NRG ONCOLOGY
NSABP PROTOCOL B-43
ClinicalTrials.gov NCT 00769379

A Phase III Clinical Trial Comparing Trastuzumab Given Concurrently with Radiation Therapy and Radiation Therapy Alone for Women with HER2-Positive Ductal Carcinoma In Situ Resected by Lumpectomy

This trial is part of the National Clinical Trials Network (NCTN) program, which is sponsored by the National Cancer Institute (NCI). The trial will be led by NRG Oncology with the participation of the network of NCTN organizations: the Alliance for Clinical Trials in Oncology, ECOG-ACRIN Cancer Research Group, NRG Oncology, and SWOG

NRG Oncology
Nova Tower 2
Two Allegheny Center – Suite 1200
Pittsburgh, PA 15212
TELEPHONE: 412-339-5300

NRG Oncology Statistics and Data Management Center
One Sterling Plaza
201 North Craig Street, Suite 500
Pittsburgh, PA 15213
TELEPHONE: 412-624-2666
FAX: 412-624-1082

Clinical Coordinating Department: 1-800-477-7227
(For clinical questions only)

KEY STUDY PERSONNEL

NRG Oncology Chairman:	Norman Wolmark, MD
NRG Oncology Breast Committee Chair	Eleftherios Mamounas, MD, MPH
Protocol Chair:	Melody Cobleigh, MD
Radiation Oncology Protocol Chair:	Douglas Arthur, MD
Protocol Officer:	Thomas Julian, MD
Radiation Oncology Protocol Officer:	David Parda, MD
Protocol Statistician:	Stewart Anderson, PhD
Protocol Pathologists:	Soonmyung Paik, MD
	Kalliopi Siziopikou, MD, PhD

Protocol B-43 (trastuzumab), sponsored by the NCI

STUDY DRUG	NSC#	DRUG SUPPLY
Trastuzumab	688097	Genentech, a Member of the Roche Group, through the NCI

NCI Version Date: June 3, 2016, (Replaces all other versions)

NRG ONCOLOGY
NSABP PROTOCOL B-43
A Phase III Clinical Trial Comparing Trastuzumab Given Concurrently with Radiation Therapy and Radiation Therapy Alone for Women with HER2-Positive Ductal Carcinoma In Situ Resected by Lumpectomy

Document History

	Version/Update Date	Broadcast Date
Amendment 8	June 3, 2016	TBD
Amendment 7	November 14, 2014	June 15, 2015
Amendment 6	April 16, 2014	July 2, 2014
Amendment 5	April 2, 2012	January 26, 2012
Amendment 4	July 18, 2011	August 11, 2011
Amendment 3	September 10, 2010	September 30, 2010
Amendment 2	May 21, 2010	September 30, 2010
Amendment 1	February 16, 2010	March 23, 2010
Activation	May 28, 2008	October 17, 2008

PROTOCOL REVISION RECORD

Original Version: May 28, 2008

Amendment #1: February 16, 2010

Sections Changed

Cover Page
Information Resources
Section 1.0
Section 4.0: 4.1
Section 6.0: 6.1, 6.2
Section 9.0: 9.3, 9.5
Section 10.0: 10.2
Section 12.0: 12.1
Section 16.0: Reference 46
Appendix B: 3.0, 4.0
Appendix C: Cover page, Pages 1, 2, 5
Appendix D: Cover page, Pages 1, 2

Amendment #2: May 21, 2010

Sections Changed

Cover Page
Section 10.0: 10.2
Appendix C: Cover page
Appendix D: Cover page, Pages 1, 5, 6

Amendment #3 September 10, 2010

Sections Changed

Cover Page
Glossary of Abbreviations and Acronyms
Section 5.0: 5.0 (Table 3)
Section 8.0: 8.1, 8.2 (Table 6)
Section 10.0: 10.1.3, 10.2, 10.3, 10.3.4 (Table 8), 10.3.5 (Table 9), 10.3.6, 10.3.7, 10.5, 10.6
Appendix B: 5.0, 5.3, 5.4
Appendix C: Cover page
Appendix D: Cover page, Pages 1, 6

Amendment #4: July 18, 2011

Sections Changed

Cover Page
CTSU Information Resources
Section 1.0
Section 2.0: 2.3
Section 5.0: 5.0 (Table 3)
Section 6.0: 6.2
Section 10.0: 10.3.4 (Table 8), 10.3.5 (Table 9), 10.3.6, 10.6

Section 14.0: 14.3 (Table 10), 14.5, 14.6, 14.7
Section 16.0: A new reference, #90, has been added.
Appendix B: 1.0, 3.0, 4.0, 6.0
Appendix C: Cover page, Pages 1, 3, 4, 5
Appendix D: Cover page, Pages 1, 2, 8, 9, 10

Amendment #5: April 2, 2012

Sections Changed:

Cover Page
CTSU Information Resources
Glossary of Abbreviations and Acronyms
Section 10.0: 10.1, 10.2 (Table 7)
Appendix B: 4.0
Appendix C: Cover page, Pages 1, 3, 4
Appendix D: Cover page, Pages 1, 5, 6, 8, 10

Amendment #6: April 16, 2014

Sections Changed:

Throughout the protocol, the following have been changed:

- *The NSABP and the NSABP Operations Center were changed to NRG Oncology where appropriate.*
- *All references to NSABP Biostatistical Center have been changed to NRG Oncology Statistics and Data Management Center (SDMC).*
- *Division has been changed to Department throughout the protocol where appropriate.*
- *References to the "Adverse Event Expedited Reporting System (AdEERS)" have been changed to "CTEP Adverse Event Reporting System (CTEP-AERS)."*

Cover Page
Information Resources
CTSU Information Resources
Glossary of Abbreviations and Acronyms
Section 4.0: 4.1
Section 5.0: 5.0 (Table 3)
Section 9.0: 9.1, 9.3, 9.7, 9.8, 9.9
Section 10.0: 10.1.1, 10.2 (Table 7), 10.3, new Section 10.3.7 added; subsequent sections renumbered, 10.3.8
Section 12.0: New Sections 12.1, 12.2, 12.3 added; subsequent sections renumbered, 12.4, 12.7
Section 13.0: 13.2, 13.5
Section 14.0: 14.3 (Table 10), 14.5, 14.7
Section 15.0: #6
Appendix B: Deleted
Pre-Entry Central HER2 Testing Sample Consent Form
Treatment Study Sample Consent Form

Amendment #7: November 14, 2014

Sections Changed:

Cover Page
Section 4.0: 4.1
Section 10.0: 10.3.8
Treatment Study Sample Consent Form

Amendment #8: June 3, 2016

Sections Changed:

Cover Page
Information Resources
Cancer Trials Support Unit (CTSU) Information Resources
Section 1.0: 1.0
Section 4.0: 4.0
Section 5.0: 5.0
Section 6.0: 6.1
Section 9.0: 9.1
Section 10.0: 10.2 (Table 7), 10.3.1, 10.3.4 (Table 8), 10.3.5 (Table 9)
Section 12.0: 12.0
Section 13.0: 13.2

[BLANK PAGE]

TABLE OF CONTENTS

INFORMATION RESOURCES

NRG Oncology http://www.nsabp.pitt.edu		
NRG Oncology	Nova Tower 2 Two Allegheny Center, Suite 1200 Pittsburgh, PA 15212	Phone: 412-339-5300
NRG Oncology Statistics and Data Management Center (SDMC)	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	NRG Oncology Department of Regulatory Affairs	Phone: 412-339-5300 E-mail: regulatory@nsabp.org
Submission of IRB approval	CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103	Phone: 1-866-651-2878 Fax: 215-569-0206 E-mail:CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)
Questions concerning eligibility and clinical aspects of the trial	NRG Oncology Clinical Coordinating Department	Phone: 1-800-477-7227 E-mail: ccdpgh@nrgoncology.org
Information concerning drug orders, shipments, transfers, and returns **(see Section 9.0)**	NRG Oncology SDMC (see above) ***For mail (USPS):*** Pharmaceutical Management Branch, CTEP, DCTD NCI Shady Grove Room 5W228, MSC 9725 9609 Medical Center Drive Bethesda, MD 20892-9725 ***For express courier:*** Pharmaceutical Management Branch, CTEP, DCTD NCI Shady Grove Room 5W228, MSC 9725 9609 Medical Center Drive Rockville, MD 20850	Phone: 412-624-2666 ——————— Phone: 240-276-6575 Fax: 240-276-7893 E-mail: PMBAfterHours@mail.nih.gov
Pre-entry submission of tumor blocks (for HER2 testing and, if patient is enrolled in B-43, for correlative studies); when sending blocks, please indicate on the package "Pathology Specimens Enclosed" **(see Section 6.0)**	Rush University Medical Center Department of Pathology Jelke Building – 5th Floor 1750 West Harrison Street Chicago, IL 60612 ATTN: B-43 Pathology Coordinator NSABP B-43 TRIAL	Phone: 312-942-5274 Refer to the B-43 Pathology Instructions in the Members' Area of the NSABP Web site or the CTSU Member Web site.
Questions regarding the results of the pre-entry HER2 testing	Rush University Medical Center Department of Pathology	Phone: 312-942-5274 Alternate Phone: 312-942-6507
Arrangement for return of blocks	NRG Oncology Biospecimen Bank-Pittsburgh	Phone: 412-697-6611 E-mail: nrgbiobankpgh@nrgoncology.org

Note: Information Resources continued on next page.

INFORMATION RESOURCES *(continued)*

Submission of expedited adverse event reports/questions concerning expedited adverse event reporting (see Section 10.0)	NRG Oncology SDMC B-43 AE Reporting Nurse	Phone: 412-383-2557 Fax: 412-622-2113 SAEReportingpgh@nrgoncology.org
Submission of data forms/questions concerning data management (see Section 13.0)	NRG Oncology SDMC B-43 Data Manager	Phone: 412-624-2666 Data Fax: 412-622-2111 Refer to the B-43 Data Forms page in the Members' Area of the NSABP Web site or the CTSU Member Web site.

Cancer Trials Support Unit (CTSU) Information Resources

<table>
<tr>
<td>To submit site registration documents:

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

Phone: 1-866-651-2878
Fax: 1-215-569-0206
E-mail: CTSURegulatory @ctsu.coccg.org (for submitting regulatory documents only)</td>
<td>For patient enrollments:

Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_ SYSTEM/ or https://OPEN.ctsu.org.

Contact the CTSU Help Desk with any OPEN-related questions at CTSURegulatory @ctsu.coccg.org.</td>
<td>Submit study data directly to the NRG Oncology SDMC through the Online Data Entry function unless otherwise specified in the protocol:

Submit study data online through the Online Data Entry function located in the Study Management Area of Coordinator Online in the Members' Area of the NSABP Web site. Contact the Support Desk at support@nrgoncology.org for an account.

NRG Oncology Statistics and Data Management 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.</td>
</tr>
<tr>
<td colspan="3">For clinical questions (i.e. patient eligibility or treatment-related), contact the Clinical Coordinating Department at NRG Oncology at 1-800-477-7227.</td>
</tr>
<tr>
<td colspan="3"><u>For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission)</u>, 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.</td>
</tr>
<tr>
<td colspan="3">The CTSU Member Web site is located at https://www.ctsu.org</td>
</tr>
<tr>
<td colspan="3">The most current version of the study protocol and all supporting documents must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password.</td>
</tr>
</table>

GLOSSARY OF ABBREVIATIONS AND ACRONYMS

AC	doxorubicin and cyclophosphamide
ACT	doxorubicin and cyclophosphamide followed by paclitaxel
ACTH	doxorubicin and cyclophosphamide followed by paclitaxel and trastuzumab
AE	adverse event
AGC	absolute granulocyte count
ANC	absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST (SGOT)	aspartate aminotransferase
AUC	area under the curve
BP	blood pressure
BCIRG	Breast Cancer International Research Group
CAEPR	Comprehensive Adverse Events and Potential Risks
CCD	Clinical Coordinating Department (NRG Oncology)
CE	cardiac event
CEF	cyclophosphamide, epirubicin, and 5-fluorouracil
CHF	congestive heart failure
CI	confidence interval
CREC	Cardiac Review and Evaluation Committee
CTCAE v3.0	Common Terminology Criteria for Adverse Events Version 3.0
CTCAE v4.0	Common Terminology Criteria for Adverse Events Version 4.0
CTEP	Cancer Therapy Evaluation Program
CTEP-AERS	CTEP Adverse Event Reporting System
CTEP-IAM	CTEP-Identity and Access Management
CTSU	Cancer Trials Support Unit
DCIS	ductal carcinoma in situ
DCTD	Division of Cancer Treatment and Diagnosis
DFS	disease-free survival
DMC	Data Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor
ER	estrogen receptor
FDA	Food and Drug Administration
FISH	fluorescence in situ hybridization
FSH	follicle stimulating hormone
GI	gastrointestinal
H&E	hematoxylin and eosin
HER2	human epidermal growth factor receptor 2
HERA	Herceptin Adjuvant Trial
HR	hazard ratio
IDC	infiltrating ductal carcinoma
IHC	immunohistochemistry
IND	investigational new drug
IRB	Institutional Review Board
IDFS-DCIS	invasive or DCIS disease-free survival
IV	intravenous
LCIS	lobular carcinoma in situ

GLOSSARY OF ABBREVIATIONS AND ACRONYMS (continued)

LV	left ventricular
LVEF	left ventricular ejection fraction
MAPK	mitogen-activated protein kinase
MRI	magnetic resonance imaging
NCCTG	North Central Cancer Treatment Group
NCI	National Cancer Institute
NCTN	National Trials Clinical Network
NOS	not otherwise specified
NSABP	National Surgical Breast and Bowel Project
NSC	National Service Center
OPEN	Oncology Patient Enrollment Network
OS	overall survival
P	probability (2P indicates probability from a 2-sided test)
PgR	progesterone receptor
PI3K	phosphatidylinositol 3-kinase
PMB	Pharmaceutical Management Branch
PTEN	phosphatidylinositol phosphate 3'-phosphatase
RR	relative risk
RT	radiation therapy
RT-PCR	reverse transcriptase polymerase chain reaction
RUMC	Rush University Medical Center
SDMC	Statistics and Data Management Center
SPEER	Specific Protocol Exceptions to Expedited Reporting
WBC	white blood cell
WBI	whole breast irradiation

1.0 SUMMARY OF THE STUDY

Note: Accrual closed on December 8, 2014, following achievement of the sample size goal.

The primary aim of this Phase III randomized clinical trial is to determine whether trastuzumab given concurrently with radiation therapy (RT) is more beneficial in preventing subsequent ipsilateral breast cancer recurrence, ipsilateral skin cancer recurrence, or ipsilateral DCIS, when compared with RT alone in women with HER2-positive ductal carcinoma in situ (DCIS) resected by lumpectomy. The secondary aims are to compare the possible benefit of trastuzumab given during RT to that of RT alone in preventing subsequent regional or distant recurrence and contralateral invasive or DCIS breast cancer. B-43 will determine if invasive or DCIS disease-free survival, recurrence-free interval, and overall survival can be improved with the addition of trastuzumab to RT. The effects of trastuzumab on ovarian function in premenopausal women will also be assessed.

Submission of a representative tumor block to Rush University Medical Center (RUMC) for HER2 testing is required prior to randomization. If the DCIS is found to be HER2-positive and the patient is enrolled in B-43, the block will also be used for B-43 correlative studies. Patients whose DCIS is determined to be HER2-negative by testing at RUMC will not be eligible for participation in B-43.

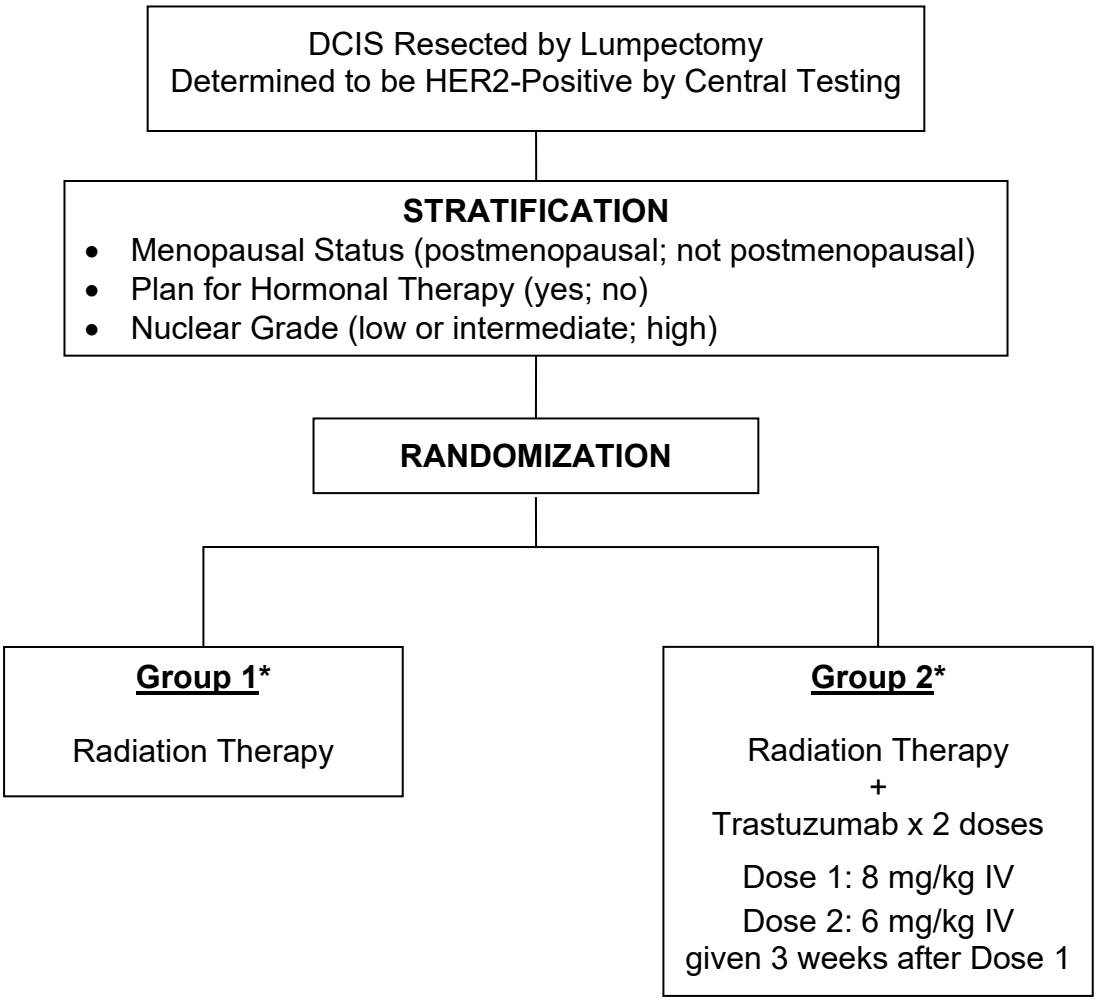
Eligible patients will be randomized to receive either RT alone or two doses of trastuzumab given concurrently with RT. The RT must be ***whole breast irradiation*** (with or without a boost) delivered over 5-6 weeks or, at the investigator's discretion, accelerated fractionation may be used.

For patients with DCIS that is hormone receptor-positive, hormonal therapy should be given for a minimum of 5 years. Selection of the hormonal therapy agent(s) is at the discretion of the investigator.

The sample size for B-43 will be 2000 patients to be accrued over a period of 7.93 years.

Figure 1

B-43 SCHEMA



* Patients with ER-positive and/or PgR-positive DCIS should receive a minimum of 5 years of hormonal therapy.

2.0 BACKGROUND

2.1 Introduction

The American Cancer Society predicts that 184,000 new invasive breast cancers will be detected in the United States in the year 2008. In addition, about 67,770 cases of breast carcinoma in situ are expected to be newly diagnosed in 2008.[1] Of these, approximately 85% are expected to be ductal carcinoma in situ (DCIS).[2] DCIS gives rise to the majority (approximately 85%) of invasive breast cancers[3], so it is an important entity to understand and to prevent.

Thus far, treatment of DCIS has been limited to mastectomy or lumpectomy plus radiotherapy, with or without tamoxifen in ER-positive cases. A substantial proportion of DCIS is ER-negative and overexpresses HER2. Targeting HER2 is a promising strategy for HER2-overexpressing DCIS. Trastuzumab, a monoclonal antibody that specifically targets the HER2 protein,[4,5] was approved by the FDA in 1998 and is now widely prescribed for patients with HER2-positive metastatic breast cancer and in combination with chemotherapy in the adjuvant setting.

