A Phase II Randomized Trial Evaluating the Effect of Trastuzumab on Disease Free Survival in Early Stage HER2-Negative Breast Cancer Patients with ERBB2 Expressing Bone Marrow Disseminated Tumor Cells

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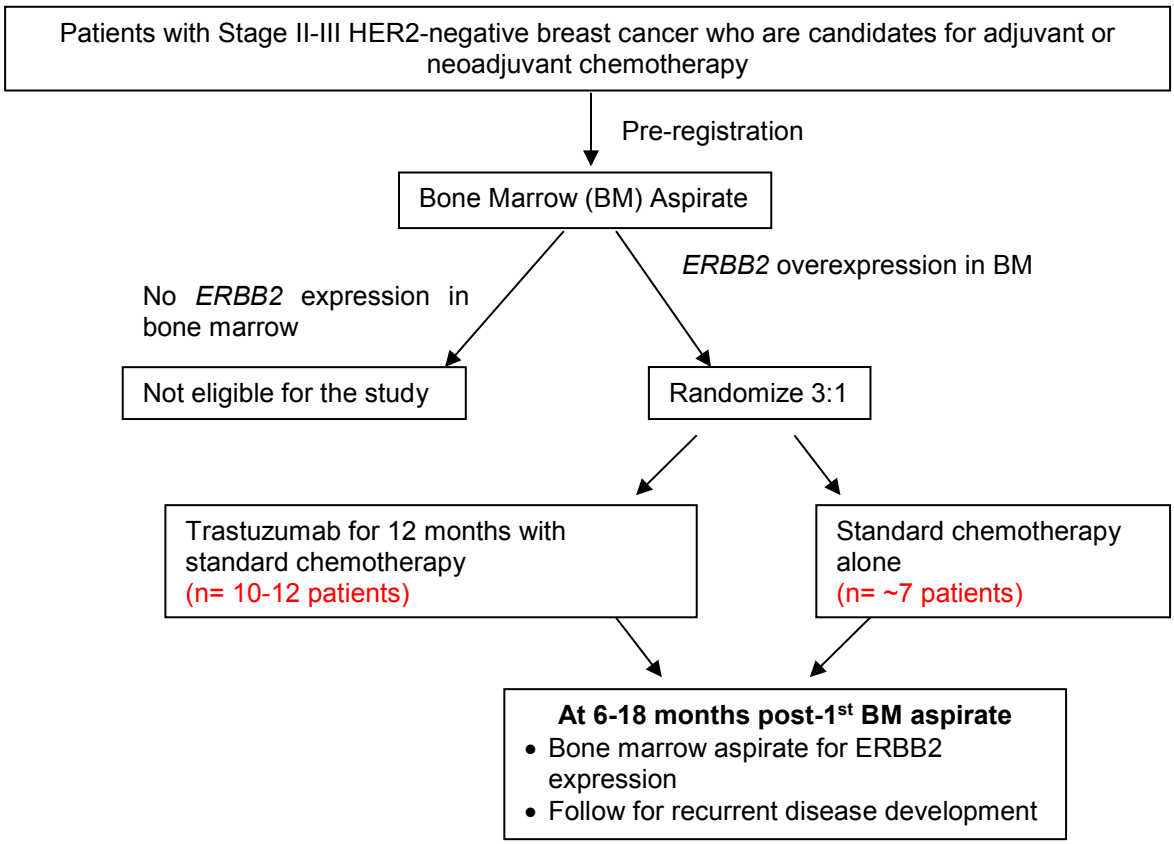

List of Amendments

Initial Approval	**09/20/13**
Amendment #1	**11/18/13**
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Amendment #8	**11/01/16**

Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BM	Bone marrow
BWFI	Bacteriostatic water for injection
CAP	College of American Pathologists
CBC	Complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
CK	Cytokeratins
CK-ICC	CK-immunocytochemical
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CSC	Cancer stem cell
CST	Central standard time
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
DOB	Date of birth
DSM	Data and Safety Monitoring
DTC	Disseminated tumor cell
ECG (or EKG)	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
EMT	Epithelial mesenchymal transition
ER	Estrogen receptor
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FISH	fluorescent in situ hybridization
FWA	Federal wide assurance
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
HHS	Department of Health and Human Services'

HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IHC	Immunohistochemical
IRB	Institutional Review Board
IV	Intravenous (i.v.)
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
MUGA	Multiple gated acquisition scan
NC	Nanostring nCounter
NCI	National Cancer Institute
NIH	National Institutes of Health
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PCR	Polymerase chain reaction
PD	Progressive disease
PI	Principal investigator
PR	Partial response
PR	Progesterone receptor
QASMC	Quality Assurance and Safety Monitoring Committee
QOL	Quality of life
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RNA	Ribonucleic acid
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease
SWFI	Sterile water for injection
TN	Triple negative
TTP	Time to progression
UPN	Unique patient number
WBC	White blood cell (count)
WHO	World Health Organization
ZA	Zoledronic acid

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1.0 BACKGROUND AND RATIONALE

1.1 Overview of Clinical Trial

Metastasis is the most significant contributor to mortality in breast cancer patients. Decades of pre-clinical research has revealed a complex cascade of key events involving cell motility, intravasation, transit in the blood or lymphatics, arrest at a secondary site, extravasation, colonization and growth at a new site. More recent data suggests that only a small, unique subset of cells within a primary tumor possess metastatic potential[1]. Thus, therapies that simply reduce primary tumor mass often fail to cure patients. Furthermore, molecular profiles of cells released from primary, heterogeneous tumors may evolve as the cells transition and progress to metastatic foci[2]. To develop new therapeutic interventions to monitor and prevent overt distant disease development, it is essential to identify and target the intermediary cells in the metastatic process since these cells likely have biological behavior and therapeutic vulnerabilities which differ from the primary tumor.

In breast cancer patients, bone marrow (**BM**) is thought to serve as a reservoir for disseminated tumor cells (**DTCs**) which are the hypothesized intermediaries in the metastatic process. DTCs are phenotypically heterogeneous and molecularly distinct from the primary tumor[2]. Currently, DTCs are identified by immunocytochemical (**CK**-**ICC**) detection of cytokeratins (**CK**) or molecular techniques that detect expression of single genes associated with DTCs[3-4]. Using CK-ICC, DTCs have been detected in the BM of up to 40% of stage I-III breast cancer patients[5]. Several large multi-institutional clinical studies have documented the independent prognostic significance of DTCs[6]. However, not all DTCs have equal metastatic potential[7]. Using an optimized gene detection platform (Nanostring nCounter) and a 38-gene expression profile, data from a pilot study provided evidence that patients with molecular subsets of DTCs overexpressing *ERBB2* are at very high risk of developing recurrent disease. Seventy-five percent of patients with Her2-negative primary tumors who harbored ERBB2-positive DTCs in their BM developed distant disease within 48 months of diagnosis. Discordance between the primary tumor and DTCs/ circulating tumor cells (CTCs)/metastatic foci have been reported by other investigators [8-10] [11]. *ERBB2* amplification seems to become more frequent in systemic progression [9-10, 12]. Targeting and eliminating *ERBB2* overexpressing DTCs may result in an improved disease-free survival (DFS) and provide a selective therapeutic intervention for these high risk patients who are not candidates for *ERBB2*-targeted therapy based on their primary tumor biomarkers. This could also provide proof of principle that a strategy of targeting DTCs based on their biomarker profile may lead to an interruption of the metastatic process by eliminating the intermediary cells of metastases formation. In addition, characterization of DTC-specific expression profiles could lead to improved prediction of the metastatic potential of DTCs and reveal their vulnerabilities to targeted therapeutics based on the expression / activation of specific signaling pathways involving key regulatory genes such as *ERBB2*[13-14].

1.2 Her2 Status of the Primary Tumor and Benefit from Trastuzumab Therapy

The Her2 protein and/or *ERBB2* gene are over expressed or amplified in approximately 25% of breast cancers[15-16]. ASCO/CAP defines Her2 positivity as immunohistochemistry (**IHC**) score of 3+ or gene amplification measure by fluorescent in situ hybridization (**FISH**)[17]. Her2 positivity of the primary tumor is associated with significantly decreased recurrence-free survival and overall survival[18-20]. Trastuzumab, a monoclonal antibody targeting Her2, is approved by for the treatment of Her2 positive cancer in the adjuvant and metastatic setting[21]. Results from multiple clinical trials demonstrate that the administration of adjuvant trastuzumab for one year concurrently with chemotherapy significantly improves DFS compared with chemotherapy alone in women with Her2 positive breast cancers leading to an approximately 50% reduction in disease events[22-23] with only minor side effects[24-26]. Treatment of women with Her2 positive early stage breast cancer with chemotherapy/trastuzumab has become the standard of care. However, it has been reported that patients whose tumors do not meet the criteria for Her2-positivity and in fact are Her2-negative by FISH and IHC may still benefit from trastuzumab treatment[27]. Paik et al examined tissue blocks from NSABP B-31, which compared standard chemotherapy of doxorubicin/cyclophosphamide followed by paclitaxel (ACT) with ACT plus trastuzumab (ACTH) in the adjuvant setting. They found that some patients with normal gene copy numbers appeared to benefit from trastuzumab treatment (relative risk for DFS, 0.40; 95% confidence interval [CI], 0.18 to 0.89; P=0.026). Possible explanations for this observation are that tumor cells exist which are dependent on the Her2 pathway for growth and survival which are not identified with conventional Her2 testing or Her2 expression is acquired as tumor cells progress along the metastatic pathway. These results have formed the basis for the NSABP B-47 trial which compares adjuvant chemotherapy with or without adjuvant trastuzumab in 3260 early stage breast cancer with Her2 expression that does not meet ASCO/CAP definition of Her2 positivity.

1.3 Molecular Classification of Primary Tumors – PAM50

Gene expression profiling has defined "intrinsic" subtypes of breast cancer which have been shown to be superior in predicting long term outcomes of breast cancer[28-31] and the likelihood of responding to therapy[31] than IHC staining for ER, PR, Her-2. The PAM50 assay is a 50-gene, second-generation breast cancer molecular profiling test based on the intrinsic gene signatures which can assign the breast cancer intrinsic subtypes to individual patient tumors. Molecular profiling of primary tumors with PAM50 will be used for the exploratory objectives in this study. Recently, Cheang et al compared response of Her2-positive tumors identified by conventional IHC/FISH versus applying the PAM50 to identify the Her2-enriched subtype[31-32]. They found that those tumors which were Her2-positive by both IHC/FISH and by PAM50 were 6-34x more likely to achieve a pCR with chemo/trastuzumab therapy than tumors which were Her2-positive by IHC/FISH alone. Within the PAM 50, there are four genes which are over-represented in the Her2-enriched subtype. 3 of the 4 genes (ERBB2, GRB7, FGFR4) are included in the 38-gene profile that we have generated for classification of DTCs[33] (see below).

1.4 Testing for Her2 Positivity of DTCs by PCR

As discussed above, presently Her2 status is defined by positivity by either IHC or FISH assays. Multiple studies have compared IHC/FISH with quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) using both fresh and paraffin fixed tissue. These have demonstrated overall concordance between the two tests of 82-100% and Her2 status by qRT-PCR was found to correlate with DFS[34-43]. In one large prospective study of 466 patients reported by Lehman-Che[43], concordance between qRT-PCR and IHC was 97%. Lack of concordance was generally due to an underestimation of Her2 positivity by qRT-PCR likely due to degradation of RNA and rare cases of intratumoral heterogeneity[43]. Thus though IHC/FISH remains the standard for assessing Her2 positivity in tumors[17]; studies indicate that Her2 testing by qRT-PCR is accurate, though it may underestimate the number of positive patients.

1.5 HER2 Negative Breast Cancers: Luminal B and Basal-Like

Gene expression profiling has defined "intrinsic" subtypes of breast cancer which have been shown to be superior in predicting long term outcomes of breast cancer[28-31] and the likelihood of responding to therapy[31] than IHC staining for ER, PR, Her-2. Based on this methodology, breast cancers have been divided into the basal-like, Her2 –positive, luminal A and Luminal B subtypes. The luminal A subtype most closely corresponds to ER+/PR+ breast cancers which are very responsive to hormonal treatment. Approximately 20% of invasive breast cancers are basal like and most closely corresponding to tumor with are negative for the expression of estrogen receptor (ER) and HER2 gene amplification (triple negative, TN) by IHC[29-30, 44]. An increased prevalence of ER-/HER2- tumors has been observed in premenopausal African American women, BCRA1 mutation carriers, and Hispanics. Luminal B tumors generally correspond to tumors which are ER+ and PR-/Her2- by IHC. Luminal B and TN are generally high grade and the lack of established targeted agents coincides with their particularly high risk of early relapse when compared to other breast cancer subtypes. Adjuvant and/or neoadjuvant chemotherapy has been shown to improve the outcome for a small subset of patients with chemotherapy-sensitive disease but is ineffective in preventing relapse in those that have residual cancer despite neoadjuvant chemotherapy. For example, a recurrence rate of 40-50% in the first 5 years is observed in patients with triple negative breast cancer who did not achieve a complete pathological response to neoadjuvant chemotherapy[45-47].

1.6 Disseminated Tumor Cells (Bone Marrow Micrometastasis or Minimal Residual Disease)

1.6.1 Clinical Significance of DTCs

Disseminated tumor cells (DTCs) detected in the bone marrow of breast cancer patients have been shown to be associated with reduced disease-free survival and overall survival in breast cancer patients at both primary diagnosis and recurrence-free follow-up after local primary local and systemic treatment [48]. DTCs, also described as bone marrow micrometastasis or minimal residual disease, can be detected in 12–45% of patients with primary operable breast cancer as determined by immunocytochemistry [6, 49-54]. The presence of DTCs has clearly been

shown to be an independent prognostic factor for patients with stage I-III breast cancer in multiple studies[6, 55]. DTCs can thus be used to select patients at increased risk for relapse who are likely to benefit from additional treatment intervention. The table below summarizes the major studies using immunocytochemical detection of DTCs in BM.

Major clinical studies of the prognostic value of DTC detection in BM by immunocytochemistry and prognostic value on disease and overall survival (univariate and multivariate analysis)

Reference	Sampling	Marker	No. patients	Detection rate (%)	Follow-up (month)	Disease free survival		Overall survival	
						Univ	Multiv	Univ	Multiv
Redding 1983[56]	Smear	MUC	110	28		NA	NA	NA	NA
Manegold 1988[57]	Biopsy Smear	CK/PKK1	50	8		NA	NA	NA	NA
Landys 1998[58]	Biopsy	CK/AE1–AE3, KL1, CAM 5-2	128	19	240	NA	NA	Yes	NA
Salvadori 1990[59]	Biopsy	CK/MBr1	121	16.5	48	No	No	NA	NA
Mathieu 1990[60]	Biopsy	MUC/EMA, HMFG2	93	1		No	No	No	No
		CK/KL1, AE1–AE3, CAM5-2							
Kirk 1990[61]	Smear	MUC/anti-milk fat globulin LICR.LON.M8.4	25	48	34	No	NA	NA	NA
Singletary 1991[62]	Smear	CK/AE1, AE3, MAK-6	71	38	11	No	No	No	No
		MUC/113F1, 260F9, 317G5							
Cote 1991[63]	Smear	MUC/C26, T16	49	36.7	30	Yes	Yes	NA	Na
		CK/AE-1							
Schlimok 1992[64]	Cytospin	CK18/CK2	187	18	39	Yes	Yes	NA	NA
Harbeck 1994[65]	Smear	CK	100	38	34	Yes	Yes	No	Yes
		MUC/EMA							
Ménard 1994[66]	Cytospin	CK/MBr1, MBr8, CK18/CK2, MUC1	197	31	NA	NA	NA	NA	NA
Molino 1997[67]	Cytospin	CK/MBr1, MBr8, MOV8, MOV16 MluC1	109	31	36	No	No	No	No
Funke 1996[68]	Cytospin	CK18/CK2	234	38	NA	NA	NA	NA	NA
Diel 1996[69-70]	Smear	MUC/TAG12 (2E11)	727	43.3	78	Yes	Yes	Yes	Yes
Mansi 1999[50, 71]	Smear	EMA	350	25.4	150	Yes	No	Yes	No
Lyda 2000[72]	Biopsy	CK/AE1–AE3, 35βH11 CAM 5-2	54	31	38	Yes	NA	NA	NA
Untch 1999[73]	Cytospin	CK18/CK2	581	28		No	No	No	No
Braun 2000[5]	Cytospin	CK/CK8,18,19 (A45 B/B3)	552	36	36	Yes	Yes	Yes	Yes
Gerber 2001[52]	Cytospin	CK/CK8,18,19 (5D3)	554	37	54	Yes	Yes	Yes	Yes
Gebauer2001[53]	Smear	CK, MUC/EMA	396	42	75	Yes	Yes	Yes	Yes
Kasimir-Bauer 2001[74]	Cytospin	CK/CK8,18,19 (A45 B/B3)	128	34	24	NA	NA	NA	NA
Naume 2004[49]	Cytospin	CK/AE1/AE3	819	13	49	Yes	Yes	Yes	Yes
Braun 2005[6]	Various	Various	4703	30.6	63	Yes	Yes	Yes	Yes
Bidard 2007[75]	Cytospin	CK/CK8,18,19 (A45 B/B3)	621	15	50	Yes	Yes	Yes	Yes

CK, cytokeratin; Muc, mucin; EMA, epithelial membrane antigen; NA, not available
Table adapted from [55].

In a pooled analysis of 9 studies comprising 4703 patients with stage I, II, or III breast cancer[6], the presence of micrometastases at diagnosis was detected in 30.6% of patients and was found to be a significant and independent prognostic factor with respect to poor overall survival (OS) and breast cancer-specific survival (univariate mortality ratios: 2.15 and 2.44, respectively; $p<0.001$ for both outcomes) and poor disease-free survival (DFS) and distant DFS during the 10-year observation period (incidence rate ratios: 2.13 and 2.33, respectively; $p<0.001$ for both outcomes).

In an institutional study (HRPO# 02-0778), bone marrow samples were collected from women with locally-advanced breast cancer undergoing neoadjuvant chemotherapy with or without zoledronic acid (ZA) to study the impact of neoadjuvant therapy on occult micrometastases and bone density. Bone marrow data was available for 119 patients prior to therapy and 112 patients at the time of surgery (at 3 months). Prior to any therapy, 46% of the ZA-treated and 41% of the no-ZA treatment group had detectable DTCs in their bone marrow ($p=0.651$). At surgery, 23% of the ZA-treated and 36% of the no-ZA treatment arm had detectable DTCs ($p=0.054$). In patients who did not achieve a pCR, about 50% were found to have bone marrow micrometastases. Patients with residual bone marrow DTCs after neoadjuvant chemotherapy were associated with a poorer prognosis than those patients without DTCs (unpublished data from Dr. Aft).

Although the application of using DTCs is still investigational according to the American Society of Clinical Oncology (ASCO) 2007 update of recommendations for the use of tumor markers in breast cancer, its incorporation into clinical management algorithms is currently the focus of much research.

