fever, chills, rash, hives, flushing, low blood pressure, swelling, and shortness of breath
- Reaction to the infusion including: fever, chills, hives, rash, joint pain, shortness of breath, low or high blood pressure, muscle stiffening, and sweating may occur during the infusion and last about 24 hours
- Kidney damage
- Coughing up blood
- Heart problems (including irregular heartbeats, changes in blood pressure, fluid collections surrounding the heart, chest pain, and possibly heart attack or heart failure)
- Infection

- Lung problems
- Reversible changes in liver function tests that may indicate liver damage
- Bowel perforation, which is an abnormal opening in the bowel wall allowing bowel contents to spill into the abdomen. This problem can lead to a life-threatening infection and usually requires surgery to repair.

  In a small study for patients with advanced lung cancer, 3 out of 17 patients who were receiving the combination of docetaxel, gemcitabine, and bevacizumab developed bowel perforations that resulted in death. Because of these deaths, the study was stopped. Bowel perforations have not been reported in patients receiving this same drug combination in 2 other studies, so it is not clear whether the bowel perforations that occurred in the lung cancer patients were related to this combination of drugs or to other medical conditions.
- In addition to bowel perforation, perforation of other organs or tissues.
- Fistula (an abnormal tube-like connection between internal organs and skin or other tissues that are not normally connected) may occur, for example, between the gastrointestinal tract and the skin or between the gastrointestinal tract and the vagina. A rare type of fistula, tracheo-esophageal fistula, which is an abnormal connection between the windpipe (trachea) and the esophagus (the tube that connects the mouth to the stomach) has been reported.
- Risk related to wound healing: There have been reports of patients receiving bevacizumab who developed problems with healing of their surgical wounds. This problem can lead to a life-threatening infection and could require surgery to repair. While you are receiving bevacizumab, your doctor will temporarily stop your bevacizumab therapy prior to any surgery to avoid possible problems with wound healing. If you need surgery for any reason while on bevacizumab, tell your doctor.

  There have also been a few reports of patients receiving bevacizumab in other trials who developed complications with wound healing due to skin problems such as leg ulcers. These wound healing problems have been associated with infections that can take a long time to heal.
- Reversible Posterior Leukoencephalopathy Syndrome (RPLS) is a medical condition related to leakiness of blood vessels in the brain. RPLS can cause headaches, confusion, vision changes or blindness, and seizure, as well as changes in brain scans. This condition is usually temporary but in very rare cases, it is potentially life-threatening and may have long-term effects on brain function.
- Severe high blood pressure that can have an effect on brain function and can be life-threatening.
- High blood pressure of the blood vessels in the lungs

Risks related to fertility and pregnancy:

The drugs in this study can affect an unborn baby. Therefore, you should not become pregnant while on this study, or if you received bevacizumab, at least 3 months after your last dose. You should ask about counseling and more information about preventing pregnancy. If you feel you might be pregnant, even though you practiced birth control, notify the study doctor immediately. A pregnancy test may be performed. Women should not breastfeed a baby while on this study, or if you received bevacizumab, at least 3 months after your last dose.

Some of the drugs used in this study may make you unable to have children in the future.

For more information about risks and side effects, ask your study doctor.

Are there benefits to taking part in this study?

Taking part in this study may or may not make your health better. While doctors hope that adding capecitabine, gemcitabine, or bevacizumab to standard chemotherapy will be more useful in treating your breast cancer compared to standard chemotherapy alone, there is no proof of this yet. We do know that the information from this study will help doctors learn more about capecitabine and gemcitabine as treatments for breast cancer. This information could help future cancer patients.

What other choices do I have if I do not take part in this study?

Your other choices may include:

- Getting treatment or care for your cancer without being in this study
- Taking part in another study
- Having surgery first and then deciding about other treatments after surgery
- Getting no treatment

Please talk with your doctor about your choices before you decide if you will take part in this study.

02/15/08 **Will my medical information be kept private?**

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Organizations that may look at and/or copy your medical records for research, for quality assurance, and data analysis include:

- the National Surgical Adjuvant Breast and Bowel Project (NSABP);
- Eli Lilly and Company (the company that makes gemcitabine);
- Roche Laboratories, Inc. (the company that makes capecitabine);
- Genentech, Inc. (the company that makes bevacizumab);
- Precision Therapeutics, Inc. (a company involved with the tissue research);
- your local Institutional Review Board (IRB), a group of people who review the research study to protect your rights;
- the Cancer Trials Support Unit (CTSU), a research group sponsored by the National Cancer Institute (NCI) to provide greater access to cancer trials; and
- government agencies, including the NCI or its authorized representatives, the FDA, the Office for Human Research Protections (OHRP), and Health Canada. These agencies may review the research to see that it is being done safely and correctly.

