Evidence Report/Technology Assessment
Number 172

HER2 Testing to Manage Patients With Breast Cancer or Other Solid Tumors

Prepared for:
Agency for Healthcare Research and Quality
U.S. Department of Health and Human Services
540 Gaither Road
Rockville, MD 20850
www.ahrq.gov

Contract No. 290-02-0026

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AHRQ Publication No. 09-E001
November 2008

This report is based on research conducted by the Blue Cross and Blue Shield Association Technology Evaluation Center Evidence-based Practice Center (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract No. 290-02-0026). The findings and conclusions in this document are those of the author(s), who are responsible for its content, and do not necessarily represent the views of AHRQ. No statement in this report should be construed as an official position of AHRQ or of the U.S. Department of Health and Human Services.

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Suggested Citation:
Seidenfeld J, Samson DJ, Rothenberg BM, Bonnell CJ, Ziegler KM, Aronson N. HER2 Testing to Manage Patients With Breast Cancer or Other Solid Tumors. Evidence Report/Technology Assessment No. 172. (Prepared by Blue Cross and Blue Shield Association Technology Evaluation Center Evidence-based Practice Center, under Contract No. 290-02-0026.) AHRQ Publication No. 09-E001. Rockville, MD: Agency for Healthcare Research and Quality. November 2008.

No investigators have any affiliations or financial involvement (e.g., employment, consultancies, honoraria, stock options, expert testimony, grants or patents received or pending, or royalties) that conflict with the material presented in this report.

Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to **epc@ahrq.gov.**

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Acknowledgments

The research team would like to acknowledge the efforts of Maxine A. Gere, M.S., for general project management and editorial assistance; Elizabeth De La Garza and Joyce Gonzalez for administrative support; Ariel Katz, M.D., M.P.H., for study selection and data abstraction; Thomas Ratko, Ph.D., for fact-checking; and Gurvaneet Randhawa, M.D., M.P.H., for advice as our Task Order Officer.

Structured Abstract

Objectives: Systematic review of trastuzumab outcomes among breast cancer patients who have negative, equivocal, or discordant HER2 assay results; use of HER2 assay results to predict outcomes of chemotherapy or hormonal therapy regimen for breast cancer; use of serum HER2 to monitor treatment response or disease progression in breast cancer patients; and use of HER2 testing to manage patients with lung, ovarian, prostate, or head and neck tumors. Also, narrative review of concordance of HER2 assays.

Data Sources: We abstracted data from: three articles plus one conference abstract on negative, equivocal, or discordant HER2 results; 26 studies on selection of chemotherapy or hormonal therapy; 15 studies on serum HER2; and 26 studies on ovarian, lung, prostate, or head and neck tumors. Foreign-language studies were included.

Review Methods: We sought randomized trials or single-arm series (prospective or retrospective) of identically treated patients that presented relevant outcome data associated with HER2 status.

Results: HER2 assay results are influenced by multiple biologic, technical, and performance factors. Many aspects of HER2 assays were standardized only recently, so inconsistencies confound the literature comparing different methods. The evidence is weak on outcomes of trastuzumab added to chemotherapy for HER2-equivocal, -discordant, or -negative patients. Evidence comparing chemotherapy outcomes in HER2-positive and HER2-negative patient subgroups may generate hypotheses, but is too weak to test hypotheses. Only a rigorous test can resolve whether HER2-positive patients (but not HER2-negative patients) benefit from an anthracycline regimen. Evidence is available only from uncontrolled series on whether HER2 status predicts complete pathologic response to neoadjuvant chemotherapy. Evidence also is weak regarding differences by HER2 status for outcomes of chemotherapy for advanced or metastatic disease; with most studies lacking statistical power. Data from studies of tamoxifen and aromatase inhibitors suggest that future studies should examine whether HER2 status predicts response to specific hormonal therapies among estrogen-receptor-positive patients. The evidence is weak on whether serum HER2 predicts outcome after treatment with any regimens in any setting, as is the evidence on use of serum or tissue HER2 testing for malignancies of lung, ovary, head and neck, or prostate.

Conclusions: Overall, few studies directly investigated the key questions of this systematic review. Going forward, cancer therapy trial protocols should incorporate elements to facilitate robust analyses of the use of HER2 status and other biomarkers for managing treatment.

Contents

Figures

Tables

Appendixes

Appendixes and Evidence Tables for this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Executive Summary

The human epidermal growth factor receptor-2 (HER2) gene is amplified and the HER2 protein overexpressed in approximately 18–20 percent of breast cancer cases. Amplification or overexpression of HER2 is associated with poor prognosis. Evidence from randomized trials demonstrates that adding trastuzumab, a therapeutic monoclonal antibody that targets HER2, to adjuvant chemotherapy regimens for HER2-positive breast cancer improves survival. HER2 also is overexpressed in other epithelial malignancies such as ovarian, thyroid, lung, salivary gland/head and neck, stomach, colon, and prostate cancers.

This report is a systematic review of the evidence on other applications of HER2 testing to the management of cancer patients including: potential for response to trastuzumab among breast cancer patients who have negative, equivocal, or discordant HER2 assay results; use of HER2 assay results to guide selection of breast cancer treatments other than trastuzumab (i.e., chemotherapy regimen or hormonal therapy regimen); the use of serum HER2 to monitor treatment response or disease progression in breast cancer patients; and use of HER2 testing to manage patients with ovarian, lung, prostate, or head and neck tumors. The concordance and discrepancy of HER2 measurement methods are discussed in a narrative review.

Methods

The review methods were defined prospectively in a written protocol. A technical expert panel provided consultation. The draft report was also reviewed by other experts and stakeholders.

A narrative review was conducted on Key Question 1, which addressed concordance and discrepancy among HER2 assays in breast cancer. HER2 assay results are influenced by multiple biologic, technical, and performance factors. Since many aspects of HER2 assays were standardized only recently, we could not isolate effects of these disparate influences on assay results and patient classification. This challenged the validity of using systematic review methods to compare available assay technologies.

For Key Questions 2-5, we sought randomized trials or single-arm series (prospective or retrospective) of identically treated patients that presented relevant outcome data associated with HER2 status. Primary outcomes were: overall survival (OS); disease-free survival (DFS); progression-free survival (PFS); time to failure (TTF) or progression; quality of life; palliation of symptoms; and treatment-related adverse effects.

Our search had no language restrictions and used these electronic databases:

- MEDLINE® (through February 2007)
- EMBASE® (through February 2007)
- Cochrane Controlled Trials Register (through February 2007)

The searches were updated in April 2008, using the Cochrane clinical trial filter.

Additional sources were the past two years of conference proceedings of the American Association for Clinical Chemistry (AACC), American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), and the San Antonio Breast Cancer Symposium (SABCS).

Of 6,337 citations, 666 articles were retrieved and 70 were selected for inclusion:

- Three articles plus one abstract on use of trastuzumab among HER2-negative or -discordant breast cancer patients;
- 26 articles on chemotherapy or hormonal therapy for breast cancer patients;
- 15 articles on plasma or serum HER2 in patients treated for breast cancer; and
- 26 articles on serum or tissue HER2 in patients with lung cancer, ovarian cancer, head and neck cancer, and prostate cancer.

A single reviewer screened citations for article retrieval; citations judged as "uncertain" were reviewed by a second reviewer. The same procedure was used to select articles for inclusion in the review. A single reviewer performed data abstraction and a second reviewed the evidence tables for accuracy. However, study quality was appraised by dual independent review. All disagreements were resolved by consensus.

The quality of predictive studies was assessed using the general approach described in the "Reporting Recommendations for Tumor Marker Prognostic Studies" (REMARK) statement (McShane, Altman, Sauerbrei, et al., 2005). In addition, we used a hierarchical framework for evaluating how informative different designs and analytic strategies would be to predictions of outcomes according to HER2 status. Most informative is a trial that randomizes patients to receive treatment guided by HER2 results or not; or, alternatively, a trial that stratifies randomized assignment to treatment groups by HER2 status (Conley and Taube, 2004). Other types of studies, in decreasing order of information value, include: randomized trials using prespecified multivariate subgroup analyses, randomized trials using post-hoc multivariate subgroup analyses, randomized trials presenting HER2 by treatment subgroup analyses, single-arm studies using prespecified multivariate analyses, single-arm studies using post-hoc multivariate analyses, and single-arm studies using univariate analyses.

Results

Key Question 1: Concordance and Discrepancy of HER2 Methods

HER2 assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2 assays were standardized only recently, these disparate influences confound the existing literature that compares results of different methods. Discordances between immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) results might arise in one of three ways. They may be artifacts of one accurate and one inaccurate test or of two inaccurate tests, as preanalytic, analytic, and postanalytic practices can vary among laboratories within a study, as well as among studies. Interobserver variability can play a role. Alternatively, discordances may reflect a threshold issue, either related to changes in threshold definitions over time, or an inherent problem of using a continuous measure to classify patients dichotomously. Finally, discordant test results might accurately reflect a variation among patients with respect to the biologic mechanisms that can increase membrane levels of the HER2 protein. This clearly affects the interpretation of evidence on the use of "HER2 status" to predict treatment or disease outcomes, which presumes accurate classification by tissue assays.

Notably, there is no recognized gold standard to determine the HER2 status of tumor tissue, which also precludes consensus on one "best" HER2 assay. Recent guidelines acknowledge

present uncertainty, permit clinicians and laboratories to choose an initial well-validated and properly performed HER2 assay method, and recommend confirming results with an alternative assay when initial tests are equivocal. The ASCO/CAP expert panel (Wolff, Hammond, Schwartz, et al., 2007a) defines equivocal HER2 assay results as IHC 2+, or HER2 gene copy number from 4.0 to 6.0, or HER2/CEP17 ratio from 1.8 to 2.2, if ISH is the first or only assay.

Key Question 2: HER2-Negative or -Discrepant Breast Cancer

Currently available evidence on outcomes of trastuzumab added to chemotherapy for most HER2-equivocal, -discordant, or -negative patients may generate hypotheses, but is too weak to test hypotheses. Most of this evidence is from post-hoc analyses on subgroups not directly randomized or stratified by HER2 status. Scant but intriguing evidence suggests the hypothesis that some patients currently classified as HER2 negative may benefit from adjuvant trastuzumab. Data reported from a post-hoc subgroup analysis of one adjuvant trial (NSABP B31) showed significantly longer DFS and relapse-free interval (RFI) in FISH-negative IHC ≤2+ patients given trastuzumab than in patients managed without trastuzumab, whether the analysis did or did not include those who were IHC 0. However, analysis of data from another similar adjuvant trial (NCCTG N9831) found no significant differences. Both were interim analyses of trials in which fewer than 25 percent of subjects had reached a failure event. Followup analyses from these trials will be of interest.

CALGB 9840 investigators also analyzed a subgroup of metastatic FISH-negative patients that either had (n=38) or did not have (n=103) polysomy 17; overall response rate (ORR) was significantly higher with versus without trastuzumab for those with polysomy 17, but was identical with or without trastuzumab for those without polysomy 17. However, a study in the adjuvant setting (Reinholz, Jenkins, Hillman, et al., 2007) reports no impact of polysomy 17 on benefit from trastuzumab. Additionally, other studies report conflicting data on association of polysomy 17 with overexpression of HER2 protein.

Key Question 3: Breast Cancer Patients Receiving Chemotherapy (3a) or Hormonal Therapy (3b)

For Question 3a, across all three treatment settings (adjuvant, neoadjuvant, or advanced/metastatic), currently available evidence comparing chemotherapy outcomes in HER2-positive and HER2-negative patient subgroups may generate hypotheses, but is too weak to test hypotheses. In the only study that prespecified multivariate subgroup analysis by HER2 status, interaction of assigned adjuvant treatment (with or without paclitaxel) with HER2 status to predict outcome was not statistically significant (ratio of hazard ratios [HRs]=0.85; p=.41). All other evidence is from post-hoc analyses on subgroups not directly randomized, selected, or stratified by HER2 status, and used data from secondary or correlative analysis on patient subgroups with archived tissue samples. It is uncertain whether these subgroups were well balanced. No studies for Question 3a used trastuzumab for HER2-positive patients.

Available evidence focuses on three types of adjuvant chemotherapy: cyclophosphamide plus methotrexate plus fluorouracil (CMF), regimens with an anthracycline, and paclitaxel after or with doxorubicin (Adriamycin®) plus cyclophosphamide (AC). Evidence from two studies (one randomized, controlled trial and one series) suggests HER2-positive patients may benefit less from CMF (smaller improvements in OS and DFS) than HER2-negative patients. Only one of

four randomized, controlled trials reports a statistically significant interaction that suggests HER2-positive patients (but not HER2-negative patients) benefit from including an anthracycline in their treatment regimen. Given the highly statistically significant result favoring anthracycline therapy for the entire population (N=14,000) of breast cancer patients included in the Early Breast Cancer Trialists' Collaborative Group (EBCTCG 2005) patient-level meta-analysis, a rigorous test of this hypothesis is necessary before one can conclude that omitting anthracyclines from adjuvant chemotherapy regimens would not worsen outcome for HER2-negative patients.

Two trials compared different doses or frequencies of anthracycline-based regimens. One reported statistically significant interaction of cyclophosphamide, doxorubicin, and fluorouracil (CAF) dose with HER2 status to predict treatment outcome, but the second showed no relationship. One study found that adding paclitaxel after AC improves OS and DFS for HER2-positive patients, but may not improve these outcomes for HER2-negative patients. In contrast, the only randomized, controlled trial with a prespecified multivariate subgroup analysis found no difference by HER2 status in outcomes of concurrently added paclitaxel. Thus, for each of the adjuvant chemotherapy regimens compared, available evidence is too weak to rule out the possibility that HER2-negative patients may benefit from using the added drug or higher dose.

Evidence on whether HER2 status predicts complete response (pCR) to neoadjuvant chemotherapy is limited to four uncontrolled series (retrospective analysis in three). Data are lacking to directly compare any neoadjuvant regimens. There is also limited evidence on differences by HER2 status for outcomes of chemotherapy for advanced or metastatic disease, with most studies lacking statistical power.

For Question 3b, four studies addressed use of tamoxifen in various breast cancer patient populations, and two compared tamoxifen with aromatase inhibitors. None of these studies included trastuzumab. There were no trials that stratified randomization by HER2 status or randomization to therapy directed by HER2 results or not. Less informative designs were used, including post-hoc multivariate analyses in five randomized trials and one post-hoc multivariate analysis in a single-arm study. Data are too weak to reach new conclusions about differences between subgroups based on HER2 status in effects of specific hormone therapies for patients who are hormone-receptor positive.

Key Question 4: Plasma or Serum HER2 (sHER2) in Patients Treated for Breast Cancer

Of 13 included studies, three were randomized trials and 11 were single-arm designs. The evidence is weak on whether sHER2 predicts outcome after treatment with any regimens in any setting. Evidence primarily focused on first-line or second- and subsequent-line treatment of metastatic disease using variety of regimens. Studies used different thresholds for a positive sHER2 result and varied on whether patient selection required positive tissue HER2 status. One randomized and two single-arm studies performed multivariate analysis, although reporting lacked sufficient detail. Univariate analyses provide very limited information value, suggesting candidate variables for future multivariate analyses. Overall, the evidence is too weak to assess whether sHER2 predicts disease progression, treatment response, or outcomes of any specific treatment regimen.