Preclinical studies have shown that trastuzumab boosts the effectiveness of radiation in xenograft models and in cell lines, without producing a detrimental effect on irradiated HER2-normal cells.[6,7] The first information in humans correlating clinical response with molecular markers in trastuzumab-treated patients shows that apoptosis occurs very quickly (within one week of beginning single agent trastuzumab) and that there is little effect on proliferation.[8] This suggests that shorter treatment durations with trastuzumab, rather than indefinite or prolonged therapy should be investigated. Ongoing adjuvant trials use trastuzumab during breast irradiation, providing ample evidence of safety.

Given the accumulated body of evidence, it is reasonable to ask whether trastuzumab, administered during radiation therapy, will improve the results of lumpectomy and irradiation in women with HER2-positive DCIS. The view that cancer is a disorder of growth and that, ultimately, surgery will be replaced by other methods of re-ordering or controlling growth will be further explored in this trial. This trial will allow us to better understand the biology of breast cancer, including its prevention, and to extend the benefits of breast-conserving surgery for women with DCIS.

2.2 Prior and ongoing clinical trials in DCIS conducted by the NSABP

Retrospective reports have shown that local recurrence rates after breast-conserving surgery for DCIS, with and without radiation therapy, were no greater for DCIS than for invasive cancer.[9-15] However, mastectomy for DCIS was still the accepted standard because there was a risk that recurrence could arise in the form of invasive cancer, which could lead to distant metastasis and death, and mastectomy would eliminate the possibility of recurrence as invasive breast cancer.

Two studies carried out by the NSABP have clarified several controversial issues about the nature of DCIS and an alternative management approach. NSABP B-17 compared lumpectomy alone with lumpectomy and radiation therapy. NSABP B-24 evaluated the addition of tamoxifen to lumpectomy and radiation therapy. Together, the two trials showed that breast-conserving surgery was an appropriate option for women with DCIS.[16-20]

Results of NSABP B-17 were published in 1993 and updated in 1995, 1998,[18] and 2001.[19] Initially, 818 patients were randomized; there was subsequent follow-up of 814 patients. The incidence of invasive breast cancer at 8 years of follow-up was reduced with the addition of radiotherapy from 13.4% to 3.9%. Mortality attributable to breast cancer for the entire cohort was only 1.7% at 8 years. Also, after 8 years of follow-up, the addition of radiotherapy continued to show a low cumulative incidence of ipsilateral recurrences, both invasive and noninvasive. Overall long-term survival rates were similar to those obtained with total mastectomy in historic retrospective studies. The conclusion was that mastectomy was not generally necessary in women with localized DCIS.

Despite these favorable statistics, others argued that wider local excision could produce similar long-term disease control without radiation therapy.[21] The idea that wider excision is better because it allows the omission of radiation therapy has logic, but is a step back toward the Halstedian idea of cancer as a constantly expanding process. Another view is that cancer is a disorder of growth and that, ultimately, surgery will be replaced by other methods of re-ordering or controlling growth. NSABP B-24 was undertaken to explore the biology of breast cancer and to extend the benefits of breast-conserving surgery for women with DCIS.

Prior NSABP adjuvant trials had shown that tamoxifen decreased the incidence of tumor recurrence in the ipsilateral breast and reduced the rate of new primary tumors in the contralateral breast,[22,23] suggesting that tamoxifen could interfere with the development of primary invasive breast cancer or with the progression of DCIS into invasive cancer. In NSABP B-24, patients were randomized to receive 5 years of adjuvant tamoxifen or placebo after lumpectomy and radiation therapy. It was postulated that the addition of tamoxifen would be more effective than radiotherapy alone in preventing recurrence of DCIS or invasive breast cancer. A total of 1804 women were randomly assigned to receive tamoxifen or placebo, and median follow-up was 74 months at the time of the primary report. Women in the tamoxifen group had fewer breast cancer events at 5 years than those on placebo (8.2% vs. 13.4%, P=0.00009).[16] The cumulative incidence of all invasive breast cancers in the tamoxifen group was 4.1% at 5 years vs. 7.2% in the placebo group (p=0.004). There was a reduction in the rate of noninvasive breast cancer, but it was not significant. The benefits of this approach are a reduction in the overall incidence of all breast cancer events (ipsilateral, contralateral, and distant) and a reduction in the extent and severity of surgery required.

Margin involvement, a frequently cited predictor of recurrence, was assessed at 5 years of follow-up in NSABP B-17. Positive margins were associated with an increased risk (2.33 RR) of ipsilateral breast tumors.[20] In NSABP B-24, positive tumor margins were associated with increased rates and RR of invasive or noninvasive ipsilateral breast tumors. However, in the tamoxifen-treated group, the risk was 22% lower than the untreated group when sample margins were negative and 44% lower when margins were positive or unknown.[16] The 8-year update of the B-17 study shows that margin status was less of an influence (1.48 RR) than indicated in the earlier report.[24]

The success of tamoxifen in lowering all rates of recurrence fosters the search for more effective and/or less toxic agents to control the stimulation and growth of precancerous and cancerous cells in the breast. This idea formed the rationale for NSABP-35 which asked whether anastrozole is more effective than tamoxifen in postmenopausal women undergoing breast-conserving treatment for DCIS. Accrual for B-35 is now completed.

Young women (age <50) treated with lumpectomy and irradiation are significantly more likely to experience an in-breast tumor recurrence or a contralateral breast cancer than older women (HR 3.3 vs. 1.3, respectively).[16] These women were not eligible for the NSABP B-35 trial. Patients with ER-negative tumors were also ineligible for NSABP B-35. Thus, the current trial complements NSABP B-35 because many of its participants will be premenopausal and/or have ER-negative tumors.

2.3 Refining therapy for subgroups of patients: The HER2 alteration in DCIS

DCIS is more likely than invasive breast cancer to overexpress HER2. As reported by the Intergroup Study 0011, HER2 was overexpressed in 56% of pure DCIS specimens (including 77% of the comedo subtype), in 22% of DCIS associated with infiltrating ductal carcinoma (IDC), and in only 11% of IDC alone.[25,26]

Recently, the expression profiles of nearly 12,000 annotated genes in 25 cases of pure DCIS and 25 cases of pure infiltrating ductal cancer were compared.[3] HER2 was upregulated 3.9-fold in DCIS compared with invasive breast cancer. HER2 expression in DCIS and in invasive breast cancer within the same neoplasm were concordant in 100% of cancers tested.[27] Thus, interrupting this signal transduction pathway early might prevent development of aggressive (HER2-positive) invasive breast cancer and could have a major impact on breast cancer mortality.

A literature review revealed considerable variability in HER2 expression in DCIS, ranging from 28% to 65% (Table 1). Most investigators used immunohistochemical (IHC) staining to measure HER2 expression. The antibodies used, the methods of fixation, the duration of tissue storage, and the antigen retrieval techniques are only some of the variables that could have affected results. Only one of the studies performed both IHC and fluorescence in-situ hybridization (FISH) testing for HER2 on the same specimens. The investigators, from Rush University Medical Center, evaluated 37 specimens: Twenty-two were HER2-positive by IHC and nineteen of these were also amplified by FISH. No specimens were FISH-amplified and IHC-negative.[28]

TABLE 1. HER2 expression in DCIS

Author	N	Accrual	Surgery	HER2 Test	HER2+ (%)	ER+ Scoring	ER+ (%)	N (%) of ER+ Cases Which Were HER2+	Institution
LeBeau[29]	45	1986-1995	Any	3B5[a]	21/45 (47)	≥ 10%	22/40 (55)		U Munchen, Munchen, Germany
Cornfield[30]	151	1982-2000	BCT	ns[b]	(33)		(75)		Thomas Jefferson, Philadelphia, PA
Siziopikou K[28]	37	ns	Any	CB11[c] & FISH[d]	22/37 (59) 19/37 (51)				Rush, Chicago, IL
Barnes[31]	129	1979-2004	Any	A0485[e]	84/129 (65)				South Manchester U and Christie Hospital, England
Collins[32]	148	2000-2003	Any	A0485[f]	42/148 (28)	≥ 10%	114/148 (77)	14/114 (12)	Beth Isreal, Boston
Provenzano[33]	82	1988-1992	BCT		17/41 cases (41) 5/41 controls (12)	≥ 10%	14/37 cases (35) 24/37 controls (68)		Victorian Breast Cancer Consortium, Melbourne, Victoria, Australia
Rodrigues[34]	54	1973-2000	BCT + RT	A0485[g]	26/54 (48)	≥ 10%	45/54 (83)	18/45 (40)	Yale, New Haven, CT
Claus[35]	255	1982-1994			(28)		(60)	(19)	Yale, New Haven, CT
Van de Vijver MJ[36]	45	1976-1988	Any	3B5[h]	19/45 (42)				The Netherlands Cancer Inst Amsterdam
Perin T[37]	49	1984-1992	Any	CB11[i]	(55)	Any	(54)		C.R.O., Aviano. Italy
Ringberg A[38]	187	1987-1992	BCT, no RT	CB11[j]	92/171 (54)	≥ 10%	97/163 (60)		South Sweden Breast Cancer Groupe
Bijker N[39]	71		BCT	3B5[k]	30/65 (46) in primary 25/65 (38) in recur	≥ 1%	44/70 (63) in primary 42/66 (63) in recur		EORTC 10853 Trial
Ramachandra S[40]	74			21N	44/74 (59)				The Royal Marsden, U.K.
Schimmelpenning H[41]	107			OA-11-854[l]	51/107 (48)				Karolinska Inst, Stockholm, Sweden
Bobrow L[42]	105	1975-1991	Any	21N[m]	47/105 (45)				ICRF, Guy's Hospital, U.K.
Leal CB[43]	40	1985-1993	Any	see[n]	18/40 (45)		23/36 (64)		Instituto Portuges de Oncologia Francisco, Porto, Portugal
Somerville JE[44]	50		Any	pAbl[o] ICR12[o]	23/50 (46)				Belfast City Hospital, Belfast, Ireland
Poller DN[45]	112		Any	21N[p]	62/112 (55)	Any			City Hospital, Nottingham, U.K.

[a]Oncogene Science; any staining considered positive. [b]Signet Labs, Dedham, MA. Positive cases included those with strong membrane staining regardless of the percent of cells staining. Median f/u 65 mo (86 for those w/o recurrence). 42 recurrences w/median TTR of 28.5 mo for those who recurred. Patients received no RT and no tamoxifen. No correlation of markers with recurrence. [c]Ventana. 2-3+ was considered positive. This was not otherwise defined. [d]Vysis. Barnes: Case-controlled study. Cytoplasmic and membrane staining of HER2 was considered positive. 2+ and 3+ were considered positive. [e]HER2-staining was scored 0 (absent) to 3 (maximum cyto-membranous staining seen, comparable to a 3+ positive invasive cancer control), with a score 2 considered HER2-positive. [f]The envision double stain system (Dako Cytomation, Carpinteria, CA) for simultaneous assessment of ER and HER2 was used. Only 3+ per HercepTest definition was considered positive. Provenzano case-control series so ER% may be falsely low as half of these patients recurred. [g]Dako Hercep Test Kit. 2-3+ was considered positive. [h]Only unequivocal membrane staining was considered positive. All positive cases were comedo; 66% if comedos stained positively. A polyclonal Ab against the cytoplasmic domain of HER2. Cambridge Research Biochemicals, Northwich, UK. [i]Novocastra Labs, UK. HER2 was considered positive if any membrane staining was observed. [j]Novocastra Labs, UK. HER2 was considered positive if ≥ 10% membrane staining was observed. [k]Any + staining was considered positive. All cases analyzed were recurrence after BCT. This was a randomized trial of 1x vs 1x+RT. [m]Polyclonal Ab, Dr. W. Gullick, ICRF, London. Positive if any strong membrane staining was seen. [n]Antibody from Dako (Dakopatts, Denmark). [o]pAb1, Triton Biosciences, Inc., a polyclonal Ab against the c-terminus; ICR12, a monoclonal provided by Dr. C Dean, Institute of Cancer Res; Royal Cancer Hospital, Belmont, Sutton Surrey. Cases that showed strong membrane staining either focal (only affecting some cells) or diffuse (affecting all or most cells) were classified as positive. [p]Polyclonal Ab, Dr. W.J. Gullick, ICRF, London. Membrane staining, either homogeneous or heterogeneous was considered positive.

From NSABP B-24, 100 cases have been examined by HercepTest™ (IHC) and, if 2+ or 3+, then by FISH. Twenty-one cases were 2+ and 26 cases were 3+ by IHC. Among the 2+ cases, 12 were found to have gene amplification by FISH, and 22 of the 26 3+ cases had gene amplification (3 non-evaluable with 1 true non-amplified case). Together, 34 cases had HER2 gene amplification. cMYC was also examined among HER2 IHC 2+ or 3+ cases, and 11 of them were found to be amplified (32%). These data suggest that the incidence of HER2 amplification is at least as frequent or more frequent in DCIS as compared to invasive breast cancer.[46]

This trial will use the following HER2-expression guidelines for eligibility: From the time of study activation until April 4, 2011, central testing was based on IHC for HER2 (HercepTest) for all cases, with reflex testing by FISH in 1+ and 2+ cases. An internal analysis of B-43 cases determined that the rate of IHC 2+ DCIS found to be FISH-positive was 16.8%. With an IHC score of 1+, the FISH-positive rate was 0.36%. Based on these results, it was determined that the rate of identifying FISH-positive tumors among patients with DCIS that is IHC 2+ is sufficient to warrant the continued practice of reflex FISH for possible participation in the B-43 trial. However, the very low rate of FISH-positive DCIS among IHC 1+ tumors does not justify additional testing, and central testing procedures were changed effective April 4, 2011, to discontinue reflex FISH following an IHC test result of 1+ (see Section 6.2). Patients whose tumors are 3+ by IHC or amplified by FISH will be eligible.

2.4 Refining therapy for subgroups of patients with DCIS

NSABP B-24 patients were enrolled without regard to estrogen-receptor status. Further data analysis suggested that only ER-positive patients benefited from tamoxifen.[47] Thus, women with ER-negative DCIS currently have no proven systemic therapeutic options.

Many patients have DCIS that overexpresses HER2. Among tumors from 255 women presenting to the Yale-New Haven Hospital with DCIS, HER2 expression was inversely related to ER status. Sixty percent of HER2-positive tumors were ER-negative; however, expression of HER2 was not limited to ER-negative DCIS. Nineteen percent of ER-positive tumors from patients with DCIS overexpressed HER2.[48] It is possible that tamoxifen may be deleterious in women with ER-positive, HER2-positive breast cancer.[49]

HER2-overexpression is frequently associated with large cell, comedo variety DCIS. Of 45 patients studied in the Netherlands, 42% had DCIS that overexpressed HER2, and all were of the large-cell, comedo growth type.[50] This histologic subtype of DCIS is generally associated with more aggressive behavior. For example, subgroup analysis of NSABP B-24 revealed that the presence of comedo necrosis was associated with a significantly higher relative risk of recurrence than non-comedo histology (2.7 vs. 1.3, respectively) in patients treated with lumpectomy and radiation.[16]

Other investigators have confirmed the association between high grade DCIS and HER2 overexpression using classifications systems proposed by Lagios[51], modified Lagios[52], and Holland.[53,54] Therefore, the use of trastuzumab to block signal transduction through the HER2 pathway is an attractive idea in HER2-overexpressing DCIS.

2.5 Clinical experience with trastuzumab

Three multicenter clinical trials have shown the safety and efficacy of trastuzumab combined with chemotherapy and of trastuzumab monotherapy for patients with metastatic breast cancer.[4,5,55] Four multicenter adjuvant trials also have been completed and underscore the efficacy and safety of this agent.

The NSABP B-31 trial compared doxorubicin and cyclophosphamide followed by paclitaxel every 3 weeks (group 1) with the same regimen plus 52 weeks of trastuzumab beginning with the first dose of paclitaxel (group 2) for prolonging DFS. The North Central Cancer Treatment Group (NCCTG) trial N9831 compared three regimens, only two of which (group A and group C) were comparable to group 1 and group 2 in B-31: doxorubicin and cyclophosphamide followed by weekly paclitaxel (group A), and the same regimen plus 52 weeks of trastuzumab initiated concomitantly with paclitaxel (group C). Both studies were amended to address a joint analysis comparing groups 1 and A (the control group) with groups 2 and C (the trastuzumab group). Patients in both studies with hormone receptor-positive tumors received hormonal therapy for 5 years after chemotherapy.[56] By March 15, 2005, 394 events had been reported, triggering the first scheduled interim analysis. Of these, 133 were in the trastuzumab group and 261 in the control group (HR 0.48; P<0.0001). This result crossed the early stopping boundary. The absolute difference in DFS between the trastuzumab group and the control group was 12 percent at three years. Trastuzumab therapy was associated with a 33% reduction in the risk of death (p=0.015). The authors concluded that adjuvant trastuzumab combined with paclitaxel given after doxorubicin and cyclophosphamide improves outcomes among women with surgically removed HER2-positive breast cancer.

A second international, multicenter randomized trial, the Herceptin Adjuvant (HERA) trial, compared 1 or 2 years of trastuzumab given every 3 weeks with observation alone in patients who had HER2-positive, node-negative or node-positive breast cancer and who had completed locoregional therapy and at least four cycles of neoadjuvant or adjuvant chemotherapy. Patients with hormone receptor-positive tumors received hormonal therapy for 5 years after chemotherapy.[57] Data were reported for 1694 women assigned to 1 year of trastuzumab and 1693 women assigned to observation. At the first planned interim analysis, 347 events were observed: 127 events in the trastuzumab group and 220 in the observation group. The unadjusted hazard ratio for an event in the trastuzumab group, as compared with the observation group, was 0.54 (95% CI, 0.43 to 0.67; P<0.0001 by the log-rank test, crossing the interim analysis boundary), representing an absolute benefit in terms of DFS at 2 years of 8.4 percentage points. Overall survival in the two groups was not significantly different (29 deaths with trastuzumab vs. 37 with observation). The authors concluded that 1 year of treatment with trastuzumab after adjuvant chemotherapy significantly improves DFS among women with HER2-positive breast cancer.

In a third international trial, BCIRG 006, patients with HER2-amplified axillary lymph node-positive or high risk, node-negative breast cancer were randomized to receive either doxorubicin and cyclophosphamide (60/600 mg/m^2 q3wk x 4) followed by docetaxel (100 mg/m^2 q3wk x 4) or two trastuzumab-containing regimens: doxorubicin and cyclophosphamide followed by docetaxel with trastuzumab x 1 year or docetaxel and carboplatin (75 mg/m^2/AUC 6 q3wk x 6) with trastuzumab x 1 year. Patients with hormone receptor-positive tumors received hormonal therapy for 5 years after chemotherapy.[58] A total of 3222 patients were recruited to the trial. At a median

follow-up of 23 months, the two trastuzumab-containing arms both met the DFS endpoint (HR 0.49 with doxorubicin and cyclophosphamide followed by docetaxel plus trastuzumab, P-value=0.00000048; HR 0.61 with docetaxel plus carboplatin plus trastuzumab P-value=0.00015 as compared to doxorubicin and cyclophosphamide followed by docetaxel). The authors concluded that trastuzumab either in combination with docetaxel following doxorubicin and cyclophosphamide or combined with docetaxel and carboplatin improves outcomes among women with surgically removed HER2-positive breast cancer.

In the final study, the Finland Herceptin (FinHER) trial, 1010 women with either axillary node-positive breast cancer or node-negative cancer with tumor > 20 mm were randomly allocated to receive either 3 cycles of docetaxel (100 mg/m^2) given every 3 weeks or 8 weekly cycles of vinorelbine (25 mg/m^2) as adjuvant therapy. After single-agent docetaxel or vinorelbine, patients from both arms were given 3 cycles of cyclophosphamide, epirubicin, and 5-fluorouracil (C-600 mg/m^2; E-60 mg/m^2; F-600 mg/m^2) every 3 weeks; tamoxifen was given for 5 years for ER or PgR-positive disease. Patients whose tumors showed HER2 amplification in chromogen in situ hybridization (CISH, n=232, 23%) were randomized a second time to receive 9 weekly cycles of trastuzumab given concomitantly with either docetaxel or vinorelbine, or no trastuzumab.[59] Breast cancer recurrence was less frequent among patients treated with docetaxel/CEF than among those treated with vinorelbine/CEF (39/502 vs. 68/507; HR 0.58; log-rank test, p= 0.0036). Adjuvant 9-week trastuzumab was effective in preventing any breast cancer recurrence (11/115 vs. 26/116, HR 0.46, p for recurrence-free survival = 0.0078). Three-year distant DFS of patients who received trastuzumab was 93% and that of patients who did not receive trastuzumab was 76% (p=0.0078, 11/115 vs. 26/116 events, HR 0.43). There was a trend towards superior overall survival of patients treated with trastuzumab (6/115 deaths vs. 14/116, HR 0.43, p=0.08). The authors concluded that docetaxel/CEF was more effective than vinorelbine/CEF and that trastuzumab was effective in preventing breast cancer recurrence when combined with either docetaxel or vinorelbine as adjuvant treatment of breast cancer. Trastuzumab given concomitantly with docetaxel or vinorelbine for 9 weeks was well tolerated and was not associated with a decrease of left ventricular ejection fractions (LVEFs), making monitoring of the LVEFs unnecessary for most patients.