1.6.2 DTCs as Potential Metastatic Progenitors and a Marker of Body-wide Dissemination of Invasive Cancer Cells

Micrometastases have been shown to retain clonogenic and tumorigenic capacities in many biological reports[76-78]. Clinical studies have indicated a link between BM DTCs and the onset of bone metastases[6, 75, 79], strongly supporting the idea of local growth of DTCs into macrometastases. It has also been speculated that the bone marrow may act as a long-term reservoir for tumor cells, which can re-circulate to other distant organs, leading to recurrence[80]. The high genetic heterogeneity[81] of BM micrometastatic cells might be responsible for recirculation of some cancer seeds from the bone marrow to different host organs. However, there is currently no direct evidence suggesting that bone marrow DTCs are responsible for the lung or liver metastases. On the contrary, many biological models have reported that most of the target organs harbor micrometastatic dissemination of mammary tumors[82-84]. Current literature does not provide strong evidence for a common pool of genes responsible for coupled homing to bone marrow (or flat bones) and liver. Paget was the first to describe the non-random growth of metastases[85], and the sustaining molecular determinants of cancer cell homing to different organs have been recently characterized[86-88]. Therefore, in the case of distant non-bone or local relapses predicted by BM DTCs[75], these cells mostly appear as a

marker of a body-wide dissemination of invasive cancer cells rather than the body's only long-term reservoir of disseminated cancer cells.

1.6.3 DTCs and CSCs

The role of cancer stem cells (CSCs) in the establishment of metastases remains controversial[89-92]. Experimentally, CD44+/CD24- cancer cells, a phenotype associated with a stem cell pattern, exhibit an invasive phenotype which is a prerequisite to metastasis[93-94]. In a report on 50 cases, most BM DTCs exhibited a stem cell-like immunohistochemistry (IHC) phenotype[92].

1.6.4 DTCs and Systemic Therapy

There are few trials on systemic treatments in DTC positive patients. Cytostatic treatment has no significant effect[95-96] possibly due to the low expression of proliferation markers suggesting that these cells are dormant[97-98]. Cell cycle independent agents directed against specific DTC characteristics might therefore be more promising. Bisphosphonates clear DTCs from bone marrow[95]. This finding parallels the recently observed prolongation of survival in early breast cancer patients[99-101]. Three non-randomized trials targeting treatment to DTCs showed significant DTC elimination in patients receiving edrecolomab directed against EpCAM[102-104].

1.6.5 Potential of DTCs in Monitoring Therapy Efficacy in the Adjuvant Setting

An important potential application for DTC detection is the monitoring of therapeutic efficacy in the adjuvant setting. The effectiveness of adjuvant therapy regimens can currently only be assessed retrospectively in large-scale clinical trials after an observation period of at least 5 years. Bone marrow biopsies performed before and following therapy for the presence of DTCs make real-time assessments of therapeutic efficacy possible. Persistence of DTCs in bone marrow years after diagnosis and initial therapy has been shown to be an indicator of subsequent systemic treatment failure[79, 105-106]. Persistence or disappearance of DTCs after systemic treatment could therefore be used as a surrogate marker of treatment response[107]. Beyond prognostic relevance, phenotyping of DTC can reveal targets for individualized treatment approaches

1.6.6 Rationale for Determining Expression of ERBB2 by DTCs in Patients with Her2-negative Breast Cancers

Discordance in Her2 expression between the primary tumor and disseminated cells: Several studies have shown an antigen shift from the primary tumor to distant metastases [108-110]. Discordance in Her2 expression between primary tumors and metastases has been observed in 10-20% of cases. Several studies have reported Her2 overexpression in circulating tumor cells (CTCs) from metastatic patients with Her2-negative primary tumors[8-10, 111-112]. Her2-positive DTCs in BM from early stage patients with Her2-negative primary tumors have also been reported[11, 113-114]. Her2

amplification seems to become more frequent in systemic progression [9-10, 12]. Two hypotheses have been advanced to explain the discordance of Her2 expression between the primary tumor and CTCs/DTCs. It has been proposed that Her2 amplification is acquired during dissemination and disease progression[115]. Alternatively, Her2 positive clones of the primary tumor may have a greater tendency to break away and form metastases[111-112, 116].

To determine whether Her2-positive DTCs could be eliminated by targeted therapy, Rack et al conducted a small non-randomized phase II trial evaluating the efficacy of trastuzumab in eliminating DTCs from BM[13] in 10 women with stage I-III breast cancer. Of the ten women with Her-2 positive DTCs, 4 had Her2 positive tumors. After 12 months of trastuzumab therapy, none of the ten women had detectable Her2 positive DTCs in their BM. Interestingly, 3 patients continued to have detectable Her2 negative DTCs. Thus, in this pilot study, trastuzumab was found to be effective in eliminating Her2 overexpressing DTCs. The persistence of Her2 negative DTCs illustrates the heterogeneity of DTCs and highlights the need to define multiple predictive markers.

Preliminary Data: Her2 expression in DTCs correlates with early recurrence: We have examined bilateral iliac crest BM from 20 women with newly diagnosed clinical stage II/II breast cancer collected prior to any treatment for the expression of our 38 gene panel (HRPO# 05-0648). The primary tumors of 7 patients were Her2 positive and these patients received treatment with chemo/trastuzumab. Two of these 7 patients had DTCs that were *ERBB2* positive and none of the 7 Her2-positive patients developed metastatic disease with a mean follow-up of 48 months (**Figure 1**). In contrast, of the 13 patients who had Her2-negative tumors, 4 had DTCs that were ERBB2-positive and of these 4 patients, 3 (75%) developed metastatic disease within 24 months of diagnosis. *ERBB2*-positivity in these BM specimens was confirmed with qRT-PCR with 100% patient correspondence to the nCounter assay. Although these numbers are too small to reach statistical significance the data suggest that those patients with Her2-negative primary tumors and *ERBB2 over*expression in their BM at the time of diagnosis are at high risk of early metastatic disease development and may benefit from *ERBB2*-directed therapy. Moreover, we have found that *ERBB2* levels in blood, which were very low, did not correlate with levels in the BM or metastatic disease development.

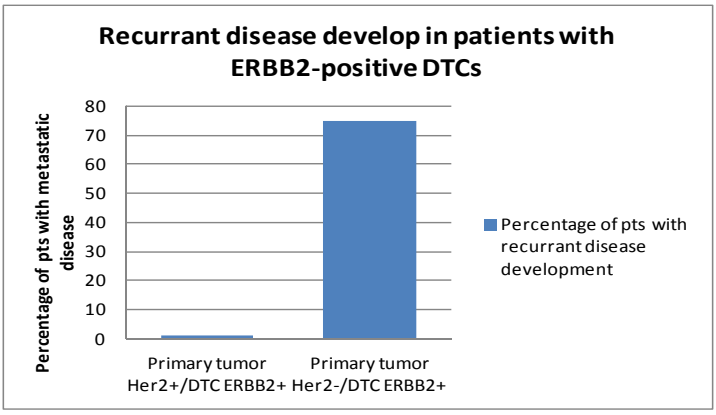


Figure 1. Recurrent disease development in patients with *ERBB2*-positive DTCs. Patients with Her2 positive tumors received chemotherapy with trastuzumab. Patients with Her2-negative tumors received cytotoxic chemotherapy alone. All patients had ERBB2-positive DTCs. All recurrences detected within 24 months (mean=19) of diagnosis.

1.6.7 Molecular Detection of Disseminated Tumor Cells

Several studies have assessed DTC detection by using molecular biology techniques such as real-time quantitative polymerase chain reaction (RT-PCR) determination with several epithelial-specific or organ specific mRNA such as CK19, MUC1[120], urokinase-type plasminogen activator receptor (uPAR), EpCAM[121] and mammaglobin[122-127]. Though expression of mammaglobin, as well as cytokeratin 19 mRNA in BM has been shown to give prognostic information[128-130], given the heterogeneity of DTC it is unlikely that there will be a single marker suitable for the detection of all DTC in all breast cancer patients.

Using single gene PCR to detect DTC highlights the difficulty of this approach. Since DTC are known to be phenotypically heterogeneous, multiple appropriate sensitive and specific markers which are predictive need to be identified. Until now, there has been technical difficulty in assessing multiple genes in the same specimen[55]. .

Multi-marker qRT-PCR has the potential to overcome some of the above concerns by allowing the detection of down-regulated/poor expression of a single genes[106, 130, 134-135]. In addition, genes associated specifically with clinically- and functionally-significant DTCs have recently been identified and validated using a conventional qRT-PCR assay[136-137]. Several of these genes when detected in bone marrow have been shown to identify patients at high risk of recurrence[137].

Nanostring nCounter™ (NC) is a sensitive, multiplex platform developed for the analysis of gene expression[138]. The NC platform can quantitatively detect expression of up to 500 genes in a single reaction with similar sensitivity and reproducibility as qRT-PCR. The NC platform counts single RNA molecules, does not require enzymatic reactions or high quality RNA

and simple to use[139]. In this technology, a multiplexed probe library is made with two sequence specific probes for each gene of interest[138]. The capture probe contains a 35-50 base sequence complementary to a particular target mRNA plus a short common sequence coupled to an affinity tag. The second reporter probe contains a second 35-50 bp sequence complementary to the target mRNA which is coupled to a color-coded tag that provides the detection signal (Figure 2). The limit of detection corresponds to 0.2-1 mRNA molecules per target cells with a dynamic range of 2.5 logs. This technology will allow the rapid, simultaneous detection of previously validated gene expression patterns analysis of hundreds of genes across many samples.

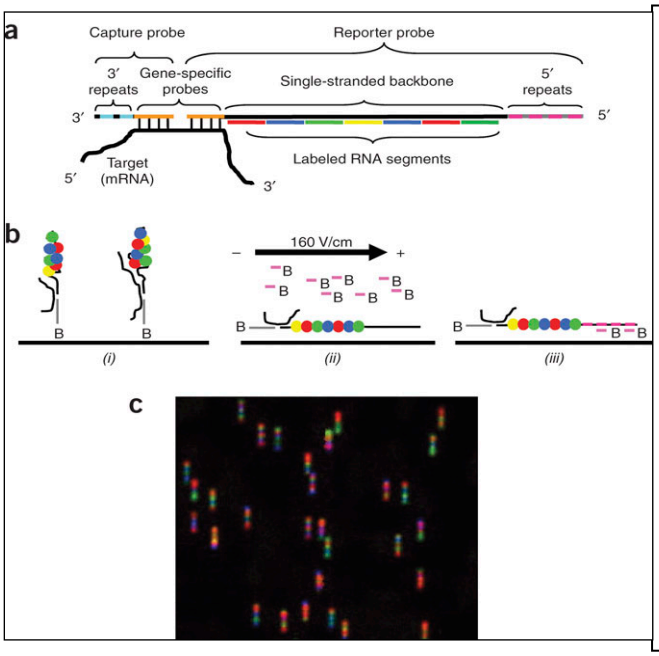


Figure 2. Mechanism of NC
a) A schematic representation of the hybridized complex (not to scale). The capture probe and reporter probe hybridize to a complementary target mRNA in solution via the gene-specific sequences (see Methods for details on capture and reporter probe construction). After hybridization, the tripartite molecule is affinity-purified first by the 3'-repeat sequence and then by the 5'-repeat sequence to remove excess reporter and capture probes, respectively. (**b**) Schematic representation of binding, electrophoresis, and immobilization. (*i*) The purified complexes are attached to a streptavidin-coated slide via biotinylated capture probes. (*ii*) Voltage is applied to elongate and align the molecules. Biotinylated anti-5' oligonucleotides that hybridize to the 5'-repeat sequence are added. (*iii*) The stretched reporters are immobilized by the binding of the anti-5' oligonucleotides to the slide surface via the biotin. Voltage is turned off and the immobilized reporters are prepared for imaging and counting. (**c**) False-color image of immobilized reporter probes. From Geiss et al

The current standard of DTC detection by IHC is labor-intensive which has limited the clinical use of DTC detection. We have validated the NC assay by measuring expression of genes which are DTC associated in patient BM. We developed a panel of 38 genes which are associated with all subtypes of breast cancer and are not expressed in normal bone marrow (**Figure 3**). These genes include those associated with epithelial cells and ER/Her2-negative tumors (EpCAM, cytokeratins 5,7,8,17, EGFR)[28], genes which have shown to be prognostic in DTC[128-130, 137, 140] (mammaglobin, K19, Twist1, PITX2) and housekeeping genes (Table 2). BM specimens were considered to be 'positive' for gene expression if expression levels in BM are at least two standard deviations above the mean expression of the 10 BM samples from healthy volunteers. Patients were considered ‘positive’ for biomarker gene expression when detected in at least one of two bone marrow samples analyzed (left or right side). For multiple gene testing by NC or Fluidigm Biomark HD (FBHD), BM is considered positive if any one of the validated genes is detected.

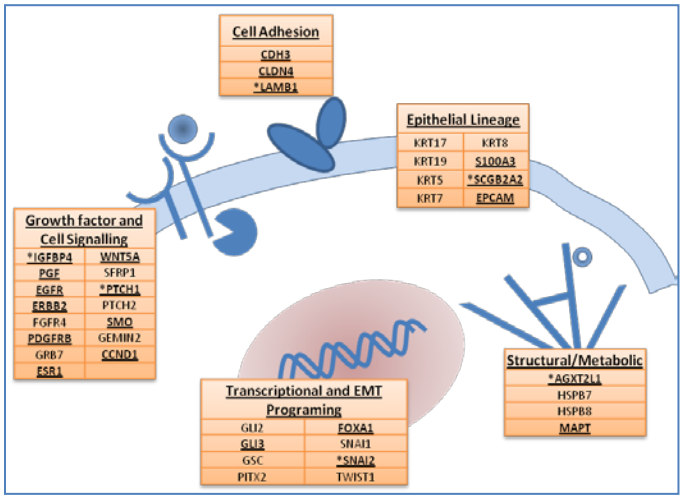


Figure 3. Genes represented in the 38-gene DTC panel and their biological pathway associations. Transcripts detected in the twenty-patient pilot cohort are highlighted in **bold underline** and those associated with early metastatic recurrence are denoted by asterisks.

We have performed several pilot studies with NC to determine the sensitivity and to optimize sample input. Using breast cancer cell lines (SKBR3, MDAMB231, ZR75) diluted into normal human bone marrow at varying concentrations, we found that the genes K19, Slug, S100A3 and EGFR could be detected at a 1:100,000 dilution using 0.5 ug of RNA and that these genes were expressed at a wide range of levels depending on the cell line. Moreover, the detection was linear over a 20-fold range (**Figure 4**). We next determined whether sensitivity would be increased by using Ficoll gradient enrichment of the DTC or by increasing the RNA. We found that Ficoll gradient enrichment resulted in a 2-fold increase in NS detected counts, while increasing the RNA to 5ug increased detection by 10-fold without increasing the background which allows us to detect certain genes at a level of 1 DTC per 1 million bone marrow cells. We next tested whether we were able to detect genes associated with DTC by NC in patient samples and determine the correlation with detection by qRT-PCR (Table 3). We found that each of the genes could be detected in at least one patient specimen and that there was good correlation with expression as detected by qRT-PCR. Our initial results indicate that NC is as sensitive as qRT-PCR for the detection of several genes which have been associated with DTCs, that the results are linear over a wide range of values, and that RNA isolated from whole bone marrow can be used for the assay.

As a preliminary test of technical feasibility, we have performed the 38-gene nCounter assay on 42 bone marrow specimens from 21 breast cancer patients and a set of 8 bone marrow specimens from healthy volunteer women. Nine patients had disease free survival (DFS) of less than 5 years while twelve patients had no evidence of metastatic disease at last follow-up. As demonstrated in **Figure 5**, all but two specimens had detectable levels (defined as two standard deviations above the mean of the control population) of at least one gene transcript in the 38-gene signature. This corresponds to a 90% positivity rate based on the expression of at least one gene transcript in at least one bone marrow sample.

Recently, the WU CLIA-certified GPS lab acquired a Fluidigm Biomark HD (FBHD) that provides quantitative analysis of cDNAs by qPCR using integrated fluidic circuit nanochips. These chips contain fluidic networks that enable automated combining of up to 96 cDNA samples with 96 gene assays to perform 9216 qPCR reactions simultaneously using 20ul sample cDNA loading volumes. This technology can detect specific targets at a minimum of 500-1,000 copies in the

original volume. Because some genes exhibit low expression, such as those associated with DTCs, resulting in more dilute target concentrations, a multiplexed 14 cycle pre-amplification of the targets of interest is performed in a primer-limited environment such that small amounts of cDNA are amplified equally without introducing bias abundances. We have employed this technology to analyze a 46-gene panel in 74 patient BM specimens. This technology will be developed as a clinical grade assay for use in the detection of DTCs in this clinical trial. From our preliminary work we have found that this technology provides excellent sensitivity and specificity for the detection of specific DTC populations. We expect this type of assay platform for clinical samples will facilitate translation of multi-gene biomarkers into the clinic, identify women at high risk of breast cancer recurrence and provide guidance on tailored therapies based on the molecular profile of micro-metastatic breast tumor cells.

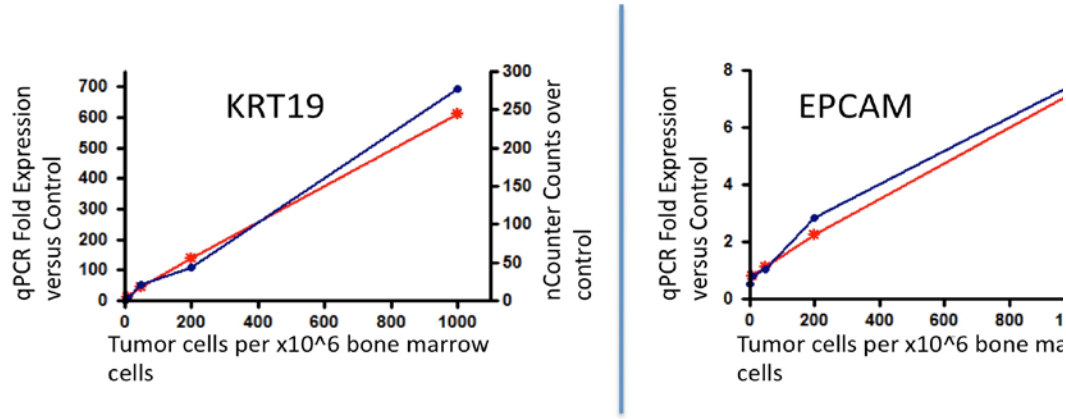


Figure 4. Comparison of assay linearity for detecting KRT19 (*left*) and EPCAM (*right*) gene expression in ZR75 breast tumor cells serially diluted into healthy volunteer bone marrow using qRT-PCR and nCounter platforms. qPCR data (left axes) was calculated using the ΔΔCt method, relative to the average signal detected in healthy control bone marrow. nCounter data (right axes) represent signal counts from each sample, after subtraction of average counts from healthy control bone marrow. Note that despite differing units of measure, both assays show roughly concordant linearity and sensitivity.

1.7 Rationale for Testing Trastuzumab for *ERBB2*-overexpressing DTCs

Evidence from the literature and our preliminary data suggest that micrometastases that persist despite chemotherapy are likely enriched with cells that have stem cell-like features that are responsible for subsequent disease recurrence[96, 141-142]. Bone marrow disseminated tumor cells (DTCs) can be used as a surrogate for systemic micrometastases Discordant Her2 expression between primary breast cancer and CTCs/DTCs have been reported.[13, 112-113] Clinical trial data has identified a yet undefined subgroup of patients with Her2-negative primary tumors who benefit from trastuzumab therapy.