What are the costs of taking part in this study?

You and/or your health plan/insurance company will need to pay for some or all of the costs of treating your cancer in this study except for those described below in this section. Some health plans will not pay these costs for people taking part in studies. Check with your health plan or

insurance company to find out what they will pay for. Taking part in this study may or may not cost your insurance company more than the cost of getting regular cancer treatment.

Tests, procedures, or drugs for which there is no charge in this study:

- MUGA scans (or echocardiograms) performed during the first 2 years after you join the study.
- All urine tests to check for protein. These tests will be done before you join the study and at the scheduled time points described earlier in this consent form.
- Costs of the blood test to check your blood counts just before beginning postoperative bevacizumab (Groups 1B, 2B, and 3B only).
- Costs of blood tests to check how well your kidneys and liver are working just before beginning postoperative bevacizumab (Groups 1B, 2B, and 3B only).
- Costs of the ***required*** additional breast tumor biopsy to collect tissue for research purposes and the ***required*** tissue collection at the time of surgery as described at the end of this consent.
- Costs of the three ***optional*** blood collections done solely for the purpose of the B-40 study as described at the end of this consent.
- Gemcitabine will be provided for this study at no cost to you by Eli Lilly and Company. Roche Laboratories, Inc. will provide the capecitabine for this study at no cost to you. Genentech, Inc. will provide the bevacizumab for this study at no cost to you. However, you or your health plan will need to pay for costs of the supplies and personnel who give you the drugs. Every effort will be made to ensure adequate supply of gemcitabine, capecitabine, and bevacizumab, free-of-charge, for all patients assigned to these drugs. Gemcitabine, capecitabine, and bevacizumab may become commercially available for your stage of breast cancer during this study. If this happens, there is a possibility that you and/or your health plan may have to pay for the drug needed to complete the study. Your study doctor will discuss this with you if this occurs.

(<u>Canadian Sites</u> must use the following information <u>in place of</u> the text above regarding costs of the investigational drugs in the study:

Eli Lilly and Company will provide the gemcitabine free-of-charge for this study. Roche Laboratories, Inc. will provide the capecitabine free-of-charge for this study. Genentech, Inc. will provide the bevacizumab free-of-charge for this study. Every effort will be made to ensure an adequate supply of gemcitabine, capecitabine, and bevacizumab, free-of-charge, for all patients assigned to these drugs. If, however, gemcitabine, capecitabine, or bevacizumab becomes commercially available for your stage of breast cancer during this study, there is a remote possibility that alternative arrangements may have to be made to pay for these drugs. Your physician will discuss this with you should this situation arise.)

Bevacizumab is the common name for the commercial drug Avastin. The bevacizumab used in this trial, however, is for use in research studies only and may be made at locations different from those where Avastin is made. Although some differences may exist, bevacizumab for research use and the commercial drug Avastin are manufactured by a similar process, meet similar standards for final product testing, and are expected to be very similar in safety and effectiveness.

Doxorubicin, cyclophosphamide, docetaxel, pegfilgrastim, and filgrastim are commercially available and will not be provided for free with this study. You and/or your health plan/insurance company will be responsible for the costs of doxorubicin, cyclophosphamide, docetaxel, and, if given, pegfilgrastim and filgrastim.

You will not be paid for taking part in this study. Taking part in this study may result in added costs to you.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site. Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____***(insert doctor's name)***, if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him or her at _____***(insert doctor's phone number).***

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

(<u>Canadian Sites</u> must insert the following paragraph <u>in place of</u> the one above:

In the case of research-related side effects or injury, medical care will be provided by your doctor or you will be referred for appropriate medical care. Although no funds have been set aside to compensate you in the event of injury or illness related to the study treatment or procedures, you do not waive any of your legal rights for compensation by signing this form.)

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

The Data Monitoring Committee (DMC), an independent group of experts, will be reviewing the data from this research throughout the study. We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study. You may be asked to sign another consent form in response to new information.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____***(insert doctor's name and phone number).***

For questions about your rights while taking part in this study, call the _____ ***(insert the institution's name)*** Institutional Review Board (IRB) (a group of people who review the research to protect your rights) at _____ ***(insert IRB phone number).***

(If your institution is using the NCI Central IRB, insert the following sentence: **You may also call the Operations Office of the NCI Central Institutional Review Board [CIRB] at 1-888-657-3711 [from the continental U.S. only].)**

Additional Tests for the NSABP B-40 Study

What about the use of my blood and tissue for research?