2.6 Rationale for not conducting baseline cardiac evaluations

Cardiac events were observed in the pivotal clinical trials that led to the approval of trastuzumab. Cardiotoxicity was most pronounced when trastuzumab was given concurrently with anthracyclines. However, all patients in the Phase III trial and most in the Phase II trial for refractory breast cancer had received anthracyclines either concurrently with trastuzumab or trastuzumab following an anthracycline.

Subsequent studies of trastuzumab in metastatic disease, which included less heavily pre-treated patients, sparked less concern. Investigators enrolled 114 patients who had HER2-positive metastatic breast cancer and who had not previously received chemotherapy for their metastatic breast cancer in a Phase II clinical trial.[60] Women with current, clinically significant cardiac disease were excluded. All cardiac events were referred to an independent, cardiac review and evaluation committee (CREC). Three women were considered by the CREC to have cardiac dysfunction. Two of the three patients had histories of significant cardiac disease, and one had received adjuvant

anthracycline therapy. The cardiac event in the third patient was attributed to underlying breast cancer. After discontinuation of trastuzumab, none of these patients required additional intervention for cardiac events.

In the study by Vogel et al, the two patients who experienced cardiac toxicity did so after cumulative doses of 56 and 104 mg/kg of trastuzumab.[60] In the adjuvant therapy trials, patients would have received at least 32 mg/kg of trastuzumab before beginning radiation therapy (RT) and an additional 12 mg/kg during RT. In the proposed trial, patients will receive a total dose of only 14 mg/kg.

NSABP B-31 compared doxorubicin and cyclophosphamide (AC) followed by paclitaxel with AC followed by paclitaxel plus 52 weeks of trastuzumab beginning concurrently with paclitaxel in patients with node-positive, HER2-positive breast cancer. Initiation of trastuzumab required normal post-AC LVEF on a multiple-gated acquisition scan. If symptoms suggestive of congestive heart failure (CHF) developed, source documents were blindly reviewed by an independent panel of cardiologists to determine whether criteria were met for a cardiac event (CE), which was defined as New York Heart Association Class III or IV CHF or possible/probable cardiac death. Frequencies of CEs were compared between arms.[61]

Among patients with normal post-AC LVEF who began post-AC treatment in NSABP B-31, 5 of 814 control patients subsequently had confirmed CEs (4 CHFs and 1 cardiac death) compared with 31 of 850 trastuzumab-treated patients (31 CHFs and no cardiac deaths). The difference in cumulative incidence at 3 years was 3.3% (4.1% for trastuzumab-treated patients minus 0.8% for control patients; 95% CI, 1.7% to 4.9%). Twenty-seven of the 31 patients in the trastuzumab arm have been followed for ≥ 6 months after diagnosis of a CE: 26 were asymptomatic at last assessment, and 18 remained on cardiac medication. CHFs were more frequent in older patients and patients with marginal post-AC LVEF. Fourteen percent of patients discontinued trastuzumab because of asymptomatic decreases in LVEF; 4% discontinued trastuzumab because of symptomatic cardiotoxicity.

In a more recent publication with four years of follow-up, a comprehensive assessment of potential risk factors for CHF in NSABP B-31 patients was conducted. Left-sided breast cancer and radiation therapy was not a risk factor for CHF.[62]

In the N9831 trial, NCCTG concluded that concurrent administration of adjuvant RT with trastuzumab in early stage breast cancer patients is not associated with an increased incidence of acute RT adverse events. Three thousand five-hundred and five (3505) patients were evaluated.[63] Women with operable, HER2-positive breast cancer were randomized to AC followed by weekly paclitaxel (T): AC→T→trastuzumab (H) or AC→TH→H. Post-lumpectomy breast ± nodal RT was recommended, as was postmastectomy chest wall + nodal RT (> 3 positive nodes); internal mammary RT was prohibited. RT started within 5 weeks of completion of T and was allowed to be administered concurrently with H. A total of 1460 patients receiving RT were available for analysis of RT-associated AEs. Also, 1286 patients on trastuzumab arms who completed T (908 who received RT and 378 who did not receive RT) were available for analysis of clinical CEs.

Rates of RT-associated AEs were compared across treatment arms, and rates of CEs were compared for RT vs. no RT within the trastuzumab arms. RT did not increase the

frequency of CEs. In the AC→T→H arm, the incidence of CEs was 2.2% in patients who received RT vs. 2.9% in patients who had not received RT. In the AC→TH→H arm, the incidence of CEs was 1.5% in the RT patients vs. 6.3% in the no-RT patients. No difference in CEs was seen between left-sided and right-sided RT fields in the RT patients in either group that included trastuzumab.

The second planned analysis of the BCIRG 006 adjuvant trial revealed that trastuzumab does not have cardiac toxicity when it is given to patients who have not received an anthracycline: trastuzumab increased the rate of cardiac toxicity only when it was added to an anthracycline-containing regimen.[64] Table 2 shows the left ventricular function as measured by LVEF before, during, and after treatment. The slope of the LVEF over time is not affected by TCH.

TABLE 2. Trastuzumab (H) impact on LVEF when no anthracyclines have been administered

	AC→T	AC→TH	TCH
N	1043	1074	1056
LVEF slope	-.78	-1.77	+.03
P-value slope	.0002	.00001	.79

In the FinHer study, wherein patients were randomized to receive only 9 weeks of adjuvant trastuzumab, one patient experienced a cardiac infarction and three had cardiac failure; none of these four patients had received trastuzumab. LVEFs were preserved in women who received trastuzumab.

In fact, trastuzumab-treated women had slightly better ejection fractions than those who did not receive trastuzumab; in an ANCOVA model, the estimated difference 12 months after the completion of chemotherapy was 1.7% (–0.1 to 3.5; p=0.06), and at 36 months it was 3.0% (0.7 to 5.4; P=0.01). Four women who were treated with trastuzumab (3.5%) and seven who were not (6.0%) had one or more measurements of ejection fraction that were more than 15 percentage points less than the pretreatment value. A decrease by more than 10 percentage points, resulting in an ejection fraction of less than 50%, occurred in three patients (none of whom had received trastuzumab).[65]

Given the fact that patients who enter this trial will not receive an anthracycline and will receive only two doses of trastuzumab, it is reasonable not to require baseline cardiac evaluation beyond the eligibility criteria, which include no serious cardiac disease and New York Heart Association Classification Class I (patients with no limitation of activities; they suffer no symptoms from ordinary activities). This approach is safe because no patient will have received prior anthracyclines and the cumulative dose of trastuzumab is low.

2.7 Implications for breast cancer prevention

Breast cancer prevention strategies have been limited to inhibiting the binding of estrogen to its receptor or to decreasing estrogen levels. This trial will allow us to study the effect of inhibiting the HER2 signal transduction pathway on the contralateral breast.

2.8 Impact of trastuzumab on ovarian function

DCIS is a precursor to invasive breast cancer and occurs months to years before invasive breast cancer. Many women who enter this trial will be premenopausal and may be concerned about the impact of trastuzumab on ovarian function. This trial will allow us to compare menstruation in control and trastuzumab-treated premenopausal women using data reported at baseline and at 18 months.

3.0 STUDY AIMS AND ENDPOINTS

3.1 Primary aim and endpoint

The *primary aim* is to determine the value of trastuzumab given during radiation therapy (RT) compared to RT alone in preventing the subsequent occurrence of ipsilateral breast cancer recurrence, ipsilateral skin cancer recurrence, or ipsilateral DCIS (IIBCR-SCR-DCIS) in women with HER2-positive DCIS resected by lumpectomy.

The *primary endpoint* is time from randomization to IIBCR-SCR-DCIS defined as ipsilateral invasive breast cancer, ipsilateral skin cancer recurrence, or ipsilateral DCIS. An IIBCR-SCR-DCIS event is defined as recurrent tumor in the ipsilateral breast parenchyma, skin of the ipsilateral breast or ipsilateral DCIS occurring after lumpectomy. In the determination of time to an IIBCR-SCR-DCIS, no statistical censoring will be performed with respect to any previous local, regional, distant recurrences or second primary cancers.

3.2 Secondary aims and endpoints

3.2.1 *Invasive or DCIS disease-free survival (IDFS-DCIS)*

Aim: Determine the value of trastuzumab given during RT compared to RT alone in prolonging IDFS-DCIS.

Endpoint: Events for analysis of IDFS-DCIS include: local recurrence in the ipsilateral breast following lumpectomy, regional recurrence, distant recurrence, contralateral breast cancer, second primary cancer (other than squamous and basal cell carcinoma of the skin, melanoma in situ, and carcinoma in situ of the colon and cervix), or death from any cause prior to recurrence or second primary cancer.

3.2.2 *Invasive or DCIS recurrence-free interval (IRFI-DCIS)*

Aim: Determine the value of trastuzumab given during RT compared to RT alone in increasing invasive or DCIS recurrence-free interval.

Endpoint: Time from randomization to first diagnosis of a local, regional or distant recurrence regardless of any intervening contralateral or other second primary cancer.

3.2.3 *Invasive regional or distant recurrence*

Aim: Determine the value of trastuzumab given during RT compared to RT alone in improving regional or distant recurrence.

Endpoint: Time from randomization to first diagnosis of regional or distant recurrence.

3.2.4 ***Contralateral breast cancer (invasive or DCIS)***

Aim: Determine the value of trastuzumab given during RT compared to RT alone in improving the incidence of contralateral invasive or DCIS breast cancer.

Endpoint: Time from randomization to first diagnosis of contralateral invasive or DCIS breast cancer.

3.2.5 ***Overall survival (OS)***

Aim: Determine the value of trastuzumab given during RT compared to RT alone in improving survival.

Endpoint: Time from randomization to death from any cause.

3.2.6 ***Ovarian function***

Aim: To explore the effect of trastuzumab on ovarian function.

Endpoint: The incidence of post-treatment amenorrhea in women who were pre-menopausal at the time of study entry. Post-treatment amenorrhea, defined as the absence of menstrual period for at least 12 months, will be assessed when the patient has been on study for 18 months.

3.2.7 ***Correlative outcomes***

- *Aim:* To determine if the benefit of trastuzumab added to RT will be significantly higher in cMYC-amplified tumors than in the cMYC non-amplified subset.

- *Aim:* To determine if the benefit of trastuzumab added to RT will be less in tumors with mutations in the PI3 Kinase gene than in tumors without PI3 Kinase gene mutations.

4.0 PATIENT ELIGIBILITY AND INELIGIBILITY

Note: Accrual closed on December 8, 2014, following achievement of the sample size goal.

4.1 Patient selection guidelines

Although the guidelines in Section 4.1 are not inclusion/exclusion criteria, investigators should consider each of these factors when selecting patients for B-43. 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 whether a patient is appropriate for B-43. These considerations should be weighed carefully, as they may make a patient an unsuitable candidate for B-43 and may increase risk to the patient.

- Pre-entry central HER2 testing (see Sections 6.1 and 6.2 and Pre-Entry Sample Consent Form) is required for all patients. The correlative studies are required for all patients enrolled in B-43 (see Treatment Study Sample Consent Form). Therefore, ***submission of a representative tumor block for the B-43 trial is required. Submission of alternative tissue samples is NOT permitted.*** The local pathology department policy regarding release of blocks must be considered when screening patients.

- ***The best representative sample for HER2 central testing may be obtained*** from the lumpectomy specimen *or* from the core biopsy specimen. However, the pre-entry central HER2 testing consent form ***must be signed and dated by the patient after her lumpectomy but before submission of the representative tumor block.***

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

- Women of reproductive potential must agree to use an effective non-hormonal method of contraception during therapy ***and for at least 7 months after completion of trastuzumab***.

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

4.2 Conditions for patient eligibility

A patient cannot be considered eligible for this study unless all of the following conditions are met:

4.2.1 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 for the pre-entry tumor block submission* for HER2 testing and B-43 correlative studies (see Section 6.1).

4.2.2 Patients must be female.

4.2.3 Patients must be 18 years of age or older.

4.2.4 Patients must have an ECOG performance status of 0 or 1 (0 = fully active, able to carry on all pre-disease performance without restriction; 1 = restricted in physically strenuous activity but ambulatory).

4.2.5 On histologic examination, the tumor must be ductal carcinoma in situ (DCIS). (Patients with mixed DCIS and lobular carcinoma in situ [LCIS] are eligible.)

4.2.6 The DCIS must be HER2-positive as ***determined by central testing*** (see Sections 6.1 and 6.2 for details).

4.2.7 Estrogen and/or progesterone receptor status must be determined prior to randomization. (Patients with DCIS that is hormone receptor positive or negative are eligible.)

4.2.8 All DCIS must have been resected by lumpectomy.

4.2.9 The margins of the resected specimen must be histologically free of DCIS. For patients in whom pathologic examination demonstrates DCIS present at the line of resection, re-excision(s) may be performed to obtain clear margins. (Patients who require mastectomy are not eligible.)

4.2.10 If axillary staging is performed, nodal staging must be pN0, pN0(i–), pN0(i+) which is defined as isolated tumor cells ≤ 0.2 mm, regardless of the method of detection, i.e., IHC or H&E, pN0(mol–), or pN0(mol+). ***Note: Axillary staging is not required.*** (Refer to AJCC Staging Criteria in the Treatment Trial Information section in the Members' Area of the NSABP Web site for TNM nomenclature and staging information.)

4.2.11 The interval between the last surgery for excision of DCIS (lumpectomy or re-excision of lumpectomy margins) and randomization must be no more than 120 days.

4.3 Conditions for patient ineligibility

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

4.3.1 Invasive (including microinvasion staged as T1mic) breast cancer. (Patients with DCIS "suspicious" for microinvasion, but not confirmed, are eligible.)

4.3.2 Nodal staging of pN1 (including pN1mi). (Note: Axillary staging is not required.)

4.3.3 DCIS present in more than one quadrant (multicentric).

4.3.4 Masses or clusters of calcification that are clinically or mammographically suspicious unless biopsied and proven to be benign. (If DCIS is found, the patient is eligible if the DCIS was in the same quadrant of the ipsilateral breast and was resected with clear margins.)

4.3.5 Contralateral breast cancer (including DCIS).

4.3.6 Whole breast irradiation administered before randomization. (Partial breast irradiation is prohibited.)

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

4.3.8 Prior anthracycline chemotherapy for any malignancy.

4.3.9 Cardiac disease that would preclude the use of the drugs included in the B-43 treatment regimens. This includes but is not confined to:

Active cardiac disease:

- angina pectoris that requires the use of anti-anginal medication;
- ventricular arrhythmias except for benign premature ventricular contractions (PVCs) controlled by medication;
- conduction abnormality requiring a pacemaker;
- supraventricular and nodal arrhythmias requiring a pacemaker or not controlled with medication; and
- clinically significant valvular disease.

History of cardiac disease:

- myocardial infarction documented by elevated cardiac enzymes or persistent regional wall abnormalities on assessment of LV function;
- documented congestive heart failure; or
- documented cardiomyopathy.

4.3.10 Uncontrolled hypertension, i.e., systolic BP greater than 180 mm/Hg and/or diastolic BP greater than 100 mm/Hg. (Patients with hypertension that is well controlled on medication are eligible.)

4.3.11 Other nonmalignant systemic disease that would preclude a patient from receiving trastuzumab or radiation therapy or would prevent prolonged follow-up.

4.3.12 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.

4.3.13 Pregnancy or lactation at the time of study entry. ***(Note: Pregnancy testing according to institutional standards should be performed for women of child-bearing potential.)***

4.3.14 Administration of any investigational agent within 30 days before study entry.

5.0 REQUIRED ENTRY AND FOLLOW-UP STUDIES

Note: Accrual closed on December 8, 2014, following achievement of the sample size goal.

TABLE 3. Studies required for study entry and during study therapy and follow-up

Required studies[a]	Prior to randomization (within 3 months unless specified otherwise)	**During RT with/without trastuzumab**		30 days following completion of RT	Years 1 through 5 from randomization	Years 6 through 10 from randomization
		Group 1	*Group 2*			
History & physical exam[b]	X	X[c]	X[c] (before trastuzumab Dose 2)	X	X (every 6 months)	X (every 12 months)
Radiation oncology evaluation	X					
Height and weight	X					
Menopausal status (See Appendix A)[d]	X (within 1 month)					
Menstrual history					X (only at 18 months)[e]	
AE assessment		X[f]	X[f]	X[f]		
Bilateral mammogram[g]	X (within 6 months)				X (every 12 months)[h]	X (every 12 months)
Pregnancy test	X[i] (within 2 weeks)					
Tumor block submission for central HER2 testing	X[j]					

a Physical examination and testing may be performed more frequently at the investigator's discretion.
b Complete H&P prior to randomization; treatment-related assessments (may be performed by physician or other healthcare professional from the radiation oncology department and/or department/clinic responsible for administering the trastuzumab) and appropriate follow-up assessments at remaining timepoints by physician or other healthcare professional.
c Timing of patient exams should be according to the radiation oncology department's usual practice. (Group 2 patients ***must be assessed*** before trastuzumab Dose 2 is administered.)
d An FSH level may be required. Refer to criteria in Appendix A.

Note: Table 3 continued on next page.

TABLE 3. Studies required for study entry and during study therapy and follow-up *(continued)*

e	Only for patients who were not postmenopausal at the time of study entry. (Information collected at 18 months will be more accurate if patients who are not postmenopausal at the time of study entry are asked to record the date of onset of their menstrual periods for the first 18 months on study.)
f	Expedited AE reporting requirements are addressed in Section 10.3. AEs requiring routine reporting will be included on one Form AE submitted 30 days following completion of RT; however, the patient should be assessed for AEs throughout therapy. (Group 2 patients must be assessed before trastuzumab Dose 2 is administered.)
g	MRI is not permitted as a substitute for a mammogram.
h	The first follow-up mammogram on study should be performed 12 months following the most recent mammogram performed prior to randomization; then every 12 months.
i	Pregnancy testing according to institutional standards should be performed for women of child-bearing potential (see Section 4.3.13).
j	After the B-43 consent form for pre-entry HER2 testing has been signed, tumor blocks ***for all patients must be submitted*** for HER2 testing at Rush University Medical Center to determine eligibility ***prior to randomization***. For patients who are subsequently enrolled in B-43, the blocks will also be used for correlative studies (see Section 6.0 and the Treatment Study Sample Consent Form).

6.0 PATHOLOGY AND CORRELATIVE STUDIES

6.1 Overview of tissue requirements

NSABP B-43 requires the submission of tumor blocks.

- Submission of a representative tumor block ***before study enrollment is required for central HER2 testing to determine eligibility for B-43. Note: Submission of alternative tissue samples is NOT permitted. The patient must sign the informed consent form for pre-entry HER2 testing, before the submission of her tissue.***

  Central testing for HER2 status will be performed at Rush University Medical Center (see Information Resources). Analysis will be performed in a real-time manner, and the results will be provided to the patient's study physician.

  Refer to the Members' Area of the NSABP Web site for the B-43 Pathology Instructions for detailed information regarding the B-43 pathology requirements, shipment and associated transmittal form, contact information, and receipt of test results.

- If the DCIS is determined to be HER2-positive by central testing and the patient is enrolled in B-43, the remaining tissue will be forwarded from RUMC to the NRG Oncology Biospecimen Bank-Pittsburgh for use in correlative studies for B-43. ***By signing the B-43 Treatment Study informed consent form, the patient is consenting to the use of her tissue for B-43 correlative studies.***

- If the DCIS is determined to be HER2-positive by central testing but the patient chooses to not enroll on the B-43 study, any tissue remaining after the HER2 testing will be returned to the pathology department that submitted the block.