Key Question 5. Serum or Tissue HER2 Testing in Malignancies of Lung, Ovary, Head and Neck, or Prostate

With respect to use of serum or tissue HER2 testing for malignancies of lung, ovary, head and neck, or prostate, the evidence is quite weak. Studies were heterogeneous regarding treatment regimens and thresholds for positive HER2 test results. Of 22 studies addressed for the four types of malignancies, there were no randomized trials that could have analyzed HER2 by treatment effect interactions. Six multivariate analyses in single-arm designs were performed, all of which were poorly described; it is unclear if they were well conducted. Data from these exploratory analyses did not consistently find that HER2 status predicts treatment results. Univariate analyses provide very limited information value, at best suggesting candidate variables for future multivariate analyses.

Discussion and Future Research

Overall, few trials directly investigated the key questions of this systematic review. Going forward, cancer therapy trial protocols should incorporate elements to facilitate robust analyses of the potential of HER2 to improve treatment management. These elements include:

- Detailed reporting of how HER2 status was ascertained.
- Stratified randomization by HER2 status or prospectively specified HER2 subgroup analysis of outcomes.
- Detailed recording of relevant data and archiving of tissue samples for all participants, and accessible to other researchers, to permit future subgroup analyses of outcomes by HER2 status.

The rationale is strongest for breast cancer therapy trials, as many therapeutic agents, classes, and regimens have been and will be tested. This approach can be generalized to other tumors, to promising biomarkers other than HER2, and to serial collection of serum samples for sHER2 levels. Maximizing data collection in trials planned for other purposes offers an opportunity to screen for potential applications of HER2 and other biomarkers.

For Key Question 2, potential for response to trastuzumab among breast cancer patients who have equivocal, discordant, or negative HER2 assay results, evidence is scant but intriguing. Whether other markers might predict response to trastuzumab for these subgroups could be explored using tissue samples from completed trials.

For Key Question 3, the most compelling question is whether anthracyclines benefit HER2-negative patients. A pragmatic approach for future research is to use individual patient data, of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis, which compared survival with anthracyclines versus CMF in 14,000 patients. However, this approach may be limited by availability of sufficient tumor samples. Also of interest is evidence to clarify whether aromatase inhibitors are more effective than tamoxifen in HER2-positive patients.

For Key Questions 4 and 5, evidence does not support conclusions about use of serum HER2 for any treatment setting within breast cancer or about any use of serum or tissue HER2 for cancer of the lung, ovary, head and neck, or prostate. Future exploratory studies in these areas using preserved or prospectively collectively specimens should be designed with attention to study quality concerns.

Conclusions

Since many technical and performance aspects of HER2 assays were not standardized until very recently, differences in preanalytic, analytic, and postanalytic practices confound the existing literature. Available evidence supports hypotheses generation but is too weak to test hypotheses. Scant but intriguing evidence suggests the hypothesis that some patients currently classified as HER2 negative may benefit from adjuvant trastuzumab. Future research should focus on biomarkers that might select such patients. Evidence suggests HER2-positive, but not HER2-negative, patients may benefit from chemotherapy regimens with an anthracycline; but rigorous testing of this hypothesis is necessary. Also worth additional testing is the hypothesis that aromatase inhibitors may be more beneficial than tamoxifen for HER2-positive, hormone-receptor-positive breast cancer patients. Overall, few trials directly investigate the key questions of this systematic review.

Going forward, cancer therapy trial protocols should incorporate elements to facilitate robust analyses of the use of HER2 status and other biomarkers for managing treatment. Given the human and financial cost of cancer therapy trials, the limited resources available, and the long duration of followup needed to assess outcomes, particularly for early stage or slowly growing cancers, it is imperative that tumor tissue blocks be collected, optimally fixed, saved, and made available for correlative tumor marker studies from all randomized patients. Agreement to share blocks with investigators should be made a condition for institutions seeking to participate in cooperative group trials.

Evidence Report

Chapter 1. Introduction

The human epidermal growth factor (EGF) receptor-2 (HER2; also referred to as HER2/*neu* and as ERBB2) gene, located at position 17q12 on chromosome 17, is amplified (i.e., gene copy number greater than 2) and/or the HER2 protein is overexpressed (i.e., cell membrane has excess of HER2 protein molecules compared to normal cells) in approximately 18 to 20 percent of breast cancer cases (Owens, Horten, and Da Silva, 2004; Yaziji, Goldstein, Barry, et al., 2004; Wolff, Hammond, Schwartz, et al., 2007a; Slamon, Clark, Wong, et al., 1987; Hanna, O'Malley, Barnes, et al., 2007). Amplification and/or overexpression of HER2 have been associated with increased tumor aggressiveness and poor prognosis. The HER2 gene is one of four (HER1 through HER4) in the EGF receptor gene family; each codes for a membrane-spanning protein that can form homodimers and heterodimers and functions in signal transduction. All but HER2 bind (EGF or another) ligand outside the cell, and all but HER3 have enzymatic activity that phosphorylates tyrosine residues in proteins (i.e., tyrosine kinase activity) and that is activated by ligand binding. Ligand-activated tyrosine kinase initially phosphorylates tyrosine residues of the receptor's intracellular domain, and subsequently can phosphorylate tyrosine residues of other intracellular proteins. HER2 also is overexpressed in varying proportions of other epithelial malignancies such as ovarian, thyroid, lung, salivary gland/head and neck, stomach, colon and prostate cancers (Baselga and Mendelsohn 1994; Blank, Chang, and Muggia, 2005; Gross, Jos, and Agus, 2004). Table 1 provides a listing of the estimated new cases and deaths in the U.S. for these cancers in 2008.

Table 1. Estimated new cases and deaths in the U.S. in 2007 for epidermal cancers (of which varying proportions overexpress HER2) (Jemal, Siegel, Ward, et al., 2008)

Cancer Type	Estimated New Cases	Estimated Deaths
Breast cancer (female)	182,460	40,480
Ovarian cancer	21,650	15,520
Thyroid cancer	37,340	1,590
Lung cancer	215,020	161,840
Head and neck		
• oral cavity/pharynx	35,310	7,590
• larynx	12,250	3,670
Stomach	21,500	10,880
Colon	108,070	49,960
Prostate	186,320	28,860

Implications of Accurately Determining HER2 Status

Laboratory assays for the HER2 gene and protein in tumor tissue are used to determine the HER2 status of patients with breast cancer (positive if either HER2 gene amplification or HER protein overexpression is present; negative if neither is present). As outlined in guideline recommendations for HER2 testing in breast cancer from the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP; Wolff, Hammond, Schwartz, et al., 2007a), and in a report from a task force of the National Comprehensive Cancer Network (NCCN; Carlson, Moench, Hammond, et al., 2006), information regarding a patient's HER2 status can contribute to treatment and other patient management decisions in several ways. HER2 overexpression has been associated with clinical outcomes in patients with breast cancer (Press,

Pike, Chazin, et al., 1993; Press, Bernstein, Thomas, et al., 1997; Yamauchi, Stearns, Hayes, 2001). Because HER2 positivity is associated with a worse prognosis in patients with newly diagnosed breast cancer who do not receive systemic adjuvant chemotherapy, HER2 status may be incorporated along with other prognostic factors into decision making regarding such therapy (Wolff, Hammond, Schwartz, et al., 2007a; Carlson Moench, Hammond, et al., 2006).

HER2 positivity also appears to be associated with relative, but not absolute, resistance to certain endocrine therapies (e.g., tamoxifen; less so for aromatase inhibitors) and lower benefit from nonanthracycline, nontaxane-containing chemotherapy regimens (Konecny, Pauletti, Pegram, et al., 2003; Ellis, Coop, Singh, et al., 2001; Menard, Valagussa, Pilotti, et al., 2001). HER2 status is also used to determine whether a patient is eligible to receive biologic therapy specifically targeted to HER2 activity, e.g., trastuzumab (Herceptin®, Genentech, San Francisco, CA) or lapatinib (Tykerb®, GlaxoSmithKline, Research Triangle Park, NC).

Additionally, therapies have been developed that specifically target the HER2 protein (Dinh, de Azambuja, Piccart-Gebhart, et al., 2007; Pal and Pegram, 2007; Viani, Afonso, Stefano, et al., 2007; Lin and Rugo, 2007). Evidence from multiple randomized trials demonstrates that trastuzumab, a therapeutic monoclonal antibody that targets HER2, decreases the risk of recurrence and mortality when added to adjuvant chemotherapy regimens for resected HER2-positive breast cancer. A recent meta-analysis (five trials; pooled N=9,117) reported an odds ratio (OR) for mortality with versus without trastuzumab of 0.52 (95 percent CI: 0.44–0.62; $p<0.00001$), while OR for recurrence was 0.53 (95 percent CI: 0.46–0.60; $p<.00001$) (Viani, Alfonso, Stefano et al. 2007). In patients with metastatic HER2-positive breast cancer, trastuzumab alone or with chemotherapy increases time to disease progression and improves survival. Thus, there is increased emphasis on accurately determining the HER2 status of patients with newly diagnosed or recurrent breast cancer.

There are several assays available to measure or detect HER2 in tissue specimens: immunohistochemistry (IHC) assays measure overexpressed protein coded for by the HER2 gene, and in-situ hybridization techniques that rely on fluorescence (FISH), chromogenic (CISH), or silver-enhanced (SISH) assays, measure gene amplification (Table 2). Additionally, these and other methods (e.g., mRNA assays) can detect or measure HER2 in circulating tumor cells (Meng, Tripathy, Shete, et al., 2004; Apostolaki, Perraki, Pallis, et al., 2007). There is also a serum-based enzyme-linked immunosorbent assay (ELISA; Immuno 1®/ADVIA Centaur®, Bayer) that measures circulating levels of extracellular domain of HER2 (Carlson, Moench, Hammond, et al., 2006; Harris, Fritsche, Mennel, et al., 2007); however, the tissue-based assays are most commonly used to establish a patient's tumor HER2 status.

Key Questions for this Systematic Review

This systematic review will address five key questions regarding HER2 testing to manage patients with breast cancer or other solid tumors:

1. What is the evidence on concordance and discrepancy rates for methods (e.g., FISH, IHC, etc.) used to analyze HER2 status in breast tumor tissue?

Table 2. HER2 assays used in tissue specimens and serum: clinical trials, clinical practice, and under development (adapted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a and including information from Carlson, Moench, Hammond, et al., 2006)

A. IHC Assays: measure HER2 protein overexpression in tissue

Assay	Mfr	Methodology	Scoring Criteria	FDA Status
Clinical Trials Assay	Developed by independent laboratory	CB11 and 4D5 MAb	0 and 1+ negative, 2+ weakly positive, 3+ strongly positive	Research assay used in trials of trastuzumab in metastatic breast cancer
HercepTest™	DAKO*	A0485 polyclonal antibody	Weakly positive (2+): weak to moderate complete membrane staining in >10% of tumor cells; strongly positive (3+): strong complete membrane staining in >10% of tumor cells*	U.S. Food and Drug Administration (FDA) approved as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered
PATHWAY™	Ventana†	CB11 MAb	Positive (2+): weak complete staining of the membrane, >10% of cancer cells; positive (3+): intense complete staining of the membrane, >10% of cancer cells†	FDA approved as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered

B. In-Situ Hybridization (ISH) Assays: measure HER2 gene amplification in tissue

Assay	Mfr	Methodology	Scoring Criteria	FDA Status
PathVysion® HER2 DNA Probe Kit (FISH)	Abbott‡	Hybridization of fluorescent DNA probes to HER2 gene (orange) and chromosome 17 centromere (green)	HER2 amplification: HER2/CEP17 ratio $\geq$2 on average for 60 cells; results at or near the cut off point (1.8–2.2) should be interpreted with caution (Persons, Tubbs, Cooley, et al., 2006; Dal Lago, Durbecq, Desmedt, et al., 2006)	FDA approved as an aid in the assessment of patients for whom Herceptin™ (trastuzumab) treatment is being considered
INFORM HER2/neu Probe (FISH)	Ventana§	Hybridization of biotin-labeled DNA probe to HER2 gene and fluorescently labeled avidin	HER2 amplification: average of >6 HER2 gene copies/nucleus; an average of >4.0 <6.0 gene copies/nucleus for 60 cells described as equivocal in one publication (Dal Lago, Durbecq, Desmedt, et al., 2006; Vera-Roman and Rubio-Martinez, 2004)	FDA approved as an adjunct to existing clinical and pathologic information currently used as prognostic indicators in the risk stratification of breast cancer in patients with a primary, invasive, localized, node-negative tumor

Table 2. HER2 assays used in tissue specimens and serum: clinical trials, clinical practice, and under development (adapted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a and including information from Carlson, Moench, Hammond, et al., 2006), continued

B. In-Situ Hybridization (ISH) Assays: measure HER2 gene amplification in tissue (continued)

Assay	Mfr	Methodology	Scoring Criteria	FDA Status
*HER*2 FISH pharmDx™ Kit	Dako[∇]	Hybridization of fluorescent DNA probes to HER2 gene (red) and PNA probes to chromosome 17 centromere (CEN-17; green)	Count 20 nuclei per tissue specimen, when possible from distinct tumor areas. Specimens with a *HER2*/CEN-17 ratio ≥2 should be considered *HER2* gene amplified (Kallioniemi, Kallioniemi, Kurisu, et al., 1992; Ellis, Dowsett, Bartlett, et al., 2000; Hanna, 2001; Tsuda, Akiyama, Terasaki, et al., 2001). Results at or near the cut-off (1.8–2.2) should be interpreted with caution. If the ratio is borderline (1.8–2.2), count an additional 20 nuclei and recalculate the ratio for the 40 nuclei	FDA approved as an adjunct to clinicopathologic information currently used for estimating prognosis in stage II, node-positive breast cancer patients and as an aid in assessment of patients being considered for Herceptin™ (trastuzumab) treatment
SPoT-Light (CISH)	Invitrogen/ Zymed¶	Hybridization of digoxigenin-labeled DNA probe to HER2 gene; detection via mouse antidigoxigenin antibody followed by antimouse-peroxidase	High HER2 amplification defined as >10 dots, or large clusters, (low if >5 dots to 10 dots, or small clusters) or mixture of multiple dots and large clusters of the HER2 gene present per nucleus in >50% tumor cells (Hanna and Kwok, 2006)	DNA probe kit not available in the U.S.
EnzMet GenePro (SISH)	Ventana	Hybridization of dinitrophenol-labeled DNA probe to HER2 gene; detection via peroxidase-labeled multimer followed by enzyme metallography	Amplification defined as six or more dots, or large clusters of dots, in 30% or more of invasive tumor cells (Downs-Kelly, Pettay, Hicks, et al., 2005)	DNA probe kit not available in the U.S.

Table 2. HER2 assays used in tissue specimens and serum: clinical trials, clinical practice, and under development (adapted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a and including information from Carlson, Moench, Hammond, et al., 2006)

C. HER2 Extracellular Domain (ECD) Assays: detect HER2 ECD in serum

Assay	Mfr	Methodology	Scoring Criteria	FDA Status
Immuno 1®/ADVIA Centaur®	Bayer	Enzyme immunoassay (EIA); primary MAbs NB-3 and TA-1 (one is labeled with fluorescein and the other is either linked to an enzyme or a chemiluminogenic molecule) specific for the ECD of HER2 added to sera; detection via binding of immunocomplex to antifluorescein antibodies in the solid phase, followed by addition of substrate in case of Immuno 1 assay	Elevated ECD concentrations often defined as >15 ng/mL (Payne, Allard, Anderson-Mauser, et al., 2000; Esteva, Cheli, Fritsche, et al., 2005)	FDA approval for followup and monitoring patients with metastatic breast cancer only

CISH: chromogenic in situ hybridization; ECD: extracellular domain; IHC: immunohistochemistry; FISH: fluorescent in situ hybridization; MAb: monoclonal antibody; Mfr: manufacturer; SISH: silver enhanced in situ hybridization;
*http://www.dakousa.com/prod_downloadpackageinsert.pdf?objectid_105073003
†http://www.ventanamed.com/products/files/ScoringGuide.pdf
∇http://www.dakousa.com/prod_downloadpackageinsert.pdf?objectid=112853001
‡http://www.vysis.com/PathVysionHER2DNAProbeKit_35793.asp
§http://www.ventanamed.com/catalog/search_detail.html?id_402&categories_id_4
¶https://catalog.invitrogen.com/index.cfm?fuseaction_viewCatalog.viewProductDetails&productDescription_10,952&CMP_LEC-GCMSSEARCH&HQS_HER2

2. For patients who are not unequivocally HER2 positive, what is the evidence on outcomes of treatment targeting the HER2 molecule (trastuzumab, etc.), or on differences in outcomes of a common chemotherapy or hormonal therapy regimen with versus without additional treatment targeting the HER2 molecule, in:
    a) Breast cancer patients characterized by discrepant HER2 results from different tissue assay methods performed adequately; and
    b) For those with HER2-negative breast cancer?