The current trial will assess if 12 months of trastuzumab in patients with expression of *ERBB2* by BM DTC will lead to the elimination of bone marrow micrometastases that persist following the standard primary and adjuvant breast cancer therapies. Women with clinical stage II/III Her2 negative primary breast cancer who are

candidates for chemotherapy will undergo right and left iliac crest BM aspiration to assess levels of *ERBB2* both by qRT-PCR and as a component of the 38 gene

Patient	ER	HER-2	MO to relapse	Side	CCND1	CLDN4	EGFR	EPCAM	ERBB2	ESR1	FOXA1	GLI3	IGFBP4	LAMB1	MAPT	PDGFRB	PGF	PTCH1	S100A3	SCGB2A2	SNAI2	TOTAL
1	pos	pos	48	L																		2
1				R																		7
2	pos	neg	34	L																		0
2				R																		2
3	neg	neg	15	L																		0
3				R																		0
4	neg	pos	2	L																		1
4				R																		1
5	pos	neg	16	L																		1
5				R																		3
6	neg	neg	8	L																		0
6				R																		4
7	neg	neg	14	L																		2
7				R																		3
8	neg	neg	24	L																		2
8				R																		5
9	pos	neg	18	L																		5
9				R																		3
10	neg	neg	NA	L																		0
10				R																		0
11	pos	pos	NA	L																		2
11				R																		2
12	neg	pos	NA	L																		3
12				R																		2
13	neg	pos	NA	L																		5
13				R																		5
14	neg	neg	NA	L																		6
14				R																		2
15	neg	pos	NA	L																		2
15				R																		3
16	pos	pos	NA	L																		1
16				R																		0
17	neg	neg	NA	L																		1
17				R																		1
18	neg	pos	NA	L																		1
18				R																		1
19	pos	neg	NA	L																		5
19				R																		4
20	pos	neg	NA	L																		1
20				R																		0
21	neg	neg	NA	L																		2
21				R																		3
TOTAL					4	1	1	7	8	2	1	3	4	5	9	15	4	10	6	3	5	

Figure 5. Simplified matrix of nCounter gene expression data showing patterns of gene expression, represented here as detected (filled box) or undetected (empty box) in paired bone marrow specimens from 21 breast cancer patients. Only 17 of the 38 genes detected in at least one patient in this trial set are shown. 'Detected' is defined as expression that was 2 SD above the mean expression in a set of 8 healthy control women. ER and Her2 status of each case is shown, as well as the number of months until relapse (NA= no relapse to date). Total samples with detectable gene expression (column sums) and total genes detected in a sample (row sum) are indicated. .

profile using multiplex gene expression analysis (NC or FBHD). Those women with *ERBB2* levels 2 standard deviations higher than control normal BM by qRT-PCR will be randomized to standard adjuvant chemotherapy with no additional treatment or 1 year of trastuzumab administered with standard chemotherapy. Results from this trial will provide critical foundation for future definitive studies assessing the effectiveness of trastuzumab in reducing breast cancer recurrence in these high-risk patients.

1.8 Correlative Studies

1.8.1 The Effects of Chemotherapy and Trastuzumab on the 38-Gene Profile

This specific aim will:

1. Prospectively identify those genes in the 38-gene signature that are associated with DTCs but are not eliminated by chemo/trastuzumab in patients with *ERBB2*-positive DTCs.
2. Determine which genes in the 38 gene profile are eliminated in parallel with the *ERBB2*-positive DTCs.

Based on the data of Rack[13], we expect up to 30% of patients to have residual *ERBB2*-negative DTCs after chemo/trastuzumab treatment. By comparing the 38-gene profile before and after chemo/trastuzumab treatment, we will be able to determine the molecular profile of those DTCs that remain after treatment and correlate expression with disease recurrence. We will examine which genes in the 38 gene profile are eliminated in parallel with the *ERBB2*-positive DTCs. For example, we will examine the relationship between *ERBB2* and the cancer stem cell/Hedgehog pathway gene *PTCH1*. In our preliminary data (**Figure 4**), we observed that all patients with Her2-negative tumors/*ERBB2*-positive DTCs also expressed *PTCH1*. Thus we should be able to determine whether these 2 genes are expressed by the same population of DTCs or by separate populations of DTCs and the possible relationship of residual *PTCH1* expression and DFS.

1.8.2 Comparison of Recurrence Rate in Trastuzumab-treated Patients with PAM50-defined Her2 Subtype versus Other Subtypes

In this specific aim, we will assign each patient's primary tumor to a corresponding intrinsic subtypes using the PAM50 gene expression signature [31]. This will be accomplished using the multiplex gene expression assays to analyze the tumor specimens for the expression of the 50 genes in the signature. This work is ongoing at Washington University. This will allow us to:

1. Compare recurrence rates in trastuzumab treated patients with PAM50 defined Her2 subtype versus other subtypes and
2. Determine whether a specific tumor subtype is associated with *ERBB2*-positive DTCs.

1.8.3 Expected Results and Alternative Strategies

We expect that *ERBB2* expression by DTCs will be a predictive biomarker identifying those patients who will benefit from trastuzumab therapy, in a population of patients who would not otherwise be candidates for this therapy based on their primary tumor. We expect that treatment of these patients with trastuzumab will confer a DFS advantage compared to the control population of patients. We expect to observe elimination of *ERBB2*-positive DTCs with trastuzumab therapy and that this will correlate with an improved DFS. Based on our pilot data, we expect >90% correlation between *ERBB2* positivity testing between qRT-PCR and multiplex assays. By analyzing the 38 gene profile on all patient BM, we expect to identify new predictive therapeutic markers for targeting by identifying genes which persist through chemotherapy. Finally, we expect to define the relationship between tumor subtype and DTC profile. We believe that many of the tumors with *ERBB2* positive DTCs will have the Her2-enriched molecular subtype and that an equal number will likely be luminal B-like tumors or basal-like. Most importantly, if successful, we will have demonstrated within the context of a clinical trial that therapeutic targeting of DTC predictive biomarkers interrupts the metastatic cascade and results in improved survival. This will provide the foundation for future prospective trials based on this paradigm.

It is possible that we will have underestimated the risk of recurrence or benefit of the proposed treatments and need to adjust the number of patients needed to accrue into the trial. To address this, we will perform an interim analysis after one year, review the results with our statistician and revise the trial accordingly. It is possible that we will observe elimination of DTCs with trastuzumab therapy but no improvement in recurrence rate. This may be due to the presence of residual subpopulations of DTCs with metastatic potential, such as those expressing the cancer stem cell/hedgehog pathway gene *PTCH1*, which are not eliminated by trastuzumab/chemotherapy. If this is the case, we will attempt to identify genes which are associated with these cells for future targeting within the context of a clinical trial. Finally, we may observe that all patients enrolled into the trial will have a Her2-enriched molecular subtype of their primary tumor, if this is the case if will obviate the need to focus on DTCs for ERBB2 analysis, but we will have identified a population of patients who will benefit from *ERBB2*-directed therapy.

1.9 Study Rationale

Data suggest that micrometastases that persist despite chemotherapy are likely enriched with cells that have stem cell-like features that are responsible for subsequent disease recurrence. Bone marrow disseminated tumor cells (DTCs) can be used as a surrogate for systemic micrometastases. Discordant Her2 expression between primary breast cancer and CTCs/DTCs have been reported. Clinical trial data has identified a yet undefined subgroup of patients with Her2-negative primary tumors who benefit from trastuzumab therapy. We hypothesize that the subgroup of patients with Her2-negative primary tumors and ERBB2-positive DTCs will benefit from trastuzumab therapy and that administering targeted trastuzumab therapy to these patients will result in the elimination of ERBB2 overexpressing DTCs and improved DFS as measured by recurrence rate. With the correlative studies, we hope to define the relationship between tumor subtype and DTC profile as well as identify new predictive therapeutic markers for targeting by identifying genes which persist through chemotherapy. Results from this trial will provide a critical foundation for future definitive studies assessing the effectiveness of trastuzumab in reducing breast cancer recurrence in these high-risk patients.

2.0 OBJECTIVES

2.1 Primary Objective

Evaluate 3 year recurrence and death rates in patients treated with trastuzumab administered with chemotherapy for 12 months versus standard chemotherapy alone

2.2 Secondary Objective

Evaluate the effect of trastuzumab administered for 12 months on the elimination of *ERBB2* overexpressing bone marrow DTCs in patients with early stage HER2-

negative breast cancer who have *ERRB2* overexpressing bone marrow DTCs in their bone marrow prior to treatment (surgery and chemotherapy).

2.3 Exploratory Objectives

- Examine primary breast cancer specimens molecular subtypes of breast cancer by PAM50 and correlate with BM expression of *ERBB2* and treatment outcome

- Examine BM for other DTC specific gene expression using multiplex gene technology and correlate with response to trastuzumab and outcome

3.0 ELIGIBILITY CRITERIA

Since bone marrow aspiration for DTCs is not routinely performed as standard clinical practice, this study includes a pre-registration phase to enroll patients who meet all eligibility criteria listed in Section 3.1. In the pre-registration phase, patients will be consented for the bone marrow aspiration to test for the presence or absence of DTC overexpressing *ERBB2.* Analysis of the bone marrow will be performed at the Washington University Molecular Diagnostics Laboratory at Barnes-Jewish Hospital and Washington University School of Medicine. Patients who are found to have DTCs overexpressing *ERBB2* in the bone marrow are eligible for further study registration and treatment. Patients who do not have DTC which overexpress *ERBB2* will not be eligible for further study intervention. Bone marrow collection and testing will be covered by research funds and will not be charged to the patient or her insurance.

Note: All patients who are eligible to enroll into the trial after their bone marrow has been screened for *ERBB2*-overexpressing DTCs will be eligible to participate in other trials if the primary and secondary endpoints of this trial will not be compromised nor the endpoints of the secondary trial which the patient is offered. The endpoints and treatments of the secondary trials will be carefully screened to ensure that there will be no interference with the interpretation of the endpoints of the primary trial. All patients who are screened for this trial but are ineligible to continue due to the status of their bone marrow may participate in other trials.

3.1 Pre-Registration Eligibility

3.1.1 Pre-Registration Inclusion Criteria

1. Histologically confirmed HER2-negative primary invasive ductal or invasive lobular breast carcinoma. For patients enrolling for neoadjuvant treatment, diagnosis must be clinical stage II or III; for patients enrolling for adjuvant treatment, diagnosis must be pathologic stage IIA to IIIC.

   Standard HER2 testing will be performed in the surgical specimen at Washington University according to the standard of care in the Department of Pathology. A HER2-negative primary breast cancer sample from a patient eligible for randomization should have a HER2 IHC score of 0 or 1+ Those patients with IHC score of 2+ should be HER2 FISH-negative in standard testing.

Patient will have undergone staging studies including a CT of the chest/abdomen/pelvis and bone scan and/or PET scan either prior to the initiation of treatment or prior to entry into the trial.

In addition, patients with non-metastatic, HER2-negative, recurrent tumors who need chemotherapy are eligible.

2. Planning to receive best practice adjuvant or neoadjuvant chemotherapy according to institutional guidelines. Adjuvant tamoxifen or aromatase inhibitors treatment will be allowed for hormone receptor-positive patients. Patients who have failed neoadjuvant endocrine therapy will also be eligible.

3. At least 18 years old.

4. ECOG performance status $\leq$ 1.

5. Patient (or legally authorized representative) must be able to understand and willing to sign a written informed consent document.

3.1.2 Pre-Registration Exclusion Criteria

1. Prior chemotherapy for this cancer (excluding initiation of best practice chemotherapy to be given as standard of care described in Section 5.3.1, which may be initiated after the pre-registration bone marrow collection but before final confirmation of eligibility and randomization).

2. Previous treatment with trastuzumab or any other Her2 targeted therapy.

3. Presence of an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2 Registration Eligibility

3.2.1 Registration Inclusion Criteria

1. Presence of bone marrow *ERBB2* overexpressing DTCs at the time of diagnosis; bone marrow aspiration will be performed in consented patients to evaluate DTCs following pre-registration provided patients meet all eligibility criteria as described in this section.

2. ECOG performance status $\leq$ 1.

3. Adequate cardiac function as demonstrated by LVEF of >55% performed no more than 4 weeks prior to randomization.

4. Normal organ and marrow function as defined below:

   - leukocytes $\geq$3,000/mcL
   - absolute neutrophil count $\geq$1,500/mcL
   - platelets $\geq$100,000/mcL
   - hemoglobin $\geq$ 10 g/dL
   - total bilirubin within institutional upper limits of normal unless related to primary disease
   - AST(SGOT)/ALT(SGPT) $\leq$2.0 X institutional upper limit of normal
   - Creatinine $\leq$ 1.5 institutional upper limits of normal OR creatinine clearance >60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

5. If a woman of childbearing potential, patient must use two forms of effective contraception for a minimum of 6 months following trastuzumab. Effective methods of birth control include use of established oral, injected, or implanted hormonal methods of birth control, IUD, IUS, and condoms.

3.2.2 Registration Exclusion Criteria

1. Evidence of distant metastasis present by CT scan, bone scan, or physical exam.

2. History of allergic reactions attributed to compounds of similar chemical or biologic composition to trastuzumab.

3. Prior chemotherapy for this cancer (excluding initiation of best practice chemotherapy to be given as standard of care described in Section 5.3.1, which may be initiated after the pre-registration bone marrow collection but before final confirmation of eligibility and randomization).

4. History of other malignancy $\leq$ 5 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix.

5. Pregnant or breastfeeding. Patient must have a negative serum pregnancy test ≤ 7 days from date of registration (if a woman of childbearing potential).

   Women of childbearing potential are defined as follows:
   - Women with regular menses
   - Women with amenorrhea, irregular cycles, or using a contraceptive method that precludes withdrawal bleeding
   - Women who have had a tubal ligation.

   Women are considered not to be of childbearing potential for the following reasons:
   - The patient has undergone hysterectomy and/or bilateral oophorectomy.
   - The patient is post-menopausal defined by amenorrhea for at least 1 year in a woman > 45 years old.

6. Clinically important history of active liver disease, including viral or other hepatitis or cirrhosis.

7. Uncontrolled hypocalcemia, hypomagnesemia, hyponatremia, or hypokalemia defined as less than the lower limit of normal for the institution despite adequate electrolyte supplementation.

8. Symptomatic intrinsic lung disease or extensive tumor involvement of the lungs resulting in dyspnea at rest.

3.3 Inclusion of Women and Minorities

Breast cancer is rare in men and children. Therefore, this trial is only open to women of all races and ethnic groups.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirmation of patient eligibility includes the information listed below:

1. Registering coordinator's name and contact information
2. Registering MD's name
3. Patient's race, sex, and DOB
4. Copy of signed consent form

5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

Patients must be registered through the Siteman Cancer Center database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Pre-Intervention Phase

Patients who meet all eligibility criteria listed in Section 3.1 with the exception of bone marrow status for DTCs (inclusion criterion #2) can be pre-registered. At that point, consent will be obtained, and bone marrow will be collected and analyzed for *ERBB2* expression. The patient will be informed whether she is eligible to continue in the study based on the results of the bone marrow testing. The patient is also to be informed that the clinical significance for the presence or absence of bone marrow DTCs is not yet clear and is used only as a marker in this trial.

Only patients with *ERBB2*-positive bone marrow DTCs will be eligible for randomization. The descriptions for the bone marrow collection procedure, sample processing, and analysis are detailed in Section 9.0. Bone marrow collection and analysis for DTCs will be covered by protocol funds and will not be charged to the patient or her insurance. Patients without *ERBB2*-positive bone marrow DTCs will be approached for enrollment to a companion trial, HRPO# 201310088.

5.2 Randomization

5.2.1 Original Randomization Plan

Upon the receipt of the bone marrow DTC status, eligibility will be confirmed and signed informed consent verified. Still-eligible and consenting patients will be randomized to receive either standard of care treatment (Arm 1) or trastuzumab (Arm 2) as recommended below. Randomization may take place before or after the start of standard of care chemotherapy as long as there is time for at least 8 weeks of overlap of SOC chemo and trastuzumab if the patient is randomized to Arm 2. Approximately the same number of patients will be assigned to each treatment group. A stratified, permuted block randomization will be used to balance as closely as possible the number of patients in each arm by lymph node status (positive vs. negative), tumor size (< 3cm vs. ≥ 3cm), estrogen receptor status of the primary tumor (positive vs. negative), and time of surgery (pre- vs. post-chemotherapy). Randomization will be in

blocks of random size. The randomization table will be uploaded in our REDCap system. Randomization will occur via an online form with entry of the patient ID number and stratum information. Once all information is entered, randomization is carried out via a submit button through REDCap. The randomization scheme will be created using a formal probability model implemented in SAS (version 9.3 or higher).

5.2.2 Current Randomization Plan (Amendment #6)

Five patients have been randomized as described above, 4 to Arm 1 (SOC treatment) and 1 to Arm 2 (trastuzumab). Following study reopening to accrual, all patients meeting screening requirements will be randomized in a 3:1 allocation ratio (trastuzumab (Arm 2) : no trastuzumab (Arm 1)). Ten to 12 patients are expected to be randomized. Randomization will be unstratified and in blocks of 4. The randomization scheme will be created using a formal probability model implemented in SAS v9.4/STAT13.1. The randomization table will be uploaded into our REDCap system. Once the randomization table is uploaded is will be locked and unalterable until the study is closed to further accrual. Randomization will be carried out by the clinical research assistant using an online form.

5.3 Agent Administration

5.3.1 Standard Chemotherapy

Patients in both arms will receive best practice standard chemotherapy according to NCCN guidelines. The 5 chemo backbone options are:

- Doxorubicin (or epirubicin) plus cyclophosphamide followed by paclitaxel (or docetaxel)
- Docetaxel plus cyclophosphamide
- Single agent paclitaxel
- Docetaxel plus carboplatin
- Fluorouracil plus epirubicin plus cyclophosphamide followed by paclitaxel (or docetaxel)

Patients are allowed to start chemotherapy after the pre-registration bone marrow collection but before confirmation of eligibility and before randomization. Patients randomized to Arm 2 must have a minimum of 8 weeks of overlap with standard of care chemotherapy and trastuzumab.

5.3.2 Trastuzumab

Patients randomized to the trastuzumab treatment arm (Arm 2) will also receive IV trastuzumab for a total of 52 weeks. Treatment with trastuzumab must be initiated such that there is a minimum of 8 weeks of overlap with the standard of care chemotherapy. The dosing of trastuzumab when given concurrently with standard of care chemo depends on the treatment cycles for the standard of care chemo. Trastuzumab may be given weekly, every 2 weeks, or every 3 weeks. If given weekly, the loading dose will be 4 mg/kg IV over 90 minutes and the subsequent doses that overlap with the standard of care chemo will be 2 mg/kg IV over 30 minutes. If given

every 2 weeks, the loading dose will be 6 mg/kg IV over 90 minutes and the subsequent doses that overlap with the standard of care chemo will be 4 mg/kg IV over 30 minutes. If given every 3 weeks, the loading dose will be 8mg/kg IV over 90 minutes and the subsequent doses that overlap with the standard of care chemo will be 6 mg/kg IV over 30-90 minutes. Please note that trastuzumab shall not be given concurrently with any anthracyclines.

After standard of care chemo has concluded, all remaining doses of trastuzumab will be given at 6 mg/kg IV over 30-90 minutes every 3 weeks. Total length of trastuzumab administration is 52 weeks, including the time period during which trastuzumab is being administered concurrently with standard of care chemo.