- If the DCIS is determined to be HER2-negative, the patient will be ineligible for B-43, and any tissue remaining after the HER2 testing will be returned to the pathology department that submitted the block.

- If HER2 testing cannot be performed because of an insufficient or inadequate tumor sample, the remaining tumor sample will be returned to the pathology department that submitted the block. If time to randomization permits and if another representative tumor block is available, it should be submitted for central HER2 testing.

6.2 Central testing to determine HER2 status

Central testing for determination of HER2 status will be performed at a central reference lab according to the ASCO College of American Pathologists (CAP) guidelines. HER2 amplification status for B-43 will be evaluated by IHC using the HercepTest™ (Dako, Carpinteria, CA) for all cases; cases with a 2+ IHC reading will be reflexed to FISH (PathVysion®, HER2 DNA probe kit, by Vysis, Inc, Downers Grove, IL). A 0, 1+, or 3+ IHC reading will be considered final for eligibility. (From the time of study activation until April 4, 2011, an IHC 1+ reading was also reflexed to FISH. This central practice was discontinued effective April 4, 2011 [see Section 2.3 for additional information]). As part of the correlative science in B-43, FISH testing will also be performed for all cases (enrolled patients) at a later date.

6.3 Correlative study hypotheses to be tested

Pre-entry submission of paraffin blocks is mandated in this study. Collected blocks (for those patients who have been enrolled in B-43) will be used to examine mRNA levels and DNA copy numbers of HER2, cMYC, and other candidate predictive genes. Additionally, we will examine mutation of the PI3K gene as a determinant of resistance to trastuzumab. We will also examine other candidate predictors of trastuzumab response as they become available, as well as candidate prognostic markers of DCIS.

In the NSABP B-35 trial for ER-positive DCIS, we were able to collect blocks from 1907 out of 2358 cases (80%). Since, in general, HER2-amplified cases are larger size, comedo lesions, we expect to be able to collect at least a similar percentage of blocks.

We propose the following hypotheses to be tested in this study:

- The benefit from trastuzumab added to radiotherapy will be significantly higher for cMYC (8q24)-amplified tumors compared to non-amplified tumors.
- The measurement of HER2 mRNA expression level will provide a better predictive assay than HER2 IHC or FISH.
- The benefit from trastuzumab added to radiotherapy will be less significant in tumors with mutations in the PI3K gene.

  Since PI3K may be integral to the mechanism of radiosensitization by trastuzumab, we hypothesize that an activating mutation in the PI3-Kinase gene will bypass the need for HER2 signaling in HER2-overexpressing cells and render them resistant to trastuzumab. Dr. Kornelia Polyak at the Dana Farber Cancer Institute will examine, by DNA sequencing, the presence of mutations in the PI3-Kinase gene using DNA extracted from paraffin blocks. This study will be complemented by a similar effort utilizing tissue blocks from the NSABP B-17 and B-24 trials.

6.4 Background and studies leading to the hypotheses

6.4.1 *Currently used assays to determine HER2 status are not optimal predictors of benefit from adding trastuzumab to adjuvant chemotherapy*

In the B-31 trial, we initially allowed local laboratories to perform HER2 testing (either IHC or FISH), but, after performing a central review of the HER2 status on the initial cohort of patients, we subsequently provided a protocol amendment which required that the test be performed by a laboratory that had been pre-approved by the NSABP. A central review of all submitted specimens from patients entered into B-31 has demonstrated a false-positive rate of 11.5% with the PathVysion FISH test and 15.4% with the Herceptest IHC assy. Table 4 summarizes the results of the centrally performed HER2 assays, which provided an opportunity to address whether HER2 status is a predictor of benefit from adjuvant trastuzumab. We had predicted that there would be no benefit in patients with tumors that have either a normal gene copy number or express normal levels of HER2 protein.[46]

TABLE 4 Relative risk of ACTH over ACT and p values from Cox univariate model of disease-free survival endpoint for each subset defined by either PathVysion FISH or Herceptest IHC or both

XCategory	RR of ACTH (95% CI)	p-value from Cox model	N	Interaction test p-value
FISH +	0.47 (0.36-0.61)	<0.0001	1588	0.60
FISH -	0.40 (0.18-0.89)	0.026	207	
IHC 3+	0.48 (0.37-0.63)	<0.0001	1488	0.26
IHC-(0-2+)	0.32 (0.16-0.65)	0.0017	299	
FISH-, IHC-	0.34 (0.14-0.80)	0.014	174	

Surprisingly, there was no statistical interaction between either HER2 gene copy numbers or expression levels and benefit from adding trastuzumab to chemotherapy, with a trend for benefit even in patients diagnosed with tumors that had 2 or less copies of the HER2 gene or expressed 2+ or 1+ levels of the HER2 protein.

These results indicate that HER2 biology is not as straightforward as expected. Response to trastuzumab in the adjuvant setting might be different from the response in advanced disease, in that it may not require a high level overexpression of HER2 protein.

Another possibility is that IHC or FISH assays do not provide a linear measurement of actual biological expression levels of HER2 protein. In our experience with estrogen receptor (ER), IHC was found to be a highly non-linear assay whereas mRNA measurement by RT-PCR was a linear predictor of response to tamoxifen.[46] Therefore, it will be important in this trial to examine mRNA levels of HER2 and other members of the HER family of receptors and their ligands as potential predictors of response.

We also examined other potential predictors. None of the clinicopathological variables examined showed interaction with trastuzumab.[46] PTEN loss as measured by IHC, Cyclin D1 by FISH, and Topoisomerase II-alpha by FISH all failed to predict benefit or lack of benefit from trastuzumab. Surprisingly, cMYC co-amplification turned out to be a strong predictor of benefit from adjuvant trastuzumab as described below.

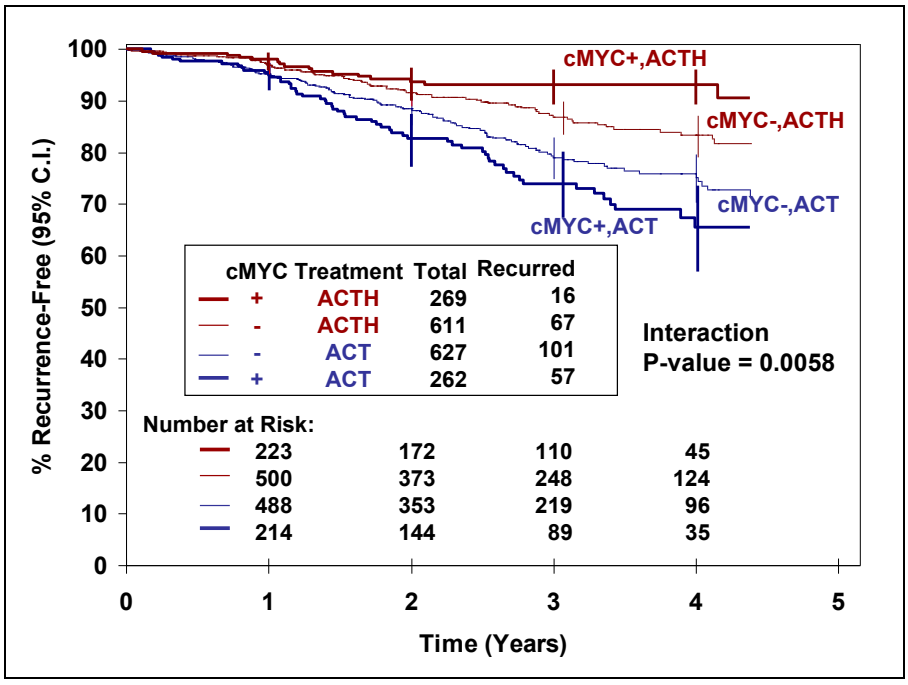
6.4.2 *cMYC (8q 24) amplification confers poor prognosis in patients with HER2-amplified breast cancer treated with AC→T but is a predictor of benefit from trastuzumab added to AC→T*

In an effort to develop a prognostic model for identifying potentially cured node-positive patients treated with standard chemotherapy, we have screened commonly amplified chromosomal loci by FISH using tissue microarrays constructed from tumor specimens from participants in NSABP B-28, in which patients with node-positive breast cancer were randomized to 4 cycles of AC or to 4 cycles of AC followed by 4 cycles of paclitaxel. We hypothesized that since gene amplification has a significant role in transcriptional regulation of the genome, profiling of commonly occurring amplifications would lead to discovery of significant prognostic factors. Twenty-seven amplicons were selected based on their known association with overexpression of genes they transcribe when amplified. This led to identification of HER2 (17q), cMYC (8q), and HTPAP

(8p) as independently prognostic amplicons in patients treated with chemotherapy. Interestingly, amplification of these three genes were independent events and there was little overlap between HER2 and HTPAP amplification. However, about 25% of HER2-amplified cases had co-amplification of cMYC, which conferred a worse prognosis than when either of the two alone were amplified. This prompted us to examine, in an independent patient cohort from the NSABP B-31 trial, whether cMYC confers poor prognosis or influences response to trastuzumab.

cMYC gene amplification was predefined as ≥ 5 FISH signals on average per tumor nucleus after counting 60 tumor nuclei. We had assay results for cMYC available from 1769 cases. cMYC was amplified in 531 cases (30%). We examined time-to-first recurrence as a primary clinical endpoint. This was a secondary endpoint used in the first reporting of the clinical results. Overall, the addition of trastuzumab to chemotherapy reduced the chance of recurrence by 53% at 4 years after randomization. In cases with absence of cMYC amplification (n=1238), there was a 38% reduction in recurrence rate in a Cox model (HR, 0.62; 95% CI, 0.45 to 0.84; 2P=0.0022). There were 101 events in 627 patients assigned to the chemotherapy only arm and 67 events in 611 patients randomized to chemotherapy plus trastuzumab. A Kaplan Meier plot is shown in Figure 2 (thin lines). Of note is that while there is a clear separation of two curves, there were continuing failures in both treatment arms during the follow-up period. In patients with cMYC amplification (n=531), there was a 74% reduction in recurrence rate (HR, 0.26; 95% CI, 0.15 to 0.45; 2P<0.0001) with 16 events in 269 patients assigned to trastuzumab in contrast to 57 events in 262 patients who received chemotherapy only. A Kaplan Meier plot is shown in Figure 2 (thick lines). The four-year recurrence-free rate of cMYC-amplified cases is 93% when trastuzumab is added and 66% in the chemotherapy-only arm. The formal statistical test for this cMYC-by-trastuzumab interaction was significant (2P-value for interaction=0.0058).[46]

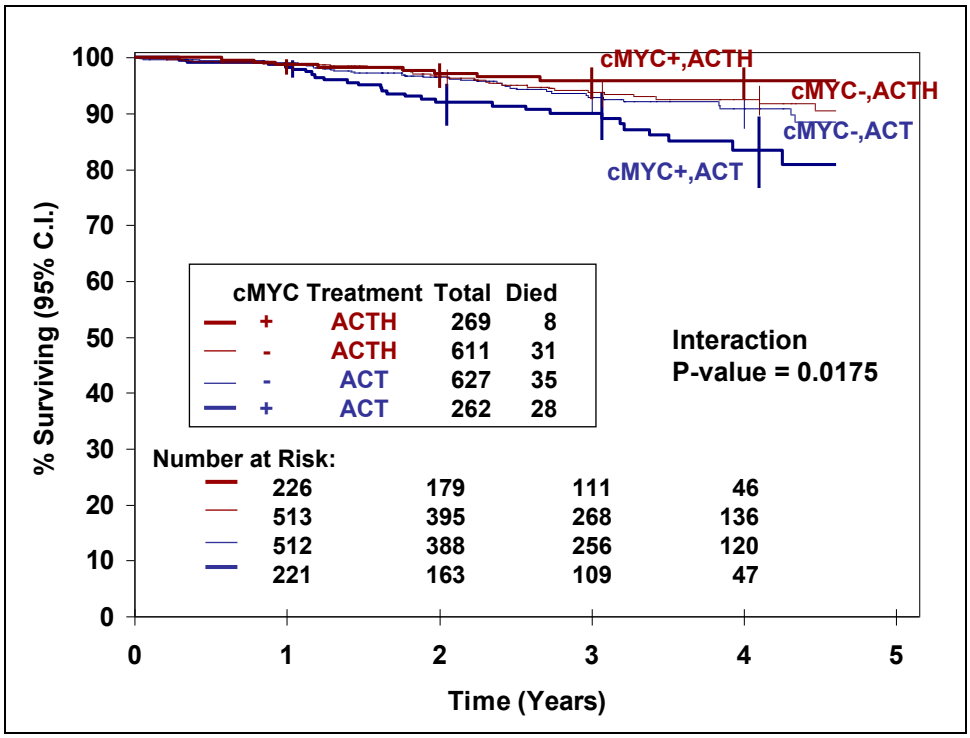
Figure 2. Kaplan-Meier plot for recurrence-free survival according to cMYC and treatment



This difference is reflected in the survival endpoint (Figure 3). There was no statistically significant benefit in the cMYC non-amplified cohort in comparison to the 72% reduction in death in the cMYC-amplifed cohort when trastuzumab was added to chemotherapy. In the cMYC non-amplified cohort (n=1238), there were 31 deaths among 611 patients randomized to the trastuzumab arm compared to 35 deaths out of 627 patients in the control arm (HR, 0.85; 95% CI, 0.53 to 1.39; 2P=0.52). In the cMYC-amplified cohort (n=531), 8 patients died among 269 who were assigned to trastuzumab plus chemotherapy versus 28 deaths among 262 who were assigned to chemotherapy only (HR, 0.28; 95% CI, 0.13 to 0.62, 2P=0.00015). This interaction between cMYC amplification and survival benefit from trastuzumab was also statistically significant (p-value for interaction = 0.0175).

The data remained consistent even when we did an analysis based on a subset of patients whose tumors were found to have HER2 gene amplification on central assay and who received at least one cycle of trastuzumab (data not shown).

Figure 3. Survival according to cMYC and treatment



6.4.3 ***Why is cMYC important?***

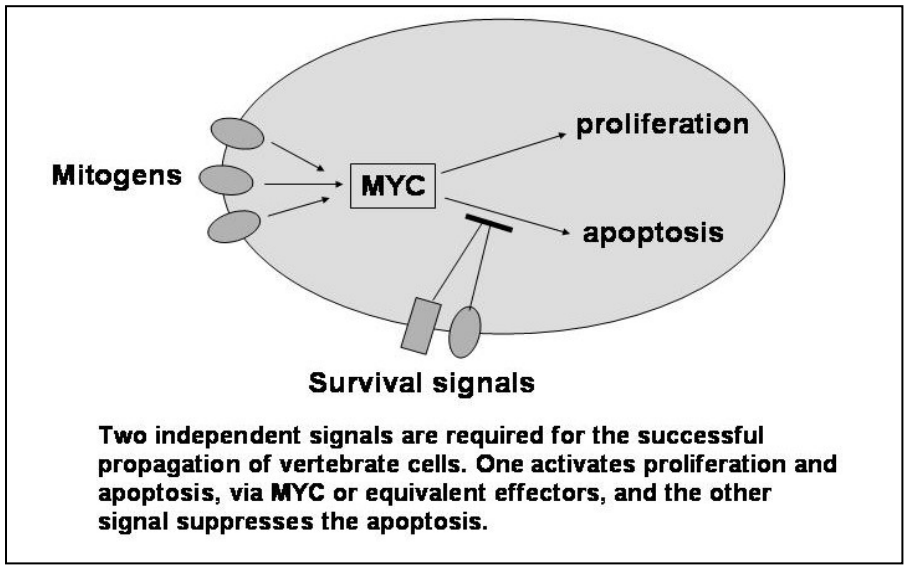
When considering the fact that cMYC amplification was confirmed to be a poor prognosticator in the chemotherapy-only arm as observed in the NSABP B-28 trial, it is remarkable that the addition of trastuzumab to chemotherapy completely reverses this trend. Why should cMYC influence response to trastuzumab in such a dramatic way? One simple hypothesis can be drawn from the known role of cMYC for apoptosis, although this model may be somewhat oversimplified.

Although the processes of cell renewal and cell death appear to be opposing and mutually contradictory, substantial evidence now indicates that the two are linked.[66] One of the first oncogenes shown to have proapoptotic activity was cMYC.[67] cMYC is one of a family of related mammalian genes that encode the MYC proteins, transcription factors of the bHLH-zip family. Deregulated expression of MYC genes is frequent in cancer, and substantial evidence implicates MYC proteins in the control of cell proliferation. MYC proteins are expressed in proliferating cells but are absent in quiescent cells. Ectopic expression of MYC is sufficient to drive many cells into the cell cycle in the absence of external mitogens. However, in certain circumstances, MYC also promotes apoptosis. The mitogenic and proapoptotic properties of cMYC are genetically inseparable. Both require an intact NH2-terminal transcriptional activation domain, DNA binding and dimerization domains, and interaction with the MYC partner protein Max. However, these two processes can be de-linked if the apoptotic pathway is suppressed by other factors. For example, expression of the anti-apoptotic protein Bcl-2 specifically abrogates cMYC-induced apoptosis

without affecting the cMYC mitogenic function.[68] The outcome of this de-linking can be profound in some systems. In a pancreatic islet cell transgenic model, overexpression of cMYC in beta-islet cells induces initial proliferation followed by involution of islets due to apoptosis.[69] Inhibition of apoptosis by introduction of Bcl-xL immediately triggers progression into cancer. In the latter model, cMYC alone, when aided by an anti-apoptotic signal, was enough to induce all necessary machinery required for expression of the malignant phenotype: proliferation, angiogenesis, and invasion. Downregulation of cMYC in this model resulted in regression of tumors. Unfortunately, the effects of withdrawing Bcl-xL on established tumors were never tested in this model since Bcl-xL was driven by a constitutive promoter.

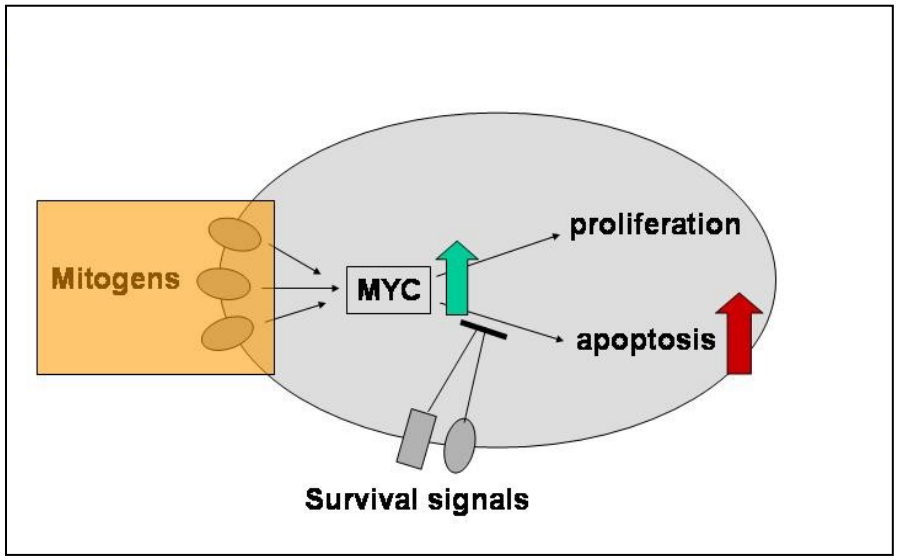
Harrington and Evan proposed a dual signaling hypothesis to explain the cooperation between cMYC and survival factors in tumorigenesis.[70] In normal cells, mitogenic signaling through cMYC will induce both the proliferation and the apoptosis pathways. Therefore, for normal cells to propagate, an apoptotic signal induced by mitogenic signaling through cMYC has to be suppressed by survival factors (Figure 4).