3. For breast cancer patients, what is the evidence on clinical benefits and harms of using HER2 assay results to guide selection of:
    a) Chemotherapy regimen; or
    b) Hormonal therapy?

4. What is the evidence that monitoring serum or plasma concentrations of HER2 extracellular domain in patients with HER2-positive breast cancer predicts response to therapy, or detects tumor progression or recurrence, and if so, what is the evidence that decisions based on serum or plasma HER2 assay results improve patient management and outcomes?

5. In patients with ovarian, lung, prostate, or head and neck cancers, what is the evidence that:
    a) Testing tumor tissue for HER2; or
    b) Monitoring serum or plasma concentrations of HER2;
either predicts response to therapy, or detects tumor progression or recurrence; and if so, what is the evidence that decisions based on HER2 assay results improve patient management and outcomes?

The first Key Question will be dealt with via a narrative review of the recent ASCO/CAP guidelines and evidence published subsequently.

Chapter 2. Methods

This report reviews and synthesizes available evidence on outcomes of using HER2 test results to manage patients with breast cancer or other solid tumors. Five Key Questions are addressed (see "Introduction"). After extensive consideration, we concluded that since a myriad of technical, biologic and performance matters influence HER2 diagnostic performance, that these variables could not be adequately captured in a systematic review. Thus, Key Question 1 will be addressed by a narrative review and Key Questions 2 through 5 will be addressed by systematic review.

This chapter describes the search strategies used to identify literature; criteria and methods used for selecting eligible articles; methods for data abstraction; methods for quality assessment; and, finally, the process for technical expert advice and peer review.

The methods of this review are generally applicable to all Key Questions except Key Question 1. However, as noted, there were variations in specific aspects of the methods as necessary to satisfy requirements of each question.

Peer Review

A technical expert panel provided consultation for the systematic review and reviewed the draft report. The draft report was also reviewed by 12 external reviewers, including invited clinical experts and stakeholders (Appendix D[*]). Revisions were made to the draft report based on reviewers' comments.

Study Selection Criteria

Types of Participants

For Key Questions 1-4, populations of interest are patients with breast cancer, with separate analyses for early stage patients receiving adjuvant therapy and those undergoing treatment for metastatic disease.

For Key Question 5, populations of interest are patients with cancers of the lung, ovary, prostate, and head and neck.

Types of Outcomes

In general, outcomes should be standard, valid, reliable, and clinically meaningful.

Two types of outcomes are relevant to Key Question 1:

- Diagnostic accuracy (e.g., analytic sensitivity, specificity, reliability, etc.);
- Concordance between assay methods; and

Multiple levels of outcomes will be addressed for Key Questions 2 through 5:

- Lead time for detection of progression, recurrence or metastasis.
- Patient management decisions, which may be altered by test results;

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

- Primary (health) outcomes, which may be affected through management changes guided by test results, such as:
    - Duration of survival, disease-free survival, progression-free survival, and/or time to failure or progression.
    - Quality of life.
    - Palliation of measurable symptoms.
    - Treatment-related adverse effects.
- Secondary (intermediate) outcomes include:
    - Objective clinical response rates (complete and partial responses; separately and summed).
    - Pathologic complete response rates in patients undergoing neoadjuvant therapy followed by surgery.
    - Response durations.

Health outcomes will be given greatest emphasis. However, it will likely be necessary to construct causal pathways to connect assay results to health outcomes through patient management decisions.

Types of Interventions

The interventions of interest for Key Questions 1, 2, 3, and 5 are tissue assays to evaluate tumor HER2 status by:

- Immunohistochemistry;
- Fluorescence in-situ hybridization;
- Chromogenic in-situ hybridization;
- Polymerase chain reaction; or
- Other methods.

The interventions of interest for Key Question 4, and also of interest for parts of Key Question 5, are assays to measure serum concentration of the HER2 extracellular domain.

Practice Settings

Interventions relevant to Key Questions 1–5 are used in the following settings:

- Pathology and laboratory medicine.
- Hospitals.
- Outpatient surgery facilities.
- Office-based practices.

Types of Studies

Following are study selection criteria specific to each key question.

HER2 assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2 assays were not standardized until very recently, we could not isolate effects of these disparate influences on assay results and patient classification.

This challenged the validity of using systematic review methods to compare available assay technologies. For that reason, we provide a narrative review of the following factors influencing HER2 test results and their use to classify patients: biologic processes, assay methods, and sources of variability.

Key Question 2. For patients who are not unequivocally HER2-positive, what is the evidence on outcomes of treatment targeting the HER2 molecule (trastuzumab, etc.), or on differences in outcomes of a common chemotherapy or hormonal therapy regimen with versus without additional treatment targeting the HER2 molecule, in:

a) Breast cancer patients characterized by discrepant HER2 results from different tissue assay methods performed adequately; and

b) For those with HER2-negative breast cancer?

Inclusion criteria:

- Randomized trials, or non-randomized studies (prospective or retrospective) on patients given a uniform chemotherapy regimen or hormonal treatment; that
- Directly compare outcomes of treatment with versus without trastuzumab (or other HER2-targeted therapy); and also
- Compare outcomes separately for one or more groups whose HER2 assay results are:
  a) equivocal, or discordant by IHC and ISH, with results separately reported for IHC 2+ and 3+ cases (IHC 0 and 1+ cases may be pooled); or
  b) unequivocally negative by both IHC and ISH.

Key Question 3. For breast cancer patients, what is the evidence on clinical benefits and harms of using HER2 assay results to guide selection of:

a) Chemotherapy regimen; or

b) Hormonal therapy?

Inclusion criteria:

- Randomized trials, prospective or retrospective studies on identically treated patients, including:
  - Identical hormonal therapy for all patients in studies on chemotherapy; and
    - Identical chemotherapy for all patients in studies on hormonal therapy; or
    - Separate reporting on identically treated groups.
- Report outcomes of a breast cancer treatment regimen separately by HER2 status;
- Report outcomes separately for patients undergoing treatment in the neoadjuvant, adjuvant or advanced (recurrent, refractory, or metastatic) settings

- Report:
  - Pathologic response (i.e. objective tumor regression) rates for studies on neoadjuvant therapy;
  - Disease-free, relapse-free, recurrence-free or progression-free survival for studies on adjuvant therapy; and
  - Progression-free or overall survival for advanced disease.

- Defined HER2 positivity consistently with the algorithm recommended in the ASCO/CAP guideline.
- Included at least 20 HE4R2-positive patients.

Separate evidence tables and analyses will focus on:

- Treatment setting (neoadjuvant, adjuvant or for advanced disease);
- Chemotherapy regimens (e.g., anthracycline-based regimens, or a taxane); and
- Hormonal therapies (e.g., tamoxifen versus aromatase inhibitors).

Key Question 4. What is the evidence that monitoring serum or plasma concentrations of HER2 extracellular domain in patients with HER2-positive breast cancer predicts response to therapy, or detects tumor progression or recurrence, and if so, what is the evidence that decisions based on serum or plasma HER2 assay results improve patient management and outcomes?

Inclusion criteria:

- Randomized trials, prospective single-arm studies, or retrospective series of identically treated patients; that
- Measure serum or plasma HER2 concentrations in breast cancer patients, either at baseline or at multiple time points; and either:
  - Associate baseline values or changes in HER2 concentration with one or more outcomes of interest (primary or secondary); or
  - Compare outcomes of treatment decisions based on assay results with outcomes of decisions made in absence of assay results.

Key Question 5. In patients with ovarian, lung, prostate, or head and neck cancers, using tumor tissue HER2 or monitoring serum or plasma concentrations of HER2 predicts response to therapy, or detects tumor progression or recurrence. Inclusion criteria:

- Randomized trials, prospective single-arm studies, or retrospective series of identically treated patients; that
- Measure HER2 in tumor tissue, serum, or plasma from patients with ovarian, lung, prostate, or head and neck cancers, and either:
  - Associate HER 2 status from tissue assays, or baseline values or changes in serum or plasma HER2 concentration, with one or more outcomes of interest (primary or secondary; see above); or
  - Compare outcomes of treatment decisions based on tumor HER2 status, or serum or plasma assay results, with outcomes of decisions made in absence of test results.

Search Strategy and Review

Search Strategy

Electronic databases. The following databases were searched for citations. The full search strategy is displayed in Appendix A[*]. The search was not limited to English-language references; however, foreign-language references without abstracts were disregarded.

The MEDLINE® search was performed through 2/23/07. The EMBASE® search was performed through 2/23/07. The Cochrane Controlled Clinical Trials Register search was performed through 2/23/07. Search updates limited by the Cochrane clinical trial filter were performed for all 3 databases on 4/25/08.

Additional sources of evidence. The Technical Expert Panel and individuals and organizations providing peer review were asked to inform the project team of any studies relevant to the key questions that were not included in the draft list of selected studies.

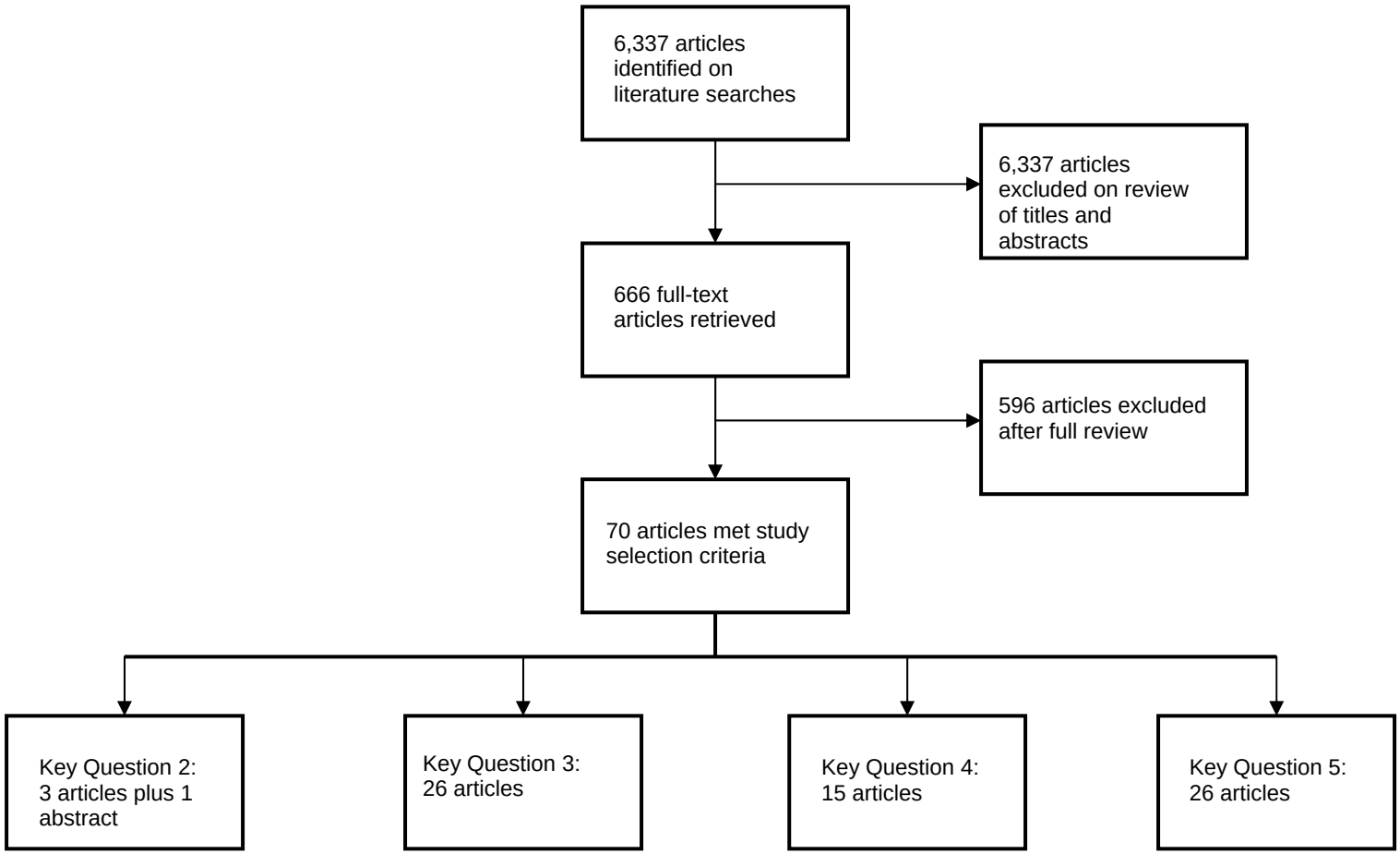
We also examined the bibliographies of all retrieved articles for citations to any relevant study that was missed in the database searches. In addition, we sought studies published in conference published in conference proceedings and abstracts from the American Association for Clinical Chemistry (AACC), American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP) and the San Antonio Breast Cancer Symposium (SABCS) over the past two years.

Search Screen

Search results were stored in a ProCite® database. Using the study selection criteria for screening titles and abstracts, a single reviewer marked each citation as either: 1) eligible for review as full-text articles; 2) ineligible for full-text review; or 3) uncertain. Citations marked as uncertain were reviewed by a second reviewer and resolved by consensus opinion, with a third reviewer to be consulted if necessary. Using the final study selection criteria, review of full-text articles was conducted in the same fashion to determine inclusion in the systematic review. Of 6,337 citations, 666 articles were retrieved and 70 selected for inclusion (Figure 1). Records of the reason for exclusion for each paper retrieved in full-text, but excluded from the review, were kept in the ProCite® database (see Appendix B, Excluded Studies).

[*] Appendixes cited in this report are available electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Figure 1. QUOROM Diagram



Data Extraction and Analysis

Data Elements

The data elements below were abstracted, or recorded as not reported, from included studies. Data elements to be abstracted were defined in consultation with the Technical Expert Panel.

Data elements from intervention studies (randomized, controlled trials, prospective single-arm studies, and retrospective consecutive series of identically treated patients) were:

- Critical features of the study design (for example, patient inclusion/exclusion criteria, number of subjects, use of blinding)
- Patient characteristics, including:
  - Age
  - Gender
  - Race/ethnicity
  - Disease and stage
  - Disease duration
  - Performance status
  - Other prognostic characteristics (e.g., estrogen or progesterone receptor status)

- HER2 assay techniques (tissue versus serum, IHC, FISH, PCR, ELISA, scoring methods, cutoffs);
- Treatment protocols (for example, regimen, dose, frequency, duration)
- Patient monitoring procedures (for example, followup duration and frequency, outcome assessment methods) and
- The specified key outcomes and data analysis methods (including techniques for assessing associations between HER2 findings and outcomes and methods for assessing treatment effect interactions)

Evidence Tables

Templates for evidence tables were created in Microsoft Excel® and Microsoft Word®. One reviewer performed primary data abstraction of all data elements into the evidence tables, and a second reviewer reviewed articles and evidence tables for accuracy. Disagreements were resolved by discussion, and if necessary, by consultation with a third reviewer. When small differences occurred in quantitative estimates of data from published figures, the values obtained by the two reviewers were averaged.