Definitive surgery may be performed prior to the initiation of chemotherapy, after the completion of part of the standard of care chemo, or after the completion of all of the standard of care chemo at the discretion of the treating surgeon.

5.4 General Concomitant Medication and Supportive Care Guidelines

Medications required to treat adverse events and manage cancer symptoms, concurrent stable disease (e.g., controlled hypertension), and pain medications are allowed.

The patient must notify a member of the research team about any new medications she takes after the start of the study medication.

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative urine pregnancy test within 7 days prior to the date of registration and again within 7 days prior to the first dose of trastuzumab (if randomized to Arm 2).

Patients are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 7 months following the last dose of trastuzumab.

If a patient is suspected to be pregnant, all study drugs should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a patient becomes pregnant during therapy or within 7 months after the last dose of trastuzumab, the investigator must be notified in order to facilitate outcome follow-up.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to AEs, treatment may continue until the end of standard of care chemo (for patients randomized to Arm 1) or approximately 14 months (time of treatment with standard of care chemo plus one full year of trastuzumab) (for patients randomized to Arm 2) or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the Investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Major violation of the study protocol
- Lost to follow-up
- Patient withdrawal
- The PI decides to remove the patient from study
- The Siteman Cancer Center decides to close the study

Subjects who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar, Section 11.0.

5.7 Duration of Follow Up

Patients will be followed every 3 months for 2 years then every 6 months for 3 years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Follow-up for all patients begins at the end of standard of care chemo or at time of surgery (depending on which is last).

6.0 DOSING DELAYS/DOSE MODIFICATIONS

6.1 Trastuzumab

6.1.1 Management of Trastuzumab Side Effects

For the first (loading) dose of trastuzumab, premedication with acetaminophen 650 mg PO will be given.

Patients should not miss more than one dose of trastuzumab consecutively. Patients do not have to make up missed doses.

6.1.2 Dose Modifications for Trastuzumab

Dose modification of trastuzumab is **not** permitted except as described in Sections 5.3.2 and 6.1.4.

6.1.3 Infusion-associated Symptoms with Trastuzumab

Infusion reactions consist of a symptom complex characterized by fever and chills, and on occasion nausea, vomiting, pain (in some cases at tumor sites), headache, dizziness, hypotension, rash, and asthenia. In postmarketing reports, serious and fatal infusion reactions have been reported. Severe reactions which include bronchospasm, anaphylaxis, angioedema, hypoxia, and severe hypotension were usually reported during or immediately following the initial infusion. However, the onset and clinical course were variable including progressive worsening, initial improvement followed by clinical deterioration, or delayed post-infusion events with rapid clinical deterioration. For fatal events, death occurred within hours to days following a serious infusion reaction.

Interrupt trastuzumab infusion in all patients experiencing dyspnea, clinically significant hypotension, and intervention of medical therapy administered, which may include: epinephrine, corticosteroids, diphenhydramine, bronchodilators, and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms. The rate of infusion should be decreased for mild or moderate (CTCAE 4.0 grade 1 or 2) infusion reactions. Permanent discontinuation should be strongly considered in all patients with severe (grade 3 or 4) infusion reactions.

6.1.4 Cardiac Dysfunction

Signs and symptoms of cardiac dysfunction were observed in a number of women who received trastuzumab alone or in combination with chemotherapy, most often anthracycline-based treatment. Cardiac dysfunction was observed most frequently among patients who received trastuzumab plus AC chemotherapy (28%), compared with those who received AC alone (7%), trastuzumab plus paclitaxel (11%), paclitaxel alone (1%), or trastuzumab alone (7%). Severe disability or fatal outcome due to cardiac dysfunction was observed in ~1% of all patients.

The nature of the observed cardiac dysfunction was similar to the syndrome of anthracycline-induced cardiomyopathy. The signs and symptoms of cardiac dysfunction usually responded to treatment. Complete and partial responses were observed among patients with cardiac dysfunction. The risk appears to be independent of tumor response to therapy. Analysis of the clinical database for predictors of cardiac dysfunction revealed only advanced age and exposure to an anthracycline as possible risk factors. In the clinical trials, most patients with cardiac dysfunction responded to appropriate medical therapy, often including discontinuation of trastuzumab. In many cases, patients were able to resume treatment with trastuzumab. In a subsequent study using weekly paclitaxel and trastuzumab as first-line treatment for metastatic breast cancer, the observed incidence of serious cardiac dysfunction was 3% (N=95) (Seidmen et al. 2001). Since the occurrence of cardiac dysfunction in the trastuzumab plus chemotherapy trial was an unexpected observation, no information is available regarding the most appropriate method for monitoring cardiac function in patients receiving trastuzumab. Significant advances in the understanding and treatment of CHF have been made in the past several years, with many of the new drugs demonstrating the ability to normalize cardiac function. Patients who develop symptoms of congestive heart failure while on trastuzumab should be treated according to the HFSA guidelines (Appendix B).

All patients randomized to Arm 2 must have a MUGA scan at baseline, and on a regular schedule throughout the course of the study. Investigators are strongly urged to schedule MUGA scans at the same radiology facility where the patient's baseline MUGA scan was done whenever possible. MUGA scans are required at protocol-specified time points and after any patient has any of the following: discontinuation of protocol therapy, congestive heart failure, breast cancer recurrence, or a second primary cancer.

Post-surgical radiation therapy may be required in patients at risk for recurrence. Whenever possible, irradiation to the internal mammary nodes should be avoided because of the concern for possible additional cardiotoxicity from the combination of trastuzumab and radiation therapy. Efforts should be taken to ensure that the volume of the heart irradiated is minimal. Investigators are encouraged to discuss cardiac toxicity concerns with their radiation oncologists to ensure careful planning of the ports of ***left-sided*** lesions.

Recommended Cardiac Monitoring

Conduct thorough cardiac assessment, including history, physical examination, and determination of LVEF by MUGA scan. The following schedule is recommended:

- Baseline LVEF measurement immediately prior to initiation of trastuzumab
- LVEF measurements every 3 months during and upon completion of trastuzumab using the same modality and same facility as used for baseline
- Repeat LVEF measurement at 4 week intervals if trastuzumab is withheld for significant left ventricular cardiac dysfunction

- LVEF measurements every 6 months for at least 2 years following completion of trastuzumab as a component of adjuvant therapy.

Asymptomatic Patients
If a patient does not have significant symptoms related to LV dysfunction, administration of trastuzumab will depend on the absolute change in LVEF between baseline and follow-up assessments.

Trastuzumab should be initiated in an asymptomatic patient if:
- The LVEF increased or stayed the same;
- The LVEF decreased by ≤ 15 percentage points but is still at or above the lower limit of normal for the radiology facility.

Trastuzumab is ***PROHIBITED*** in an asymptomatic patient if:
- The LVEF decreased ≤ 15 percentage points and is **below** the limit of normal for the radiology facility:
- The LVEF decreased by 16 percentage points or more (regardless of lower limits of normal for the radiology facility)

Withhold trastuzumab dosing for at least 4 weeks for either of the following:
- > 16% absolute decrease in LVEF from pre-treatment values
- LVEF below institutional limits of normal and > 10% absolute decrease in LVEF from pretreatment values.
- Trastuzumab may be resumed if, within 4-8 weeks, the LVEF returns to normal limits and the absolute decrease from baseline is < 15%.
- Permanently discontinue trastuzumab for a persistent (> 8 weeks) LVEF decline or for suspension of trastuzumab dosing on more than 3 occasions for cardiomyopathy.

If a patient has significant symptoms related to left ventricular (LV) dysfunction, cardiac ischemia, or arrhythmia, initiation of trastuzumab is prohibited.

6.1.5 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 or with extensive tumor involvement of the lungs, 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.

6.1.6 Hematologic Toxicity

Hematologic toxicity is infrequent following the administration of trastuzumab as a single agent, with an incidence of Grade III toxicities for

WBC, platelets, hemoglobin all < 1%. No Grade IV toxicities were observed.

6.1.7 Diarrhea

Of patients treated with trastuzumab as a single agent, 25% experienced diarrhea. An increased incidence of diarrhea, primarily mild to moderate in severity, was observed in patients receiving trastuzumab in combination with chemotherapy.

6.1.8 Infection

An increased incidence of infections, primarily mild upper respiratory infections of minor clinical significance or catheter infections, was observed in patients receiving trastuzumab in combination with chemotherapy.

6.1.9 Other Serious Adverse Events

The following other serious adverse events occurred in at least one of the 958 patients treated with trastuzumab in clinical studies:

Body as a Whole: cellulitis, anaphylactoid reaction, ascites, hydrocephalus, radiation injury, deafness, amblyopia
Cardiovascular: vascular thrombosis, pericardial effusion, heart arrest, hypotension, syncope, hemorrhage, shock, arrhythmia
Digestive: hepatic failure, gastroenteritis, hematemesis, ileus, intestinal obstruction, colitis, esophageal ulcer, stomatitis, pancreatitis, hepatitis
Endocrine: hypothyroidism
Hematological: pancytopenia, acute leukemia, coagulation disorder, lymphangitis
Metabolic: hypercalcemia, hypomagnesemia, hyponatremia, hypoglycemia, growth retardation, weight loss
Musculoskeletal: pathological fractures, bone necrosis, myopathy
Nervous: convulsion, ataxia, confusion, manic reaction
Respiratory: apnea, pneumothorax, asthma, hypoxia, laryngitis
Skin: herpes zoster, skin ulceration
Urogenital: hydronephrosis, kidney failure, cervical cancer, hematuria, hemorrhagic cystitis, pyelonephritis

6.2 Standard of Care Chemotherapy

All dose modifications for the standard of care chemotherapy regimens should be performed as per routine during standard of care administration.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 7.4. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

Genetech requires that all events be reports as outlined in Section 7.5.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html.

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgement, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB pre-approval of all protocol exceptions must be obtained prior to the event.

7.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within 10 working days of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within 1 working day of the occurrence of the event or notification to the PI of the event.

QASMC must be notified within **10 days** of receipt of IRB acknowledgement via email to a QASMC auditor.

7.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

7.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 7.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.

Report any serious, unexpected adverse experiences (Section 7.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

7.5 Reporting to Genentech

Safety assessments will consist of monitoring and reporting AEs and SAEs that are considered related to trastuzumab, all events of death, and any study-specific issue of concern.

For purposes of reporting to Genentech, an AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with breast cancer that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).
- If applicable, AEs that occur prior to assignment of study treatment associated with medication wash-out, no treatment run-in, or other protocol mandated intervention.
- Pre-existing medical conditions (other than breast cancer) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death)
- It is life-threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.)
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational medicinal product.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above.)

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the {study drug} (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of trastuzumab and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to trastuzumab; and/or the AE abates or resolves upon discontinuation of trastuzumab or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than trastuzumab (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to trastuzumab administration (e.g., cancer diagnosed 2 days after first dose of trastuzumab).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

7.5.1 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

<u>Diagnosis vs. Signs and Symptoms</u>
If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death”.

Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 90 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the {study drug} should be reported as an SAE.

Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior {study drug} exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently

conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

Reconciliation
The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

7.5.2 Reporting Instructions

Investigators must report all SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682
OR
(650) 225-5288

- Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.
- Serious AE reports that are related to the trastuzumab (regardless of causality) will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
- Serious AE reports that are unrelated to the trastuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
- Additional Reporting Requirements to Genentech include the following:
    - Any reports of pregnancy following the start of administration with the trastuzumab will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
    - All Non-serious Adverse Events originating from the Study will be forwarded in a semi-annual report to Genentech.

All written IND safety report submitted to the FDA by the investigator must also be faxed to Genentech Drug Safety at one of the numbers above. Additionally, all IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety at one of the numbers above. Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations Contact for the study.

7.6 Timeframe for Reporting Required Events

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study procedures and ends 30 days following the last day of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

8.0 PHARMACEUTICAL INFORMATION

8.1 Trastuzumab

8.1.1 Description

Trastuzumab (Herceptin) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of HER2 (Kd = 5 nM)[143-144]. 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.

8.1.2 Pharmacokinetics and Drug Metabolism

Trastuzumab administered once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 1.7 and 12 days at the 10 and 500 mg dose levels, respectively. Trastuzumab's volume of distribution was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 mcg/mL.

In studies using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, a mean half-life of 5.8 days (range = 1 to 32 days) was observed. Between Weeks 16 and 32, trastuzumab serum concentrations reached a steady state with a mean trough and peak concentrations of approximately 79 microgram/mL and 123 microgram/mL, respectively.

Data suggest that the disposition of trastuzumab is not altered based on age or serum creatinine (up to 2.0 mg/dL). No formal interaction studies have been performed.

8.1.3 Supplier(s)

Trastuzumab will be purchased from Genentech.

8.1.4 Dosage Form and Preparation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 400 mg of trastuzumab, 9.9 mg of L-histidine HCl, 6.4 mg of L-histadine, 400 mg of α,α-trehalose dihydrate, and 1.8 mg of 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 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6.

Use appropriate aseptic technique. Each vial of trastuzumab should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multidose 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.

If the patient has known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with Sterile Water for Injection (see PRECAUTIONS). Trastuzumab which has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided.

Determine the dose of trastuzumab needed. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed.

Refer to Section 5.3.2 for dosing information.

8.1.5 Storage and 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 or polyethylene 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, since diluted trastuzumab contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated (2-8°C).

8.1.6 Administration

Treatment may be administered in an outpatient setting by intravenous (IV) infusion over 90 minutes. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** Patients should be observed for fever and chills or other infusion-associated symptoms.

If trastuzumab is being administered concomitantly with chemotherapy, trastuzumab administration should precede chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms. If prior infusions are well tolerated, subsequent doses may be administered over 30 minutes.

8.1.7 Special Handling Instructions

None.

9.0 BONE MARROW ANALYSIS FOR STUDY ENTRY AND PRIMARY ENDPOINT

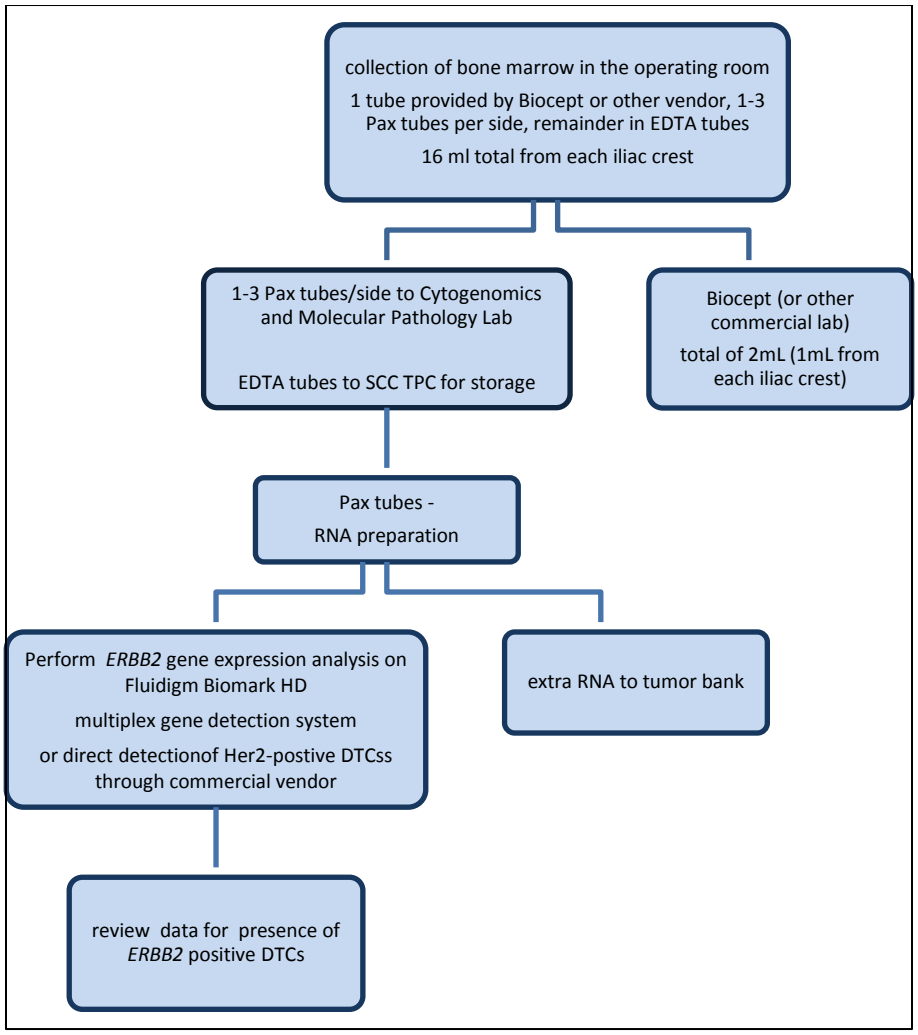
9.1 Collection of Bone Marrow Specimens

Bone marrow aspirations for DTCs will be obtained at two time points:

- Baseline (in order to determine eligibility for randomization)
- 6-18 months after 1st bone marrow aspiration

Bone marrow collection and analysis will be paid for by research funds and will not be charged to the patient or her insurance.

The baseline bone marrow aspiration will be performed in the operating room at the time of portacath placement (for patients receiving neoadjuvant chemotherapy) or at the time of surgery (for patients receiving adjuvant chemotherapy). The 6-18 month bone marrow aspiration will be performed in the operating room at the time of portacath removal (for patients randomized to Arm 2) or at a scheduled appointment in the Center for Advanced Medicine (for patients randomized to Arm 1 who do not keep the portacath for the full year).



Sixteen cc of bone marrow will be collected from the right and/or left anterior iliac crest. After the collection of 8 cc the needle will be redirected to minimize the collection of blood. If the patient tolerates the collection of bone marrow from one side, then bone marrow will be collected from the contralateral side.

Subjects will be in the supine position, the right or left posterior superior iliac crest region or the anterior iliac crest will be identified, and the area will be sterilely prepped with an alcohol based solution.

The region to be entered will be anesthetized with lidocaine and/or lidocaine/bupivacaine mixture and, following adequate anesthesia, an Illinois needle will be inserted into the iliac crest region.

The bone marrow cavity will be penetrated and approximately 16cc of bone marrow will be obtained from each iliac crest. If BM is collected from both iliac crests, then 1-4 cc's of BM from each side will be combined and placed in a tube provided by a commercial vendor for DTC enumeration/Her2+ determination. The remainder of the BM will be divided between 1-3 PAXgene bone marrow RNA tubes (2 ml each) (Qiagen) and EDTA tubes.

The procedure will be repeated on the contralateral side. After aspiration, the needle will be removed.

The puncture sites will be cleaned and bandaged. Each participant will receive printed wound care instructions containing contact information for medical assistance if the participant experiences any adverse symptoms or problems following the procedure.

9.2 Handling of Bone Marrow Specimens

All tubes containing bone marrow will be labeled with an assigned code number and the site of collection.

All PAXgene tubes from each side (1-3 per side, or 2-6 total) will be transported to the Molecular Diagnostics Laboratory (**MDL**) at Washington University and stored until analysis is requested. If analyzed, two of the tubes will be used for RT-PCR analysis, and one tube will be stored. The Cee Sure tubes or tubes from other commercial vendors will be shipped in the kits provided by the vendor to Biocept or other commercial vendor according to the vendor's instructions. The EDTA tubes will be transported to the Siteman Cancer Center Tissue Procurement Core for processing and storage. (Please refer to sample flow diagram.)