About using blood and tissue for research: One of the main reasons for doing this study is to test blood and tissue samples to find out if doctors in the future will be able to predict which patients will benefit from specific drugs or combinations of drugs. Not all patients will respond the same way to chemotherapy. We hope the samples collected in this study will help doctors be able to select the best drugs for patients in the future.

The research that will be done with your blood and tissue samples is not designed to specifically help you. It might help people who have cancer in the future. Reports about research done with your samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your blood and tissue samples will not affect your care. These blood and tissue samples will not be used for genetic research about diseases that are passed on in families.

The blood and tissue samples will be stored at the NSABP and will be used for the purpose of the B-40 study. Some of the research tests will be done soon, but others will be done in the future when the best methods are ready to test the samples. The NSABP will study the samples and may give them to other researchers approved by the NSABP. Any research using your samples must also be approved by an Institutional Review Board (IRB). An IRB is a group of people who review the research to determine if it is being done correctly and safely.

People who do research with your blood and tissue samples may need to know more about your health. While the NSABP may give them reports about your health, they will not give them your name, address, phone number, or any other information that will let the researchers know who you are.

Your blood and tissue samples will be used only for research and will not be sold. The research done with your samples may help to develop new products in the future, but you will not be paid.

Collection of tissue samples is required if you take part in this study. Collection of blood samples is optional. All of the samples are important to answer questions about how breast cancer responds to different combinations of chemotherapy. Each of these tissue and blood collections is described in more detail on the following pages.

Required collection of tissue before chemotherapy begins: These samples must be collected before you join the study.

By signing this consent form, you are agreeing to have an additional biopsy before you join the study. You will have small samples of your tumor removed and sent to the NSABP. This is required for you to participate in this study because it is a very important part of this study. You or your insurance company will not be charged for the tissue sample procedure.

You are also agreeing to allow the NSABP to use the samples for this research study. Since these samples are required for this study, if you do not agree, you cannot be in the study.

02/15/08

Required collection of tissue following your surgery: Tissue removed during your surgery will be examined in the hospital's standard manner. If any tumor 1 cm (about half an inch) or larger is remaining, small samples of the tumor will then be sent to the NSABP. Researchers will compare the results of these samples to the samples collected before you joined the study. This will help answer the question of how tumors respond to chemotherapy.

Optional collection of blood: The NSABP would also like to have samples of your blood collected at three times during the study. If you agree, these collections will usually be taken at the same times that other blood tests that are done for your cancer treatment. They will not usually require a separate collection. The blood samples will be collected:

- before you start your chemotherapy,
- before your third cycle of chemotherapy that includes docetaxel, and
- after you have finished all of your chemotherapy (before your surgery).

Researchers will look at markers in the blood that may give them information about how the chemotherapy regimens work for different patients.

If you decide now that your blood and tissue samples can be kept for this research, you can change your mind at any time. Just *contact your study doctor* and let him or her know that you do not want the NSABP to use your blood and tissue samples and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until the NSABP decides to destroy them.

Benefits and risks: The possible benefits of research from your blood and tissue include learning more about what causes cancer and how to treat it.

There are risks to having a biopsy performed. These include discomfort during the biopsy, bleeding at the site of the biopsy, and infection. In addition, there is a risk of the release of information from your health records. The NSABP will protect your records so that your name, address, and phone number will be kept private. The chance that this information will be given to someone else is very small.

Costs of the sample collections: You and/or your health plan/insurance company will need to pay for all of the costs of your first breast biopsy which was done to diagnose your cancer. Check with your health plan or insurance company to find out what they will pay for. *There will be no cost to you for the additional breast tumor biopsy required for this study, the required tissue collection at the time of surgery, or for the collection and storage of the optional blood collections in this study.*

Making your choices

Please read each question below and think about your choice. After reading each question, circle "yes" or "no." If you have questions, please talk to your doctor or health care team member.

Participation in the optional blood collection: Remember, no matter what you decide about the ***optional*** collection and use of the blood samples in this research study, you may still take part in the B-40 study.

1. I agree to have blood samples collected three times during this study. These samples will be sent to the NSABP for the research purposes of the B-40 study.

   YES NO

Contact in the future for other research: Remember, no matter what you decide, you may still take part in the B-40 study.

2. My study doctor (or someone he or she chooses) may contact me in the future to ask me to take part in more research.

   YES NO

Where can I get more information about cancer and its treatment?