Assessment of Study Quality

For this systematic review we constructed a hierarchy of evidence quality for studies assessing HER2 status in predicting outcome. As addressed below, the continuum ranged from more informative specially designed randomized trials to less informative single-arm studies using univariate analyses. In addition to the hierarchy of evidence, we adapted acknowledged frameworks for evaluating the quality of prognostic or predictive studies. For assessing the quality of randomized trials, the general approach to grading evidence developed by the U.S.

Preventive Services Task Force (Harris, Helfand, Woolf, et al., 2001) was applied. To assess the quality of predictive studies, we adapted the "Reporting Recommendations for Tumor Marker Prognostic Studies" (REMARK) statement (McShane, Altman, Sauerbrei, et al., 2005). The quality of included prospective, single-arm intervention studies and retrospective consecutive series of identically treated patients was assessed based on a set of study characteristics proposed by Carey and Boden (2003). The quality of the abstracted studies was assessed by two independent reviewers. Discordant quality assessments were resolved with input from a third reviewer, if necessary.

Evidence Hierarchy

Table 3 shows the framework for evaluating how informative different designs and analytic strategies would be to predictions of outcomes according to HER2 status. The most informative scenario would be a trial in which randomized assignment to treatment groups would be stratified by HER2 status or patients were randomized to receive treatment guided by HER2 results or not (Conley and Taube, 2004). An adequately powered stratified randomization would allow valid inferences of treatment by HER2 interactions. Randomized trials generally are preferred because they convey the possibility of determining differences in the relative efficacy of two treatments, whereas single-arm studies can only assess the association between HER2 status and outcomes after a single treatment regimen. Subgroup analyses in randomized trials should ideally assess the significance of treatment effect interactions. Prespecified subgroups analyses guard against the problems of data dredging.

Post-hoc subgroup analyses may generate hypotheses, but may not support strong inferences about differential effectiveness. Multivariate subgroup analyses in randomized trials may be useful if the subgroup variable introduces imbalances between different variable by treatment combinations, particularly when only a subset of patients have tumor or serum specimens available. An alternative to multivariate subgroup analysis is cross tabulation of treatment by HER2 level results. The weakness of this approach is failure to control for imbalances in any important prognostic factors, particularly if the patients analyzed are a subset of those randomized. A formal test of interaction is preferred for any trial subgroup analysis. In single-arm (identically treated) studies, multivariate analyses may identify whether a variable is a significant independent predictor of treatment outcome while taking into account the separate influences of other predictors. The least informative situation would be a single-arm study that presents univariate comparisons of HER2 groups.

Table 3. Hierarchy of study design and conduct for assessing HER2 status prediction of outcome

More informative	Randomized trial, randomization stratified on HER2 status OR patients randomized to HER2-guided treatment or non-HER2-guided treatment
↑	Randomized trial, prespecified multivariate subgroup analysis
↑	Randomized trial, post-hoc multivariate subgroup analysis
Continuum	Randomized trial, treatment by HER2 subgroup analysis
↓	Single-arm study, prespecified multivariate analysis
↓	Single-arm study, post-hoc multivariate analysis
Less informative	Single-arm study, univariate analysis

Assessment of Study Quality

As stated, to assess the quality of predictive studies, we adapted the REMARK statement (McShane, Altman, Sauerbrei, et al., 2005). A checklist based on portions of REMARK and other sources (Gould Rothberg, and Bracken, 2006; Altman and Riley, 2005; Altman, 2001a, 2001b; Altman and Lyman, 1998; Brocklehurst and French, 1998; Altman, Lausen, Sauerbrei, et al., 1994; Simon and Altman, 1994) was developed. Table 4 identifies good quality characteristics that we looked for in predictive studies, including: prospective design; prespecified hypotheses about relation of marker to outcome; large, well-defined, representative study population; marker assay methods well-described; blinded assessment of marker in relation to outcome; homogeneous treatment(s), either randomized or rule-based selection; low rate of missing data ($\leq$15 percent); sufficiently long followup; well-described, well-conducted multivariate analysis of outcome. Decision rules for evaluating each quality item are described in the table.

For assessing the quality of randomized trials, the general approach to grading evidence developed by the U.S. Preventive Services Task Force (Harris, Helfand, Woolf, et al., 2001) was applied.

a. The quality of randomized, controlled trials will be assessed on the basis of the following criteria:

- Initial assembly of comparable groups: adequate randomization, including concealment and whether potential confounders (e.g., other concomitant care) were distributed equally among groups.
- Maintenance of comparable groups (includes attrition, crossovers, adherence, contamination).
- Important differential loss to followup or overall high loss to followup.
- Measurements: equal, reliable, and valid (includes masking of outcome assessment).
- Clear definition of interventions.
- All important outcomes considered.
- Analysis: Adjustment for potential confounders, intention-to-treat analysis.

Table 4. Interpretation rules for assessing quality of predictive studies

Quality Criterion	Rule
Prospective design	Applies to original study design, whether predictive aspect was part of original focus or not.
Prespecified hypotheses about relation of marker to outcome	Article must clearly state that investigation of relation of marker to outcome was prespecified primary or secondary objective of study. Must be coded no if original study design is retrospective. Retrospective analysis of originally prospective design is not a prespecified analysis (e.g., use of banked specimens).
Large, well-defined, representative study population	At least 100 participants and must have at least 10 events (not participants) per candidate predictor variable.
Marker assay methods well-described	Details or references available for detailed assay protocol including reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, scoring and reporting.
Blinded assessment of marker in relation to outcome	Were individuals assessing assay results blinded to outcomes?
Homogeneous treatment(s), either randomized or rule-based selection	All patients within a study arm must be given the same treatment regimen (no differences in type and number of modalities). Exceptions made for members of a class within a modality or combinations that have been show to have comparable efficacy. Heterogeneity of treatment regimens allowable up to 5% of patient population.
Low rate of missing data (≤15%)	Refers to number of participants originally enrolled.
Sufficiently long followup	Depends on natural history of disease for patient population defined by stage and other prognostic factors.
Well-described, well-conducted multivariate analysis of outcome:	
1) clear candidate variable selection	Methods for selecting candidate variables should be clearly described.
2) clear, appropriate model-building guidelines	Model building strategies should be based on previous evidence of predictive factors, not on arbitrary univariate significance levels or stepwise procedures.
3) assumptions tested	Mention should be made, for example, that the proportional hazards assumption of the Cox regression was tested.
4) standard prognostic variables included	A final model should include standard prognostic/predictive variables regardless of significance in univariate analysis.
5) continuous variables well handled	Arbitrary cutoffs should be avoided, optimal cutoffs should be clearly explained, multiple analytic methods explored including keeping variable continuous and more than 2 categories.
6) validation	Was a validation procedure mentioned?

Definition of ratings based on above criteria:

The rating of intervention studies encompasses the three quality categories described here.

- *Good:* Meets all criteria: Comparable groups are assembled initially and maintained throughout the study (followup at least 80 percent); reliable and valid measurement instruments are used and applied equally to the groups; interventions are spelled out clearly; all important outcomes are considered; and appropriate attention is given to confounders in analysis. In addition, for randomized, controlled trials, intention to treat analysis is used.
- *Fair:* Studies will be graded "fair" if any or all of the following problems occur, without the fatal flaws noted in the "poor" category below: In general, comparable groups are assembled initially but some question remains whether some (although not major) differences occurred with followup; measurement instruments are acceptable (although not the best) and generally applied equally; some but not all important outcomes are considered; and some but not all potential confounders are accounted for. Intention to treat analysis is done for randomized, controlled trials.
- *Poor:* Studies will be graded "poor" if any of the following fatal flaws exists: Groups assembled initially are not close to being comparable or maintained throughout the study; unreliable or invalid measurement instruments are used or not applied at all equally among groups (including not masking outcome assessment); and key confounders are given little or no attention. For randomized, controlled trials, intention to treat analysis is lacking.

b. The quality of included prospective single-arm intervention studies and retrospective consecutive series of identically treated patients was assessed based on a set of study characteristics proposed by Carey and Boden (2003), as follows:

- Clearly defined question.
- Well-described study population.
- Well-described intervention.
- Use of validated outcome measures.
- Appropriate statistical analyses.
- Well-described results.
- Discussion and conclusion supported by data.
- Funding source acknowledged.

Chapter 3. Results and Conclusions

Narrative Review for Key Question 1

What is the evidence on concordance and discrepancy rates for methods (e.g., FISH, IHC, etc.) used to analyze HER2 status in breast tumor tissue?

HER2 assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2 assays have not been standardized until very recently, the effects of these disparate influences could not be isolated. This challenged the validity of using systematic review methods to compare available assay technologies. For that reason, we provide a narrative review of the following factors influencing HER2 test results and their use to classify patients: biologic processes, assay methods, and sources of variability.

Biologic Processes that Influence Cell Membrane Levels of HER2 Protein

Genes such as those in the epidermal growth factor (EGF) receptor family (HER1 through HER4) affect cellular function through the proteins they encode. The HER2 gene is expressed and HER2 protein is found in membranes of all breast and other epithelial cells, and cut-points between "normal" and "overexpressed" levels of HER2 protein are imprecise. Nevertheless, studies have associated increased amounts of HER2 protein in cell membranes with more aggressive behavior of breast and other epithelial cancers and may predict treatment outcomes (Slamon, Clark, Wong, et al., 1987; Esteva, Pusztai, Symmans, et al., 2000; Rowinsky, 2004; Hynes and Lane, 2005; Ettinger, 2006; Serrano-Olvera, Duenas-Gonzalez, Gallardo-Rincon, et al., 2006).

Expression of HER2 and similar genes is a sequential process that (in a simplified overview) includes the following steps: transcription of DNA to messenger RNA (mRNA); processing mRNA to mature, translatable messages; and translation of mature mRNA to synthesize the protein's amino acid sequence. For many proteins (including HER2), additional steps required to produce functional molecules include: post-translational modification (e.g., glycosylation), three-dimensional folding, assembly of multi-subunit proteins, and movement to the relevant cellular site or organelle (not necessarily in this sequence).

We will discuss each of the following biologic mechanisms that potentially may increase the amount of HER2 protein in cell membranes:

A. Increased gene copy number (i.e., more than diploid amounts of HER2 DNA in cell nuclei), by:
    1. HER2 gene amplification, or
    2. Chromosome 17 polysomy;
B. Elevated HER2 protein levels in cells with diploid amounts of HER2 DNA, by
    1. Increased rate of HER2 gene expression; or
    2. Decreased degradation (increased stability) of HER2 mature message and/or protein.

Increased gene copy number.

Gene amplification. In most HER2-positive cases, increased levels of HER2 protein in breast cancer cell membranes are attributable to an amplified HER2 gene (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007; Slamon, Clark, Wong, et al., 1987). Gene amplification increases the copy number for a segment from one arm of a chromosome (Albertson, 2006; Myllykangas and Knuutila, 2006); amounts of the central portion (centromere) and the chromosome's other arm remain unaltered. The amplified DNA segment (amplicon) can include one or several genes. It can be organized as extrachromosomal elements, as repeated units at a single locus (which lengthens the affected chromosome arm), or repeats can be spread throughout the genome. Typically, all or most copies of the amplified gene(s) are expressed, and amounts of the excess protein increase nearly exponentially with gene copy number per cell (Szollosi, Balazs, Feurenstein, et al., 1995; Konecny, Pegram, Venkatesan , et al., 2006).

The HER2 gene has been mapped to the long arm of chromosome 17, at position 17q12 (Vanden Bempt, Drijkoningen, and De Wolf-Peeters, 2007; Jarvinen and Liu, 2006; Kauraniemi and Kallioniemi, 2006; Mano, Rosa, De Azambuja, et al., 2007). Amplicon size can vary, with from two to ten (or more) other amplified genes mapping to the region from 17q12 to 17q21. Although not relevant to assays used to classify HER2 status of patients with breast cancer, note that the gene coding for the enzyme topoisomerase II-α (TOPIIA, a target of the anthracyclines) also is located in this segment. Co-amplification of these genes may be more relevant to predict outcomes of therapy with an anthracycline regimen than amplification of the HER2 gene alone, since excess TOPIIA activity is a potential mechanism of anthracycline resistance (see "Results and Conclusions, Key Question 3").

Chromosome 17 polysomy. HER2 gene copy number also may rise if cells have more than two copies of chromosome 17. Obviously, cells that have replicated their DNA but not yet divided have four rather than two copies of each chromosome, thus also of the HER2 gene. But some breast or other cancer cells may have extra copies of one or more whole chromosomes (termed polysomy), and may stably pass this characteristic to daughter cells. Cells with chromosome 17 polysomy have extra copies of the HER2 gene, although the ratio of HER2 copy number to centromere copy number is the same as in diploid cells unless HER2 also is amplified. However, it is uncertain whether chromosome 17 polysomy is associated with overexpression of the HER2 protein (Vanden Bempt, Drijkoningen, and De Wolf-Peeters, 2007; Beser, Tuzlali, Guzey, et al., 2007; Corzo, Bellosillo, Corominas, et al., 2007; Hyun, Lee, Kim, et al., 2008; Torrisi, Rotmensz, Bagnardi, et al., 2007; Downs-Kelly, Yoder, Stoler, et al., 2005; Ma, Lespagnard, Durbecq, et al., 2005).

Elevated HER2 protein in cells with diploid HER2 DNA. Although uncommon, clinical investigators have reported breast cancer cases with elevated HER2 protein levels in malignant diploid cells (i.e., cells lacking amplified HER2 genes or polysomy 17; e.g., Mass, Press, Anderson, et al., 2005; Vogel, Cobleigh, Tripathy, et al., 2002; Pauletti, Godolphin, Press, et al., 1996). This probably arises through increased expression of the HER2 gene, although decreased rates of degradation for either the mRNA or protein are at least theoretically possible. Increased expression may involve enhanced rates of transcription, message processing, translation, and/or post-translational modification (selectively for the HER2 gene). Detailed review of mechanisms that may increase rates of these processes is outside this report's scope.

It is uncertain whether tumors with increased membrane HER2 protein but diploid HER2 DNA respond differently to therapies (targeted to the HER2 protein, or to others) than do tumors

with amplified HER2 DNA that increases HER2 protein. It is also unknown if the route to excess HER2 protein (i.e., whether from increased mRNA production, protein synthesis, or decreased degradation of either) affects tumor biology and aggressiveness or treatment outcomes. In vitro data suggest that increased membrane HER2 protein affects cell physiology, proliferation, and treatment responses in the same way, regardless of how the excess is produced (Pierce, Arnstein, DiMarco, et al., 1991).

Tissue Assays Routinely Used in Clinical Practice to Determine HER2 Status of Breast Tumors

In current clinical practice, assays used to classify breast cancer patients with respect to HER2 status detect either HER2 protein or HER2 DNA (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Research laboratories use assays for HER2 mRNA to study molecular mechanisms and biologic regulation. They are technically more difficult than protein and DNA assays, and measure less-stable molecules. Although real-time reverse transcription polymerase chain reaction (RT-PCR) methods recently were adapted to measure HER2 mRNA in fixed, paraffin-embedded tissues and compared with IHC and ISH assays (Capizzi, Gruppioni, Grigioni, et al., 2008), RT-PCR assays for HER2 mRNA are still uncommon in clinical management of patients with breast cancer and thus are not included in this review.