Figure 4. Dual signal hypothesis



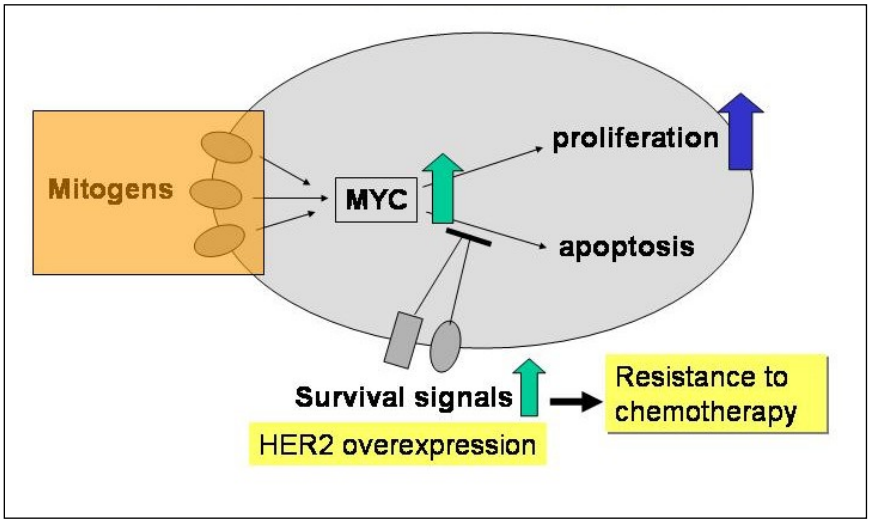
In cells with deregulated cMYC expression, a mitogenic signal is no longer needed and cells will go through initial proliferation but will soon go through apoptosis (Figure 5).

Figure 5. Cells with amplification and deregulated expression of cMYC



Only when there is help from overactive survival signals can the cells default to proliferation (Figure 6). We hypothesize that in breast cancer with coamplification of cMYC and HER2, HER2 provides an anti-apoptotic signal to help cMYC function as a proliferation switch. Transgenic mouse models have demonstrated cooperation between EGFR (a dimerization partner for HER2) and cMYC in mammary tumorigenesis, which is mediated by induction of Bcl-xL through EGFR activation of Akt, thus suppressing the proapoptotic function of cMYC. Once the apoptotic pathway is de-linked, cMYC becomes a full oncogene capable of inducing all hallmarks of the cancer phenotype: proliferation, invasion, and angiogenesis.

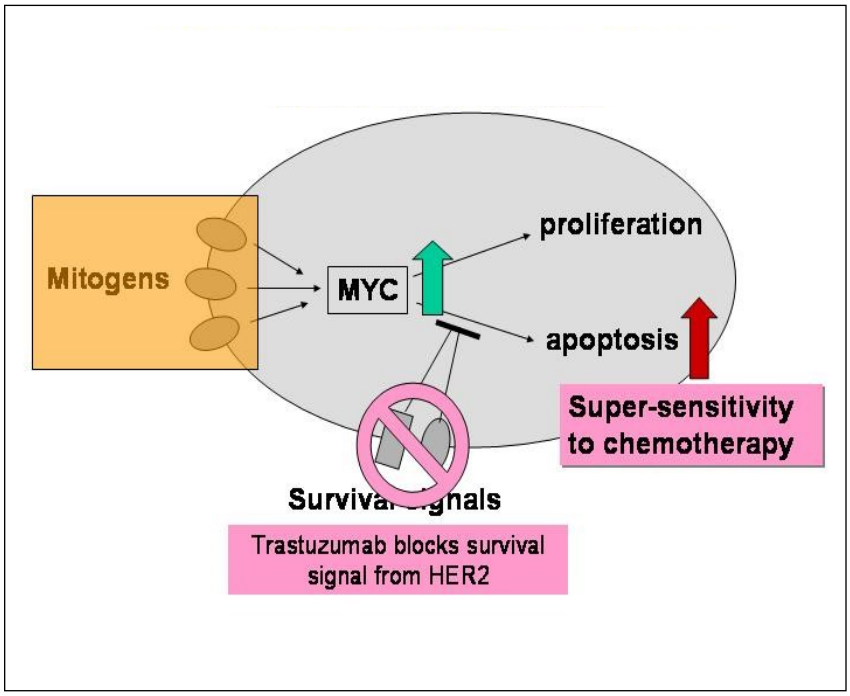
Figure 6. Cells with cMYC and HER2 co-amplification



From a cMYC-centric view of the process, HER2 is only a helper, not the instigator of tumorigenesis. Therefore, while cells primed for malignant transformation by cMYC-amplification will be able to escape the fate of

apoptosis with the help of HER2-amplification, this also makes them dependent upon HER2-signaling to survive, making them supersensitive to trastuzumab (Figure 7). Inhibition of the HER2 signal by trastuzumab will trigger the proapoptotic function of cMYC in such cancer cells. In human tumors, studies have demonstrated that Bcl-2 and survivin are upregulated by HER2 overexpression, and trastuzumab was shown to cause apoptosis of HER2-positive tumor cells in the preoperative treatment setting.[71]

Figure 7. Cells with cMYC and HER2 co-amplification treated with trastuzumab



Why is chemotherapy not effective in treating cMYC-amplified tumors? It could be due to the obligatory presence of strong anti-apoptotic signaling from HER2 (and other survival factor receptors) to suppress cMYC-induced apoptosis in such cells. Therefore, chemotherapy-induced apoptosis is enhanced by suppression of the anti-apoptotic signal coming from HER2 in the B-31 study. Whether cMYC alone is sufficient to induce apoptosis or whether cMYC is simply enhancing chemotherapy-induced apoptosis is not clear and needs further investigation.

In this trial, trastuzumab will be tested as a radiosensitizer. We hypothesize that cMYC will have an interaction with radiotherapy similar to its interaction with chemotherapy due to an enhancement of the survival pathway in cMYC-amplified cells. We will use the exact same assay methods and scoring rates used in the B-31 study. cMYC gene amplification is predefined as ≥ 5 FISH signals on average per tumor nucleus after counting 60 tumor nuclei.

Obviously, the above model is somewhat oversimplified since the cMYC amplicon is fairly big and there are other genes in that amplicon. It is possible that some of the genes coordinately overexpressed with the 8q24 amplicon may

be involved in the HER2 signaling pathway, therefore, making tumor cells with both HER2 and cMYC amplified tumors overdependent or addicted to the HER2 signaling pathway. This would result in sensitivity to trastuzumab. However, the expected end result would be the same as that deduced from the apoptosis hypothesis, and from the clinical point of view, it should not really matter. However, it will have an impact on how we interpret the data and how we extrapolate the results from this trial to other studies. Therefore, it will be important to also examine the interaction between trastuzumab and expression levels of genes other than cMYC in or around the 8q24 amplicon. We plan to achieve this aim by using real-time reverse transcription polymerase chain reaction or the branched DNA amplification method.

6.4.4 *Role of PI3K in radiosensitization by trastuzumab*

PI3K may have an important role in tumor cell responses to trastuzumab. Trastumab-induced inhibition of PI3K is required for the anti-tumor action of trastuzumab on HER2-overexpressing breast cancer cells. Furthermore, genes affecting the cell cycle, growth, maintenance, as well as chemosensitivity, are preferentially regulated by anti-HER2 antibody through PI3K-Akt signaling.[72] Direct inhibition of PI3K with an inhibitor markedly reduced expression of 14 genes that were also affected by the anti-HER2 antibody.[73] Further evidence that PI3K is important in trastuzumab action is demonstrated by its effect on survivin expression. Asanuma et al examined regulation of survivin expression by HER2 and EGFR: trastuzumab reduced the expression of survivin in HER2-overexpressing cells and this reduction of survivin expression was blocked by inhibition of PI3K but not by inhibition of MEK1.[74]

Liang et al examined the mechanism of radiosensitization of HER2-overexpressing breast cancer cells by trastuzumab. Using six breast cancer cell lines with various levels of HER2 (BT474, SKBR3, MDA453, MCF7, ZR75B, and MDA468), the investigators found that trastuzumab inhibits breast cancer cell proliferation but does not induce apoptosis when used alone; it also enhanced radiation-induced apoptosis of the cells in a HER2 level-dependent manner. They furthered this study in MCF7 cells transfected for high levels of HER2 (MCF7HER2). Compared with parental or control vector-transfected MCF7 cells, MCF7HER2 cells showed increased phosphorylation of at least two important HER2 downstream molecules, protein kinase B/Akt and mitogen-activated protein kinase (MAPK), and increased resistance to radiotherapy, as shown by reduced induction of apoptosis and increased cell clonogenic survival after radiation. Exposure of the cells to trastuzumab downregulated the levels of HER2, reduced phosphorylation levels of Akt and MAPK in MCF7HER2 cells, and sensitized these cells to radiotherapy. When specific inhibitors of the PI3-K and MAPK kinase (MEK) pathways were used, we found that exposure of MCF7HER2 cells to the PI3-K inhibitor LY294002 inhibited Akt phosphorylation and radiosensitized the cells; the radiosensitization effect by the MEK inhibitor PD98059 was relatively weaker, even though the phosphorylation of MAPK was reduced by PD98059 treatment. These results indicate that the PI3-K pathway might be the major pathway for trastuzumab-mediated radiosensitization of breast cancer cells.[7]

6.4.5 *PI3-Kinase gene mutation in DCIS*

In support of the importance of the PI3-K-Akt pathway in human cancer, genetic alterations in several components of the pathway including deletion of PTEN, amplification of Akt and PI3-Kinase gene, and somatic mutations of PI3-Kinase gene have been reported in various tumor types. In human breast cancer, mutations in PI3-Kinase gene have been reported to occur in 8-40% of tumors, making PI3-Kinase gene one of the most frequently mutated genes besides TP53 in this tumor type. Expression of cancer derived PI3K mutants in immortalized human mammary epithelial cells was sufficient to induce colony formation in soft agar and tumorigenicity *in vivo*, suggesting that mutations in PI3-Kinase gene may play a role in the early stages of breast tumorigenesis. However, somatic mutations in PI3-Kinase gene have been reported to occur at higher frequency in advanced stage tumors suggesting a role in tumor progression.

Only two published studies of human breast cancer analyzed PI3-Kinase gene mutations in pre-invasive tumors, including DCIS. In both reports, a limited number of DCIS tumors were analyzed. Campbell et al performed Single-Strand Conformational Polymorphism (SSCP) analysis of PI3-Kinase gene exons 1-20 in 70 breast tumors, including 3 DCIS cases, and detected mutations in 28/67 invasive tumors and 0/3 DCIS cases; Lee et al analyzed exons 9 and 20 also by SSCP in 93 breast tumors and identified mutations in 24/78 of invasive breast tumors and only 2/15 for DCIS. Neither report provided information on the type of DCIS that was analyzed (pure or adjacent to invasive tumor, histologic type, etc.), but these findings implicate PI3K in the in situ-to-invasive carcinoma progression. Correlating with this, analysis of PTEN expression in DCIS and invasive tumors by IHC found down regulation of PTEN only in 2/18 pure DCIS and 9/26 DCIS adjacent to invasive cancer. Importantly, in the 26 DCIS-IDC pairs, the expression of PTEN was the same in the in situ and invasive components in all but two cases confirming the clonal relatedness of the two lesions. Immunohistochemical analysis of the activity and expression of several components of the PI3-K-Akt pathway in breast tumors of different stages also detected more frequent loss of PTEN expression, increased S6, AT, mTOR, and 4E-BP1 expression in invasive tumors compared to preinvasive and premalignant lesions.[75,76]

To determine if the frequency of PI3-Kinase gene mutations is different in pure DCIS compared to DCIS adjacent to invasive cancer, the Polyak laboratory sequenced exons 9 and 20 of the PI3-Kinase gene in 22 pure, 1 microinvasive, and 5 concomitant (adjacent to invasive cancer) DCIS cases. Correlating with a potential role of PI3K in progression to invasiveness, only 2/22 pure DCIS cases, compared to 3/5 DCIS adjacent-to-invasive-tumor cases had mutations in exons 9 or 20 of the PI3-Kinase gene. Due to the small number of cases in each group, this difference has not reached statistical significance (p=0.085 two-sided Fisher's exact test). Thus, the analysis of additional cases is necessary.

6.5 Examination of future candidate predictive markers for trastuzumab response

Many studies to identify predictors of trastuzumab response are ongoing. By the time the accrual for this trial is completed, we will have many candidate markers of response. We may elect to use a real time RT-PCR assay to interrogate the mRNA expression levels of such molecules. One alternative method we are currently developing is based on a branched DNA-amplification method and monitored using a luminometer or Luminex® bead array. We may also use IHC and/or variations of the method (such as reverse phase protein array) to examine such molecules depending on the availability and reliability of the assays. We are currently exploring a Sequencing by Synthesis approach to parallel sequence multiple gene targets at the same time. If successful, we may sequence candidate cancer genes in addition to PI3-Kinase gene in this trial. Statistical interaction between each marker as a continuous variable and trastuzumab-treatment effect will be examined. If multiple genes are examined, appropriate adjustments will be made for multiple comparisons. These studies will be regarded as exploratory in nature.

7.0 TREATMENT REGIMEN

7.1 Radiation therapy for Group 1 (RT alone) and Group 2 (RT + trastuzumab)

All B-43 patients will receive post-lumpectomy **whole breast irradiation (WBI)** in accordance with institutional standards and the NSABP Radiation Therapy Guidelines posted on the NSABP Web site.

7.1.1 *RT treatment schedule*

RT (WBI) must begin following randomization. Radiation can be delivered in 25+ fractions or with accelerated fractionation (16-17 fractions).

7.1.2 *Boost to tumor bed*

RT boost, including brachytherapy, may be administered at the radiation oncologist's discretion.

7.1.3 *Prohibited RT*

- Partial breast irradiation
- Regional nodal irradiation

7.2 Trastuzumab (only Group 2 patients)

Administer trastuzumab according to instructions outlined in Table 5.

TABLE 5. Trastuzumab treatment regimen for **Group 2** patients

Trastuzumab	**Dose* and Administration**	**Treatment Schedule**	
		RT is planned for 25+ fractions	**RT is planned for 16-17 fractions (accelerated fractionation)**
Dose 1	8 mg/kg IV over 90 minutes	Administer within 1 week before RT begins or within the first 5 days of RT (on or before **Day 5**)	Administer within 1 week before RT begins or within the first 2 days of RT (on or before **Day 2**)
Dose 2	6 mg/kg IV over 30 minutes *(if the patient tolerates the initial 90-minute infusion)*	Administer 3 weeks following trastuzumab Dose 1	

* The rounding of trastuzumab doses is optional. If the treating physician decides to round the dose(s), trastuzumab should be rounded to the nearest 1 mg.

7.3 Hormonal therapy for all B-43 patients with hormone-receptor positive DCIS

Patients with ER-positive and/or PgR-positive DCIS should receive hormonal therapy, which may begin before, during, or after radiation therapy and should be administered for a minimum of 5 years. Choice of the drugs(s) to be used for hormonal therapy is at the physician's discretion. The dose and schedule should be consistent with the drug package insert instructions.

7.4 Non-protocol therapy

- See Section 7.1.3 for RT techniques that are prohibited.
- Cancer therapy other than that specified in the B-43 protocol is prohibited until the time of first cancer recurrence or second primary cancer.
- If a patient is considering participation in another clinical trial (including supportive therapy trials), contact the Clinical Coordinating Department (see Information Resources).

8.0 TREATMENT MODIFICATIONS

8.1 General instructions

- The NCI Common Terminology Criteria for Adverse Events Version 3.0 (CTCAE v3.0) must be used through September 30, 2010, to grade the severity of adverse events (AEs). CTCAE v4.0 will be utilized for AE reporting beginning October 1, 2010.
- Treatment decisions should be based on the AE requiring the greatest modification.

8.2 Trastuzumab treatment modifications (Group 2 patients)

- If RT is held due to RT-related adverse events, trastuzumab should be administered according to the B-43 treatment regimen (see Table 5 in Section 7.2).
- If RT is never initiated, trastuzumab may not be given. (If Dose 1 was administered before the decision to not initiate RT, Dose 2 may not be given.)
- If AEs occur related to trastuzumab, follow trastuzumab treatment modification instructions listed in Table 6.

TABLE 6. Trastuzumab modifications

CTCAE v4.0 Adverse Event	CTCAE Grade	Action to be Taken
Cardiac Disorders		
Acute coronary syndrome	2, 3, 4	Do not administer trastuzumab Dose 2.
Heart failure	2, 3, 4	Do not administer trastuzumab Dose 2.
Left ventricular systolic dysfunction	3, 4	Do not administer trastuzumab Dose 2.
Myocardial infarction	3, 4	Do not administer trastuzumab Dose 2.
General Disorders		
Infusion-related reaction	1	Slow the infusion and assess the patient; management is at the investigator's discretion.
	2, 3, 4	Do not administer trastuzumab Dose 2.
Immune System Disorders		
Allergic reaction	1	Slow the infusion and assess the patient; management is at the investigator's discretion.
	2, 3, 4	Do not administer trastuzumab Dose 2.
Anaphylaxis	3, 4	Do not administer trastuzumab Dose 2.
Cytokine release syndrome	2, 3, 4	Do not administer trastuzumab Dose 2.
Investigations		
Ejection fraction decreased	2, 3, 4	Do not administer trastuzumab Dose 2.
Other		
Other clinically significant AEs*	3, 4	Do not administer trastuzumab Dose 2.
* Determination of "clinically significant" is at the investigator's discretion and applies to those AEs that ***are attributed to trastuzumab and are not related to RT alone***.		

8.3 Radiation therapy treatment modifications

Management of adverse events related to RT is at the discretion of the radiation oncologist.

9.0 DRUG INFORMATION

9.1 Trastuzumab (NSC #688097)

Investigators with an affiliation with either NRG Oncology or the CTSU may request an Investigator's Brochure by emailing the Pharmaceutical Management Branch's IB Coordinator at ibcoordinator@mail.nih.gov or by calling PMB at 240-276-6575 and providing:

- the investigator's full name (first, middle, last)
- the investigator's NCI investigator number
- the agent name (i.e., "trastuzumab")
- the NSC (i.e., "688097")
- the protocol (i.e., "NSABP B-43")
- the requestor's name, email address, and phone number

9.2 Description of trastuzumab (Herceptin®) (NSC #688097)

Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd=5 nM) to the extracellular domain of the human epidermal growth factor receptor 2 protein, HER2. The antibody is an IgG_1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary [CHO]) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. The nominal content of each trastuzumab vial is 440 mg trastuzumab, 400 mg α,α-trehalose dihydrate, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI), USP, containing 1.1% benzyl alcohol as a preservative, yields a multi-dose solution containing 21 mg/mL trastuzumab, at a pH of approximately 6.

9.3 Procurement of trastuzumab

Trastuzumab will be supplied free of charge by Genentech, Inc., and distributed by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI).

Trastuzumab (NSC #688097) may be requested by the principal investigator (or his/her authorized designee[s]) for NSABP B-43 at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that trastuzumab be shipped to the treating investigator. The responsible investigator at each participating institution must be registered with CTEP, DCTD through the annual submission of an FDA Form 1572 (Statement of Investigator), a Curriculum Vitae, a Supplemental Investigator Data Form (IDF), and a Financial Disclosure Form (FDF).

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order

Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call 240-276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email ibcoordinator@mail.nih.gov anytime.

9.4 Shipping

Vials of trastuzumab are shipped at room temperature by overnight express delivery ***Monday through Thursday***, and must be placed in a 2°- 8°C (36°- 46°F) refrigerator immediately upon receipt to ensure optimal retention of physical and biochemical integrity.

9.5 Storage/stability

Vials of trastuzumab are stable at 2° - 8°C (36°- 46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2° - 8°C (36° - 46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. ***Do not freeze trastuzumab that has been reconstituted.*** The solution of trastuzumab for infusion diluted in polyvinylchloride, polyethylene, or polypropylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2° - 8°C (36° - 46°F) for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at room temperature 2° - 25°C. However, because diluted trastuzumab contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated 2° - 8°C.

9.6 Reconstitution and administration

9.6.1 *Reconstitution*

The diluent provided has been formulated to maintain the stability and sterility of trastuzumab for up to 28 days. Other diluents have not been shown to contain effective preservatives for trastuzumab. Each vial of trastuzumab should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multi-dose solution containing 21 mg/mL trastuzumab.

Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after:" with the future date that is 28 days from the date of reconstitution.

Note: When administering trastuzumab to a patient with a known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with Sterile Water for Injection (SWFI), and only one dose per trastuzumab vial should be used. ***Trastuzumab which has been reconstituted with SWFI must be used immediately and any unused portion must be discarded. Use of other reconstitution diluents should be avoided***.

Shaking the reconstituted trastuzumab or causing excessive foaming during the addition of diluent may result in problems with dissolution and the amount of

trastuzumab that can be withdrawn from the vial. Use appropriate aseptic technique when performing the following reconstitution steps:

- Using a sterile syringe, slowly inject the 20 mL of diluent into the vial containing the lyophilized cake of trastuzumab. The stream of diluent should be directed into the lyophilized cake.
- Swirl the vial gently to aid reconstitution. Trastuzumab may be sensitive to shear-induced stress, e.g., agitation or rapid expulsion from a syringe. ***Do not shake.***
- Slight foaming of the product upon reconstitution is not unusual. Allow the vial to stand undisturbed for approximately 5 minutes. The solution should be essentially free of visible particulates, clear to slightly opalescent and colorless to pale yellow.