9.3 Bone Marrow Processing

RT-PCR detection of bone marrow DTCs will be performed at the CLIA-certified Genomic and Pathology Service/Molecular diagnostics Laboratory (**MDL**) at Washington University (GPS@WU) (CLIA# 26D0698685). Direct detection of Her2+ DTCs will be performed by Biocept or other commercial vendor.

ERBB2 status of bone marrow will be determined by a clinical grade qRT-PCR assay for *ERBB2* expression in BM developed in the CLIA certified Molecular Diagnostic Laboratory using guidelines for the development of biomarkers[145]. RNA will be processed and purified from 2 PAXgene tubes per iliac crest side (right and left) using a commercially available protocol/kit designed for PAX tubes (Qaigen). Her2-expressing cell lines diluted into normal bone marrow will serve as the positive control. Commercially available primers/probe for *ERBB2 and ERBB2* related genes will be used (Applied Biosystems). PCR reactions will be run on a Fluidigm Biomark HD. Levels of expression relative to normal BM will be calculated as previously described using an established gene as an endogenous control[137]. Samples will be considered positive/over expressed if *ERBB2* overexpression levels are 2 standard deviations above a pool of control bone marrows. Validation and interassay variation coefficients will be determined using the breast cancer cell line SKBr3 cells (Her2 expressing) diluted into normal BM. Only patients *ERRB2* expression in their BM will be eligible to continue on the study and receive

trastuzumab for 12 months. A second BM aspirate will be performed after the completion of drug treatment. The unused portion of the BM specimens will be banked.

9.3.1 Analysis of Her2+ DTCs by Commercial Vendors

DTCs will be analyzed by Biocept or other commercial vendor for Her2+ expression by FISH. Briefly, after Ficoll gradient enrichment, DTCs are immunomagnetically isolated using a cocktail of 10 antibodies. The retrieved cells are stained for standard epithelial markers and for Her2+ amplification by FISH. Any BM specimen that contains a Her2+ overexpressing DTC will be considered positive and the patient enrolled into the trial.

9.3.2 RNA Quantification and Quality Control

Aglient Bioanalyzer with Agilent Nano 6000 kits will be used to assess the quality of each RNA sample. The Nanodrop 1000 spectrophotometer will be used to assess the quantity of each sample. All RNAs to be used for qRT-PCR or NC analysis will have demonstrated a RIN score of > 7.0.

cDNA will be prepared from RNA according to standard procedures.

9.3.3 Analysis of Gene Expression

(If funding is available only.)

Samples of 300 ng will be loaded onto a Fluidigm Biomark HD Each sample will be run in duplicate, for a total of 8 samples per patient (2 from each iliac crest analyzed in duplicate).

For each sample, each gene duplicate will be averaged and the ddCT will be calculated using reference genes. Fold overexpression from normal bone marrow will be calculated. Any gene 2 STD over the expression of the normal bone marrow pool from healthy volunteers will be considered overexpressed in that bone marrow. If a gene is overexpressed in both bone marrow specimens from the same side, the patient's bone marrow will be considered positive for expression. Bone marrows will be considered negative if the all of the bone marrow samples are less than 2 STD above normal bone marrow pool or if only one specimen on each side is 2STD above the normal bone marrow pool.

10.0 CORRELATIVE STUDIES

10.1 Existing FFPE or Frozen Tumor Blocks

Existing FFPE or frozen tumor blocks from a prior diagnostic biopsy or breast surgery will be requested and logged onto the Siteman Cancer Center Tissue Procurement Core.

Tumor blocks will then be processed in the Tissue Procurement Core for tumor DNA/RNA extraction and tissue sectioning for IHC analysis. H&E staining will be performed before the tissue processing to ensure high tumor content for future analysis. If needed, micro or macro dissection will be performed to isolate tumor cells from the surrounding stroma.

The following analyses will be performed for their potential predictive value in treatment response to trastuzumab:

- Targeted DNA sequencing will be performed on tumor DNA for somatic mutations which could affect treatment response
- To define the breast cancer molecular subtype; tumor RNA will be analyzed by at the Washington University Human Genome Sequencing Center for PAM50 classification of the tumor.

10.2 Optional Fresh Pre-Treatment Tumor

A tumor biopsy using a 14-gauge needle may be performed prior to initiation of treatment if the tumor is easily accessible and the patient consents to this optional biopsy. The tissue specimen will be taken to the Tissue Procurement Core for storage and shared with other investigators who have appropriate IRB approvals.

10.3 Multiplex Gene Detection of Bone Marrow

(If funding is available only.)

All bone marrow specimens will be analyzed by a multiplex gene detection system for expression of 38-53 genes associated with the presence of DTCs. Gene expression analysis will be correlated with trastuzumab treatment either before or after microfilter enrichment for DTCs.

Analysis to be performed based on genes expression and analysis of tumor specimens include:

1. To correlate time to recurrence with reduction in *ERBB2* gene expression in bone marrow
2. Correlate time to recurrence with the elimination of DTCs
3. Assess the effect of trastuzumab on alterations in expression of other DTCs genes/pathways using multiplex gene detection

10.3.1 Analysis of DTC Expression Using Multiplex Gene Expression Assays

RNA will be prepared from BM nucleated cells and examined for expression of a panel of genes using NC or FBHD. The assay will be performed using the manufacturer's methods. Expression data will be normalized and analyzed using the manufacturer's standard procedures[139, 146]. All analyses will be performed in duplicate. Positive and negative controls will be included in each assay to normalize for assay based variation (differences in hybridization, purification and binding efficiency). Additionally, the data will be normalized against internal reference genes (housekeeping genes) to correct for differences in number of cells, absolute mRNA content and sample preparation. In replicate measurements, the average, the standard deviation and coefficient of variation percentage (%CV) is calculated using the normalized data. BM specimens will be considered to be 'positive' for gene expression if expression levels in BM are at least two standard deviations above the mean expression of the 20 BM samples from healthy volunteers. Patients will be considered 'positive' for biomarker gene expression when detected in at least one of two bone marrow samples analyzed (left or right side). For multiple gene testing by NC, BM will be considered positive if any one of the panel of genes is detected.

10.4 Serial Research Blood Collection (required)

Thirty mL of blood (10 mL in a red top silica clot activator serum tube for serum, 10 mL in a lavender top EDTA tube for plasma, and 10 mL in the cell-free DNA BCT tube for plasma circulating DNA) will be collected for future analysis of circulating markers at the following time points:

- Baseline
  Note: an additional 10 mL of blood in a lavender top EDTA tube will be collected at this time point for germline DNA
- Pre-treatment
- Every 6 months (+/- 1 month) post-initiation of treatment for 5 years (or until disease relapse or end of study participation, whichever comes first)

These samples will be transported to the Siteman Cancer Center Tissue Procurement Core for further processing and storage as described below.

10.4.1 Serum Processing Instructions

Ten mL blood in red top silica clot activator serum tube will be centrifuged at 1200G for 10 minutes at 4°C. The serum should then be stored as 3 to 5 vials of 1 mL aliquots at -70°C.

10.4.2 Plasma Processing Instructions

Ten mL blood in lavender top EDTA tube is mixed several times to ensure adequate anticoagulation and placed on ice. Deliver tube to laboratory within 30 minutes of draw and spin at 1000G for 10 minutes at 4°C. The

plasma will then be aspirated off in 3 to 5 vials of 1 mL aliquots at -70°C.

10.4.3 Germline DNA Processing Instructions

Ten mL blood in lavender top EDTA tube should be transported at room temperature and processed to cell pellet.

10.4.4 Cell-free Plasma DNA Processing Instructions

Ten mL blood in the cell-free DNA BCT (from Streck Clinical Laboratory Products) is immediately mixed by gentle inversion 8 to 10 times and transported at room temperature and processed to cell-free plasma for storage at -70°C. Spin twice to ensure the plasma layer has no cellular contamination.

10.5 Quality of Life Assessments

The EORTC QLQ-C30 and BR23 will be administered by a research coordinator to assess quality of life at the following time points:

- Before initiation of chemotherapy
- At one year (at the same time as the bone marrow aspiration)

11.0 STUDY CALENDAR

11.1 Arm 1 Study Calendar

If the patient's condition is deteriorating, laboratory evaluations should be repeated no more than 48 hours before the next cycle of therapy.

	Pre-Reg	Baseline	Day 1 of each cycle	Follow-up[a]	Off Study
Informed consent	X				
Demographics	X				
Medical history	X			X	X
Con meds	X		X	X	X
Adverse events	X		X	X	
Physical exam, VS, ECOG PS	X		X	X	X
Height	X				
Weight	X		X		X
CBC w/diff, plts		X[m]	X	X	X
CMP		X[m]	X	X	X
β-HCG[d]		X[m]			X
ECHO (preferred) or MUGA	X[k]	X[k]			
CT/bone scan or PET scan	X[j]	X[j]			
Bone marrow collection	X[e]			X[c]	
Research blood draw	X	X	X[n]		
Tumor tissue		X[h]			
Definitive surgery[f]		X[f]		X[f]	
Portacath placement		X			
Chemotherapy[b]					
QOL assessments		X[m]		X[c]	
Mammogram[g]	X			X	

a. Q 3 mo for yrs 1-2, q 6 mo for yrs 3-5 starting at the end of standard of care chemo or surgery (depending on which is last)
b. Best practice standard of care chemo to be given according to NCCN guidelines. May start any time after the baseline bone marrow collection. See Section 5.3.1 for details.
c. 6-18 months after 1st bone marrow aspiration and, for research blood, one year following that 6-18-month time point.
d. Women of childbearing potential only.
e. After eligibility is confirmed.
f. Surgery may take place prior to initiation of chemotherapy, at any point during standard of care chemo, or at the end of standard of care chemo.
g. Annual mammograms for patients with remaining breast tissue
h. Archival tissue required, fresh tissue optional.
j. This imaging need only occur once at either the pre-registration or baseline time point.

k. Only one ECHO or MUGA need take place; this must occur no more than 4 weeks prior to randomization.
m. Must occur no more than 4 weeks prior to initiation of chemotherapy
n. Q 6 mo after start of treatment for 5 yrs.

11.2 Arm 2 Study Calendar

In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Reg	Baseline	Active Treatment		Follow-up[a]	1 year from the date of trastuzumab completion	Off Study
			Day 1 of each cycle	12 mo after start of trastuzumab			
Informed consent	X						
Demographics	X						
Medical history	X				X	X	X
Con meds	X		X		X	X	X
Adverse events	X		X	X	X		
Physical exam, VS, ECOG PS	X		X[q]		X	X	X
Height	X						
Weight	X		X				X
CBC w/diff, plts		X[o]	X[r]	X	X	X	X
CMP		X[o]	X[r]	X	X	X	X
β-HCG[e]		X[o]	X[e]			X	X
ECHO (preferred) or MUGA[b]	X[n]	X[n]	X[b]		X[b]		
CT/bone scan or PET scan	X[m]	X[m]					
Bone marrow collection	X[f]			X[g]			
Research blood draw	X	X	X[p]				
Tumor tissue		X[k]					
Definitive surgery[h]							
Portacath placement and removal		X		X			
Chemotherapy[c]							
Trastuzumab[d]							
QOL assessments		X[o]		X[g]			
Mammogram[j]	X				X		

a. Q 3 mo for yrs 1-2, q 6 mo for yrs 3-5 starting at the end of standard of care chemo or surgery (depending on which is last).
b. Repeat LVEF using the same methodology as at baseline will be obtained every 3 months following initiation of trastuzumab, or every 4 weeks if trastuzumab is held for significant left ventricular cardiac dysfunction. LVEF measurements should be taken after completion of trastuzumab as clinically indicated.
c. Best practice standard of care chemo to be given according to NCCN guidelines. May start any time after the baseline bone marrow collection. See Section 5.3.1 for details.
d. Patients randomized to receive trastuzumab must have a minimum of 8 weeks of overlap with standard of care

chemo and trastuzumab. Trastuzumab is to be administered first on days when it is given with standard of care chemo. See Section 5.3.2 for details.
e. Women of childbearing potential only – at screening and within 7 days of the first dose of trastuzumab.
f. After eligibility is confirmed.
g. 6-18 months after 1st bone marrow aspiration.
h. Surgery may take place prior to initiation of chemotherapy, at any point during standard of care chemo, or at the end of standard of care chemo.
j. Annual mammograms for patients with remaining breast tissue
k. Archival tissue required, fresh tissue optional.
m. This imaging need only occur once at either the pre-registration or baseline time point.
n. Only one ECHO or MUGA need take place; this must occur no more than 4 weeks prior to randomization.
o. Must occur no more than 4 weeks prior to initiation of chemotherapy.
p. Q 6 mo after start of treatment for 5 yrs.
q. Patient should be seen on Day 1 of each cycle while receiving SOC chemo, but may be seen every 3rd cycle once SOC chemo has concluded and patient is receiving trastuzumab only.
r. CBC and CMP should be drawn on Day 1 of each cycle while receiving SOC chemo, and then every 3rd cycle once SOC chemo has concluded and patient is receiving trastuzumab only.

12.0 DATA SUBMISSION SCHEDULE

Form	Submission schedule
Original Consent Form Bone Marrow Form	Prior to registration
Eligibility Checklist On Study Form Prior Therapy Form Research Blood Form EORTC QLQ-C30 Form EORTC BR23 Form	At baseline
Randomization Form	Time of randomization
On Study Standard Chemo Summary Form	End of standard chemotherapy
Trastuzumab Treatment Record	Within 4 weeks of each cycle during trastuzumab therapy
Bone Marrow Form EORTC QLQ-C30 Form EORTC BR23 Form	6-18 months after first BM aspiration 1 year after second BM aspiration (blood only)
Research Blood Form	Screening Baseline Every 6 months for 5 years after start of treatment
Treatment Summary Form	End of treatment
Follow-up Form	Every 3 months for the first 2 years, then every 6 months for the next 3 years
Adverse Events Form	ongoing
SAE Reporting Form	At time of any SAE

13.0 MEASUREMENT OF EFFECT

The primary endpoints are 3 year recurrence rate and 3 year death rate. Disease recurrence is defined as the documented appearance of local (breast, chest wall, axillary, supraclavicular nodes) or distant disease. Assessment for recurrence will begin at the time at which the patient is considered to be disease-free; for the purposes of this protocol, this time will be the time point at which the patient has completed both standard chemotherapy and definitive surgery. Surgery may take place either before, during, or

after standard chemotherapy. Patients will be followed for 3 years after the completion of standard chemotherapy + surgery for the detection of any recurrent disease. For patients randomized to Arm 2, some of this assessment for recurrent disease will take place during the time when they are receiving trastuzumab.

The secondary endpoints of this study are the effectiveness of trastuzumab in clearing *ERBB2*-positive bone marrow DTCs. Bone marrow DTCs are evaluated by RT-PCR performed on specimens collected 6-18 months apart (one before and one after therapy). The proportion of samples turned negative after therapy will be calculated. Samples will be considered negative for ERBB2-expression if expression from bone marrow collected from each iliac crest is less than 2 standard deviations above the ERBB2-level in pooled normal bone marrow specimens.

The exploratory endpoints are the primary tumor molecular subtype by PAM50 assay, DTC-specific gene expression by multiplex gene expression analyses and response to trastuzumab. Their association with BM expression of ERBB2, response to trastuzumab and treatment outcome (disease progression and death) will be examined.

14.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark). This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by arm
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by arm
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study Endpoints

The primary objective is to determine the 3-year recurrence and death rates in patients treated with trastuzumab administered with chemotherapy for 12 months versus standard chemotherapy alone. The primary outcome measures for each patient are: 1) disease free survival, defined as the time from completion of standard chemotherapy + definitive surgery to the detection of local or distant recurrence, death, or last follow-up; and 2) time to death, defined as time from diagnosis to death from any cause or last follow-up. The secondary endpoint is *ERBB2* expression (positive vs. negative) in bone marrow samples from right and left iliac crests after one year of therapy. All eligible patients will have detectable *ERBB2* overexpression in the bone marrow at time of enrollment, so baseline ERBB2 status is not an endpoint. Exploratory endpoints include molecular breast cancer subtype measured by PAM50 assay, determination of DTC genes associated with recurrent disease development and response to trastuzumab and treatment outcome.

15.2 Justification of Sample Size

Based on our preliminary data, we estimated that 25% of stage II/IIIHer2-negative breast cancer patients harbor *ERBB2*-positive DTCs in their bone marrow. Approximately 200 patients were expected to have their bone marrow screened for *ERBB2*-positive DTC to enroll 50 patients into the trial. Less than 10% of patients were expected to be lost to follow-up before primary and secondary endpoints were measured, leaving 46 patients for analysis of primary and secondary endpoints. Based on the literature and preliminary data, the expected 3-year recurrence rate is 75% in the standard chemotherapy arm and 40% in the trastuzumab arm.

15.2.1 Revised Sample Size Justification without Interim Analysis

All patients who meet screening requirements during the next 12 months will be enrolled in addition to the 5 patients already on study. No more than one patient is expected to be lost to follow-up, providing 10-12 patients for analysis of primary and secondary endpoints. The primary endpoint is 3 year progression free survival, estimated to be 25% without trastuzumab and 60% with trastuzumab. The power to detect this difference will be < .50, given a two-sided log rank test at a .05 significance level (power ~ .30 with 13 patients, .25 with 10 patients and .20 with 8 patients).

The detectable difference with 12 patients, assuming balanced or nearly balanced numbers in the two arms, is a hazard ratio ~ .076, corresponding to 25% 3 year PFS in the no-trastuzumab arm vs. 90% in the trastuzumab arm.

The principal secondary endpoint is the proportion of patients who have no evidence of disseminated tumor cells in their bone marrow. The expected proportion to eliminate DTCs is <10% in the no-trastuzumab arm and >80% in the trastuzumab arm. Given 12 patients, 6 in each arm, a one-sided Fisher's Exact test with significance level .05 will have power = .73 to detect this difference and power = .82 to detect a 5% greater difference (5% vs. 80% or 10% vs. 85%).

15.3 Analysis Plan

15.3.1 Original Analysis Plan

Cox proportional hazards models were to be used to estimate 3-year recurrence and death rates adjusted for the 4 stratification factors. Median time to recurrence and time to death was also to be calculated by these models, as were hazard ratios by study arm. Based on our data and the published DFS advantage observed with trastuzumab treatment, we estimated the expected 3-year recurrence rate will be 75% in the placebo arm and 40% in the trastuzumab arm. One interim analysis was planned after the first 15 patients (~ 1/3 of the total) had completed 3 years of follow-up after the end of standard chemotherapy + definitive surgery and 3 year recurrence rates had been determined. Conditional study power was to be used to evaluate the estimates on which study power has been calculated, and the sample size was to be adjusted, if necessary. Analysis of the primary endpoints was to be carried out at a significance level of 0.0052 for the interim analysis and 0.048 for the final analysis in order to preserve an overall significance level of 0.05.

Logistic regression was to be used to compare the proportion of patients who eliminate *ERBB2*-positive DTCs from BM in the two study arms adjusted for the four stratification factors. Heatmaps, cluster diagrams and histograms were to be used to display expression changes by study arm and *ERBB2* status. Change in the 38-gene signature was to be analyzed. Based on Fisher's Exact test (because the smaller sample size may provide sparse or empty cells), the minimum detectable difference was estimated to be 60% (if ~10% are expression positive), 43% (if 30% expression positive) or 40% (if 50% expression positive).