Each method used to determine HER2 status applies results of a quantitative or semiquantitative assay to assign a binary ("yes/no") classification. Thus, test results with each assay can vary with different scoring systems and thresholds for positivity. As discussed in a following section ("Postanalytic Factors"), scoring and thresholds may depend on choice of reagents to detect, visualize, and quantitate analytes. Scoring systems and thresholds also have changed over time, with standardized approaches recommended quite recently (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Data are lacking to determine whether differences in treatment outcome as a function of HER2 status are affected by reclassifying patients with currently recommended scoring systems and thresholds.

Methods to detect/measure amount of HER2 protein. Immunohistochemistry (IHC) is the assay used most widely for classifying HER2 status of breast cancer patients, since it uses techniques and equipment long used by most clinical pathology laboratories for other proteins such as estrogen and progesterone receptors (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). The assay incubates thin slices of fixed tissue on a microscope slide with an antibody to HER2, washes off unbound antibody, then visualizes bound antibody. Because IHC preserves tissue architecture and cellular structure (morphology), it permits scoring to focus on antibody specifically bound to membranes of invasive breast cancer cells. IHC also permits permanent storage of stained slides if later re-evaluation is needed.

IHC scoring systems consider the proportion of antibody-stained invasive cancer cells and the intensity of staining, a partly subjective judgment. Besides the U.S. Food and Drug Administration (FDA) -approved IHC kits (HercepTest™ and PATHWAY™; see Table 2, Introduction), various antibodies to HER2 protein are commercially available as analyte-specific reagents that can be used for independently developed (so-called "home-brew") assays (Wolff,

Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003; Hicks and Kulkarni, 2008). Some are polyclonal, with a mix of antibody molecules that may recognize different binding site (epitopes) on the HER2 protein. Others are monoclonal, homogeneous molecules that recognize a single epitope. These differences may lead to discrepant results with different antibody reagents (Press, Hung, Godolphin, et al., 1994). Other sources of variability in IHC results are discussed in the following section, "Sources of Variability in Classifying HER2 Status."

Protein assays on homogenized tissue may use antibody to visualize HER2 after separating proteins in a solid matrix (Western blots), or quantitate HER2 by enzyme-linked immunosorbent assay (ELISA). These assays destroy the analyzed tissue samples. Additionally, tissue extracts may mix proteins from cytosol, membranes, and other organelles; and also from multiple cell types: normal breast, inflammatory cells, in situ tumor, and invasive cancer. HER2 levels of in situ breast tumor cells often are elevated, for uncertain reasons and with inadequately studied clinical implications (Allred, Clark, Tandon, et al., 1992; Hoque, Sneige, Sahin, et al., 2002; Collins and Schnitt, 2005). Guidelines stress avoiding areas of ductal carcinoma in situ when scoring assay results (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Nearly all clinical studies on HER2 protein assays to predict treatment outcomes used IHC on tissue slices rather than assays on tissue homogenates, and assigned HER2 status by amount of HER2 protein in membranes of invasive breast cancer cells.

Methods to detect/measure HER2 gene copy number or amount of HER2 DNA. In situ hybridization (ISH) is the most commonly used method to measure HER2 gene copy number in tissue samples from breast cancer patients (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007; Ross, Fletcher, Linette, et al., 2003; Hicks and Kulkarni, 2008). It uses a labeled probe complementary to the DNA sequence of interest (here, a unique segment from the HER2 gene). Double-stranded DNA in cell nuclei of the fixed tissue sample is denatured so the probe can hybridize (bind) to its complementary sequence, then unbound probe is washed away. As with IHC, tissue preparation for ISH preserves tissue and cell morphology, and scoring focuses on invasive breast cancer cells.

The gene-specific probes are visualized in one of three ways: by fluorescence (FISH), a chromogenic reaction (CISH; uses digoxigenin), or silver deposition (SISH; uses dinitrophenol for enzymatic metallography). FISH requires a fluorescence microscope (more expensive and unavailable in some smaller pathology laboratories), while CISH and SISH use routine light (brightfield) microscopy. Three FDA-approved kits are available for HER2 testing by FISH (PathVysion®, Inform™, and *HER2* FISH pharmDx™), while kits for CISH (SPoT-Light) and SISH (EnzMet GenePro™) are not yet approved (see Table 2, Introduction). Slides prepared for FISH testing lose fluorescence, thus, cannot be stored for later review. In contrast, slides prepared for either CISH or SISH can be archived and re-evaluated. Additionally, it is sometimes difficult to identify invasive tumor cells with fluorescence microscopy. All three ISH methods require more time per sample than IHC for slide scoring. Because they were developed recently, fewer clinical studies used CISH or SISH than FISH to classify HER2 status of breast cancer patients.

In ISH assays, pathologists count fluorescent (FISH) or dark-colored (CISH, SISH) spots visible above the nucleus to measure HER2 gene copy number: two in diploid cells; more in cells

with amplified HER2 or polysomy 17. Typically, one determines gene copy number for multiple invasive cancer cells on the slide, and averages results for the tissue sample. In some ISH assays, slides are hybridized simultaneously with two probes that fluoresce in or show different colors, to permit copy number measurement for the HER2 gene and chromosome 17 centromere (CEP17). With this approach, HER2 gene status is defined by the ratio of HER2 to CEP 17 copy numbers: greater than 2 if amplified, but approximately 2 if unamplified whether chromosome 17 polysomy is absent or present.

Early research studies extracted DNA from tissue homogenates and measured amounts of the HER2 gene by Southern or slot blots, or by quantitative polymerase chain reaction (PCR) assays. Southern blots first separate DNA molecules by their mobility in a matrix, while slot blots use the mixed extract. Each selectively visualizes the DNA sequence of interest by hybridizing to labeled probes as in ISH. PCR assays amplify (selectively replicate) DNA sequences of interest in vitro, detect them by fluorescent or other probes, and quantify the starting amount using standard curves. As with protein assays on tissue homogenates, these techniques dilute DNA from invasive cancer cells with DNA from surrounding normal tissues and inflammatory cells (Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). They also consume the samples they analyze. Southern and slot blots are less sensitive than PCR and require substantially larger amounts of DNA. Southern blot assays also are labor intensive and less widely available in clinical pathology labs. The remainder of this review focuses on IHC and ISH methods, the only HER2 assays with FDA-approved kits available for clinical use.

Sources of Variability in Classifying HER2 Status

Accurately determining HER2 status depends on proper performance of preanalytic, analytic, and postanalytic steps (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hicks and Kulkarni, 2008; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). Preanalytic steps are those involved in obtaining, preserving (fixing), and storing tissue samples prior to staining and analysis. Analytic steps prepare and stain fixed tissue samples with antibody to HER2 for IHC, or prepare and hybridize them to HER2 gene probe for ISH, then visualize tissue-bound antibody or probe. Postanalytic steps score test results, classify patients, and assure test quality, consistency, and reproducibility. Some processes for these steps are the same for IHC or ISH, but many differ.

Preanalytic: tissue processing and storage. HER2 tests can use tissue from core (incisional) biopsy or tumor excised for biopsy, lumpectomy, or mastectomy (Wolff, Hammond, Schwartz, et al., 2007a). Tissue sources can be the primary tumor or a lymph node or distant metastasis (Carlson, Moench, Hammond, et al., 2006). While uncommon, studies have reported discordances in HER2 status between primary tumor and metastases (for references, see Carlson, Moench, Hammond, et al., 2006). Retesting HER2 status if metastases develop after a long disease-free or progression-free interval may be warranted, depending on where and how HER2 status of the primary tumor was determined.

Tissues are prepared and preserved for assays by slicing larger samples, fixing in a denaturing solution, and embedding fixed tissue for long-term storage (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Factors that may influence test results include: edge, retraction, or crush artifacts with some core needle biopsies; time from excision to slicing, and to fixation; type of and time in

fixative; choice of embedding material; and conditions and duration of storage for fixed and embedded tissues.

Guidelines seeking to standardize methods were not published until recently (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007), although prior consensus conferences (cited in the guidelines) recommended many of the same methods. Importantly, the recommended preanalytic steps are identical for tissues to be tested by IHC or ISH; these are summarized in a following section (see Table 5 in "Current Guideline Recommendations"). Systematic reviews conducted for the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) and National Comprehensive Cancer Network (NCCN) guidelines (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006) reported data were lacking to evaluate effects of nonadherence on test results for some aspects of tissue processing. The published guidelines did not include evidence tables summarizing effects of nonadherence on test results for those aspects of tissue processing that have been evaluated comparatively.

Notably, the literature review for this report showed that most studies reporting concordance and discordance rates of different IHC and ISH assays used archived samples, fixed and embedded elsewhere than the laboratory performing the HER2 assays. With exceptions, most publications did not report adequately on adherence to guideline or prior (consensus) recommendations for tissue processing.

Analytic: performing HER2 assays. Analytic steps for processing thin sections of fixed and embedded tissue cut onto glass slides differ for IHC and ISH assays (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Each begins by deparaffinizing thin tissue sections, but IHC assays use an antigen retrieval step that optimizes antibody binding to HER2 protein while ISH assays first unwind (denature) cells' double-stranded DNA so that the probe can hybridize to its complementary sequence. The temperature and duration of heating used to bake tissue sections on slides, as well as the conditions used for antigen retrieval, can introduce variability in IHC results. Each assay incubates slides with an analytic reagent (antibody for IHC; probe for ISH), removes unbound reagent in one or more washing steps, and incubates with other reactants to visualize bound analytic reagent. Some steps can be automated, which improves consistency and reproducibility if equipment is well-maintained and regularly calibrated. In addition to reagent choice (which antibody, for IHC; which DNA probe, for ISH), varying the conditions (temperatures, durations, etc.), solutions, and reactants used for each step can affect test results, as can poorly maintained or calibrated automated equipment.

While FDA-approved kits include protocols with optimized methods for each analytic step, guideline publications report that approximately half of surveyed laboratories did not adhere completely to protocol methods (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006). The guidelines stress the need to train and periodically assess the skills of staff conducting these assays, and that each run should include standardized positive and negative controls. They also emphasize that each laboratory offering HER2 testing services should validate its test results against a previously validated test, and that laboratories departing from protocol-specified methods with FDA-approved kits, and those using independently developed assays with analyte-specific reagents, should validate test results against established methods and develop their own standard protocols.

As with preanalytic steps, most published studies did not adequately report information needed to evaluate complete adherence with guideline or prior (consensus) recommendations on

all analytic steps. Studies that used FDA-approved kits rarely commented on protocol adherence in the methods sections of their reports, and studies that used independently developed assays rarely described assay validation against approved kits.

Postanalytic factors. IHC scoring systems and positivity thresholds have changed over time, and these changes likely alter the proportion of patients classified as HER2 positive (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hicks and Kulkarni, 2008; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). Some studies on archived tissues classified tumors as HER2 positive if any invasive cells showed strong, complete membrane staining (e.g., Paik, Bryant, Park, et al., 1998; Houston, Plunkett, Barnes, et al., 1999; Paik, Bryant, Tan-Chiu, et al., 2000). Others classified samples as HER2 positive if 1 percent or more of invasive cells were stained (e.g., MacGrogan, Mauriac, Durand, et al., 1996; Elledge, Green, Ciocca, et al., 1998; Di Leo, Larsimont, Gancberg, et al., 2001); yet others, only if 50 percent or more were stained (e.g., Agrup, Stal, Olsen, et al., 2000; Berry, Muss, Thor, et al., 2000; Colozza, Sidoni, Mosconi, et al., 2005). Few studies adopted (or adapted) Allred's system (Harvey, Clark, Osborne, et al., 1999; developed for IHC assays of estrogen receptors), which rates the proportion of stained invasive cells (from 0 to 5) and the intensity of staining (from 0 to 3), then adds for a final score between 0 and 8.

The scale recommended in FDA-approved IHC kits (0 to 3+; developed for HercepTest™ but also used with PATHWAY™) requires membrane staining in 10 percent or more of invasive cells for scores greater than 0. The scale assigns positive scores by staining intensity and totality of membrane staining: 1+ is faint or barely perceptible staining that is incompletely circumferential; 2+ is moderate intensity but complete circumferential staining; and 3+ is strong intensity and complete circumferential staining (www.dakousa.com/prod_downloadpackageinsert.pdf?objectid_105073003). However, some studies that used this scale defined HER2-positive cases as those scored 2+ or 3+, while others classified only those with a score of 3+ as HER2 positive. The ASCO/CAP guideline retains the original definitions for scores of 0 to 2+, but recommends scoring IHC 3+ only if more than 30 percent of invasive breast cancer cells show dark, homogeneous, circumferential membrane staining in a "chicken wire" pattern (Wolff, Hammond, Schwartz, et al., 2007a). Adequate data are lacking to compare accuracy or concordance for this wide variety of scoring systems and thresholds used to classify patients' HER2 status by IHC alone. However, in one recent study (Hameed, Chhieng, and Adams, 2007), three pathologists blinded to FISH results scored IHC-stained slides from 98 breast cancer cases separately using cut-offs of 10 percent, 30 percent, and 50 percent of stained cells to classify samples as HER2+. Specificity of IHC versus FISH was 82 percent, 86 percent, and 87 percent, respectively, for the three increasing cut-offs, while concordance rates of 3+ cases with FISH were 59 percent, 64 percent, and 65 percent.

Scoring and categorizing results of ISH assays also varies (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hicks and Kulkarni, 2008; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). Guidelines stress that precision and accuracy depend on the number of cells counted and averaged, on accurately identifying and only counting invasive cells, and on counting invasive cells from two or more separate areas of each tumor on either the same or sequential slide(s) (Wolff, Hammond, Schwartz, et al., 2007a). With assays estimating gene copy number per cell without normalizing to a CEP17 probe, most published studies using FISH classified tissues averaging more than 4.0 copies per cell as HER2 positive (for references, see Wolff,

Hammond, Schwartz, et al., 2007; Carlson, Moench, Hammond, et al., 2006; Laudadio, Quigley, Tubbs, et al., 2007a). Most published studies using CISH scored samples HER2 positive if the average gene copy number per cell was greater than 5, although some followed the manufacturer's recommendation and defined low-level amplification as copy numbers between 6 and 10. In contrast to published studies with FISH or CISH, recent guidelines consider average scores greater than 6.0 as FISH positive, scores less than 4.0 as FISH negative, and scores between 4.0 and 6.0 as equivocal (ASCO/CAP) or borderline (NCCN). Most studies that normalized to CEP17 classified HER2 to CEP17 ratios greater than 2.0 as HER2 positive (for references, see Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Laudadio, Quigley, Tubbs, et al., 2007). The guidelines consider a HER2/CEP17 ratio greater than 2.2 as positive, a ratio less than 1.8 as negative, and ratios between 1.8 and 2.2 as equivocal (ASCO/CAP) or borderline (NCCN). As with IHC scoring and thresholds, data are lacking to evaluate consequences of the newer classification criteria on accuracy or concordance.

Guidelines and reviews caution that assigning HER2 status is partially subjective and potentially inconsistent because IHC and FISH scoring criteria are variably interpreted and applied by different raters (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hicks and Kulkarni, 2008; Hanna, O'Malley, Barnes, et al., 2007; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). Expert panels and reviewers emphasize that image analysis methods, using digital microscopy and automated cellular imaging systems (e.g., Bloom and Harrington, 2004; McCabe, Dolled-Filhart, Camp, et al., 2005; Tubbs, Pettay, Swain, et al., 2006; Ciampa, Xu, Ayata, et al., 2006; Tawfik, Kimler, Davis, et al., 2006; Moeder, Giltnane, Harigopal, et al., 2007), can decrease inter-rater variability and thus improve scoring consistency, accuracy, and precision, particularly for IHC assays. However, this requires careful validation and periodic recalibration of automated systems against standardized positive, negative, and equivocal control samples. Nevertheless, a study testing agreement between pathologists reported that use of digital microscopy to score IHC improved concordance with FISH and also decreased inter-rater variability (Bloom and Harrington, 2004).