9.6.2 ***Administration***

See Section 7.2.

9.7 Transfer of trastuzumab

PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). Trastuzumab may not be used outside the scope of this protocol, nor can trastuzumab be transferred or licensed to any party not participating in this clinical study. To obtain an approval for transfer, investigators should complete and submit to the PMB (fax number 240-276-7893) a Transfer Investigational Agent Form available on the NCI home page (http://ctep.cancer.gov) or call the PMB at 240-276-6575. The participating institution should also inform the NRG Oncology SDMC of the transfer.

9.8 Return of unused trastuzumab

- **For U.S. sites**

  At the completion of accrual and treatment, all unused (unopened) vials of trastuzumab must be **returned to the PMB.** When it is necessary to return study drug (e.g., unused vials remaining when the protocol is closed to accrual and treatment at a participating clinical site, unopened expired vials), investigators should return the study drug to the PMB using the NCI Return Agent Form available on the NCI home page (http://ctep.cancer.gov) or call the PMB at 240-276-6575.

- **For non-U.S. sites**

  Undispensed or expired study drug may be destroyed in the Pharmacy department of each center as per each center's policy and guidelines or returned to the NCI Clinical Repository in cases where it is not possible for the site to destroy the drug locally. Sites must contact the PMB at 240-276-6575 or by e-mail PMBAfterhours@mail.nih.gov to request and obtain authorization for local destruction of undispensed or expired study drug, prior to local destruction of undispensed or expired study drug.

9.9 Drug accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return of all drugs received from the PMB using the NCI Investigational Agent Accountability Record Form, available on the NCI home page (http://ctep.cancer.gov) or call the PMB at 240-276-6575. (Refer to the NCI Investigator's Handbook for procedures for Drug Accountability and Storage.)

9.10 Warnings and contraindications

9.10.1 *Cardiotoxicity*

Administration of trastuzumab can result in the development of ventricular dysfunction and congestive heart failure. Signs and symptoms of cardiac dysfunction, such as dyspnea, increased cough, paroxysmal nocturnal dyspnea, peripheral edema, S_3 gallop, or reduced ejection fraction, have been observed in patients treated with trastuzumab. Congestive heart failure associated with trastuzumab therapy may be severe and has been associated with disabling cardiac failure, death, and mural thrombosis leading to stroke. ***See*** Section 2.6 ***for information related to cardiotoxicity for B-43 Group 2 patients.***

9.10.2 *Hypersensitivity reactions including anaphylaxis*

Severe hypersensitivity reactions have been infrequently reported in patients treated with trastuzumab. Signs and symptoms include anaphylaxis, urticaria, bronchospasm, angioedema, and/or hypotension. In some cases, the reactions have been fatal. ***Trastuzumab infusion should be interrupted in all patients with severe hypersensitivity reactions***. In the event of a hypersensitivity reaction, appropriate medical therapy should be administered, which may include epinephrine, corticosteroids, diphenhydramine, bronchodilators, and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms.

9.10.3 *Infusion reactions*

In the postmarketing setting, rare occurrences of severe infusion reactions leading to a fatal outcome have been associated with the use of trastuzumab. In clinical trials, infusion reactions consisted of a symptom complex characterized by fever and chills, and on occasion included nausea, vomiting, pain, headache, dizziness, dyspnea, hypotension, rash, and asthenia. These reactions were usually mild to moderate in severity.

However, in postmarketing reports, more severe adverse reactions to trastuzumab infusion were observed and included bronchospasm, hypoxia, and severe hypotension. These severe reactions were usually associated with the initial infusion of trastuzumab and generally occurred during or immediately following the infusion. However, the onset and clinical course were variable. Delayed post-infusion events with rapid clinical deterioration have also been reported. Rarely, severe infusion reactions culminated in death within hours or up to one week following an infusion.

9.10.4 ***Pulmonary events***

Severe pulmonary events leading to death have been reported rarely with the use of trastuzumab in the postmarketing setting. Signs, symptoms and clinical findings include dyspnea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary edema, pulmonary insufficiency and hypoxia, and acute respiratory distress syndrome. These events may or may not occur as sequelae of infusion reactions. Patients with symptomatic intrinsic lung disease resulting in dyspnea at rest, may be at greater risk of severe reactions.

Other severe events reported rarely in the postmarketing setting include pneumonitis and pulmonary fibrosis.

10.0 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.

10.1 B-43 definitions for adverse event reporting

10.1.1 *Investigational agent*

The investigational agent administered in NSABP B-43 is ***trastuzumab***. Trastuzumab is being made available under an IND sponsored by the National Cancer Institute (NCI). For patients who receive trastuzumab, prior expectedness of adverse events is based on the current NCI Specific Protocol Exceptions to Expedited Reporting (SPEER) List.

10.1.2 *Investigational combination therapy*

This study includes an investigational agent and radiation therapy (RT). When an ***investigational agent*** (trastuzumab) is administered concurrently with ***RT*** and an adverse event occurs that is expected for RT, but is not listed for trastuzumab, 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 trastuzumab rather than the ***RT***, the adverse event should then be considered unexpected for the combination.

10.1.3 *Adverse event characteristics*

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting beginning October 1, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

10.2 Adverse events reported for trastuzumab

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with **bold** and ***italicized*** text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 4621 patients.*

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

The National Cancer Institute (NCI) holds an IND for trastuzumab, thus, they require the entire CAEPR list be included within the protocol document (see Table 7).

TABLE 7. Comprehensive Adverse Events and Potential Risks list (CAEPR) for trastuzumab
(Herceptin, NSC 688097) **Version 2.4, April 14, 2016[1]**

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 4.0 Term) [n= 4621]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 2)***
	Febrile neutropenia[2]		
CARDIAC DISORDERS			
	Cardiac disorders - Other (cardiomyopathy)		
	Heart failure		
	Left ventricular systolic dysfunction		***Left ventricular systolic dysfunction (Gr 3)***
	Pericardial effusion		
	Pericarditis		
	Sinus tachycardia[3]		***Sinus tachycardia[3] (Gr 2)***
	Supraventricular tachycardia[3]		
EYE DISORDERS			
	Conjunctivitis		
	Watering eyes		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Diarrhea		***Diarrhea (Gr 3)***
	Mucositis oral		***Mucositis oral (Gr 2)***
	Nausea		***Nausea (Gr 3)***
		Pancreatitis	
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills[3]		***Chills[3] (Gr 2)***
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
	Fever[3]		***Fever[3] (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
	Infusion related reaction		***Infusion related reaction (Gr 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 2)***
	Pain		***Pain (Gr 2)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction[4]	
		Anaphylaxis	
INFECTIONS AND INFESTATIONS			
	Infection[5]		***Infection[5] (Gr 3)***

Note: Table 7 is continued on the next page.

TABLE 7. Comprehensive Adverse Events and Potential Risks list (CAEPR) for trastuzumab *(continued)*

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 4.0 Term) [n= 4621]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
INVESTIGATIONS			
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Cardiac troponin I increased		
	Ejection fraction decreased		***Ejection fraction decreased (Gr 3)***
	GGT increased		***GGT increased (Gr 2)***
	Neutrophil count decreased[2]		***Neutrophil count decreased[2] (Gr 4)***
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		***Back pain (Gr 2)***
	Bone pain		***Bone pain (Gr 2)***
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		
	Myalgia		***Myalgia (Gr 2)***
	Pain in extremity		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Tumor pain		***Tumor pain (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		
	Headache		***Headache (Gr 2)***
	Peripheral sensory neuropathy		
PSYCHIATRIC DISORDERS			
	Depression		
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
		Adult respiratory distress syndrome[3, 4]	
	Allergic rhinitis		***Allergic rhinitis (Gr 2)***
		Bronchospasm[3, 4]	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Nail loss		
	Rash acneiform		***Rash acneiform (Gr 2)***
	Rash maculo-papular		***Rash maculo-papular (Gr 2)***
	Urticaria[3]		***Urticaria[3] (Gr 2)***

Note: Table 7 is continued on the next page.

TABLE 7. Comprehensive Adverse Events and Potential Risks list (CAEPR) for trastuzumab *(continued)*

Adverse Events with Possible Relationship to Trastuzumab (Herceptin) (CTCAE 4.0 Term) [n= 4621]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
VASCULAR DISORDERS			
	Hot flashes		
	Hypertension[3]		
	Hypotension[3]		
	Lymphedema		
	Vascular disorders - Other (vasodilation)		

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

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

[3]Associated with infusion-related reactions or administration-related reactions (ARRs).

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

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

Adverse events reported on trastuzumab (Herceptin) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that trastuzumab (Herceptin) caused the adverse event:

Cardiac Disorders - Asystole; Atrial fibrillation; Atrial flutter; Cardiac disorders - Other (edema); Chest pain - cardiac; Myocardial infarction; Myocarditis; Palpitations; Sinus bradycardia; Ventricular arrhythmia; Ventricular tachycardia

Ear and Labyrinth Disorders - Hearing impaired; Vertigo

Eye Disorders - Dry eye; Extraocular muscle paresis

Gastrointestinal Disorders - Ascites; Constipation; Dyspepsia; Enterocolitis; Esophagitis; Gastritis; Gastrointestinal disorders - Other (ischemic bowel); Small intestinal perforation; Typhlitis

General Disorders and Administration Site Conditions - Injection site reaction; Malaise; Multi-organ failure; Sudden death NOS

Injury, Poisoning and Procedural Complications - Fracture

Investigations - Alanine aminotransferase increased; Blood bilirubin increased; Creatinine increased; Platelet count decreased; Weight gain; White blood cell decreased

Metabolism and Nutrition Disorders - Dehydration; Hyperkalemia; Hypoalbuminemia; Hypokalemia; Hypophosphatemia

Musculoskeletal and Connective Tissue Disorders - Arthritis; Chest wall pain; Flank pain; Generalized muscle weakness; Muscle weakness left-sided; Neck pain

Nervous System Disorders - Amnesia; Depressed level of consciousness; Dizziness; Encephalopathy; Leukoencephalopathy; Paresthesia; Seizure; Syncope

Psychiatric Disorders - Anxiety; Confusion

Renal and Urinary Disorders - Acute kidney injury; Proteinuria

Respiratory, Thoracic and Mediastinal Disorders - Epistaxis; Nasal congestion; Pharyngolaryngeal pain; Pleural effusion[4]; Pulmonary hypertension; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (oropharyngeal pain); Wheezing[4]

Skin and Subcutaneous Tissue Disorders - Dry skin; Erythema multiforme; Hyperhidrosis; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Stevens-Johnson syndrome

Vascular Disorders - Hematoma; Thromboembolic event

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

- Additional adverse events from the FDA-approved October 2010 Prescribing Information for Herceptin® (trastuzumab)
  - *General Disorders* – edema limbs, edema trunk
  - *Infections and Infestations* – pharyngitis
  - *Nervous System Disorders* – dizziness
  - *Psychiatric Disorders* – depression, insomnia
  - *Renal and Urinary Disorders* – other (glomerulopathy)

10.3 Expedited reporting of adverse events

NRG Oncology follows procedures for centralized reporting of adverse events. Centralized reporting requires that adverse events be reported to the NRG Oncology SDMC. NRG Oncology forwards reports to the appropriate regulatory agencies and the pharmaceutical company involved in the trial. Expedited reporting for B-43 utilizes the CTEP Adverse Event Reporting System (CTEP-AERS).

The NRG Oncology is identified in CTEP-AERS as the Lead Group for NRG Oncology protocols that require CTEP-AERS reporting. **Expedited AE reporting for this study must be submitted to the NRG Oncology Lead Group** using CTEP-AERS, accessed via the CTEP home page https://eapps-ctep.nci.nih.gov/ctepaers. In the rare event when Internet connectivity is disrupted, a 24-hour notification is to be made to the NCI by telephone at: 301-897-7497. An electronic report must be submitted immediately upon re-establishment of the Internet connection.

10.3.1 *Expedited reporting methods*

- **CTEP-AERS 24-Hour Notification:** requires that a CTEP-AERS 24-hour notification is electronically submitted to the NCI **within 24 hours** of learning of the adverse event. Each CTEP-AERS 24-hour notification must be followed by either a CTEP-AERS 3 Calendar Day Report (see Table 8) or a CTEP-AERS 5 Calendar Day Report (see Table 9).
- **CTEP-AERS 3 Calendar Day Report:** requires that a complete report is electronically submitted to the NRG Oncology Lead Group **within 3 calendar days** of submission of the CTEP-AERS 24-hour notification.

- **CTEP-AERS 5 Calendar Day Report:** requires that a complete CTEP-AERS report is electronically submitted to the NRG Oncology Lead Group **within 5 calendar days** of the investigator learning of the adverse event.
- **Supporting documentation** is required for all expedited (CTEP-AERS) reports. Include the protocol number, patient's study number, and CTEP-AERS ticket number on each page, and **fax supporting documentation to the NRG Oncology SDMC (412-622-2113)**.

10.3.2 ***Expedited reporting requirements – CTEP-AERS 24-hour notification, CTEP-AERS, and other protocol requirements***

- Expedited reporting requirements begin with the first RT treatment or with the administration of the first trastuzumab dose. Expedited reporting requirements for all Group 2 patients who receive trastuzumab are provided in Table 8. Expedited reporting requirements for Group 1 patients are provided on Table 9.
- There may be protocol specific requirements and exceptions for expedited reporting. Refer to Table 8 and Table 9 for instructions.

10.3.3 ***Other recipients of adverse event reports***

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

10.3.4 ***Expedited adverse event reporting requirements for Group 2 patients receiving the investigational agent (trastuzumab) are listed in Table 8.***

TABLE 8. Phase 2 and 3 trials utilizing an agent under a CTEP IND: CTEP-AERS expedited reporting requirements for adverse events that occur within **30 days[1] of the last dose of the investigational agent**

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

1 **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND (trastuzumab) require reporting as follows:**

CTEP-AERS 24-hour notification followed by complete report within 3 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 5 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

2 **Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.**

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 3 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report to the NRG Oncology Lead Group within 3 calendar days of the initial 24-hour report.
  - "5 calendar days" - A complete CTEP-AERS report on the AE must be submitted to the NRG Oncology Lead Group within 5 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disability/incapacity, congenital anomaly, or birth defect must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Please note that Table 8 is continued on the next page.

TABLE 8. Phase 2 and 3 trials utilizing an agent under a CTEP IND: CTEP-AERS expedited reporting requirements for adverse events that occur within 30 days[1] of the last dose of the investigational agent (continued)

Additional Instructions or Exceptions to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent under a CTEP-IND:

a Reports submitted via CTEP-AERS 24-hour notification are available for review by both the NCI and NRG Oncology after submission. All other CTEP-AERS reports are first sent to the NRG Oncology Lead Group and then are forwarded to the NCI. The timelines in the table above have been set so that the information can be forwarded to the NCI in a timely manner per the NCI/CTEP's guidelines.

b On all reports, use the NCI protocol number, CTEP-AERS ticket number, and the protocol-specific ID provided during the trial registration. **Fax supporting documentation to the NRG Oncology SDMC**.

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

d Refer to Section 10.1.2 for instructions regarding assignment of attribution and expectedness for ***investigational combination therapy***.

e CTEP-AERS reporting is required for grade 2 unexpected adverse events and grade 3 unexpected adverse events without hospitalization **only** if the adverse event is possibly, probably or definitely related to the **investigational agent**.

f **Protocol-specific expedited reporting requirements:** For this study, the adverse events listed below require expedited reporting via CTEP-AERS to the NRG Oncology Lead Group within 5 calendar days of learning of the event.

- Cardiac disorders regardless of attribution: ≥ grade 3 acute coronary syndrome; ≥ grade 2 heart failure; ≥ grade 3 left ventricular systolic dysfunction; ≥ grade 3 myocardial infarction ***(NOTE: Requires expedited reporting via CTEP-AERS from the first dose of study therapy until 30 days after the last dose of study therapy.)***
- Secondary malignancies as defined in Section 10.3.6 ***(Note: Secondary malignancies require expedited reporting via CTEP-AERS from the first dose of study therapy until the end of the patient's follow-up):***
  - Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy

g **Protocol-specific expedited reporting exceptions:** For this study, the adverse events listed below, including hospitalizations for these events, do not require expedited reporting via CTEP-AERS:

- Neoplasms-malignant (i.e., a second primary malignancy) determined by the investigator to NOT be most probably related or definitely related to treatment for malignancy. (See footnote f and Section 10.6.)

10.3.5 *Expedited adverse event reporting requirements for Group 1 patients (RT alone) are listed in Table 9.*

TABLE 9. Phase 2 and 3 trials: CTEP-AERS expedited reporting requirements for adverse events that occur **within 30 days of the last dose of RT**

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				CTEP-AERS		CTEP-AERS	CTEP-AERS	
Possible, Probable, Definite		CTEP-AERS report if hospitalized		CTEP-AERS 24-hour and CTEP-AERS		CTEP-AERS 24-hour and CTEP-AERS		

CTEP 24-hour: Indicates a CTEP-AERS 24-hour notification must be electronically submitted to the NCI *within 24 hours* of learning of the event.

CTEP Expedited Report: Indicates a complete expedited report must be electronically submitted to the NRG Oncology 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, CTEP-AERS ticket number, and the protocol-specific patient ID provided during trial registration. ***Fax supporting documentation to the NRG Oncology SDMC.***

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

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

- CTEP-AERS 24-hour notification followed by a complete CTEP-AERS report within 5 calendar days of learning of the event for:
  - grade 4 unexpected events
  - grade 5 unexpected events
- CTEP-AERS 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 CTEP-AERS if the event occurs following treatment.

d Protocol-specific expedited reporting requirements: For this study, the adverse events listed below require expedited reporting via CTEP-AERS to the NSABP Lead Group within 5 calendar days of learning of the event.

- Cardiac disorders regardless of attribution: ≥ grade 3 acute coronary syndrome; ≥ grade 2 heart failure; ≥ grade 3 left ventricular systolic dysfunction; ≥ grade 3 myocardial infarction ***(NOTE: Requires expedited reporting via CTEP-AERS from the first dose of study therapy until 30 days after the last dose of study therapy.)***
- Secondary malignancies as defined in Section 10.3.6 ***(Note: Secondary malignancies require expedited reporting via CTEP-AERS from the first dose of study therapy until the end of the patient's follow-up):***
  - Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy

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 CTEP-AERS:

- Neoplasms-malignant (i.e., a second primary malignancy) determined by the investigator to NOT be most probably related or definitely related to treatment for malignancy. (See footnote d and Section 10.6.)

10.3.6 *Reporting a secondary malignancy*

A ***secondary malignancy*** is a cancer caused by a treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation, or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

All secondary malignancies that occur on NCI-sponsored trials either during or following treatment must be reported via CTEP-AERS within 5 days of learning of the secondary malignancy (see either Table 8 or Table 9). Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Supporting documentation, including pathology and cytogenetics reports which confirm the secondary malignancy, must be faxed to the NRG Oncology SDMC expedited fax at 412-622-2113. Each page of supporting documentation must include the NCI protocol number, the CTEP-AERS ticket number, and the protocol-specific patient ID number provided during trial registration.

Note: All secondary malignancies should also be reported on the B-43 Form F (see Section 10.6).

10.3.7 *Second malignancy*

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting online on the B-43 Form F (see Section 10.6).

10.3.8 *Expedited reporting of pregnancy, fetal death, and death neonatal occurring during study therapy*

Any pregnancy, fetal death, or death neonatal occurring while the patient is receiving study therapy or within 7 months following the last dose of study therapy (trastuzumab) must be reported via CTEP-AERS as a medically significant event. Definitions and reporting instruction for these events are provided in the Cancer Therapy Evaluation Program's (CTEP) revised NCI Guidelines for Investigators: Adverse Event Reporting Requirements (Section 5.5.6) located at the following CTEP website: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf).