A linear model or nonparametric alternative was to be used to compare mean or median expression of ERBB2 expression in BM, by molecular subtype. Stratification factor-adjusted Cox proportional hazards models were to be used to compare the hazard of recurrent disease and death of any cause by molecular subtype. The frequency of patients with *ERBB2* positive DTCs in each primary tumor PAM50 Her2 subtype was to be tabulated, plotted using histograms and described with a relative risk of remaining *ERBB2* positive and 95% confidence interval. Difference of proportions was to be tested with Fisher's Exact test and with stratification-adjusted logistic regression models.

15.3.2 Current Analysis Plan

Unstratified Cox proportional hazards regression will be used to estimate the primary endpoint, 3-year progression-free survival, with 95% confidence intervals in the two study arms. If median times are reached, medians time to recurrence and time to death will be calculated along with hazard ratios and 95% confidence by study arm. The principal secondary endpoint is the proportion of patients who eliminate *ERBB2*-positive DTCs from BM. Fisher's Exact test will be used to compare the proportion of patients who eliminate *ERBB2*-positive DTCs from BM in the two study arms. Heatmaps, cluster diagrams and histograms may be used to display expression changes by study arm and *ERBB2* status. Change in the 38-gene signature will be examined in an exploratory fashion gene by gene.

15.4 Timeline and Feasibility

15.4.1 Original Timeline and Feasibility

We evaluate and treat approximately new 500 patients with early stage breast cancer each year at Siteman Cancer Center (data from year 2008). Among these patients, approximately 350 patients each year will have HER2- disease (data from year 2008) and can be offered the trial. Conservatively, if 25% of these patients (87 patients) are pre-registered for the bone marrow testing, we can enroll approximately 21 patients (25% of patients) with ERBB2 positive bone marrow DTCs each year. In addition, since the interest to this trial for this high risk population will likely be high, we anticipate that a significant number of patients will be referred from community oncologists for consideration of this trial. Therefore, the accrual goal of 50 patients with positive DTCs to this trial will be easily reached in 2.5 years.

15.4.2 Current Timeline and Feasibility

Once the study reopens for accrual all patients who meet screening requirements during the 12 months will be enrolled. We evaluate and treat approximately 500 patients with early stage breast cancer each year at the Siteman Cancer Center (data from 2008). Among these patients approximately 350 patients each year are expected to have HER2 negative disease. Conservatively, if 25% of these patients (87 patients) are pre-registered for bone marrow testing and 10%-15% have confirmed presence of DTCs at diagnosis, the study can enroll 8-13 new patients during the next year.

15.5 Stopping Rules

15.5.1 Original Stopping Rule

A 2012 meta-analysis of five major trials plus three others enrolling 11,991 women with HER2-positive early breast cancer confirmed a significantly increased risk for severe heart failure (2.5 versus 0.4 percent, relative risk [RR] 5.11, 90% CI 3.00-8.72) and reduction in left ventricular ejection

fraction (RR 1.83, 90% CI 1.36-2.47) in patients treated with trastuzumab versus non-trastuzumab-based adjuvant or neoadjuvant chemotherapy. There was no difference in the cardiotoxicity profile in trials with concurrent as compared to sequential administration of chemotherapy and trastuzumab.

Based on these data, the incidence of severe cardiac dysfunction would be 0.1 in the 25 patients with no trastuzumab; that is, 1/10th of 1 event. It would be 0.625 in the 25 patients with trastuzumab (or about 6/10ths of 1 event). Based on these estimates of risk, the probability of 0 events is 0.53 (slightly greater than ½) in the trastuzumab arm and 0.97 (about 97 in 100) in the control arm. The probability of >2 events in the trastuzumab arm is .024 (or 2-3 chances in 100) and .0001 (about 1 in 10,000) in the control arm.

If the relative risk is higher than expected, for example, as high as 9 (the upper limit of the 95% confidence interval for the relative risk), the risk in the trastuzumab group would be about 3.6, instead of 2.5, and the probability of > 2 events is .059 (about 6 in 100). The study would expect to see 0.9 events (9/10th of 1 event). That is, at the maximum estimated risk, there might be 1 event in the trastuzumab group, whereas it's unlikely that any will be observed in the no-trastuzumab group. Based on this analysis, if more than 2 patients in the treatment arm develop severe cardiac dysfunction the study will be halted.

15.5.2 Current Stopping Rule

A 2012 meta-analysis of five major trials plus three others enrolling 11,991 women with HER2-positive early breast cancer confirmed a significantly increased risk for severe heart failure (2.5 versus 0.4 percent, relative risk [RR] 5.11, 90% CI 3.00-8.72) and reduction in left ventricular ejection fraction (RR 1.83, 90% CI 1.36-2.47) in patients treated with trastuzumab versus non-trastuzumab-based adjuvant or neoadjuvant chemotherapy. There was no difference in the cardiotoxicity profile in trials with concurrent as compared to sequential administration of chemotherapy and trastuzumab.

Based on these data, the incidence of severe cardiac dysfunction would be .024-.028 in the 6-7 patients with no trastuzumab; that is, 2/100ths-3/100ths of 1 event. It would be .15-.175 in the 6-7 patients with trastuzumab (or about 1/10th-2/10ths of 1 event). Based on these estimates of risk, the probability of 0 events is .84-.86 in the trastuzumab arm and .97-.98 in the control arm. The probability of >2 events in the trastuzumab arm is .009-.012 (or 1 chance in 100) and .0002-.0003 (about 2-3 in 10,000) in the control arm.

If the relative risk is higher than expected, for example, as high as 9 (the upper limit of the 95% confidence interval for the relative risk), the risk in the trastuzumab group would be about 3.6, instead of 2.5, and the probability of > 2 events is .018-.024 (about 2-3 in 100). The study would expect to see .2 events (2/10th of 1 event). That is, at the maximum estimated risk, there might be 1 event in the trastuzumab group, whereas

it’s unlikely that any will be observed in the no-trastuzumab group. Based on this analysis, if more than 2 patients in the treatment arm develop severe cardiac dysfunction the study will be halted.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: HFSA Guidelines
Recommendations for Pharmacological Therapy: Left Ventricular Systolic Dysfunction

ß-Adrenergic Receptor Blockers

Background for Recommendations

The single most significant addition to the pharmacological management of heart failure since the publication of previous guidelines involves the use of ß-receptor antagonists. This represents a noteworthy departure from traditional doctrine in which ß-blocking agents were classified as contraindicated in the setting of left ventricular systolic dysfunction. A solid foundation of both clinical and experimental evidence now firmly supports their use in heart failure with the aim of reducing both morbidity and mortality (16,22,23).

ß-Blocker therapy for heart failure has been advocated by some investigators since the 1970s (24). During the subsequent 2 decades, many small- to medium-sized placebo-controlled trials, which used a variety of agents, showed several common findings: 1) the use of ß-blockers in mild to moderate heart failure was generally safe when initiated at low doses and gradually uptitrated under close observation; 2) improvement in left ventricular ejection fraction was observed in all trials that lasted at least 3 months; and 3) there was wide variability in the effects of ßblockade on exercise tolerance but improvement in outcome and symptomatic benefits was noted in many studies. These generally positive findings stimulated additional, large-scale clinical trials that have provided an impressive body of evidence that supports the use of ß-blockers in patients with heart failure caused by left ventricular systolic dysfunction. The recommendations that follow are derived from nearly 2 decades of research that include basic science data, animal models, and clinical trial experience in over 10,000 patients (25,26).

Although this is a major advance in efficacy, identification of appropriate candidates for ßblocker therapy is essential to ensure safe and effective treatment. Prescribing physicians should understand the potential risks of ß-blocker therapy, as well as the benefits. The interested practitioner who is unfamiliar with ß-blocker initiation and titration may first seek further education and counsel from sources such as the Heart Failure Society of America or local and regional heart failure specialty centers.

> **Recommendation 1. ß-blocker therapy should be routinely administered to clinically stable patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) and mild to moderate heart failure symptoms (ie, NYHA class II-III, Appendix A) who are on standard therapy, which typically includes ACE inhibitors, diuretics as needed to control fluid retention, and digoxin (Strength of Evidence = A).**

The most persuasive outcome in heart failure management remains all-cause mortality. Combined endpoints, including mortality or hospitalization and mortality or hospitalization for heart failure, have also emerged as key outcomes. These latter endpoints reflect a more comprehensive assessment of the influence of therapy on quality of life and disease

progression and are assuming more importance as mortality rates decline with treatment advances. The substantial beneficial effect of ß-blocker therapy on these endpoints has been well shown in clinical trials of symptomatic patients (NYHA class II - III) treated with carvedilol, bisoprolol, or metoprolol controlled release/extended release (CR/XL) (27-29). Trials with these agents encompass the combined, worldwide experience with ß-blocker therapy in patients with chronic
heart failure who were stable on background therapy, including ACE inhibitors (over 90%) and diuretics (over 90%). Digoxin was common as background therapy, particularly in studies conducted in the United States. Trial results indicate that both selective and nonselective ßblockers, with and without ancillary properties, have significant efficacy in heart failure. ß-Blocking agents with intrinsic sympathomimetic activity appear to have a negative impact on survival and should not be used in heart failure patients.

Metoprolol. The MDC Study was an early trial that included 383 patients with heart failure caused by nonischemic causes, NYHA class II-III symptoms, and a left ventricular ejection fraction of less than or equal to 40% (30). Patients with coronary artery disease were excluded. Study results showed a 34% reduction in risk in patients treated with metoprolol, although this strong trend toward benefit (P = .058) was entirely attributable to a reduction in the frequency of cardiac transplantation listing in the treatment group. In fact, the absolute number of deaths in the metoprolol group was higher than in the placebo group (23 *v* 19, P = .69).

The MERIT-HF Trial evaluated the effect of metoprolol CR/XL with all-cause mortality as the primary endpoint. The trial included 3,991 patients with NYHA class II-IV heart failure, although 96% of the study patients were functional class II or III (31). In this study, investigators were allowed to select the starting dose of metoprolol CR/XL. Seventy-nine percent chose 25 mg as the starting dose for class II patients, and 77% chose 12.5 mg for class III-IV patients. The target dose was 200 mg and doses were up-titrated over a period of 8 weeks. Premature discontinuation of blinded therapy occurred in 13.9% of those treated with metoprolol CR/XL and 15.3% of those in the placebo group (P = .90). The study results revealed a 34% reduction in mortality in the metoprolol group (relative risk of .66; 95% confidence interval [CI], .53 to .81; p=.0062 after adjustment for interim analyses), with annual mortality rates of 11% in the placebo

and 7.2% in the metoprolol CR/XL group (29).

Bisoprolol. The CIBIS Study evaluated the effects of bisoprolol in 641 patients with left ventricular systolic dysfunction caused by ischemic or nonischemic causes and NYHA class III-IV heart failure (32). The primary endpoint was all-cause mortality, and hospitalization for worsening heart failure was one of the secondary outcomes of interest. The initial bisoprolol dose was 1.25 mg/day, which was increased to a maximum dose of 5 mg/day. The trial found no significant reduction in all-cause mortality in patients treated with bisoprolol (20% reduction bisoprolol *v* placebo, P = .22) (32). The risk of hospitalization was significantly reduced by 34% (28% placebo group *v* 19% bisoprolol group, $P < .01$).

The favorable trends seen in CIBIS led to the larger CIBIS II Study, which ultimately was prematurely terminated as a result of a significant reduction in mortality in the bisoprolol arm (28). These results were obtained in 2,647 patients who were followed for

an average of 1.3 years. Over 80% of the patients were judged to be NYHA class III at enrollment. Background therapy included ACE inhibitors in 96% and diuretic in 99% of the study patients, whereas 52% were taking digoxin. In contrast to the original CIBIS study, CIBIS II had a similar starting dose of 1.25 mg but had a greater target dose of 10 mg daily of bisoprolol. More stringent criteria for defining ischemic cardiomyopathy were used. Treatment with bisoprolol reduced the annual mortality rate by 34% (13.2% placebo *v* 8.8% bisoprolol; hazard ratio .66; 95% CI, .54 to .81; *P*< .0001). Hospitalizations for worsening heart failure were also decreased by 32% (18% placebo *v* 12% bisoprolol, hazard ratio .64; 95% CI, .53 to .79; *P*< .0001). Although a post hoc
analysis of the CIBIS Study had suggested benefit might be consigned to patients without coronary disease, the survival benefit, with significant reductions apparent in both ischemic or nonischemic patients, was not influenced by disease origins.

Carvedilol. Carvedilol, a nonselective ß-blocker and α-blocker, has been extensively investigated for treatment of heart failure caused by left ventricular systolic dysfunction. In the United States carvedilol trials, 4 separate study populations were examined and the data from 1,094 patients were combined to evaluate the effect of carvedilol therapy on the clinical progression of heart failure (27). Clinical progression was defined as worsening heart failure leading to death, hospitalization, or, in one study, a sustained increase in background medications. Patients with a left ventricular ejection fraction of 35% or less and NYHA class II-IV were eligible if they tolerated 6.25 mg of carvedilol twice per day for a 2-week, open-label, run-in period. Although this run-in phase biased the ultimately randomized patient population, less than 8% of eligible patients failed the open-label challenge. Target dosages for the studies were 50 to 100 mg/day of carvedilol that were administered in divided doses twice daily. Patients completing the run-in period were randomized based on results from their 6- minute walk test into mild, moderate, or severe trials. These studies were prematurely terminated (median follow-up 6.5 months) by the Trial Data and Safety Monitoring Board because of reduced mortality across the 4 combined trials of patients treated with carvedilol.

Data from these combined trials indicated a substantial benefit from carvedilol treatment. The risk of mortality was 65% lower (7.8% placebo *v* 3.2% carvedilol; 95% CI, 39% to 80%; *P*< .001) and the combined risk of hospitalization or death was reduced by 38% (20% on placebo *v* 14% on carvedilol; 95% CI, 18% to 53%; *P*< .001). A significant mortality reduction was also noted when deaths that occurred in the run-in period were included in the analysis. The statistical validity of the survival analysis across the trials has been questioned because mortality was not the primary endpoint, and only 1 of the 4 trials achieved a significant result when analyzed based on the primary endpoint. Nevertheless, the magnitude of the survival benefit and the reduction in hospitalization were impressive. The survival benefit was not influenced by the cause of disease, age, gender, or baseline ejection fraction. Overall, 7.8% of the placebo group and 5.7% of the carvedilol group discontinued study medication. Data from the individual trials, PRECISE and MOCHA, which evaluated patients with moderate to severe heart failure, found that carvedilol reduced the risk of the combined endpoint of mortality or heart failure hospitalization by 39% to 49% (33,34). The MOCHA Study provided strong evidence for increased benefit from higher dosages (25 mg twice per day) versus lower dosages (6.25 mg twice per day) of carvedilol, so uptitration of carvedilol dosages to 25 mg twice per day is generally recommended. However, favorable effects were noted at 6.25 mg twice per day, so intolerance of high doses should not be a reason for discontinuation of therapy.

The Australia-New Zealand Carvedilol Trial enrolled 415 patients with ischemic cardiomyopathy and a left ventricular ejection fraction of less than 45% (35). Although patients with NYHA functional classes I-III were eligible, the majority enrolled were NYHA functional class I (30%) or II (54%). ACE inhibitors were used in 86% of the participants, whereas 76% were on diuretic therapy, and 38% were on digoxin. This trial also had a run-in phase during which 6% of the patients discontinued ß-blocker therapy. During an average follow-up of 19 months, carvedilol decreased the combined risk of all-cause mortality or any hospitalization by 26% (relative risk .74; 95% CI, .57 to .95; *P*= .02). Overall mortality was 12.5% in the placebo group and 9.6% in the carvedilol group which was not statistically significant (relative risk .76; 95% CI, .42 to 1.36; *P* > .10).

Unreported or Ongoing Trials. Studies that are underway will provide additional data concerning specific aspects of the efficacy of ß-blocker therapy in heart failure. The effect of bucindolol on mortality and morbidity in patients with moderate to severe heart failure has been evaluated in the BEST Study. This study enrolled a substantial number of women so the potential influence of gender on the efficacy of ß-blocker therapy can be investigated. The trial has been stopped, and no results are available for analysis.

The COPERNICUS Trial is designed to assess the effect of carvedilol treatment on disease progression and survival in patients with advanced heart failure with symptoms at rest or on minimal exertion. The COMET protocol is a 3,000 patient study that directly compares the survival benefit of carvedilol versus metoprolol. This trial will provide important data concerning the relative efficacy of a selective ß-blocker versus a nonselective ß-blocker with ancillary properties.

Recommendation 2. ß-blocker therapy should be considered for patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) who are asymptomatic (ie, NYHA class I) and standard therapy, including ACE inhibitors (Strength of Evidence = C).

Data from the SOLVD Prevention Trial prospectively illustrated the efficacy of ACE inhibitors in delaying the onset of heart failure symptoms and the need for treatment or hospitalization for heart failure in asymptomatic patients with a left ventricular ejection fraction less than or equal to 35% (36). Similar controlled, clinical trial data that support the use of a ß-blocker in this clinical circumstance are not available. However, significant support for the use of ßblocker therapy in patients with asymptomatic left ventricular dysfunction can be derived from clinical trials in coronary artery disease and hypertension. Previous data indicate that ß-blocker therapy should be used in patients after myocardial infarction (MI) and in patients with myocardial revascularization who have good symptomatic and functional recovery but residual ventricular systolic dysfunction. Trials in hypertension indicate that ß-blocker therapy decreases the risk of developing heart failure. Given the potential of ß-blockers to retard disease progression and improve ventricular function, the risk to benefit ratio seems sufficiently low to support ß-blocker use in asymptomatic patients with left ventricular dysfunction, especially when the dysfunction is marked, and coronary artery disease is present.

Recommendation 3. To maximize patient safety, a period of clinical stability on standard therapy should occur before ß-blocker therapy is instituted. Initiation of ß-blocker therapy in patients with heart failure requires a careful baseline evaluation of clinical status (Strength of Evidence = B).

Initiation of ß-blocker therapy has the potential to worsen heart failure signs and symptoms. This risk increases with the underlying severity of the heart failure that is present. To minimize the likelihood of worsening failure, a period of treatment with standard therapy and evidence of clinical stability without acute decompensation or fluid overload is recommended before initiation of ß-blocker therapy. The majority of the large-scale, ß-blocker heart failure trials required that chronic heart failure be present 3 months or more before initiation of ß-blocker therapy. Patients enrolled in these trials were typically treated with ACE inhibitors (if tolerated), diuretic, and digoxin for at least 2 months and were observed to be clinically stable for 2 to 3 weeks before beginning ß-blocker therapy. Thus, many heart failure clinicians favor a minimum of 2 to 4 weeks of clinical stability on standard therapy before ß-blocker therapy is instituted. Likewise, most clinicians discourage the initiation of ß-blocker therapy in the hospital setting after treatment for new or decompensated heart failure (with or without associated inotrope administration). Some experienced clinicians initiate ß-blocker therapy in the hospital in selected patients who have responded well to inpatient treatment and who can be followed closely after discharge.

Recommendation 4. There is insufficient evidence to recommend the use of ß-blocker therapy for inpatients or outpatients with symptoms of heart failure at rest (ie, NYHA class IV) (Strength of Evidence = C).