Postanalytic steps also include reporting elements that should be provided to clinicians ordering HER2 testing, as well as quality assurance procedures (laboratory accreditation and proficiency testing; competency assessment for pathologists). However, these issues are outside the scope of this report. Readers are referred to recommendations in current guidelines (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007).

Is There a "Best" Method to Determine HER2 Status from Breast Tumor Tissue?

Although many studies reported concordance and discrepancy rates for collections of breast tumor tissue tested for HER2 status by IHC with different antibodies, or by IHC and ISH assays, or by multiple ISH assays, current evidence does not suggest one HER2 assay is superior to all others (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hicks and Kulkarni, 2008; Laudadio, Quigley, Tubbs, et al., 2007; Ross, Fletcher, Linette, et al., 2003). As described previously, preanalytic, analytic and postanalytic methods varied between studies, and all studies preceded guidelines for standardizing these methods. Additionally, data are lacking to fully evaluate effects of nonadherence with certain guideline recommendations on test results. Thus, it is difficult (perhaps impossible) to isolate effects of individual factors that contribute to discordance. As detailed above, these include differences in:

- Fixing and embedding tissues, preparing and staining them for assays, or scoring and classifying test results;
- Inherent differences in antibody binding, epitope stability, or antigen retrieval when comparing different antibodies used for IHC;
- Different biologic mechanisms that can increase membrane HER2 protein, when comparing IHC assays versus ISH assays; or differences in sensitivity and specificity of diverse DNA probes and visualization techniques when comparing different ISH methods.

Identifying one "best" HER2 test clearly requires better comparative data than presently available, with assays that standardized key aspects of preanalytic, analytic, and postanalytic steps in HER2 assay methods.

The lack of a gold standard to determine breast tumors' HER2 status also prevents agreement on one "best" HER2 assay. Furthermore, seeking a single gold standard may be unrealistic, since HER2 status is used in different ways. The optimal assay (or combination of assays) may differ for HER2 as a prognostic marker, as a marker to predict clinical benefit from trastuzumab, or as a marker to predict benefit from a chemotherapy drug class (e.g., an anthracycline or a taxane). For example, HER2 gene amplification may best predict tumor aggressiveness hence prognosis, while membrane density of HER2 protein may best predict trastuzumab binding to tumor cells and thus clinical response. Furthermore, HER2 may only be a surrogate marker for other molecular alterations that more directly impact tumor cell sensitivity to certain chemotherapy drugs (e.g., anthracyclines).

Outcomes of well-designed and adequately powered comparative clinical trials with sufficient followup duration may be a gold standard to evaluate HER2 assays as predictors of treatment benefit. However, even the large randomized, controlled trials on adjuvant trastuzumab (Romond, Perez, Bryant, et al., 2005; Piccart-Gebhart, Procter, Leyland-Jones, et al., 2005; Slamon, Eiermann, Robert, et al., 2005; Joensuu, Kellokumpu-Lehtinen, Bono, et al., 2006) may not have adequately standardized preanalytic steps at local hospitals, did not test all patients with at least two assays, treated few patients with discordant results by different assays conducted in central laboratories; and presently lack sufficient followup to compare outcomes in subgroups of the main treatment arms (see "Results and Conclusions, Key Question 2").

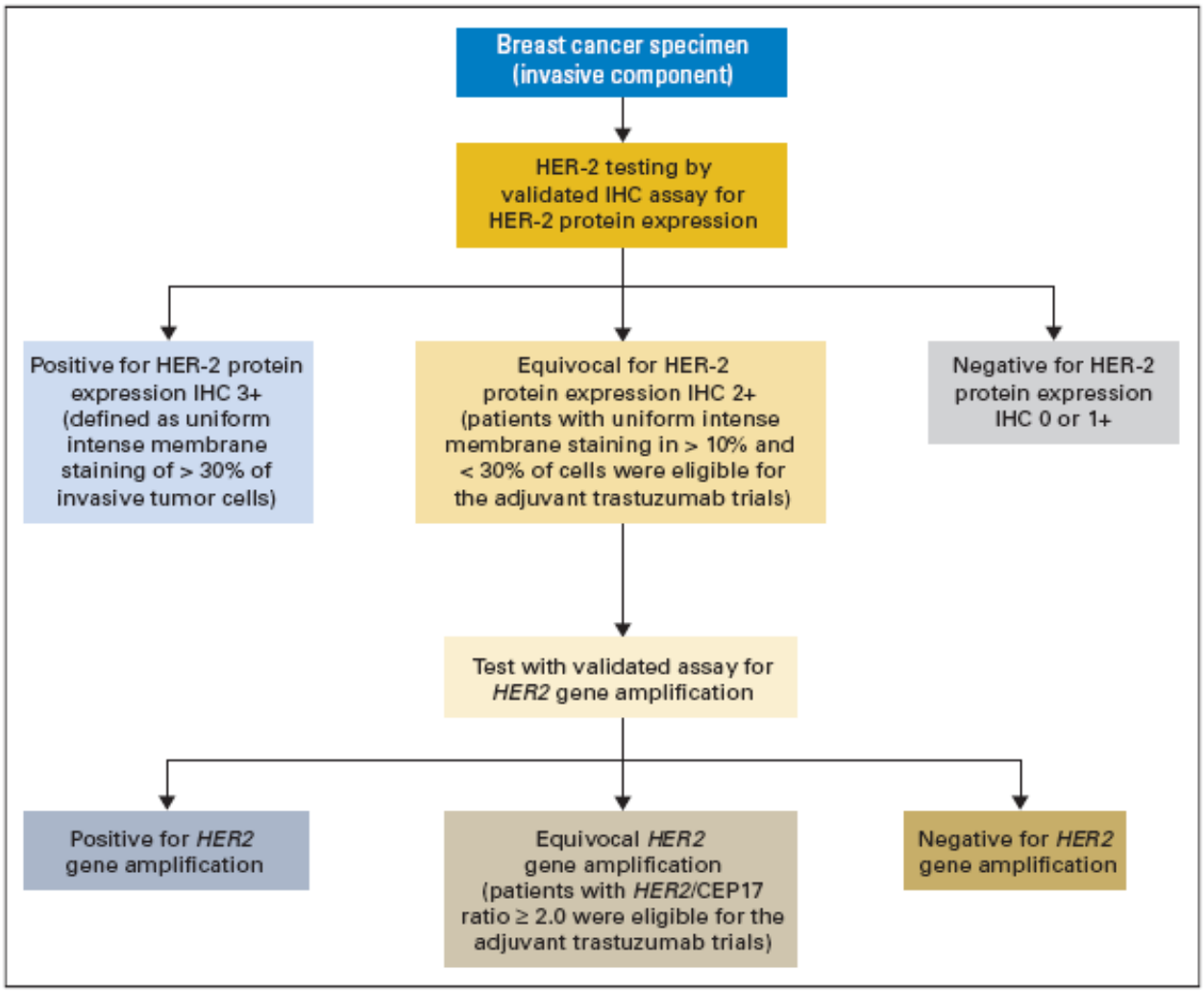
Current guidelines acknowledge present uncertainty, permit clinicians and laboratories to choose an initial HER2 assay method, and recommend confirming results with an alternative

assay when initial tests are equivocal (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007).

Current Guideline Recommendations

Current guidelines recommend very similar algorithms for using well-validated IHC and ISH assays to classify breast cancer patients with respect to HER2 status (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). The algorithm shown in Figure 2, describes possible results, decision-making, and

Figure 2. Algorithm for immunohistochemistry (IHC). (Reprinted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007b)



confirmatory testing when IHC is the initial test. All three guidelines agree that an IHC score of 3+ is definitively HER2 positive, a score of 0 or 1+ is definitively HER2 negative, and a score of 2+ is equivocal and requires ISH followup testing to determine HER2 status. In contrast to the other guidelines, the NCCN Task Force (Carlson, Moench, Hammond, et al., 2006) did not specify that an IHC 3+ score requires complete membrane staining in more than 30 percent of invasive cells. The ASCO/CAP expert panel recommended this change from FDA labeling

(which requires staining in more than 10 percent of invasive cells), primarily to decrease the number of patients with false-positive results who might be given trastuzumab but are unlikely to benefit (Wolff, Hammond, Schwartz, et al., 2007a). This recommendation anticipates that true positives with equivocal IHC results will be correctly classified by followup ISH. However, data are currently lacking to test this hypothesis.

Figure 3 provides a similar algorithm if FISH is the initial test. The guidelines suggest that well-validated alternatives (CISH or SISH, currently available in the U.S. only as independently developed assays) probably can replace FISH. The algorithm considers HER2 gene copy numbers from 4.0 to 6.0 or HER2/CEP17 ratios between 1.8 and 2.2 as equivocal ISH results. It recommends additional cell counting, retesting by a reference laboratory, or followup testing by IHC before classifying equivocal cases. The other guidelines agree with this recommendation (Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). No studies reviewed for this report followed this recommendation; thus, data are lacking to determine whether confirmatory followup testing on patients with equivocal ISH results improves the accuracy of HER2 status as a predictor for treatment outcomes.

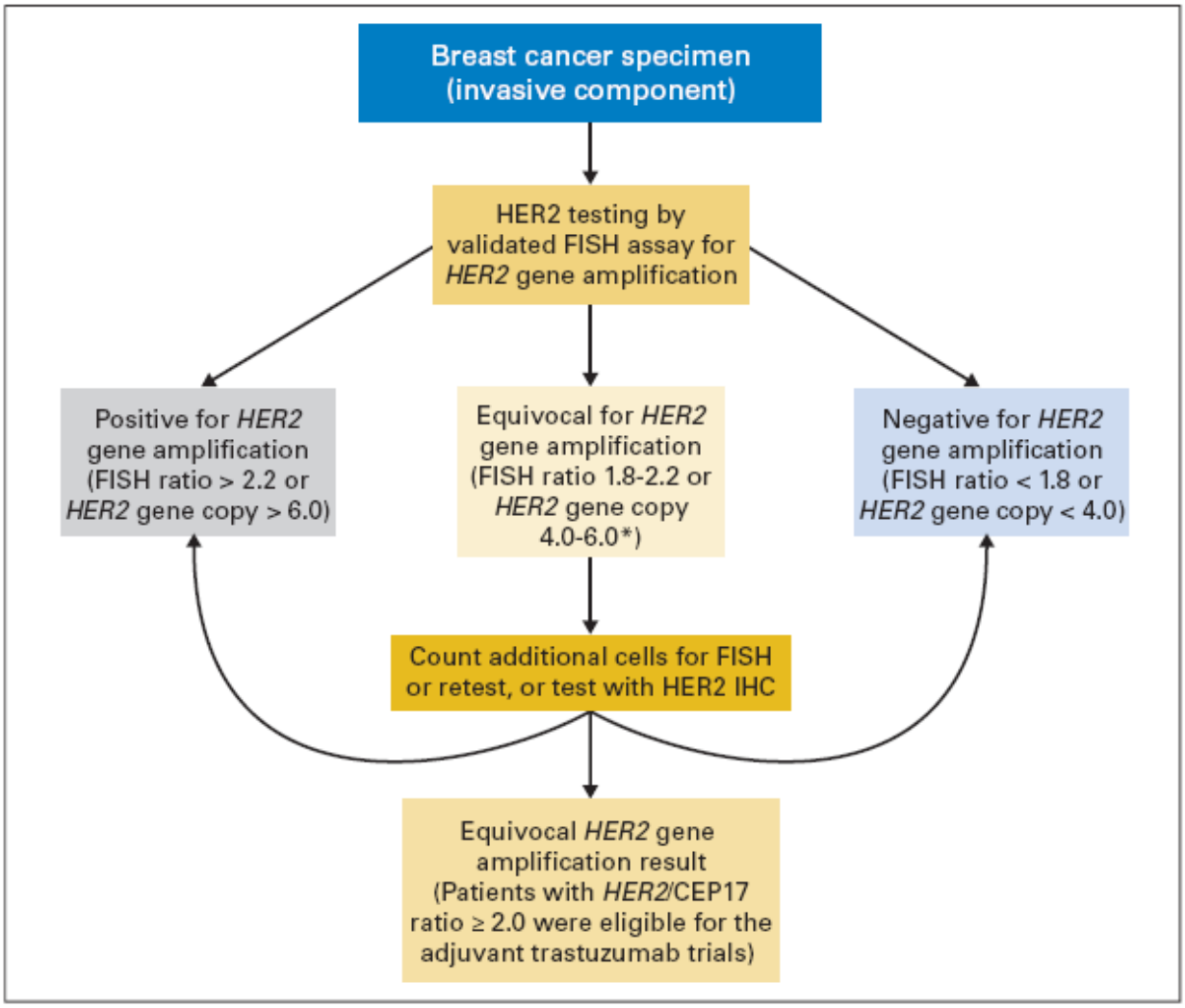
Importantly, the guidelines' treatment recommendations are not identical for all patients whose assay results remain in the equivocal range after additional cells are counted, a different assay method is used, and/or testing is repeated on another tumor section. The recommendation depends on whether the patient would have been included in or excluded from key randomized, controlled trials. For example, patients with HER2/CEP17 ratios 2.0 or greater but less than 2.2 were included and randomized in the adjuvant trastuzumab trials. Therefore, the guidelines view current evidence as too weak to deny such patients adjuvant therapy that includes trastuzumab. In contrast, patients with HER2/CEP17 ratios 1.8 or greater but less than 2.0 were excluded from these trials, and the guidelines view current evidence as too weak to support including trastuzumab in their adjuvant therapy regimens. Figures 2 and 3 include information on trial eligibility of patients whose test results are equivocal by each HER2 assay.

Interestingly, a recent study reported on 17 patients with breast core biopsy specimens showing invasive carcinoma and equivocal FISH results (HER2/CEP17 ratios between 1.8 and 2.2) (Striebel, Bhargava, Horbinski, et al., 2008). These patients were subsequently re-evaluated by IHC and FISH testing on resection specimens. For 10 of the 17 cases, equivocal results obtained with biopsy specimens were definitively resolved by retesting of resection specimens. Four patients were classified HER2 positive and treated with trastuzumab, while six were classified HER2 negative and managed without trastuzumab.

Other recommendations in the ASCO/CAP guideline focus on good laboratory practices for each preanalytic, analytic, and postanalytic step of IHC and ISH assays (Wolff, Hammond, Schwartz, et al., 2007a). They provide a more explicitly detailed set of recommendations than included in the other two guidelines (Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Table 5 reprints the summary of recommendations from the ASCO/CAP guideline. The remainder of this narrative review for Key Question 1 summarizes evidence published after these guidelines on the following four topics, and discusses unresolved issues and uncertainties:

- Concordance and discordance of different assay methods
- Discordance between central and local laboratory results
- Validation and proficiency testing
- Reports on polysomy 17

Figure 3. HER2 testing algorithm when ISH is the initial test (Reprinted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a)



(For additional information on the adjuvant trastuzumab trials, see Romond, Perez, Bryant, et al., 2005; Piccart-Gebhart, Procter, Leyland-Jones, et al., 2005; Slamon, Eiermann, Robert, et al., 2005; Joensuu, Kellokumpu-Lehtinen, Bono, et al., 2006.)