Upon learning of a pregnancy, fetal death, or death neonatal that occurs during study or within 7 months following the last dose of study therapy (trastuzumab) the investigator is required to:

- Call the NRG Oncology Clinical Coordinating Department (see Information Resources). ***Patients must immediately discontinue receiving study (trastuzumab) therapy.***

- Within 5 working days of learning of the event, and as required by the NCI Guidelines for Investigators: Adverse Event Reporting Requirements (Section 5.5.6):

  - Create and submit a CTEP-AERS report;
  - Complete the Pregnancy Information Form (located in the NSABP Members' Area in Protocol B-43 "Forms and Supporting Documents"); and
  - Fax the completed Pregnancy Information Form with all available supporting documentation to the NRG Oncology SDMC's expedited fax number at 412-622-2113.

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

- For questions concerning AE reporting, contact the AE Reporting Nurse (see Information Resources).

10.4 Routine reporting of adverse events

10.4.1 *Online reporting on the B-43 Adverse Event Report Form*

- Direct online entry of adverse events is done through the Online Data Entry function located in the Study Management Area of Coordinator Online in the Members' Area of the NSABP Web site.
- **Specified grade 2 AEs and all ≥ grade 3 adverse events not reported via CTEP-AERS** that occurred during RT and trastuzumab (if given) or during the 30 days following completion of RT must be reported on the B-43 Adverse Event Report Form. Only one B-43 Adverse Event Report Form will be completed and submitted.
- Supporting documentation for each adverse event reported online on the B-43 Adverse Event Report Form must be maintained in the patient's research record. When submission of supporting documentation to the NRG Oncology SDMC is required, the online software will provide a transmittal form that must be printed. Fax this transmittal form with the supporting documentation to 412-622-2111. Remove patient names and identifiers such as social security number, address, telephone number, etc. from reports and supporting documentation.

10.4.2 *Submission of the B-43 Adverse Event Report Form*

The B-43 Adverse Event Report Form is submitted online to the NRG Oncology SDMC 30 days after completion of RT.

10.5 Reporting selected adverse events on the B-43 Follow-up Form

The selected late adverse events listed below will be reported online on the B-43 Follow-up Form. Note: Supporting documentation must be submitted for each adverse event listed below and maintained in the patient's research record. When submission of supporting documentation to the NRG Oncology SDMC is required, the online software will provide a transmittal form that must be printed. Fax this transmittal form with the

supporting documentation to 412-622-2111. Remove patient names and identifiers such as social security number, address, telephone number, etc. from reports and supporting documentation.

- ≥ grade 3 acute coronary syndrome
- ≥ grade 2 heart failure
- ≥ grade 3 left ventricular systolic dysfunction
- ≥ grade 3 myocardial infarction

10.6 Reporting cancer recurrence, secondary malignancy, and second primary cancer

Report breast cancer recurrence, secondary primary malignancies (including leukemia secondary to oncology chemotherapy, myelodysplastic syndrome, and treatment-related secondary malignancy previously reported through CTEP-AERS), and second primary cancer (a malignancy which is unrelated to the treatment of a prior malignancy and which is not a metastasis from the initial malignancy) online on the B-43 Follow-up Form. Fax supporting documentation that confirms the breast cancer recurrence or second primary cancer diagnosis with the transmittal form (provided by the online software and printed) to 412-622-2111.

11.0 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.

11.1 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 ipsilateral breast must have a biopsy of the suspicious lesion to confirm the diagnosis. (Note: Invasive includes microinvasion defined as the extension of cancer cells beyond the basement membrane into the adjacent tissues with no focus more than 0.1 cm in greatest dimension. When there are multiple foci of microinvasion, the size of only the largest focus is used to classify the microinvasion. The presence of multiple foci of microinvasion should be noted, as it is with multiple larger invasive carcinoma.[77])

- Acceptable: positive histologic biopsy (positive cytology is not acceptable)

11.1.1 *Ipsilateral invasive breast cancer or ipsilateral skin cancer recurrence or DCIS (IIBCR-SCR-DCIS)*

An IIBCR-SCR-DCIS event is defined as recurrent tumor in the ipsilateral breast parenchyma, skin of the ipsilateral breast, or ipsilateral DCIS occurring after lumpectomy.

11.1.2 *Other local recurrence*

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

11.2 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

11.3 Distant recurrence

Defined as evidence of tumor in any area of the body, with the exception of those described in Sections 11.1 and 11.2.

11.3.1 ***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

11.3.2 ***Bone marrow metastasis***

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

11.3.3 ***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.

11.3.4 ***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.

11.3.5 ***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.

11.3.6 ***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)

11.4 **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. (Note: Invasive includes microinvasion defined as the extension of cancer cells beyond the basement membrane into the adjacent tissues with no focus more than 0.1 cm in greatest dimension. When there are multiple foci of microinvasion, the size of only the largest focus is used to classify the microinvasion. The presence of multiple foci of microinvasion should be noted, as it is with multiple larger invasive carcinoma.[77])

- Acceptable: positive histologic biopsy

11.5 Second primary cancer (non-breast)

The diagnosis of a second primary non-breast invasive cancer must be confirmed histologically whenever possible.

11.6 Documentation requested following death

- Autopsy reports should be secured whenever possible and should be submitted to the NRG Oncology SDMC.
- A copy of the death certificate should be forwarded to the NRG Oncology SDMC 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.

12.0 REGISTRATION, STUDY ENTRY, AND WITHDRAWAL PROCEDURES

Note: Accrual closed on December 8, 2014, following achievement of the sample size goal.

12.1 CTEP investigator registration procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

12.2 CTEP associate registration procedures / CTEP-IAM account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

12.3 CTSU registration procedures

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

12.3.1 *IRB approval*

Each 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 be approved to enroll patients. Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU members' website by entering credentials at https://www.ctsu.org. For sites under the CIRB initiative, IRB data will automatically load to RSS.

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

12.3.2 *Downloading site registration documents*

Site registration forms may be downloaded from the NSABP B-43 protocol page located on the CTSU members' website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the NCTN Groupname link to expand, then select trial protocol NSABP B-43
- Click on the Site Registration Documents link

12.3.3 *Requirements for protocol number site registration*

- CTSU IRB Certification (for sites not participating via the NCI CIRB)
- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)

12.3.4 *Submitting regulatory documents*

Submit completed forms along with a copy of your IRB Approval and Model Informed Consent, if applicable, to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103
Phone: 1-866-651-2878
Fax: 215-569-0206
E-mail: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

12.3.5 *Checking your site's registration status*

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

12.4 Required pre-entry tumor block submission for central HER2 testing

Pre-entry HER2 testing will be performed for all patients in order to determine eligibility for B-43 (see the Pre-Entry Sample Consent Form). ***A representative tumor block must be submitted to Rush University Medical Center*** (see Information Resources). All tumor specimens will be identified by the NSABP Specimen ID for Central Testing (HER2) (a unique coded number provided by the NRG Oncology SDMC). Patients eligible for, and consenting to participate in, the B-43 treatment study, must have the NSABP Specimen ID for Central Testing (HER2) number recorded on Form ENTRY. Patients will not be able to be enrolled in B-43 until the investigator receives confirmation that the DCIS is HER2-positive. For more information regarding central testing for HER2 status, shipment and associated transmittal form, contact information, and receipt of test results, refer to Section 6.2 and the "B-43 Pathology Instructions" located in the Members' Area of the NSABP Web site.

12.5 Patient consent form

Before the patient is enrolled, the consent form for the Treatment Study, including any addenda, must be signed and dated by the patient and the person who explains the study to that patient.

12.6 Patient enrollment

Patient registration can occur only after pre-treatment evaluation is complete, eligibility criteria have been met, and the study site is listed as 'approved' in the CTSU RSS. Patients must have signed and dated all applicable consents and authorization forms.

12.7 Oncology Patient Enrollment Network (OPEN)

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the LPO or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. (Site staff should use the registration forms provided on the NRG Oncology or CTSU Web site as a tool to verify eligibility.)
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).
- DCIS must be determined to be HER2-positive by pre-entry HER2 central testing (See Section 6.1 and Section 6.2 and the Pre-Entry Sample Consent Form.)

  Note: The OPEN system will provide the site with a printable confirmation of registration, including the Patient ID number for the study, and treatment information. Please print this confirmation for your records. Additionally, a transmittal form to be used when faxing the signed consent form to the NRG Oncology SDMC will be provided. If it is necessary to reprint the randomization confirmation or the transmittal form, they can be reprinted through Coordinator Online via the ***View a Patient Entry Report*** under Patient Entry.

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

12.8 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 drug or other treatments for breast cancer becomes available.

If study therapy is stopped, study data and other materials should be submitted according to the study schedule unless the patient withdraws from the study (see Section 12.10).

12.9 Patient-initiated discontinuation of study therapy

Even after a patient agrees to take part in this study, she may stop study therapy 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.

12.10 Patient-initiated withdrawal from the study

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

13.0 REQUIRED FORMS AND MATERIALS

13.1 Data collection

A table of required forms and materials is provided in the "Forms and Supporting Documents" section in the Members' Area of the NSABP Website for Protocol B-43 http://www.nsabp.pitt.edu. (CTSU investigators should also refer to the NSABP B-43 Web page located on the CTSU Member Web site.)

Data will be collected on patient characteristics, menstrual history in patients who were premenopausal at the time of study entry, DCIS characteristics, trastuzumab therapy, radiation therapy, adverse events, ipsilateral breast tumor recurrence, invasive regional and distant breast cancer, contralateral breast cancer, second non-breast primary cancer, disease-free survival, and overall survival.

13.2 Instruction for completion and submission of B-43 forms and materials

- Submit all completed CRFs (with the exception of patient enrollment forms), clinical reports, and other documents directly to the NRG Oncology SDMC. Submission of study data directly to NRG Oncology is done through the Online Data Entry function located in the Study Management Area of Coordinator Online in the Members' Area of the NSABP Web site. Contact the Support Desk at support@nrgoncology.org for an account. When submission of supporting documentation to the NRG Oncology SDMC 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.
- The NRG Oncology SDMC will send query notices and delinquency reports directly to the site for reconciliation. Please send query responses and delinquent data to the NRG Oncology SDMC and do not copy the CTSU Data Operations. If the query is sent with fax transmittal form, return the data to the fax number on the transmittal form, otherwise fax to 412-624-1082.
- B-43 data form worksheets and specimen transmittal forms, as well as instructions for completion and submission of B-43 data and materials, are available in the Members' Area of the NSABP Web site, http://www.nsabp.pitt.edu. (CTSU investigators should refer to the NSABP B-43 Web page located on the CTSU Member site.) Sites must use the current form versions and adhere to the instructions and submission schedule outlined in the protocol.

13.3 Adverse event reporting

Routine and expedited adverse event reporting requirements are addressed in Section 10.0.

13.4 Pathology specimens

See Section 6.0 for required tumor block submission.

13.5 **Data monitoring for CTEP**

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. CTEP has assigned abbreviated CDUS reporting: no AE reporting (routine or expedited) is required via any of the CDUS mechanism. Cumulative CDUS data will be submitted quarterly by the NRG Oncology SDMC to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

14.0 STATISTICAL CONSIDERATIONS

14.1 Endpoints

14.1.1 *Primary endpoint*

The primary endpoint for analysis is time from randomization to an ipsilateral invasive breast cancer, ipsilateral skin cancer recurrence, or DCIS (IIBCR-SCR-DCIS). In the determination of time to an IIBCR-SCR-DCIS, no statistical censoring will be performed with respect to any previous local, regional, or distant recurrences or second primary cancers. For example, if a patient experiences a regional metastasis then her time to regional metastasis will be recorded as her event time for the determination of invasive or DCIS disease-free survival (IDFS-DCIS), and she will continue to be monitored for determining the time to an IIBCR-SCR-DCIS.

14.1.2 *Secondary endpoint*

Secondary endpoints are: a) IDFS-DCIS, b) invasive or DCIS recurrence, c) contralateral invasive or DCIS breast cancer, d) overall survival (OS), and e) ovarian function via the determination of post-treatment amenorrhea.

Events for calculation of IDFS-DCIS are any recurrence, whether invasive or DCIS, second primary cancer, and death from any cause. LCIS, basal cell carcinoma, squamous cell carcinoma, melanoma in situ, carcinoma in situ of the colon, and carcinoma in situ of the cervix will not be included as recurrences or second primaries. For calculation of OS, any death (cancer-related or not) is considered an event.

14.2 Stratification and randomization

Assignment of treatment to patients will be balanced with respect to menopausal status (postmenopausal, not postmenopausal), hormonal therapy (yes, no), and nuclear grade (low or intermediate, high). Patients will be randomized to Group 1 (radiation therapy alone) or Group 2 (radiation therapy plus trastuzumab). Upon verification of inclusion and exclusion criteria, the NRG Oncology SDMC will stratify and randomize the patient to either Group 1 or Group 2 using a biased-coin-minimization approach.[78]

14.3 Sample size estimation

Our design calls for accrual of 2000 patients during a period of roughly 8 years (see Table 10). A definitive analysis of the primary endpoints will be performed when 163 ipsilateral breast cancer events are observed, which is expected to be achieved between 10 and 10.5 years after the start of the protocol. This number of events affords 80% power to detect a hazard reduction of 36%, from 1.73 ipsilateral breast cancer events per 100 patient-years to 1.11 events per 100 patient-years. The 36% observed reduction in the hazard of IIBCR-SCR-DCIS on the trastuzumab arm is based on a projection of 40% hazard reduction if the compliance were perfect, with a 10% noncompliance rate. In those 10% of the patients, regardless of when and why they stopped study medication, we conservatively assume that no effect would be observed, thus attenuating the hazard reduction to 0.9 x 40%=36%. The power calculations also take into account a 1% loss to follow-up in each arm of the study.

TABLE 10. Revision of accrual time and time to definitive analysis (see Section 14.7)

Accrual Scenario	1st Year Average Monthly Accrual	Average Subsequent Monthly Accrual	Total Years of Accrual	Approximate Years From the Start of the Study to Definitive Analysis	Approximate Cutoff Date for Definitive Analysis*
As originally projected in the protocol	30	60	3.33	8.00	12/31/2016
Revised using observed accrual to 12/31/2013	14.167	28	6.03	11.15	12/31/2019
* Time of cutoff date estimated based on quarterly generation of summary files.					

To estimate the rate of IIBCR-SCR-DCIS in the control arm of the proposed study, we used the rates for patients in B-24 with comedo necrosis (as a proxy for HER2+ status). Table 11 displays the sub-group proportions and IIBCR-SCR-DCIS event rates for patients in B-24. We also assumed that 80% of hormone receptor-positive patients will be treated with tamoxifen, and that no hormone receptor-negative patient will be treated with tamoxifen. Furthermore, it was conservatively assumed that 60% of the patients would have comedo necrosis and 40% would not. To obtain the anticipated rate of breast cancer events among hormone receptor-negative patients, we used the rate for placebo-treated B-24 patients because hormone receptor status is only known for a subset of B-24 patients, and there were too few known hormone receptor-negative patients (with comedo necrosis) to allow rate estimation among age groups. Furthermore, the rates of breast cancer events among placebo-treated patients were nearly the same between the known hormone receptor-negative and hormone receptor-positive patients.

TABLE 11. Rates of ipsilateral invasive and skin cancer and DCIS breast cancer recurrences (IIBCR-SCR-DCIS) in selected subsets of NSABP B-24

Sub-group of proposed study	Percentage expected (%)	Sub-group of B-24 used for rate estimation	Rates* of IIBCR-SCR-DCIS (per 100 patient-years)
Pre-menopausal, not receiving tamoxifen	15%	Comedo, age < 50, placebo Non-comedo, age < 50, placebo	4.384 1.967
Pre-menopausal, receiving tamoxifen	23%	Comedo, age < 50, tamoxifen Non-comedo, age < 50, tamoxifen	2.517 1.240
Post-menopausal, not receiving tamoxifen	28%	Comedo, age ≥ 50, placebo Non-comedo, age ≥ 50, placebo	1.698 1.022
Post-menopausal, receiving tamoxifen	34%	Comedo, age ≥ 50, tamoxifen Non-comedo, age ≥ 50, tamoxifen	1.244 0.775
* Rate calculated over the first 5 years of follow-up			

The anticipated overall rate in the proposed study is therefore 1.73 IIBCR-SCR-DCIS events per 100 patient-years.

14.4 Statistical analysis plan

The intention-to-treat principle will be used for primary analyses of the endpoints. Accordingly, the analyses will be performed on all patients with follow-up and will use the treatment assignments made at randomization. As secondary analyses, these analyses will be repeated on patients with follow-up who are eligible for the study.

For all statistical analyses, statistical significance will be determined by a two-sided P-value less than or equal to 0.05. Adjustment will be made to the significance level for the primary definitive analysis to account for the interim analyses, as described below.

Time to IIBCR-SCR-DCIS will be compared across treatment arms using cumulative incidence curves,[79,80] Cox proportional hazard models,[81] and the Kaplan-Meier method.[82] For formal comparison of cumulative incidence curves, the method by Fine and Gray[83] will be employed. In secondary analyses, Cox proportional hazards models[81] will be used to evaluate the effect of treatment on time to any breast cancer event, OS and IDFS-DCIS, controlling for the stratification factors as well as other variables including comedo necrosis (presence or absence), palpable masses (presence or absence), and hormone receptor status (ER+ and/or PgR+ versus neither). If an imbalance occurs in variables (such as the presence or absence of a radiation boost) not included in the stratification scheme, we will adjust for that imbalance analytically in a secondary analyses.[84] The distributions of time to any breast cancer, IDFS-DCIS and OS will be estimated by the Kaplan-Meier method[82] for each treatment group and will be compared between treatments by simple and stratified log-rank tests[84,85] (the latter adjusted for menopausal status and hormonal therapy). Invasive breast cancer, ipsilateral recurrence, and contralateral breast cancer will be compared across treatment arms using cumulative incidence functions.[79,80,83] Furthermore, tests for treatment by covariate interactions will be performed.[86] If the interaction between a covariate and treatment is significant, the treatment effect will be reported within each level of the covariate, with 95% confidence intervals. We will test the proportional hazards assumption by creating an artificial time-dependent covariate and testing its interaction with other covariates, as described in Klein and Moeschberger.[87] Confidence intervals for the risk ratios will be computed by assuming that the events follow a binomial distribution, conditioning on the total number of events and person-years at risk. Amenorrhea will be summarized by treatment arm in terms of incidence rates and the ranges, medians, and quantiles of duration.

14.5 Interim analyses

Toxicity and accrual information will be monitored monthly and will be presented at every semiannual Data Monitoring Committee (DMC). Three formal interim analyses and one definitive analysis are projected and each will test the equality of proportions of patients who are free of ipsilateral invasive breast cancer recurrence, ipsilateral skin cancer recurrence, or DCIS (IIBCR-SCR-DCIS) over time between the treatment arms. The first analysis will occur at a point when roughly one quarter (41) of the total expected number of IIBCR-SCR-DCIS events are observed. Subsequent interim analyses will occur when 82 and 123 IIBCR-SCR-DCIS events are reported with the definitive analysis occurring when 163 IIBCR-SCR-DCIS events are reported. Based on 12/31/2013 data, the timing of the interim analyses will most likely be at years 6.5, 8, and 12.25 with the definitive analysis most likely being performed between 15 and 15.5 years after the start of the study. If we have not observed 163 events 5 years after the last patient is enrolled,

we will still analyze the data at that time (projected to be 12/31/2019), using whatever events (IBTRs) we have observed. The file closure and analysis dates for all interim analyses may be adjusted to coincide with Data Monitoring Committee meetings. If early results indicate that the treatment is efficacious with respect to IIBCR-SCR-DCIS, then Fleming-Harrington-O'Brien α-levels[88] for the four analyses will be used to make formal recommendations to the Data Monitoring Committee. The corresponding two-sided α-levels for the three interim analyses and the definitive analysis are 0.00167, 0.00194, 0.00233 and 0.0484, respectively. Asymmetric stopping boundaries will be employed using a modification of the method proposed by Wieand, et al.[89] The lower interim boundaries are constructed so that if a detrimental effect is rejected at the one-sided 0.025 level in the first interim analysis or if any detrimental effect (significant or not) is observed in any subsequent interim analyses, then the lower boundary would be crossed. If the difference in IIBCR-SCR-DCIS results in the upper or lower boundaries as defined above being crossed, then the DMC will consider this and any other information relevant to the trial to decide if it is appropriate to make a recommendation of early reporting of results.