ß-Blocker therapy cannot be routinely recommended for NYHA class IV patients because there are currently no clinical trial data to indicate favorable long-term efficacy and safety of ßblocker therapy in this patient population. A substantial body of observational data indicates that successful institution of ß-blocker therapy in patients with this degree of heart failure is problematic. If used, these agents may precipitate deterioration, and patients so treated should be monitored by a physician who has expertise in heart failure.

The number of patients with class IV heart failure at the time of ß-blocker initiation in controlled clinical trials is small. Available trials, which report data on patients with severe heart failure mostly labeled as NYHA class III, show the potential problems of ß-blocker therapy in this part of the heart failure spectrum. This experience is reflected in a 14-week study that evaluated the effects of ß-blocker therapy in 56 patients (51 NYHA class III and 5 NYHA class IV at randomization) with severe left ventricular dysfunction (average left ventricular ejection fraction of 16% ± 1% and left ventricular filling pressure of 24 mm Hg ± 1 mm Hg) (37). These patients had significant impairment of exercise capacity (mean VO2 max of 13.6 mL/kg/min ± 0.6 mL/kg/min) despite ACE-inhibitor, digoxin and diuretic therapy. Patients were believed to be clinically stable (requiring no medication adjustments) for a 2-week period before an open-label challenge was conducted. Seven patients (12%) failed to complete the open-label, run-in period, during which 5 died and 2 had nonfatal adverse reactions. Clinical parameters did not distinguish these patients from

those who were able to continue in the trial. Eighteen of the 49 patients (37%) completing the run-in period experienced worsened dyspnea or fluid retention during this phase. Also, 22% experienced dizziness and required medication adjustment, which delayed up-titration during the run-in. Subsequently, an additional 12% of the patients randomized to carvedilol withdrew from the blinded arm of the study. One of the United States carvedilol trials studied patients with severe left ventricular dysfunction who had markedly reduced exercise capacity as assessed by the 6-minute walk test (38). In this trial, 131 patients with a mean left ventricular ejection fraction of 22% and severe impairment in quality of life underwent a 2-week, open-label challenge phase of 6.25 mg of carvedilol twice per day. Ten of these 131 patients (8%) were unable to complete this run-in phase, most because of worsening heart failure, dyspnea, or dizziness. Subsequently, 11% of the patients randomized to carvedilol withdrew, as did a similar number of patients (11%) in the placebo group. In the recently completed large-scale BEST Trial, the mortality trend in NYHA class III-IV patients favored the ß-blocker bucindolol, but the difference from placebo was not significant. Further analysis of these preliminary findings is necessary, but the data suggest that the striking benefit of ß-blockers in mild-to-moderate heart failure may not be extrapolated to those with severe symptoms.

Recommendation 5. ß-Blocker therapy should be initiated at low doses and up-titrated slowly, generally no sooner than at 2-week intervals. Clinicalreevaluation should occur at each titration point and with worsening of patient symptoms. Patients who develop worsening heart failure or other side effects after drug initiation or during titration require adjustment of concomitant medications. These patients may also require a reduction in ß-blocker dose and, in some cases, temporary or permanent withdrawal of this therapy (Strength of Evidence = B).

ß-Blocker therapy should be initiated at doses substantially less than target doses. Clinical trials required patient reassessment at up-titration of each dose. This careful evaluation by trained nurses and/or heart failure specialists likely contributed to the relatively low withdrawal rates and safety profiles observed in the clinical trials.

Treatment for symptomatic deterioration may be required during ß-blocker titration, but with appropriate adjustments in therapy, most patients can be maintained and generally achieve target doses. There is a risk of worsening heart failure, and vasodilatory side effects may occur with certain agents. Worsening heart failure is typically reflected by increasing fatigue, lower exercise tolerance, and weight gain. Increased diuretic doses may be required for signs and symptoms of worsened fluid retention. Treatment options also include temporary down-titration of the ß-blocker to the last tolerated dose. Abrupt withdrawal should be avoided. A minimum period of stability of 2 weeks should occur before further up-titration is attempted. Hypotensive side effects may often resolve with reduction in diuretic dose. Temporary reductions in ACE inhibitor dose may be helpful for symptomatic hypotension not obviated by staggering the schedule of vasoactive medications. Administration of carvedilol with food may alleviate vasodilatory side effects as well.

If ß-blocker treatment is interrupted for a period exceeding 72 hours and the patient is still judged a candidate for this therapy, drug treatment should be reinitiated at 50% of the previous dose. Subsequent up-titration should be conducted as previously described.

Recommendation 6. In general, patients who experience a deterioration in clinical status or symptomatic exacerbation of heart failure during chronic maintenance treatment should be continued on ß-blocker therapy (Strength of Evidence = C).

Clinical decompensation that occurs during stable maintenance therapy is less likely caused by chronic ß-blocker therapy than other factors (diet or medication noncompliance, ischemia, arrhythmia, comorbid disease, infection, or disease progression). In these situations, maintaining the current ß-blocker dose while relieving or compensating for the precipitating factor(s) is most often the best course. Data from patients randomized to continue or discontinue ß-blocker therapy in this setting are not currently available. However, studies of the withdrawal of ß-blocker therapy in patients with persistent left ventricular systolic dysfunction but improved and stable clinical heart failure have revealed a substantial risk of worsening heart failure and early death after discontinuation of ß-blocker therapy (39,40).

Recommendation 7. Patient education regarding early recognition of symptom exacerbation and side effects is considered important. If clinical uncertainty exists, consultation with clinicians who have expertise in heart failure and/or specialized programs with experience in ß-blocker use in patients with heart failure is recommended (Strength of Evidence = B).

In certain patients, frequent return visits for dose-titration may be difficult to accommodate in a busy clinical practice. Trained personnel, including nurse practitioners, physicians' assistants, and pharmacists with physician supervision, may more efficiently perform patient education and reevaluation during up-titration. Heart failure specialty programs are more likely to have the resources to provide this follow-up and education (41). Consultation or referral may be particularly beneficial when the clinical heart failure status of the patient is uncertain or problems arise during initiation of therapy or dose-titration that may cause unwarranted discontinuation of therapy. Ideal patients for ß-blocker therapy should be compliant and have a good understanding of their disease and their overall treatment plan. Patients should be aware that symptomatic deterioration is possible early in therapy and that symptomatic improvement may be delayed for weeks to months.

Unresolved Therapeutic Issues

Combining ß-Blocking Agents With Amiodarone Therapy. Concomitant use of amiodarone was generally precluded in the trials evaluating carvedilol and most other ß-blockers. However, the use of this agent for rate control of atrial arrhythmia or for maintenance of sinus rhythm is common in heart failure patients. Drug interactions between ß-blockers and amiodarone are possible, including symptomatic bradycardia, and may limit the maximum tolerated dose of the ß-blocker. When the combination is used, the smallest effective dose of amiodarone should be employed. Given the lack of a clear survival benefit, amiodarone is not a substitute for ß-blocker therapy in heart failure patients who are candidates for this therapy.

Implantation of Cardiac Pacemakers. Given the strength of evidence that supports ß-blocker therapy in patients with symptomatic heart failure, some physicians would consider

pacemaker implantation when symptomatic bradycardia or heart block occur during the initiation of this therapy, although no data are available to support such use. Consideration should be given, after weighing risks and benefits, to the withdrawal of other drugs that may have bradycardia effects.

Duration of Therapy. Whether patients experiencing marked improvement in left ventricular systolic dysfunction and heart failure symptoms during therapy can be successfully withdrawn from ß-blocker therapy remains to be established. Concern continues that such patients would experience worsening after ß-blocker withdrawal, either in systolic function or symptoms, over a time period that is undefined. Until clinical trial data indicate otherwise, the duration of ßblocker therapy must be considered indefinite.

Digoxin

Background for Recommendations

Although little controversy exists as to the benefit of digoxin in patients with symptomatic left ventricular systolic dysfunction and concomitant atrial fibrillation, the debate continues over its current role in similar patients with normal sinus rhythm. Recent information regarding digoxin's mechanism of action and new analyses of clinical data from the DIG Trial and the combined PROVED and RADIANCE Trial databases provide additional evidence of favorable efficacy that was unavailable to previous guideline committees (42-47). In fact, this information has recently formed the basis of Food and Drug Administration (FDA) approval of digoxin for the treatment of mild to moderate heart failure (48). Digoxin, a drug that is inexpensive and can be given once daily, represents the only orally effective drug with positive inotropic effects approved for the management of heart failure. The committee's consensus is that digoxin, when used in combination with other standard therapy, will continue to play an important role in the symptomatic management of the majority of patients with heart failure.

The efficacy of digoxin for the treatment of heart failure caused by systolic dysfunction has traditionally been attributed to its relatively weak positive inotropic action that comes from inhibition of sodium-potassium adenosine triphosphatase (ATPase) that results in an increase in cardiac myocyte intracellular calcium. However, in addition to positive inotropy, digitalis has important, neurohormonal-modulating effects in patients with chronic heart failure, including a sympathoinhibitory effect that cannot be ascribed to its inotropic action (49,50). Digoxin also ameliorates autonomic dysfunction as evidenced by studies of heart rate variability, which indicates increased parasympathetic and baroreceptor sensitivity during therapy (51).

> **Recommendation 1. Digoxin should be considered for patients who have symptoms of heart failure (NYHA class II-III, Strength of Evidence = A and NYHA class IV, Strength of Evidence = C) caused by left ventricular systolic dysfunction while receiving standard therapy.**

Digoxin increases left ventricular ejection fraction and alleviates symptomatic heart failure as evidenced by drug-related improvement in exercise capacity and reductions in heart-failureassociated hospitalization and emergency room visits. Digoxin should be used in conjunction with other forms of standard heart failure therapy including ACE inhibitors, diuretics and ß-blockers.

The DIG Trial, a randomized, double-blind, placebo-controlled trial in over 7,000 patients with heart failure, showed a neutral effect on the primary study endpoint and mortality from any cause during an average follow-up of approximately 3 years (42). In the main trial, 6,800 patients with left ventricular ejection fraction less than or equal to 45% were randomized to digoxin or placebo, in addition to diuretics and ACE inhibitors. A total of 1,181 deaths occurred on digoxin (34.8%) and 1,194 on placebo (35.1%) for a risk ratio of .99 (95% CI, .91 to 1.07; $P = .80$). These results differ from other oral agents with inotropic properties that have been associated with an adverse effect on mortality. In addition, the need for hospitalization and cointervention (defined as increasing the dose of diuretics and ACE inhibitors or adding new therapies for worsening heart failure) was significantly lower in the digoxin group, even in those patients who were not previously taking digoxin. Fewer patients on digoxin compared with placebo were hospitalized for worsening heart failure (26.8%*v* 34.7%; risk ratio .72; 95% CI, .66 to .79; $P < .001$). These long-term data are consistent with recent results obtained from an analysis of the combined PROVED and RADIANCE databases (45). In this analysis, patients who continued digoxin as part of triple therapy with diuretics and an ACE inhibitor were much less likely to develop worsening heart failure (4.7%) than those treated with a diuretic alone (39%, $P < .001$), diuretic plus digoxin (19%, $P = .009$) or diuretic plus an ACE inhibitor (25%, $P = .001$).

Although there are no clinical trial data (level A evidence) for the efficacy of digoxin in patients with NYHA Class IV heart failure, there is evidence that digoxin works across the spectrum of left ventricular systolic dysfunction. A prespecified subgroup analysis of patients enrolled in the DIG Trial with evidence of severe heart failure (as manifested by left ventricular ejection fraction less than 25%, or cardiothoracic ratio [CTR] greater than .55) showed the benefit of digoxin (48). The following reductions in the combined endpoint of all-cause mortality or hospitalization were seen on digoxin compared with placebo: 16% reduction (95% CI, 7% to 24%) in patients with a left ventricular ejection fraction of less than 25%, and a 15% reduction (95% CI, 6% to 23%) in patients with a CTR of greater than .55 (43). Reductions in the risk of the combined endpoint of heart-failure related mortality or hospitalization were even more striking: 39% (95% CI, 29% to 47%) for patients with left ventricular ejection fraction less than 25%, and 35% (95% CI, 25% to 43%) for patients with a CTR greater than .55 (48).

Evidence for the efficacy of digoxin in patients with mild symptoms of heart failure has been provided by a recent retrospective, cohort analysis of the combined PROVED and RADIANCE data (52). The outcome of patients in these trials who were randomized to digoxin withdrawal or continuation was categorized by using a prospectively obtained heart failure score based on clinical signs and symptoms. Patients in the mild heart failure group (heart failure score of 2 or less) who were randomized to have digoxin withdrawn were at increased risk of treatment failure and had deterioration of exercise capacity and left ventricular ejection fraction compared with patients who continued digoxin (all $P < .01$). Patients in the moderate heart failure group who had digoxin withdrawn were significantly more likely to experience treatment failure than either patients in the mild heart failure group or patients who continued digoxin (both $P < .05$). These data suggest that patients with left ventricular systolic dysfunction benefit from digoxin despite only mild clinical evidence of heart failure.

In summary, a large body of evidence supports the efficacy of digoxin in patients with symptomatic heart failure caused by left ventricular systolic dysfunction. Digoxin has been shown to decrease hospitalizations, as well as emergency room visits; decrease the need

for cointervention; and improve exercise capacity (42-44,53,54). Taken as a whole, these clinical trial data provide support for digoxin's beneficial effect on morbidity and neutral effect on mortality (42).

Recommendation 2. In the majority of patients, the dosage of digoxin should be .125 mg to .25 mg daily (Strength of Evidence = C).

Recent data suggest that the target dose of digoxin therapy should be lower than traditionally assumed. Although higher doses may be necessary for maximal hemodynamic effects (55), beneficial neurohormonal and functional effects appear to be achieved at relatively low serum digoxin concentrations (SDC) typically associated with daily doses of .125 mg to .25 mg of digoxin (55-57). The utility of lower SDC is supported by recent clinical trial data; the mean SDC achieved in the RADIANCE Trial was 1.2 ng/mL and in the DIG Trial was 0.8 ng/mL (42,44). Recent retrospective, cohort analysis of the combined PROVED and RADIANCE databases indicates that patients with a low SDC (less than .9 ng/mL) were no more likely to experience worsening symptoms of heart failure on maintenance digoxin than those with a moderate (.9 to 1.2 ng/mL) or high (greater than 1.2 ng/mL) SDC (41). All SDC groups were significantly less likely to deteriorate during follow-up compared with patients withdrawn from digoxin.

Therefore, patients with left ventricular systolic dysfunction and normal sinus rhythm should be started on a maintenance dosage of digoxin (no loading dose) of .125 or .25 mg once daily based on ideal body weight, age, and renal function For patients with normal renal function, a dosage of digoxin of .25 mg/day will be typical. Many patients with heart failure have reduced renal function and should begin on .125 mg daily. In addition, patients with a baseline conduction abnormality, or who are small in stature or elderly, should be started at .125 mg/day, which can be up-titrated if necessary. Once dosing has continued for a sufficient period for serum concentration to reach steady state (typically in 2 to 3 weeks), some clinicians consider the measurement of a SDC, especially in elderly patients or those with impaired renal function in which the digoxin dose is often not predictive of SDC. SDC measurements may be considered when 1) a significant change in renal function occurs; 2) a potentially interacting drug (amiodarone, quinidine, or verapamil) is added or discontinued; or 3) confirmation of suspected digoxin toxicity is necessary in a patient with signs or symptoms and/or electrocardiographic changes consistent with this diagnosis. Samples for trough SDC should be drawn more than 6 hours after dosing. Otherwise, the result is difficult to interpret because the drug may not be fully distributed into tissues.

Recommendation 3. In patients with heart failure and atrial fibrillation with a rapid ventricular response, the administration of high doses of digoxin (greater than .25 mg) for the purpose of rate control is not recommended. When necessary, additional rate control should be achieved by the addition of ß-blocker therapy or amiodarone (Strength of Evidence = C).

Digoxin continues to be the drug of choice for patients with heart failure and atrial fibrillation. However, the traditional practice of arbitrarily increasing the dose (and SDC) of digoxin until ventricular response is controlled should be abandoned because the risk of digoxin toxicity increases as well. Digoxin alone is often inadequate to control ventricular response in patients with atrial fibrillation, and the SDC should not be used to guide dosing

to achieve rate control. Therefore, digoxin should be dosed in the same manner as in a patient with heart failure and normal sinus rhythm.

Digoxin slows ventricular response to atrial fibrillation through enhancement of vagal tone. However, with exertion or other increases in sympathetic activity, vagal tone may decrease and ventricular rate accelerate. Addition of a ß-blocker or amiodarone 1) complements the pharmacological action of digoxin and provides more optimal rate control; 2) allows the beneficial clinical effects of digoxin to be maintained; and 3) limits the risk of toxicity that may occur if digoxin is dosed to achieve a high SDC (58). For patients who have a contraindication to ßblockers, amiodarone is a reasonable alternative. If amiodarone is added, the dose of digoxin should be reduced, and the SDC should be monitored so that the serum concentration can be maintained in the desired range. Some clinicians advocate the short-term, intravenous administration of diltiazem for the acute treatment of patients with very rapid ventricular response, especially those with hemodynamic compromise. This drug is not indicated for long-term management because its negative inotropic effects may worsen heart failure.

Unresolved Therapeutic Issues

Combination With ß-blockers. ß-Blocker therapy has become pivotal in the management of heart failure. However, the majority of patients enrolled in controlled clinical trials that study the efficacy of digoxin were not taking ß-blockers. Therefore, it is uncertain whether or not digoxin should be routinely included as part of a ß-blocker regimen for symptomatic heart failure caused by left ventricular systolic dysfunction. There are attractive features of combining digoxin with ß-blocker therapy in the treatment of heart failure. The majority of heart failure patients have coronary artery disease and may be at risk for transient episodes of myocardial ischemia that could cause catecholamine release and sudden cardiac death. Combining digoxin with a ß-blocker may preserve the beneficial effects of digoxin on the symptoms of heart failure while minimizing the potential detrimental effects of this therapy on catecholamine release in the setting of ischemia (47).

Combination with Diuretics. Non-potassium-sparing diuretics can produce electrolyte abnormalities such as hypokalemia and hypomagnesemia, which increases the risk of digoxin toxicity. The combination of digoxin with a potassium- sparing diuretic would be a potentially safer alternative. Further study will be necessary to carefully elucidate the efficacy and safety of combining digoxin with these agents.

Anticoagulation and Antiplatelet Drugs

Background for Recommendations

Patients with heart failure are recognized to be at increased risk for thromboembolic events that can be arterial or venous in origin. In addition to atrial fibrillation and poor ventricular function (which promote stasis and increase the risk of thrombus formation), patients with heart failure have other manifestations of hypercoagulability. Evidence of heightened platelet activation; increased plasma and blood viscosity; and increased plasma levels of fibrinopeptide A, ßthromboglobulin, D-dimer, and von Willebrand factor (59-61) have been found in many patients. Despite a predisposition, estimates regarding the incidence of thromboemboli in patients with heart failure vary substantially between 1.4 and 42 per 100 patient years (62-65). Although variability in the reported incidence likely results from differences in the populations studied and the methods used to identify these events, the consensus is that pulmonary and systemic emboli are not common in heart

failure patients. Traditionally, the issue of anticoagulation in patients with heart failure centered on warfarin. Growing recognition of the importance of ischemic heart disease as a cause of heart failure suggests that the role of antiplatelet therapy must be considered in patients with this syndrome as well.