- You may call the National Cancer Institute's (NCI's) Cancer Information Service at: 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615
- You may also visit the NCI Web site at http://cancer.gov
- For the NCI's clinical trials information, go to: http://cancer.gov/clinicaltrials
- For the NCI's general information about cancer, go to: http://cancer.gov/cancerinfo
- You may also visit the NSABP Web site at http://www.nsabp.pitt.edu

You will receive a copy of this form. If you want more information about this study, ask your study doctor.

(NSABP institutions may insert or attach a list of materials that they can provide locally to patients regarding clinical trials, drug information, the institution/investigator, and/or the NSABP.)

Signatures

I have been given a copy of all twenty-five pages of this form. I have read the consent form or it has been read to me. This information was explained to me and my questions were answered.

I agree to take part in this research study, including the collection of the required tissue samples.

_____	_____
Date	Patient's signature
_____	_____
Date	Signature of person conducting the informed consent discussion

NSABP SAMPLE CONSENT FORM ADDENDUM #1

Consent Form Addendum #1
for
A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

[This information was not provided in the original consent form that patients signed. It is provided here as new information to be given to patients after local approval of Amendment #2.]

When you joined the NSABP B-40 study, the NSABP promised to tell you about new information that might affect your participation in the trial. The NSABP would like to inform you of some changes to the study and some additional side effects related to the drugs used in this study.

New information about side effects related to bevacizumab:

You were told in the original consent form you signed that the drug bevacizumab may cause wound healing problems for patients who undergo surgery while receiving the drug. There have also been a few reports of patients receiving bevacizumab in other trials who developed complications with wound healing due to skin problems such as leg ulcers. These wound healing problems have been associated with infections that may take a long time to heal. Therefore, if you develop any skin problems, such as open sores or wounds, tell your study doctor.

You were also told in the original consent form you signed that some patients with cancer in the abdomen or pelvis have developed bowel perforations while receiving bevacizumab. (A bowel perforation is an abnormal opening in the bowel wall allowing bowel contents to spill into the abdomen.) The company that makes bevacizumab recently reported that patients receiving bevacizumab for cancers that are not in the abdomen and pelvis have also had bowel perforations.

In a small study for patients with advanced lung cancer, 3 out of 17 patients who were receiving the combination of docetaxel, gemcitabine, and bevacizumab developed bowel perforations that resulted in death. Because of these deaths, the study was stopped. Bowel perforations have not been reported in patients receiving this same drug combination in 2 other studies, so it is not clear whether the bowel perforations that occurred in the lung cancer patients were related to this combination of drugs or to other medical conditions.

Since you signed the original consent form, there have also been reports of patients receiving bevacizumab who experienced the following side effects:

- In addition to bowel perforation, perforation of other organs or tissues (rare, occurring in less than 3% of patients receiving bevacizumab).
- Fistula (an abnormal tube-like connection between internal organs and skin or other tissues that are not normally connected) may occur, for example, between the gastrointestinal tract and the skin or between the gastrointestinal tract and the vagina. A rare type of fistula, tracheo-esophageal fistula, which is an abnormal connection between the windpipe (trachea) and the esophagus (the tube that connects the mouth to the stomach) has been reported. (rare, occurring in less than 3% of patients receiving bevacizumab)
- High blood pressure of the blood vessels in the lungs (rare, occurring in less than 3% of patients receiving bevacizumab).
- Low red blood cell count (may lead to anemia, tiredness, shortness of breath); (less likely, occurring in 3-9% of patients receiving bevacizumab).
- Allergy type symptoms like stuffy nose and sneezing (less likely, occurring in 3-9% of patients receiving bevacizumab).
- Abnormally slow bowel contraction (less likely, occurring in 3-9% of patients receiving bevacizumab)
- Bleeding from the lining of the vagina (less likely, occurring in 3-9% of patients receiving bevacizumab)

Information about side effects related to gemcitabine and docetaxel:

You were told in the original consent form you signed that the drug docetaxel alone and the combination of capecitabine and docetaxel may cause the side effects listed below. However, these risks were accidentally not included in the list of risks related to the combination of gemcitabine and docetaxel. Thus, the NSABP would like to inform you that the risks listed below are also related to the combination of gemcitabine and docetaxel.