Table 5. Summary of ASCO/CAP guideline recommendations (Reprinted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a)

	Recommendation
Optimal algorithm for *HER2* testing	Positive for *HER2* is either IHC *HER2* 3+ (defined as uniform intense membrane staining of > 30% of invasive tumor cells) or FISH amplified (ratio of *HER2* to CEP17 of > 2.2 or average *HER2* gene copy number > six signals/nucleus for those test systems without an internal control probe) Equivocal for *HER2* is defined as either IHC 2+ or FISH ratio of 1.8-2.2 or average *HER2* gene copy number four to six signals/nucleus for test systems without an internal control probe Negative for *HER2* is defined as either IHC 0-1+ or FISH ratio of < 1.8 or average *HER2* gene copy number of < four signals/nucleus for test systems without an internal control probe These definitions depend on laboratory documentation of the following: 1. Proof of initial testing validation in which positive and negative *HER2* categories are 95% concordant with alternative validated method or same validated method for *HER2* 2. Ongoing internal QA procedures 3. Participation in external proficiency testing 4. Current accreditation by valid accrediting agency
Optimal FISH testing requirements	Fixation for fewer than 6 hours or longer than 48 hours is not recommended Test is rejected and repeated if ● Controls are not as expected ● Observer cannot find and count at least two areas of invasive tumor ● >25% of signals are unscorable due to weak signals ● >10% of signals occur over cytoplasm ● Nuclear resolution is poor ● Autofluorescence is strong Interpretation done by counting at least 20 cells; a pathologist must confirm that counting involved invasive tumor Sample is subjected to increased counting and/or repeated if equivocal; report must include guideline-detailed elements
Optimal IHC testing requirements	Fixation for fewer than 6 hours or longer than 48 hours is not recommended Test is rejected and repeated or tested by FISH if ● Controls are not as expected ● Artifacts involve most of sample ● Sample has strong membrane staining of normal breast ducts (internal controls) Interpretation follows guideline recommendation ● Positive *HER2* result requires homogeneous, dark circumferential (chicken wire) pattern in > 30% of invasive tumor ● Interpreters have method to maintain consistency and competency Sample is subjected to confirmatory FISH testing if equivocal based on initial results Report must include guideline-detailed elements

Table 5. Summary of ASCO/CAP Guideline Recommendations (Reprinted with permission from the American Society of Clinical Oncology; Wolff, Hammond, Schwartz, et al., 2007a), continued

	Recommendation
Optimal tissue handling requirements	Time from tissue acquisition to fixation should be as short as possible; samples for *HER2* testing are fixed in neutral buffered formalin for 6-48 hours; samples should be sliced at 5-10 mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of neutral buffered formalin Sections should ideally not be used for *HER2* testing if cut >6 weeks earlier; this may vary with primary fixation or storage conditions Time to fixation and duration of fixation if available should be recorded for each sample
Optimal internal validation procedure	Validation of test must be done before test is offered Initial test validation requires 25-100 samples tested by alternative validated method in the same laboratory or by validated method in another laboratory Proof of initial testing validation in which positive and negative *HER2* categories are 95% concordant with alternative validated method or same validated method for *HER2* Ongoing validation should be done biannually
Optimal internal QA procedures	Initial test validation Ongoing quality control and equipment maintenance Initial and ongoing laboratory personnel training and competency assessment Use of standardized operating procedures including routine use of control materials Revalidation of procedure if changed Ongoing competency assessment and education of pathologists
Optimal external proficiency assessment	Participation in external proficiency testing program with at least two testing events (mailings)/year Satisfactory performance requires at least 90% correct responses on graded challenges for either test ● Unsatisfactory performance will require laboratory to respond according to accreditation agency program requirements
Optimal laboratory accreditation	Onsite inspection every other year with annual requirement for self-inspection ● Reviews laboratory validation, procedures, QA results and processes, results and reports ● Unsatisfactory performance results in suspension of laboratory testing for *HER2* for that method

Abbreviations: *HER2*, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; QA, quality assurance.

Evidence Reported Post-ASCO/CAP Guidelines on Concordance and Discrepancy of HER2 Assay Results

Concordance/discordance of different assay methods. Evidence reviewed by the ASCO/CAP expert panel (Appendixes C and G in Wolff, Hammond, Schwartz, et al., 2007a) led to consensus definitions for unequivocal IHC and ISH results. As shown in Figures 2 and 3, and in Table 5, the panel defined unequivocal HER2-positive results by IHC (i.e., 3+) as greater than 30 percent of invasive cells strongly stained in a homogeneous, circumferential "chicken-wire" pattern, and by ISH as HER2 gene copy number per cell greater than 6 or HER2/CEP17 ratio greater than 2.2. They defined unequivocal HER2-negative results by IHC as scores of 0 or 1+, and by ISH as HER2 gene copy number per cell less than 4.0 or HER2/CEP17 ratio less than 1.8. Equivocal results (defined as 2+ by IHC, HER2 gene copy number from 4.0 to 6.0, or HER2/CEP17 ratio from 1.8 to 2.2) probably imply low-level HER2 amplification and/or overexpression, and should not be considered discordant, whether results of followup testing are positive or negative. Some but not all of these samples may actually have an amplified HER2 gene, but require additional testing to define the patient's correct HER2 status. The ASCO/CAP expert panel found insufficient evidence to determine whether breast cancer patients with equivocal HER2 results benefit from HER2-targeted therapy, although as discussed above, some patients included in adjuvant trastuzumab trials fit this category (also see "Results and Conclusions, Key Question 2").

For purposes of this review, discordant results are operationally defined as unequivocally positive results by one assay method and unequivocally negative results by a different assay method on sections from the same tumor, with both assays conducted using good laboratory practices, as recommended in the ASCO/CAP guideline (Wolff, Hammond, Schwartz, et al., 2007a). Presently, evidence is lacking to estimate discordance rates from studies that followed all ASCO/CAP recommendations on tissue preparation, testing practices, scoring systems, and thresholds to classify HER2 status of breast cancer patients. Therefore, in the following sections, we summarize evidence on discordance rates reported after the guideline was published by studies that used scoring systems and thresholds similar to those originally specified in U.S. Food and Drug Administration (FDA) -approved kits for IHC and ISH assays.

Investigators from the National Surgical Adjuvant Breast and Bowel Project's (NSABP) central pathology laboratory and colleagues at NSABP-approved reference laboratories conducted IHC (HercepTest™) and FISH (PathVysion®) assays on formalin fixed, paraffin embedded tumor blocks (Paik, Kim, Jeong, et al., 2007; Paik, Kim, and Wolmark, 2008). They reported results with both assays for 1,787 of 2,043 patients enrolled in the NSABP B31 randomized, controlled trial on adjuvant therapy with versus without trastuzumab (Romond, Perez, Bryant, et al., 2005). Of these, they found FISH-negative, IHC 3+ discordant results in 31 cases (1.7 percent). They also reported FISH-positive, IHC 0, 1+, or 2+ results in another 125 cases (7 percent), but did not separately report the proportion of those who tested FISH positive and IHC 0 or 1+.

Central and reference laboratory results with both IHC (HercepTest™) and FISH (PathVysion®) assays also are available (Perez, Romond, Suman, et al., 2007) for 1,779 of the 2,535 patients registered in a similar randomized, controlled trial conducted by the North Central Cancer Treatment Group (NCCTG N9831; Romond, Perez, Bryant, et al., 2005). Investigators reported discordant IHC 3+, FISH-negative results in 53 cases (3 percent), and FISH-positive, IHC 0, 1+, or 2+ results in 218 cases (12.3 percent). Here again, separate results were not

reported for the proportion who tested FISH positive and IHC 0 or 1+. Data presently are unavailable on IHC/ISH discordance rates from three other randomized, controlled trials of adjuvant trastuzumab (Piccart-Gebhart, Procter, Leyland-Jones, et al., 2005; Slamon, Eiermann, Robert, et al., 2005; Joensuu, Kellokumpu-Lehtinen, Bono, et al., 2006).

In a retrospective study, a Canadian central reference laboratory used HercepTest™ and three other HER2 antibody IHC assays to retest tumors from patients diagnosed with metastatic breast cancer between 1999 and 2002, and compared the IHC results with central lab FISH using PathVysion® (O'Malley, Thomson, Julian, et al., 2008). Among 505 patients initially classified HER2 positive by IHC in local labs and treated with trastuzumab for metastatic disease, concordance between central IHC and central FISH ranged from 88.9 percent to 90.9 percent, depending on the HER2 antibody used. Concordance between IHC and FISH was highest (92.2 percent) when all four HER2 antibody assays were used to test each sample, and tumors were only classified IHC positive if positive by 2 or more assays. In a sequential sample of 205 invasive breast tumors locally classified IHC negative, from patients diagnosed with metastasis, concordance of central IHC and central FISH ranged from 93.7 percent to 99 percent for individual antibody assays, and was 98.1 percent if tumors were only classified IHC negative if negative by 2 or more assays. However, this study did not report FISH/IHC discordance rates separately by IHC score.

A study from Greece that separately compared IHC results (using HercepTest™ and two other methods) from central and regional laboratories versus central FISH (PathVysion®) reported on 375 breast tumors tested centrally by IHC and FISH (Papadopoulos, Kouvatseas, Skarlos, et al., 2007). FISH-positive, IHC 0/1+ discordances were seen in six cases (1.6 percent; 11.5 percent of 52 IHC 0/1+ cases), while FISH-negative, IHC 3+ discordances were seen in three cases (0.8 percent; 9.4 percent of 32 IHC 3+ cases). Another study from three Greek hospitals compared IHC results (CB11 antibody) with FISH (PathVysion®) for 194 resected breast cancer patients, and also with CISH (SpoT-Light) for 159 of these patients (Kostopoulou, Vageli, Kaisaridou, et al., 2007). This study reported no FISH-positive cases and only one CISH-positive case among 94 IHC 0/1+ patients. Of 30 patients with IHC 3+ results, one (3.3 percent) was FISH negative and CISH negative.

A study from Germany on patients evaluated for inclusion in a trial of trastuzumab for metastatic breast cancer reported central IHC (HercepTest™) and FISH (PathVysion®) results for 289 patients (Hofmann, Stoss, Gaiser, et al., 2008). Investigators reported no FISH-positive cases among 100 patients scored IHC 0/1+, and nine FISH-negative but IHC 3+ cases (8.4 percent of 107 scored IHC positive; 3.1 percent of all patients evaluated).

A small study (n=55) compared two dual-probe (i.e., for HER2 and CEP17) FISH kits (PathVysion® and *HER2* FISH pharmDx), a single-probe FISH kit (Inform; HER2 only) and the SpoT-Light CISH kit versus two IHC assays (HercepTest™ and an independently developed test) (Cayre, Mishellany, Lagarde, et al., 2007). Investigators reported results with each assay (and with different positivity thresholds for Inform and SpoT-Light) separately for each sample. Four of 55 (7.3 percent) cases tested IHC 3+ with HercepTest™ and ISH-negative by all assays (other than a threshold of more than four signals for Inform). Three of the four were scored less than 3+ by independently developed IHC. All cases scored FISH positive by two or more kits also were scored IHC 3+ by HercepTest™.

Another small study (n=54) used the HercepTest™ and PathVysion® kits on all samples (Kuo, Wang, Chang, et al., 2007). Three cases (5.6 percent) that tested FISH negative were scored 3+ by IHC. In contrast, no cases that tested FISH positive were scored IHC 0 or 1+.

A systematic review abstracted data from 17 studies (all published before the ASCO/CAP guideline; pooled N=8,419) on FISH/IHC concordance (Dendukuri, Khetani, McIsaac, et al., 2007). Selection criteria sought studies that included consecutive patient series or a random sample, reported agreement between IHC and FISH using standard thresholds, and used assays licensed in Canada to select patients for trastuzumab therapy. All studies used PathVysion® for FISH; 16 used HercepTest™ and one used PATHWAY™ for IHC. Ten combined results for patients scored IHC 0 or 1+, and separately for those scored IHC 2+ or 3+ (pooled N=4,641); seven reported results separately for each IHC score (pooled N=3,778). Using Bayesian meta-analysis, they estimated proportions of breast cancer patients with each of the four possible IHC scores and proportions with each IHC score with positive results by FISH. Table 6 summarizes estimated IHC/FISH discordance rates based on results of the Dendukuri and coworkers' meta-analysis.

Table 6. Estimated discordance rates from meta-analysis of 17 studies on IHC and FISH

IHC Score	median % of patients	95% credible interval	expected # per 1,000 screened by IHC	95% credible interval	% discordant by FISH[a]	95% credible interval	expected # of discordances by FISH per 1,000 screened by IHC	95% credible interval
0	36.1	4.4–64.3	362	44–642	1.6	0.9–2.8	6	1–13
1+	35.5	7.4–67.4	355	74–674	4.9	2.6–17.9	18	8–30
2+	12.0	3.5–21.4	120	35–214	NA[b]	NA[b]	NA[b]	NA[b]
3+	16.2	10.7–22.9	162	107–230	7.6	3.8–12.9	12	6–21

[a] percentages shown are of expected # patients with IHC score listed in left column; [b] NA = not applicable, since IHC 2+ is considered an equivocal result, thus defined as not discordant regardless of subsequent FISH result.

Three small studies (combined N=211) conducted outside North America compared results of different ISH methods. An Australian study on 49 breast cancer samples reported that each case (n=20) scored highly positive (greater than 10 signals/cell) by FISH, and seven of 10 cases scored low-positive (5–10 signals/cell) by FISH, also scored positive by CISH (Bilous, Morey, Armes, et al., 2006). Each sample scored IHC 3+ by HercepTest™ also tested CISH positive. A study from Germany reported agreement in 95 of 99 breast tumor samples tested by FISH (PathVysion®) and SISH, an overall concordance of 96 percent (Dietel, Ellis, Hofler, et al., 2007). Finally, a study from Poland compared FISH, CISH, and SISH on 63 breast tumor specimens selected for 2+ or 3+ staining by IHC (Sinczak-Kuta, Tomaszewska, Rudnicka-Sosin, et al., 2007). Investigators reported and interpreted multiple statistical tests (Pearson chi-square tests with $p<0.01$; gamma correlation coefficients of 0.89 to 0.96; Spearman rank correlation coefficients of 0.70 to 0.79; and Kappa coefficients of 0.38 to 0.58) for separate two-way comparisons of assay results (i.e., CISH versus FISH, FISH versus SISH, and SISH versus CISH) as evidence for good agreement between the methods, but did not report concordance or discordance rates. Larger studies are needed to estimate more reliably rates of concordance and discordance between FISH or IHC and newer ISH methods (CISH, SISH). Furthermore, FDA-approved kits for CISH or SISH are not yet available.

To summarize, evidence from seven studies and a meta-analysis reported after the ASCO/CAP guideline (Wolff, Hammond, Schwartz, et al., 2007a) suggests variable but perhaps non-negligible rates for FISH-negative, IHC 3+ discordance (albeit by the older definition of strong, complete membrane staining in greater than 10 percent of invasive cells), ranging from 0.5 percent to 7.3 percent of breast cancer cases. The meta-analysis also estimated that 0.6 percent (95 percent CI: 0.1–1.3 percent) of cases might be scored IHC 0 and FISH positive,

while 1.8 percent (95 percent CI: 0.8–3.0 percent) of cases might be scored IHC 1+ and FISH positive. However, data are unavailable to estimate discordance rates for either group using the current ASCO/CAP definition of IHC 3+ (greater than 30 percent of invasive cells stained).