14.6 Power considerations for correlative studies

The main hypothesis to be tested in the correlative science aspect of this trial is that cMYC is a predictive marker for benefit from adding trastuzumab to radiotherapy. We conservatively estimate that 80% of the patients accrued to this trial will have cMYC information leaving 1600 patients with evaluable information. Using data from NSABP Protocol B-31, we estimate that 70% of the patients will be cMYC negative and 30% will be cMYC positive. Thus, we project that we will have information available for 1120 cMYC negative patients and 480 cMYC positive patients. The hazard ratios comparing breast cancer related events for the trastuzumab arm versus the placebo arm in B-31 were 0.62 and 0.26, for the cMYC negative and cMYC positive populations, respectively. If these hazard ratios are observed in this study, then at the time of the definitive analysis (10 to 10.5 years after the beginning of the study), using a two-sided α=0.05, we would have 71.8% power to detect a 38% reduction in the breast cancer related events in cMYC negative patients and 95.7% power to detect a 74% reduction in the breast cancer related events in cMYC positive patients. For the IIBCR-SCR-DCIS endpoint, the power for detecting the above reductions at the time of the definitive analysis would be 60.1% and 89.5%, for the cMYC negative and cMYC positive populations, respectively. All of the above calculations assume that there is a 1% loss to follow-up rate among all patients in the study. Other markers are regarded as exploratory in nature.

14.7 Accrual rates

The original estimated rate of accrual to this trial was based on the observed accrual to NSABP Protocol B-35, with modifications to account for the expected distribution of patients by menopausal and ER status. We also made modifications to account for the proportion of patients who would be HER2-positive using data from DCIS patients whose tumors were HER2-positive in the study from the Netherlands.[49] This resulted in an estimated accrual rate of 60 patients per month. At this rate, it was originally projected that it would require 3 years and 4 months to complete accrual.

The actual accrual rate experienced in the first years of the trial did not meet our expectations and was substantially less than 60 per month. The reason for this was two-fold. First, the initial accrual rate was based on the estimate from the Netherlands

study that 42% of DCIS cases would be found to be HER2-positive. In actuality, the proportion was 33.8%.[90] Second, our original projections regarding the number of patients meeting the eligibility criteria and likely to participate in the trial was optimistic. Thus, in accordance with National Cancer Institute requirements for trials accruing at a slow rate, with the approval of the Data Monitoring Committee and the National Cancer Institute, the protocol was amended to modify the rate and duration of accrual to the study. This change reflects a more realistic goal based on an internal review of HER2-positive rates and accrual to date. The accrual rate was originally changed to 22 patients per month, but based on 12/31/2013 accrual, was revised to 28 per month and the duration was changed to 6.03 years (see Table 10). The latter calculation was made by taking into consideration the accrual rate from 2013 which was just over 38 patients per month.

14.8 Issues relating to racial and ethnic differences

TABLE 12. Expected racial and ethnic composition of NSABP B-43

Ethnic Category	Total
Hispanic or Latino	86
Not Hispanic or Latino	1914
Ethnic Category: Total of all subjects	2000
Racial Category	
American Indian or Alaskan Native	2
Asian	52
Black or African American	165
Native Hawaiian or other Pacific Islander	20
White	1761
Racial Category: Total of all subjects	2000
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

15.0 PUBLICATION INFORMATION AND ADMINISTRATIVE AGREEMENTS

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

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. *If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.*

2. For a clinical protocol where there is an investigational Agent used in combination with (an) other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):

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

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

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

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

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase III studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. *Press releases and other media presentations must also be forwarded to CTEP prior to release.* Copies of any manuscript, abstract and/or press release/media presentation should be sent to:

   E-mail: ncicteppubs@mail.nih.gov

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

16.0 **REFERENCES**

1. Jemal A, Siegel R, Ward E, et al. Cancer Statistics, 2008. CA Cancer J Clin 2008; 58:71-96.

2. Cancer Facts and Figures, 2008. American Cancer Society, Inc. www.cancer.org.

3. Allred C, Tsimelzon A, Mohsin SK. Genetic differences between ductal carcinoma in situ and invasive breast cancer. Breast Cancer Res Treat 2003; 82:S10, Abstract 13.

4. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999; 17(9):2639-2648.

5. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344(11):783-792.

6. Rodrigues L, Mansi J, and Griffiths J. Radiation enhances the antitumor effect of herceptin on c-neu/HER2 mammary carcinomas in transgenic oncomice. Proc American Assoc Cancer Res 2003; 44:30, Abstract 132.

7. Liang K, Lu Y, Jin W, et al. Sensitization of breast cancer cells to radiation by trastuzumab. Mol Cancer Ther 2003; 2(11):1113-1120.

8. Chang JC, Mohsin S, Weiss H, et al. Induction of apoptosis without change in cell proliferation in primary breast cancers with neoadjuvant trastuzumab. Breast Cancer Res Treat 2003; 82:S13, Abstract 24.

9. Silverstein MJ, Cohlan BF, Gierson ED, et al. Duct carcinoma in situ: 227 cases without microinvasion. Eur J Cancer 1992; 28:630-634.

10. Arnesson L-G, Smeds S, Fagerberg G, Grontoft O. Follow up of two treatment modalities for ductal carcinoma in situ of the breast. Br J Surg 1989; 76:672-675.

11. Carpenter R, Boulter PS, Cooke T, Gibbs NM. Management of screen detected ductal carcinoma in situ of the female breast. Br J Surg 1989; 76:564-567.

12. Fourquet A, Zafrani B, Campana F, et al. Breast-conserving treatment of ductal carcinoma in situ. Semin Radiat Oncol 1992; 2:116-124.

13. Recht A. Intraductal carcinoma of the breast: Results of treatment with excisional biopsy and irradiation. J Clin Oncol 1985; 3:1339-1343.

14. Stotter AT, McNeese M, Oswald MJ, et al. The role of limited surgery with irradiation in primary treatment of ductal in situ breast cancer. Rad Onc Biol Phys 1990; 18:283-287.

15. Zafrani B, Fourquet A, Vilco JR, et al. Conservative Management of intraductal breast carcinoma with tumorectomy and radiation therapy. Cancer 1986; 57:1299-1301.

16. Fisher B, Dignam J, Wickerham DL, et al. Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomized controlled trial. Lancet 1999; 353:1993-2000.

17. Fisher B, Costantino J, Redmond C, et al. Lumpectomy compared with lumpectomy and radiation therapy for the treatment of intraductal breast cancer. New Eng J Med 1993; 328:1581-1586.

18. Fisher B, Dignam J, Wolmark N, et al. Lumpectomy and Radiation Therapy for the Treatment of Intraductal Breast Cancer: Findings from National Surgical Adjuvant Breast and Bowel Project
B-17. J Clin Oncol 1998; 16:441-452.

19. Fisher B, Land S, Mamounas E, et al. Prevention of invasive breast cancer in women with ductal carcinoma in situ: An update of the National Surgical Adjuvant Breast and Bowel Project experience. Semin Oncol 2001; 28(4):400-418.

20. Fisher ER, Costantino J, Fisher B, et al. Pathologic findings from the National Surgical Adjuvant Breast Project (NSABP) Protocol B-17. Cancer 1995; 75:1310-1319.

21. Silverstein MJ, Lagios MD, Groshen S, et al. The influence of margin width on local control of ductal carcinoma in situ of the breast. N Engl J Med 1999; 340(19):1455-1461.

22. Fisher B, Costantino J, Redmond C, et al. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors. New Engl J of Med 1989; 320:479-484.

23. Fisher B, Dignam J, Bryant J, et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. J Natl Cancer Inst 1996; 88(21):1529-1542.

24. Fisher ER, Dignam J, Tan-Chiu E, et al. Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) eight-year update of Protocol B-17. Cancer 1999; 86:429-438.

25. Allred DC, Clark GM, Tandon AD, et al. HER2 in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of in situ carcinoma. J Clin Oncol 1992; 10:566-605.

26. Allred DC, Clark GM, Molina R, et al. Overexpression of HER2 and its relationship with other prognostic factors change during the progression of in situ to invasive breast cancer. Hum Pathol 1992; 23:974-979.

27. Storm FK, Gilchrist KW, Warner TF, and Mahvi DM. Distribution of Hsp-27 and HER-2/neu in in situ and invasive ductal breast carcinomas. Ann Surg Oncol 1995; 2(1):43-48.

28. Siziopikou KP and Khan S. Correlation of HER2 gene amplification with expression of the apoptosis-suppressing genes bcl-2 and bcl-x-L in ductal carcinoma in situ of the breast. Appl Immunohistochem Mol Morphol 2005; 13(1):14-18.

29. Lebeau A, Unholzer A, Amann G, et al. EGFR, HER-2/neu, cyclin D1, p21 and p53 in correlation to cell proliferation and steroid hormone receptor status in ductal carcinoma in situ of the breast. Breast Cancer Res Treat 2003; 79(2):187-198.

30. Cornfield DB, Palazzo JP, Schwartz GF, et al. The prognostic significance of multiple morphologic features and biologic markers in ductal carcinoma in situ of the breast: a study of a large cohort of patients treated with surgery alone. Cancer 2004; 100(11):2317-2327.

31. Barnes NL, Khavari S, Boland GP, et al. Absence of HER4 expression predicts recurrence of doctal carcinoma in situ of the breast. Clin Cancer Res 2005; 11(6): 2163-2168.

32. Collins LC and Schnitt SJ. HER2 protein overexpression in estrogen receptor-positive ductal carcinoma in situ of the breast: frequency and implications for tamoxifen therapy. Mod Pathol 2005; 18(5):615-620.

33. Provenzano E, Hopper JL, Giles GG, et al. Biological markers that predict recurrence in ductal carcinoma in situ of the breast. Eur J Cancer 2003; 39(5):622-630.

34. Rodrigues NA, Dillon D, Carter D, et al. Differences in the pathologic and molecular features of intraductal breast carcinoma between younger and older women. Cancer 2003; 97(6):1393-1403.

35. Claus EB, Chu P, Howe CL, et al. Pathobiologic findings in DCIS of the breast: morphologic features, angiogenesis, HER-2/neu and hormone receptors. Exp Mol Pathol 2001; 70(3):303-316.

36. van de Vijver MJ, Peterse JL, Mooi WJ, et al. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med 1988; 319(19):1239-1245.

37. Perin T, Canzonieri V, Massarut S, et al. Immunohistochemical evaluation of multiple biological markers in ductal carcinoma of the breast. Eur J Cancer 1996; 32A(7):1148-1155.

38. Ringberg A, Anagnostaki L, Anderson H, et al. Cell biological factors in ductal carcinoma in situ (DCIS) of the breast-relationship to ipsilateral local recurrence and histopathological characteristics. Eur J Cancer 2001; 37(12):1514-1522.

39. Bijker N, Peterse JL, Duchateau L, et al. Histological type and marker expression of the primary tumour compared with its local recurrence after breast-conserving therapy for ductal carcinoma in situ. Br J Cancer 2001; 84(4):539-544.

40. Ramachandra S, Machin L, Ashley S, et al. Immunohistochemical distribution of c-erbB-2 in in situ breast carcinoma--a detailed morphological analysis. J Pathol 1990; 161(1):7-14.

41. Schimmelpenning H, Eriksson ET, Pallis L, et al. Immunohistochemical c-erbB-2 protooncogene expression and nuclear DNA content in human mammary carcinoma in situ. Am J Clin Pathol 1992; 97(5 Suppl 1):S48-52.

42. Bobrow LG, Happerfield LC, Gregory WM, et al. Ductal carcinoma in situ: assessment of necrosis and nuclear morphology and their association with biological markers. J Pathol 1995; 176(4):333-341.

43. Leal CB, Schmitt FC, Bento MJ, et al. Ductal carcinoma in situ of the breast. Histologic categorization and its relationship to ploidy and immunohistochemical expression of hormone receptors, p53, and c-erbB-2 protein. Cancer 1995; 75(8):2123-2131.

44. Somerville JE, Clarke LA, Biggart JD. c-erbB-2 overexpression and histological type of in situ and invasive breast carcinoma. J Clin Pathol 1992; 45(1):16-20.

45. Poller DN, Hutchings CE, Galea M, et al. p53 protein expression in human breast carcinoma: relationship to expression of epidermal growth factor receptor, c-erbB-2 protein overexpression, and oestrogen receptor. Br J Cancer 1992; 66:583-588.

46. Kim C, Pogue-Geile K, Jeong J-H, et al. Adjuvant trastuzumab in the treatment of HER2-negative breast cancer. Manuscript in preparation.

47. Allred DC, 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. Breast Cancer Res Treat 2002; 76:S36, Abstr 30.

48. Claus EB, Chu P, Howe CL, et al. Pathobiologic findings in DCIS of the breast: morphologic features, angiogenesis, HER-2/neu and hormone receptors. Exp Mol Pathol 2001; 70(3):303-316.

49. De Laurentiis M, Carlomagno C, Perrone F, et al. Adjuvant tamoxifen efficacy is influenced by ErbB2 expression: a study in node negative breast cancer patients (Meeting abstract). Breast Cancer Res Treat 1996; 37(Suppl).

50. Van de Vijver MJ, Peterse JL, Moor WJ, et al. Association with Comedo-type Ductal Carcinoma in Situ and Limited Prognostic Value in Stage II Breast Cancer. N Eng J Med 1988; 319:1239-1245.

51. Leal CB, Schmitt FC, Bento MJ. Ductal carcinoma in situ: Histologic categorization and its relationship to ploidy and immunohistochemical expression of hormone receptors, p53, c-erbB-2 protein. Cancer 1995; 75:2123-2131.

52. Mack L, Kerkvliet N, Doig G, et al. Relationship of a new histological categorization of ductal carcinoma in situ of the breast with size and the immunohistochemical expression of p53, c-erb b2, bcl-2 and ki-67. Hum Pathol 1997; 28:974-979.

53. Bobrow LG, Happerfield LC, Gregory WM. The classification of ductal carcinoma in situ and its association with biological markers. Semin Diagn Pathol 1994; 11:199-207.

54. Zafrani B, Leroyer A, Fourquet A. Mammographically detected ductal carcinoma in situ of the breast analyzed with a new classification: A study of 127 cases: Correlation with estrogen and progesterone receptors, p53 and c-erbB-2 proteins and proliferative activity. Semin Diagn Pathol 1994; 11:208-214.

55. Vogel C, Cobleigh MA, Tripathy D, et al. Genentech H0650 study investigators. First-line, single-agent Herceptin(trastuzumab) in metastatic breast cancer: a preliminary report. Eur J Cancer 2001; 37 Suppl 1:S25-9.

56. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353(16):1673-84.

57. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353(16):1659-1672.

58. Slamon D, Eiermann W, Robert N, et al. Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (ACT) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (ACTH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study. Breast Cancer Res and Treatment 2005; 94:Supplement; abstract 1, p S5.

59. Joensuu H, Kellokumpu-Lehtinen P-L, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 2006; 354(8):789-790.

60. Vogel C L, Cobleigh MA, Tripathy D, et al. Efficacy and Safety of Trastuzumab as a Single Agent in First-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer. J Clin Oncol 2002; (20):719-726.

61. Tan-Chiu E, Yothers G, Romond E, et al. Assessment of Cardiac Dysfunction in a Randomized Trial Comparing Doxorubicin and Cyclophosphamide Followed by Paclitaxel, With or Without Trastuzumab As Adjuvant Therapy in Node-Positive, Human Epidermal Growth Factor Receptor 2–Overexpressing Breast Cancer: NSABP B-31. J Clin Oncol 2005; 23(31):7811-7819.

62. Geyer CE, Bryant JL, Romond, EH, et al. Update of cardiac dysfunction on NSABP B-31, a randomized trial of sequential doxorubicin/cyclophosphamide (AC)→paclitaxel (T) vs. AC→T with trastuzumab (H). American Society of Clinical Oncology Annual Meeting 2006. Abstr. 581.

63. Halyard MY, Pisansky TM, Solin LJ, et al. Adjuvant radiotherapy (RT) and trastuzumab in stage I-IIA breast cancer: Toxicity data from North Central Cancer Treatment Group Phase III trial N9831. J Clin Oncol, 2006; 24(18S) (June 20 Supplement), 523.

64. Personal communication, Vicente Velaro 6/10/05.

65. Joensuu H, Kellokumpu-Lehtinen P, Bono P, et al. Adjuvant Docetaxel or Vinorelbine with or without Trastuzumab for Breast Cancer. N Engl J Med 2006; 354:809-820.

66. Evan GI, Littlewood TD. The role of c-myc in cell growth. Curr Opin Genet Dev 1993; 3(1): 44-49.

67. Evan GI, Wyllie AH, Gilbert CS, et al. Induction of apoptosis in fibroblasts by c-myc protein. Cell 1992; 69(1):119-128.

68. Fanidi A, Harrington EA, Evan GI. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature 1992; 359(6395):554-556.

69. Pelengaris S, Khan M, Evan GI. Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. Cell 2002; 109(3):321-334.

70. Harrington EA, Fanidi A, Evan GI. Oncogenes and cell death. Curr Opin Genet Dev. 1994; 4(1):120-129.

71. Mohsin SK, Weiss HL, Gutierrez MC, et al. Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. J Clin Oncol. 2005; Apr 10;23(11):2460-2468. Epub 2005 Feb 14.

72. Yakes FM, Chinratanalab W, Ritter CA, et al. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res. 2002 Jul 15;62(14):4132-41.

73. Le XF, Lammayot A, Gold D, et al. Genes affecting the cell cycle, growth, maintenance, and drug sensitivity are preferentially regulated by anti-HER2 antibody through phosphatidylinositol 3-kinase-AKT signaling. J Biol Chem 2005; 280(3):2092-2104

74. Asanuma H, Torigoe T, Kamiguchi K, et al. Survivin expression is regulated by coexpression of human epidermal growth factor receptor 2 and epidermal growth factor receptor via phosphatidylinositol 3-kinase/AKT signaling pathway in breast cancer cells. Cancer Res 2005; 65(23):11018-11025.

75. Campbell IG, Russell SE, Choong DY, et al. Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 2004; 64(21):7678-7681.

76. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 2005; 24(8):1477-1480.

77. Green FL, Page DL, Fleming ID, et al., Ed. AJCC Cancer Staging Handbook from the American Joint Committee on Cancer Staging Manual. Springer-Verlag, New York, 2002, pp. 225.

78. White SJ, Freedman LS. Allocation of patients to treatment groups in a controlled clinical study. Br J Cancer 1978; 37:849-857.

79. Gaynor JJ, Feuer EJ, Tan CC, et al. On the use of cause-specific failure and conditioned failure probabilities: examples from clinical oncology data. J Am Stat Assoc 1993; 88:400-409.

80. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann of Statistics 1988; 16:1141-1154.

81. Cox DR. Regression models and life tables (with discussion). J Royal Stat Soc 1972; B 3: 248-275.

82. Kaplan EL, Meier, P. Nonparametric estimation from incomplete observations. J Amer Stat Assoc 1958; 53:457-481.

83. Fine JP and Gray RJ. A proportional hazards model for the subdistribution of a competing risk. JASA 1999; 94:496-509.

84. Kalbfleisch JD and Prentice RL. The Statistical Analysis of Failure Time Data, John Wiley & Sons, New York, 1980.

85. Robins J, Breslow N, and Greenland S. Estimators of the Mantel-Haenszel variance consistent in both sparse data and large-sample limiting models. Biometrics 1986; 42:311-323.

86. Gail M, Simon R. Testing for qualitative interactions between treatment effects and patient subsets. Biometrics 1985; 41:361-372.

87. Klein JP, Moeschberger ML. Survival Analysis: Techniques for Censored and Truncated Data, Spring-Verlag, New York, 1997; pp. 276-278.

88. Fleming TR, Harrington DP, and O'Brien PC. Designs for group sequential tests. Control Clin Trials 1984; 5:348-361.

89. Wieand S, Schroeder G, and O'Fallon JR. Stopping when the experimental regimen does not appear to help. Statistics in Medicine 1994; 13:1453-1458.

90. Siziopikou K, Cobleigh M, Anderson SJ, et al. Preliminary results of centralized HER2 testing in DCIS of the breast: NSABP B-43. J Clin Oncol 2011; 29: Abstr 588.

APPENDIX A

DETERMINATION OF PRE-ENTRY MENOPAUSAL STATUS

The following criteria will be used to define ***postmenopausal***:

- A prior documented bilateral oophorectomy, **or**
- A history of at least 12 months without spontaneous menstrual bleeding, **or**
- Age 55 or older with a prior hysterectomy, **or**
- Age 54 or younger with a prior hysterectomy without oophorectomy (or in whom the status of the ovaries is unknown), with a documented FSH level demonstrating confirmatory elevation in the lab's postmenopausal range.

Women failing to meet one of these criteria will be classified as ***not postmenopausal at study entry***.