Previous guidelines have recommended warfarin anticoagulation in patients with heart failure complicated by atrial fibrillation and in heart failure patients with prior thromboembolic events (18,19). Warfarin anticoagulation specifically was not recommended in patients with heart failure in the absence of these indications. There have been no randomized, controlled trials of warfarin in patients with heart failure. Therefore, recommendations regarding its use, in the absence of atrial fibrillation or clinically overt systemic or pulmonary thromboemboli, must be made on the basis of cohort data and expert opinion. The likely incidence of thromboembolic events and the possibility of averting them with warfarin are important considerations for any guideline recommendation. In addition, the potential beneficial effects of warfarin on coronary thrombotic events, independent of embolic phenomenon, must be taken into account. The substantial clinical trial data that reflect the beneficial effects of antiplatelet therapy in patients with ischemic heart disease suggest that new guideline recommendations for heart failure should address the role of this form of therapy in patients with left ventricular dysfunction.

Anticoagulation

> **Recommendation 1. All patients with heart failure and atrial fibrillation should be treated with warfarin (goal, international normalized ratio (INR) 2.0 to 3.0) unless contraindicated (Strength of Evidence = A).**

The committee agrees with previous guideline recommendations that concern warfarin therapy in patients with heart failure complicated by atrial fibrillation. The benefit of warfarin anticoagulation in this setting is well established through several randomized trials (66). Patients with heart failure commonly have atrial fibrillation. Warfarin anticoagulation should be implemented in all of these patients unless clear contraindications exist.

> **Recommendation 2. Warfarin anticoagulation merits consideration for patients with left ventricular ejection fraction of 35% or less. Careful assessment of the risks and benefits of anticoagulation should be undertaken in individual patients (Strength of Evidence = B).**

Cohort analyses examining the relationship between warfarin use and noncoronary thromboembolism in patients with heart failure have not consistently yielded positive findings (62,63,65,67-69). It is possible that the lack of consistent benefit was related to the low incidence of identifiable embolic events in these populations. However, these studies do not make a convincing argument for the use of warfarin to prevent embolic events in the absence of atrial fibrillation or a previous thromboembolic episode.

In contrast, a recent cohort analysis of the SOLVD population focused on the relation between warfarin use and the risk of all-cause mortality rather than risk for embolic events (70). After adjustment for baseline differences, patients treated with warfarin at baseline had a significantly lower risk of mortality during follow-up (adjusted hazard ratio .76; 95%

CI, .65 to .89, *P* = .0006). In addition to a mortality benefit, warfarin use was also associated with a significant reduction in the combined endpoint of death or hospitalization for heart failure (adjusted hazard ratio .82; 95% CI, .72 to .93, *P* = .002). In the SOLVD population, the benefit associated with warfarin use was not significantly influenced by 1) presence or absence of symptoms (treatment trial *v* prevention trial), 2) randomization to enalapril or placebo, 3) gender, 4) presence or absence of atrial fibrillation; 5) age, 6) ejection fraction, 7) NYHA class, or 8) origins of disease.

The benefit associated with warfarin use in the cohort analysis of the SOLVD population was related to a reduction in cardiac mortality. Specifically, there was a significant reduction among warfarin users in deaths that were identified as sudden, in deaths associated with heart failure, and in fatal MI. In contrast (yet in agreement with previous cohort analyses), there was no significant difference in deaths considered cardiovascular but noncardiac, including pulmonary embolism and fatal stroke. Some caution is needed in consideration of this finding because the number of cardiovascular deaths that were noncardiac was far less than the number of cardiac deaths.

Reduction in ischemic events is one potential explanation for the apparent benefit from warfarin in the SOLVD Study. Warfarin users showed a reduced rate of hospitalization for unstable angina or nonfatal MI. Prior investigations of patients after acute MI showed that warfarin anticoagulation, when started within 4 weeks, reduces the incidence of fatal and nonfatal coronary events, as well as pulmonary embolus and stroke (71).

As with other post hoc, cohort analyses, it is possible that the findings from the SOLVD Study may result from differences between the treatment groups that were not identified and for which statistical correction could not adequately adjust. For this reason, evidence from any cohort study must be considered less powerful compared with evidence derived from randomized, controlled trials. Nevertheless, in the absence of randomized data, the SOLVD cohort analysis represents reasonable evidence to support more aggressive use of warfarin anticoagulation than previously recommended in patients with reduced left ventricular ejection fraction and sinus rhythm. The data from this analysis provide no information regarding the ideal warfarin dose in this patient population. Therefore, the dosing recommendation should likely conform to that derived from previous randomized trials performed in patients without mechanical prosthetic valves (INR 2.0 to 3.0).

Antiplatelet Drugs

> **Recommendation 1. With regard to the concomitant use of ACE inhibitors and acetylsalicylic acid (ASA), each medication should be considered on its own merit for individual patients. Currently, there is insufficient evidence concerning the potential negative therapeutic interaction between ASA and ACE inhibitors to warrant withholding either of these medications in which an indication exists (Strength of Evidence =** C).

Strong evidence supports the clinical benefit of aspirin in ischemic heart disease and atherosclerosis (72-75). However, recent post hoc analyses of large randomized trials involving ACE inhibitors in heart failure and post-MI suggest the possibility of an adverse drug interaction between ASA and ACE inhibitors (76-78). A retrospective cohort analysis of the SOLVD Study found that patients on antiplatelet therapy (assumed to be ASA in the

great majority of patients) derived no additional survival benefit from the addition of enalapril. Data from CONSENSUS II and GUSTO-1 in post-MI patients, suggest not only no additive benefit, but the possibility of a negative effect on mortality from the combination of ASA and ACE inhibition. In contrast, an unadjusted, retrospective registry study in patients with chronic coronary artery disease did not support an adverse interaction (79). Interestingly, in an adjusted analysis of the subset of patients with heart failure in this study, the beneficial effects of aspirin seemed less evident in patients taking ACE inhibitors. Despite these provocative post hoc findings, no prospective studies have yet been reported that concern the possible adverse interaction between ACE inhibitors and aspirin. To date, there is no clear evidence of harm from the combination of ASA and ACE inhibitors in patients with heart failure (76).

There is also some evidence that the potential interaction between ASA and ACE inhibitors may be dose related. A recent meta-analysis of all hypertension and heart failure patients who have received both ASA and ACE inhibitors suggests that ASA at doses equal to or less than 100 mg showed no interaction with ACE inhibitors (80). Any interaction, if observed, occurred at higher doses of aspirin.

A potential mechanism for the hypothesized adverse interaction between ASA and ACE inhibitors in patients with heart failure involves prostaglandin synthesis. ACE inhibition is believed to augment bradykinin which, in turn, stimulates the synthesis of various prostaglandins that may contribute vasodilatory and other salutary effects. In the presence of ASA, the bradykinin-induced increase in prostaglandins should be attenuated or blocked, which potentially reduces the benefits of ACE inhibition. Invasive hemodynamic monitoring has shown that the acute hemodynamic effect of enalapril is blunted by concomitant administration of aspirin (81). Another possibility is that ASA and ACE inhibitors act in a similar fashion in heart failure, therefore no added benefit is gained from the combination. ACE inhibitors appear to reduce ischemic events in heart failure patients possibly through antithrombotic effects, which could mimic those of antiplatelet agents. Recent study results that suggest ASA may have independent beneficial action on ventricular remodeling support the hypothesis of similar mechanisms of action for ACE inhibitors and ASA (82).

Development of the adenosine diphosphate (ADP) antagonists, ticlopidine and clopidogrel, provides alternative therapy for platelet inhibition that does not appear to influence prostaglandin synthesis (83). In direct comparison with aspirin, large-scale clinical trial results have established the efficacy of clopidogrel in the prevention of vascular events in patients with arteriosclerotic disease (84). Clinical data are limited with ADP antagonists in heart failure. However, hemodynamic evaluation found a similar reduction in systemic vascular resistance in heart failure patients treated with the combination of ACE inhibitors and ticlopidine versus ACE inhibitors alone, which suggests no adverse hemodynamic interaction with ACE inhibition with this type of antiplatelet compound (85). Definitive resolution of the therapeutic implications of the ASA/ACE inhibitor interaction and the appropriate alternative therapy, if any, in heart failure awaits the results of additional clinical research studies.

Angiotensin II Receptor Blockers

Background for Recommendations

Angiotensin ll (AT) receptor blockers (ARBs) differ in their mechanism of action compared with ACE inhibitors. Rather than inhibiting the production of AT by blockade

of ACE, ARBs block the cell surface receptor for AT. ARBs that are currently available are selective and only effectively inhibit the AT1 subtype of this receptor. Theoretical benefits of ARBs include receptor blockade of AT produced by enzymes other than ACE and maintenance of ambient AT to maintain or increase stimulation of AT2 receptors. AT1 receptor antagonism is important because this receptor appears to mediate the classical adverse effects associated with AT in heart failure. In contrast, the AT2 receptor subtype appears to counterbalance AT1 receptor stimulation by causing vasodilation and inhibiting proliferative and hypertrophic responses (86). Thus, the selective receptor blockade of the current ARBs may be particularly advantageous. Theoretical concerns about ARB therapy include the potential deleterious effects of increased AT levels and AT2 receptor-mediated enhancement of apoptosis. Whether ARBs have beneficial effects similar to ACE inhibitors on the course of coronary artery disease remains to be determined. ARBs may or may not influence bradykinin concentrations, which are anticipated to rise with ACE inhibitor therapy and may contribute to their efficacy.

The hemodynamic actions of ARBs have, thus far, been similar to ACE inhibitors for reduction of blood pressure in hypertension and lowering of systemic vascular resistance in heart failure (87). ARBs have a similar mild-to-modest effect on exercise capacity and produce a comparable reduction in norepinephrine relative to ACE inhibitors (88).

Recommendation 1. ACE inhibitors rather than ARBs continue to be the agents of choice for blockade of the renin-angiotensin system in heart failure, and they remain the cornerstone of standard therapy for patients with left ventricular systolic dysfunction with or without symptomatic heart failure (Strength of Evidence = A).

At present, it is not possible to predict where ARBs will ultimately reside among accepted therapies for heart failure. Although the initial small ELITE Trial suggested a greater benefit from a losartan dosage of 50 mg daily than from a captopril dosage of 50 mg 3 times daily on mortality in elderly patients with heart failure (89), the ELITE II Mortality Trial, which included more than 3,000 patients (90), showed no comparative benefit from losartan and a trend for a better outcome and fewer sudden deaths with captopril (91). This result provides no evidence that the low dose (50 mg) of losartan that was tested is better than an ACE inhibitor for treating heart failure, but it does not exclude the efficacy of a higher dose designed to provide continuous inhibition of the AT1 receptor. Tolerability of losartan was better than of captopril, primarily because of an ACEinhibitor cough. But the well-established efficacy of the ACE inhibitors on outcome in the post-MI period, in diabetes, in atherosclerosis, and in heart failure mandates that this drug group remains agents of choice for inhibiting the renin-angiotensin system in heart failure. The RESOLVD Trial suggested no major differences in efficacy of candesartan and enalapril, with a trend favoring enalapril during the study period of 43 weeks (92). The OPTIMAAL and VALIANT Studies will provide information specifically about the role of ARBs versus ACE inhibitors in the post-MI population.

Currently, ACE inhibitors continue to be regarded as the therapy of choice to inhibit the renin-angiotensin system in patients with asymptomatic and symptomatic left ventricular dysfunction. There is no current rationale to recommend initiating ARBs in patients with new onset heart failure or for switching from a tolerated ACE-inhibitor regimen to an ARB in patients with chronic heart failure.

Recommendation 2. All efforts should be made to achieve ACE inhibitor use in patients with heart failure caused by left ventricular dysfunction. Patients who are truly intolerant to ACE inhibitors should be considered for treatment with the combination of hydralazine and isosorbide dinitrate (Hyd-ISDN) (Strength of Evidence = B) or an ARB (Strength of Evidence = C).

Previous large-scale trials do not specifically address the role of ARB and Hyd-ISDN in patients who are intolerant to ACE inhibitors. One arm of the CHARM Study has been specifically designed to test the effectiveness of candesartan in patients with systolic dysfunction who are intolerant to ACE inhibitors. The primary endpoint in this study will be a composite of cardiovascular death and time until first hospitalization for heart failure. For now, ARBs offer a reasonable alternative in the heart failure or post-MI patient who is truly intolerant to ACE inhibition. Intolerance because of cough should always trigger a careful reevaluation for congestion. If congestion is present, cough should abate with increases in diuretic that should allow ACE-inhibitor use to continue (93). It should be emphasized that patients intolerant to ACE inhibitor because of renal dysfunction, hyperkalemia, or hypotension are often intolerant to ARBs as well. ACE inhibitor intolerance because of persistent symptomatic hypotension in advanced heart failure may represent severe dependence on the hemodynamic support of the renin-angiotensin system, which generally would predict hypotension with ARB use as well.

The combination of Hyd-ISDN has not been studied in the post-MI population, but sufficient experience exists to support its use in the ACE-inhibitor-intolerant patient with symptomatic heart failure. Hydralazine blocks the development of nitrate tolerance, which argues for the use of combination therapy. Although they were not studied alone in a heart failure mortality trial, oral nitrates represent another reasonable alternative for patients intolerant to both ACE inhibitors and hydralazine.

Unresolved Therapeutic Issues

Combination Therapy With ACE Inhibitors and ARBs. Interest has grown in the potential utility of combining ACE inhibitors and ARBs in patients with heart failure. Initial data suggest that the combination yields more vasodilation and decreased blood pressure than either agent alone. The addition of losartan to an ACE inhibitor has been found to improve exercise capacity compared with an ACE inhibitor alone (94). Preliminary data from the RESOLVD Trial suggest that ventricular dilation and neuroendocrine activation may be best reduced with combination therapy, but other endpoints were not clearly affected. Trials are currently underway to determine the safety, as well as benefit, of more complete blockade of the renin-angiotensin system. The Val-HeFT Trial is a large-scale investigation of the effect of valsartan in addition to ACE inhibitors on morbidity and mortality in symptomatic patients with heart failure caused by systolic dysfunction. One arm of the CHARM Study will also examine the effect of the addition of candesartan in patients with symptomatic, systolic dysfunction treated with an ACE inhibitor. Preliminary data from the RESOLVD Trial suggest that combination therapy may be even more efficacious when used in conjunction with ß-blocker treatment.

Results from Val-HeFT and CHARM in the subset of patients treated with ß-blocker therapy will provide more information concerning this strategy.

Combination therapy represents a rational option when treating severe hypertension or other vasoconstriction but cannot, at present, be recommended as routine therapy in the absence of a proven superiority to ACE-inhibitor therapy alone.

HFSA Guidelines
Criteria for NYHA functional classification for chronic heart failure patients, functional capacity (130)

CLASS 1	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea.
CLASS 2	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnea.
CLASS 3	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation or dyspnea.
CLASS 4	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

HFSA Guidelines
Glossary of Clinical Trials

AVID	Antiarrhythmics Versus Implantable Defibrillators
BEST	Beta-blocker Evaluation of Survival Trial
CAMIAT	Canadian Amiodarone Myocardial Infarction Arrhythmia Trial
CAPRIE	Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events
CASH	Cardiac Arrest Study Hamburg
CHF-STAT	Congestive Heart Failure-Survival Trial of Antiarrhythmic Therapy
CHARM	Candesartan Cilexetil in Heart Failure Assessment of Reduction in Mortality and Morbidity
CIBIS	Cardiac Insufficiency BIsoprolol Study
CIBIS II	Cardiac Insufficiency Bisoprolol Study II
CIDS	Canadian Implantable Defibrillator Study
COMET	Carvedilol or Metoprolol European Trial
CONSENSUS	Cooperative North Scandinavian Enalapril Survival Study
CONSENSUS II	Cooperative New Scandinavian Enalapril Survival Study II
COPERNICUS	Carvedilol Prospective Randomized Cumulative Survival Trial
DEFINITE	Defibrillators in Nonischemic Cardiomyopathy Treatment Evaluation
DIAMOND	Danish Investigation of Arrhythmia and Mortality on Dofetilide
DIG	Digitalis Investigation Group
ELITE	Evaluation of Losartan In The Elderly
ELITE II	Losartan Heart Failure Survival Study - ELITE II
EMIAT	Infarction Amiodarone Trial
GESICA	Grupo de Estudio de Sobrevida en Insuficiencia Cardiaca en Argentina
GUSTO 1	Global Utilization of Streptokinase and TPA for Occluded coronary arteries
MADIT	Multicenter Automatic Defibrillator Implantation Trial
MADITII	Multicenter Automatic Defibrillator Implantation Trial II
MDC	Metoprolol in Dilated Cardiomyopathy trial
MERIT-HF	Metoprolol CR/XL Randomized Intervention Trial in Heart Failure
MOCHA	Multicenter Oral Carvedilol in Heart-failure Assessment
MTT	Myocarditis Treatment Trial
OPTIMALL	Optimal Therapy in Myocardial Infarction with the Angiotensin II Antagonist Losartan
PRECISE	Prospective Randomized Evaluation of Carvedilol In Symptoms and Exercise
PROVED	Prospective Randomized study Of Ventricular failure and the Efficacy of Digoxin
RADIANCE	Randomized Assessment of Digoxin on Inhibitors of the Angiotensin Converting Enzyme
RALES	Randomized Aldactone Evaluation Study
RESOLVD	Randomized Evaluation of Strategies for Left Ventricular Dysfunction
SAVE	Survival And Ventricular Enlargement
SCD-HeFT	Sudden Cardiac Death in Heart Failure: Trial of prophylactic amiodarone versus implantable defibrillator therapy
SOLVD	Studies Of Left Ventricular Dysfunction
SWORD	Survival With Oral D-sotalol
ValHeFT	Valsartan Heart Failure Trial
VALIANT	Valsartan in Acute Myocardial Infarction

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EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____

Your birthdate (Day, Month, Year): ____

Today's date (Day, Month, Year): 31 ____

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ - BR23

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Did you have a dry mouth?	1	2	3	4
32. Did food and drink taste different than usual?	1	2	3	4
33. Were your eyes painful, irritated or watery?	1	2	3	4
34. Have you lost any hair?	1	2	3	4
35. Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
36. Did you feel ill or unwell?	1	2	3	4
37. Did you have hot flushes?	1	2	3	4
38. Did you have headaches?	1	2	3	4
39. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
40. Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
41. Did you find it difficult to look at yourself naked?	1	2	3	4
42. Have you been dissatisfied with your body?	1	2	3	4
43. Were you worried about your health in the future?	1	2	3	4

During the past four weeks:	Not at All	A Little	Quite a Bit	Very Much
44. To what extent were you interested in sex?	1	2	3	4
45. To what extent were you sexually active? (with or without intercourse)	1	2	3	4
46. Answer this question only if you have been sexually active: To what extent was sex enjoyable for you?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
47. Did you have any pain in your arm or shoulder?	1	2	3	4
48. Did you have a swollen arm or hand?	1	2	3	4
49. Was it difficult to raise your arm or to move it sideways?	1	2	3	4
50. Have you had any pain in the area of your affected breast?	1	2	3	4
51. Was the area of your affected breast swollen?	1	2	3	4
52. Was the area of your affected breast oversensitive?	1	2	3	4
53. Have you had skin problems on or in the area of your affected breast (e.g., itchy, dry, flaky)?	1	2	3	4