- Peeling of the skin (including hands and feet); (less likely, occurring in 3-9% of patients receiving gemcitabine and docetaxel)
- Blurred vision (less likely, occurring in 3-9% of patients receiving gemcitabine and docetaxel)
- Heart damage (rare, occurring in less than 3% of patients receiving gemcitabine and docetaxel)
- Gastrointestinal problems (such as bleeding, blockage, or perforation which is an abnormal opening in the stomach or bowel); (rare, occurring in less than 3% of patients receiving gemcitabine and docetaxel)

Information about required tissue collection following your surgery:

You were told in the original consent form you signed that small samples of the tissue removed during your surgery would be sent to the NSABP. They now will only receive samples if any tumor remaining at the time of your surgery is 1 cm (about half an inch) or larger.

Information about breast reconstruction:

You were told in the original consent form you signed that if you were randomized to Group 1B, 2B, or 3B and you have a mastectomy, that you would not be able to have breast reconstruction until at least 3 months after your bevacizumab is completed. If you have not yet had your mastectomy, you may have breast reconstruction at the same time as your mastectomy. However, if tissue expanders are used, the expander may only be expanded slightly and only up until 2 weeks before you resume bevacizumab. No further expansion will be performed until after you complete your bevacizumab therapy.

If you have questions about any of this information, you should discuss them with your study doctor ***(insert name and phone number here)***.

Signatures

I have been given this new information that was not in the original consent form.

_____	_____
Date	Patient's Signature
_____	_____
Date	Signature of person conducting the informed consent discussion

NSABP SAMPLE CONSENT FORM ADDENDUM #2

Consent Form Addendum #2
for
A Randomized Phase III Trial of Neoadjuvant Therapy in Patients with Palpable and Operable Breast Cancer Evaluating the Effect on Pathologic Complete Response (pCR) of Adding Capecitabine or Gemcitabine to Docetaxel when Administered Before AC with or without Bevacizumab and Correlative Science Studies Attempting to Identify Predictors of High Likelihood for pCR with Each of the Regimens

[This information was not provided in the original consent form that patients signed. It is provided here as new information to be given to patients enrolled on the study and randomized to receive bevacizumab.]

When you joined the NSABP B-40 study, the NSABP promised to tell you about new information that might affect your participation in the trial.

You are being provided with this information because you were assigned to receive bevacizumab as part of the B-40 study. The purpose of this addendum is to inform you about recent information reported about the use of bevacizumab.

- A review of studies using bevacizumab in patients with a *variety of advanced cancers* showed that more patients died from the side effects of treatment when they received bevacizumab along with chemotherapy than when they received chemotherapy without bevacizumab. However, when only the studies in patients with *advanced breast cancer* were considered, this difference was not present. We do not know how this information applies to patients like you with *early stage breast cancer*.
- The FDA did an updated review of the results from four studies using bevacizumab with chemotherapy in patients with *advanced (metastatic) breast cancer*. On the basis of this review, the FDA has stated that the benefit of adding bevacizumab to chemotherapy in patients with *advanced breast cancer* did not outweigh the risks of adding bevacizumab in terms of patients living longer and slowing the spread of their cancer. We do not know what the results mean for patients like you with *early stage breast cancer*.
- Two large studies (NSABP C-08 and AVANT) in which patients with *colon (bowel) cancer* received either standard chemotherapy alone or standard chemotherapy with bevacizumab following surgery have recently been reported. The purpose of these studies was to determine if bevacizumab could improve results achieved with standard chemotherapy. The NSABP C-08 study data showed that adding bevacizumab to chemotherapy did not change the activity of the chemotherapy alone. The AVANT study showed that bevacizumab did not improve the results of chemotherapy, and some of the colon cancer patients appeared to be doing better with chemotherapy alone. Side effects reported for the NSABP C-08 and the AVANT studies were in line with what is known for chemotherapy and bevacizumab. The B-40 study should provide important information about adding bevacizumab to chemotherapy for patients like you with *early stage breast cancer*.

The Data Monitoring Committee (an independent group of scientific experts) monitors the data from B-40 and data from other trials as it becomes available. These scientific experts carefully evaluate the well-being of all patients participating in B-40 on a regular basis. These scientific experts have recommended that the B-40 study continue with no change to either treatment or follow-up visits.

If you have any questions or concerns, you can discuss them with your doctor at any time ***(insert name and phone number here)***. I understand that I may withdraw from this study at any time, and it will not affect my future care.

Signatures

I have been given this new information that was not in the original consent form. I have been given a copy of this consent form.

_____	_____
Date	Patient's signature
	_____
	Print patient's name
_____	_____
Date	Signature of person conducting the informed consent discussion
	_____
	Print name of person conducting the informed consent discussion

[Additional signature lines may be added as required by local policies or regulations.]