Disagreement between central and local laboratory results. Evidence reviewed by the ASCO/CAP expert panel demonstrated disagreement between central and local laboratory HER2 test results in approximately 20 percent of cases (Wolff, Hammond, Schwartz, et al., 2007a). This included data from the first 104 patients registered for NSABP B31, showing disagreement in 18 percent of cases (Paik, Bryant, Tan-Chiu, et al., 2002), which resulted in a protocol amendment limiting HER2 testing to 23 approved laboratories. The evidence also included data from NCCTG N9831 showing agreement in 88.1 percent of 813 cases rated FISH positive, 81.6 percent of 1,063 cases scored IHC 3+ by HercepTest™, and 75.0 percent of 636 cases scored IHC 3+ by non-HercepTest™ assays (Perez, Suman, Davidson, et al., 2006). Finally, it included data from a community-based clinical study on trastuzumab for metastatic breast cancer showing 77 percent agreement on samples scored IHC 3+ by local laboratories, but only 26 percent agreement on samples locally scored IHC 2+ (Reddy, Reimann, Anderson, et al., 2006). Based on the available evidence, the panel recommended specific measures for assay validation, self-assessment, accreditation, and proficiency testing by laboratories conducting HER2 assays. In the following section, we summarize new evidence comparing local versus central laboratory results, published since the ASCO/CAP review. Although published after the ASCO/CAP guideline, these studies preceded the guideline and scored samples as originally recommended by manufacturers and FDA labeling.

Final data from NSABP B31 showed disagreement on HER2 status in 174 of 1,787 cases (9.7 percent) classified HER2 positive by local laboratories but HER2 negative by both FISH (PathVysion®) and IHC assays in central or reference laboratories (Paik, Kim, Jeong, et al., 2007; Paik, Kim, and Wolmark, 2008). Data presently are unavailable on rates of disagreement between local and central laboratories from three other randomized, controlled trials of adjuvant trastuzumab (Piccart-Gebhart, Procter, Leyland-Jones, et al., 2005; Slamon, Eiermann, Robert, et al., 2005; Joensuu, Kellokumpu-Lehtinen, Bono, et al., 2006).

A small study compared central and local laboratory IHC results on breast tumor samples initially scored IHC 2+ locally and found FISH positive after referral for central laboratory confirmation (Barrett, Magee, O'Toole, et al., 2007). Investigators reported that of 153 IHC 2+ cases referred to the central laboratory for FISH confirmation, 29 (19 percent) had amplified HER2 genes. With repeat IHC in 25 of the 29, the central laboratory scored 18 cases (72 percent) as IHC 3+ and agreed with the local laboratory score of IHC 2+ in only 7 cases (28 percent). Since the central laboratory did not repeat IHC testing for the 124 cases with nonamplified HER2 genes by FISH, the overall rate of agreement with local results cannot be determined.

A larger study compared IHC results in local (regional) and central laboratories (Papadopoulos, Kouvatseas, Skarlos, et al., 2007). Of 458 available samples, 369 were tested by IHC both regionally and centrally and scores agreed for 296 (80.2 percent). Disagreement was greatest among samples (n=11) scored IHC 3+ by regional laboratories (63 percent concordance). Concordance was better among those (n=20) scored IHC 0 or 1+ and those scored IHC 2+ (n=338) at regional laboratories (85 percent and 80 percent, respectively).

A central reference laboratory analyzed tumor specimens from 315 of 399 (79 percent) patients randomized to capecitabine with or without lapatinib, using both IHC (antibody not reported) and FISH (PathVysion®), seeking confirmation of local laboratory results that classified these patients HER2 positive thus eligible for this randomized, controlled trial

(Cameron, Casey, Press, et al., 2008). Central testing found 241 of 315 (77 percent) HER2 positive, including 211 with IHC 3+ results and 30 with IHC 2+, FISH-positive results.

In the Canadian study cited previously, central laboratory testing of breast tumor tissue samples confirmed the IHC-positive status of 79.3 percent to 89.6 percent of 505 cases found IHC positive by local laboratory results (O'Malley, Thomson, Julian, et al., 2008). Among 205 cases found IHC negative by local labs, central IHC testing confirmed local results in 94.8 percent to 100 percent of cases. The concordance rates varied, depending on which of four IHC assays the central laboratory used.

To summarize, data reported after publication of the ASCO/CAP guideline (Wolff, Hammond, Schwartz, et al., 2007a) confirm the estimate of approximately 20 percent disagreement between local (or regional) and central laboratories with respect to HER2 assay results. Data are presently lacking to evaluate the effects of adherence to guideline recommendations for preanalytic, analytic, and postanalytic steps on rates of local/central disagreement.

Validation and proficiency testing. Since these issues are outside the scope of this evidence report, interested readers are referred to current guidelines for specific recommendations on best practices to validate assays and test laboratory proficiency (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). Evidence reviewed by the expert panel included a summary of results from 2004 and 2005 surveys of laboratories participating in CAP-sponsored interlaboratory comparisons of IHC results, using tissue microarrays as the test material (Fitzgibbons, Murphy, Dorfman, et al., 2006). The key finding was that 97 of 102 laboratories (95 percent) in 2004 and 129 of 141 laboratories (91 percent) in 2005 correctly scored 90 percent or more of the test cases. In the following section, we briefly summarize evidence published after the ASCO/CAP guideline. Again, these studies scored samples as originally recommended by manufacturers and FDA labeling.

An international study compared five pathology reference centers (from Netherlands, Canada, France, Belgium, and Germany) on assay scoring and HER2 status classification for separate samples tested by IHC (n=20) or by FISH (n=20) (Dowsett, Hanna, Kockx, et al., 2007). Agreement was uniform among centers on HER2 status classifications for all 20 IHC test cases, although some scoring differences were noted, and some equivocal cases (i.e., those scored IHC 2+) required FISH confirmation to determine HER2 status. Agreement was uniform among centers 16 of 20 (80 percent) FISH test cases. Each of the other four cases was scored in the equivocal range (HER2/CEP17 ratio 1.7–2.3).

A similar international study (from Netherlands, Australia, Canada, France, and Germany) compared results from five central laboratories on 211 breast cancer specimens tested by CISH, FISH and IHC (van de Vijver, Bilous, Hanna, et al., 2007). Each central laboratory sent unstained sections from samples they tested to four other ("outside") central laboratories. Investigators reported uniform agreement by CISH in the "outside" laboratories on 73 of 76 cases (96 percent) scored highly amplified (HER2/CEP17 greater than 4.0) by FISH in the initial laboratory. Similarly, "outside" CISH uniformly agreed with 94 of 100 (94 percent) cases initially scored as not amplified by FISH (HER2/CEP17 less than 2.0). Among 35 cases scored as equivocal by initial FISH testing (HER2/CEP17 2.0–4.0), 20 were scored as CISH positive and 15 were scored as CISH negative. Overall interlaboratory concordance was 95 percent for cases with normal HER2 gene copy number (1–5) and was 92 percent for cases with 6 or more copies of the HER2 gene.

A brief report by investigators from the Italian Network for Quality Assessment of Tumor Biomarkers (INQUAT) and the United Kingdom National External Quality Assessment Service (U.K. NEQAS) highlighted the importance of including both preanalytic and analytic steps in proficiency testing programs (Paradiso, Miller, Marubini, et al., 2007). The U.K. NEQAS program for HER2 testing focuses on preanalytic aspects of the IHC assay, while the INQUAT program focuses on intra- and interlaboratory variability in scoring a set of fixed and stained IHC slides. Twelve Italian laboratories participated in both quality control programs during 2003, and only one achieved high-quality performance in preanalytic processing steps and in intra- and interlaboratory reproducibility. Some laboratories that achieved high-quality performance in preanalytic steps did not score slides reproducibly, or vice versa. Three of the 12 laboratories did not perform adequately on either preanalytic or analytic steps.

A recent study covalently attached fixed and unfixed samples of synthetic HER peptide to glass microscope slides with unstained sections of invasive breast carcinomas (Vani, Sompuram, Fitzgibbons, et al., 2008). The peptide fragments were used as positive analyte controls on slides distributed to 192 laboratories participating in the CAP 2006 HER2-B proficiency testing survey. Stained slides were returned and centrally reviewed (n=109 laboratories), permitting participants to evaluate sources of variability in HER2 staining performance. Investigators reported suboptimal staining in 20 of 109 slides (18.3 percent). Of these, seven cases (35 percent of the 20 failures) were attributable to errors in the antigen retrieval step, four (20 percent) were attributable to problems with the antibody staining protocol, and nine (45 percent) had problems with both.

In summary, two studies published subsequent to the ASCO/CAP review (Wolff, Hammond, Schwartz, et al., 2007a) reported similar results on interlaboratory comparisons. Overall, the available evidence shows 90 percent or greater agreement between high-volume reference laboratories in North America, Europe, and Australia. Scoring differences between laboratories occur most often with cases of low-level amplification or low-level overexpression. Results reported before and after the ASCO/CAP review (and other guidelines) support considering such cases as equivocal results, with confirmatory testing needed to classify HER2 status. Collaborative data from Italy and the United Kingdom suggest that quality control programs must evaluate all steps (preanalytic, analytic, and postanalytic) in HER2 testing. Positive analyte controls confirmed that antigen retrieval and antibody staining are persistent sources of interlaboratory variability in IHC results.

Reports on polysomy 17. The ASCO/CAP expert panel (Wolff, Hammond, Schwartz, et al., 2007a) interpreted evidence from two studies (Downs-Kelly, Yoder, Stoller, et al., 2005; Ma, Lespagnard, Durbecq, et al., 2005) as not supporting an association of polysomy 17 (defined as three or more copies of CEP 17) with HER2 protein or mRNA overexpression. However, one of these (Ma, Lespagnard, Durbecq, et al., 2005) reported increased HER2 protein (IHC 3+) in a subset of patients with polysomy 17 and HER2/CEP 17 ratios less than 2. In the following section, we summarize evidence published subsequent to the ASCO/CAP guideline.

Nine studies have reported data on polysomy 17 and HER2 status of breast cancer patients since the ASCO/CAP review. Of these, seven have been published in full (Dal Lago, Durbecq, Desmedt, et al., 2006; Torrisi, Rotmensz, Bagnardi, et al., 2007; Corzo, Bellosillo, Corominas, et al., 2007; Beser, Tuzlali, Guzey, et al., 2007; Hyun, Lee, Kim, et al., 2008; Kostopoulou, Vageli, Kaisaridou, et al., 2007; Hofmann, Stoss, Gaiser, et al., 2008) and two were reported at meetings with slides or video available on line (Kaufman, Broadwater, Lezon-Geyda, et al., 2007; Reinholz, Jenkins, Hillman, et al., 2007). Three studies reported no association of polysomy 17

with HER2 protein and/or mRNA overexpression (Dal Lago, Durbecq, Desmedt, et al., 2006; Torrisi, Rotmensz, Bagnardi, et al., 2007; Corzo, Bellosillo, Corominas, et al., 2007). In contrast, five other studies reported increased levels of HER2 protein in some cases with polysomy 17 and unamplified HER2 genes (Hyun, Lee, Kim, et al., 2008; Kaufman, Broadwater, Lezon-Geyda, et al., 2007; Reinholz, Jenkins, Hillman, et al., 2007; Kostopoulou, Vageli, Kaisaridou, et al., 2007; Hofmann, Stoss, Gaiser, et al., 2008). The ninth study did not report data on overexpression of HER2 protein or mRNA; this study reported chromosome 17 polysomy in two of 11 patients with HER2 gene amplification and in seven of 39 patients with unamplified HER2 genes (Beser, Tuzlali, Guzey, et al., 2007). In one study (Hofmann, Stoss, Gaiser, et al., 2008), seven of nine discordant IHC 3+/FISH-negative patients had chromosome 17 polysomy, and six of 26 patients with polysomy 17 responded to trastuzumab therapy for metastatic disease. However, all six responders were scored 3+ by IHC.

In contrast to conclusions of the ASCO/CAP review (Wolff, Hammond, Schwartz, et al., 2007a), evidence published subsequently reopens the question of whether chromosome 17 polysomy has implications for classifying patients' HER2 status. Five of eight new studies found polysomy 17 to be associated with protein (and/or mRNA) overexpression in at least some patients with nonamplified HER2 genes, while three of eight found no association.

Implications for Remainder of this Report

Discordances between IHC and FISH results might arise in one of three ways. They may be artifacts of one accurate and one inaccurate test. Alternatively, they may reflect a threshold issue, either related to the changes in threshold definitions over time, or an inherent problem of using a continuous measure to classify patients dichotomously. Finally, discordant test results might accurately reflect a small number of different patients with respect to the biologic mechanism that increases membrane levels of the HER2 protein. Present data could not tease apart the many factors reviewed here (preanalytic, analytic and postanalytic) that might have contributed to discordances in HER2 assay results. This clearly affects the interpretation of evidence on key questions that address use of "HER2 status" to predict treatment outcomes, even in nonbreast malignancies (Key Questions 2, 3, and 5). Furthermore, it also affects interpretation of evidence on the added clinical utility of serum measurements for patients with known tissue status, since this presumes accurate classification by tissue assays. Future studies reporting outcomes as a function of HER2 status should report separately on patients with concordant, equivocal, and discordant assay results.

Key Question 2

For patients who are not unequivocally HER2 positive, what is the evidence on outcomes of treatment targeting the HER2 molecule (trastuzumab, etc.), or on differences in outcomes of uniform chemotherapy or hormonal therapy regimens with versus without additional treatment targeting the HER2 molecule, in:

a) Breast cancer patients characterized by equivocal or discordant HER2 results from different tissue assay methods performed adequately; and
b) For those with HER2-negative breast cancer?

Study Selection

The search strategy for studies on HER2 testing in breast cancer yielded 3,218 citations. Initial review selected 74 citations potentially relevant to Key Question 2 for retrieval and review as full articles. We used the ASCO/CAP expert panel's definition (Wolff, Hammond, Schwartz, et al., 2007a) of equivocal HER2 assay results: IHC 2+, or HER2 gene copy number from 4.0 to 6.0 or HER2/CEP17 ratio from 1.8 to 2.2 if ISH is the first or only assay. We defined discordant results as unequivocally positive results by one assay method (i.e., IHC 3+, HER2 gene copy number greater than 6.0, or HER2/CEP17 ratio greater than 2.2) and unequivocally negative results by a different assay method on another tissue section from the same tumor. Four trials (eleven reports; see Table 7 and "Available Studies" for citations) met selection criteria for data abstraction and compared outcomes with versus without a drug targeting HER2, for breast cancer patients with equivocal, discordant, or unequivocally negative HER2 assay results. Three trials randomized patients to chemotherapy with versus without trastuzumab; the fourth randomized patients to chemotherapy with versus without lapatinib, a tyrosine kinase inhibitor active against HER1 and HER2. Trials and their results are summarized in Tables 7–9; detailed abstraction data can be found in Appendix Tables II-A–II-I[*].

Available Studies and Reports

Table 7 includes two trials on adjuvant trastuzumab with data for Key Question 2 (NSABP B31 and NCCTG N9831). Each reported post-hoc analyses on interim results for small subgroups of resected breast cancer patients inadvertently randomized to chemotherapy with or without trastuzumab in trials seeking to randomize only HER2-positive patients. Similarly, a trial on chemotherapy with or without lapatinib for locally advanced or metastatic disease (EGF100151) also intended to randomize only HER2-positive patients (Cameron, Casey, Press, et al., 2008; Geyer, Forster, Lindquist, et al., 2006). In each of these trials, local laboratory HER2 testing initially classified all randomized patients as HER2 positive. However, central or reference laboratory retests subsequently identified small subsets as equivocal, discordant, or HER2 negative. Only one trial (CALGB 9840) intentionally randomized HER2-negative metastatic breast cancer patients (referred to as "HER2 non-overexpressors" by study authors), and directly tested whether adding trastuzumab to chemotherapy improved outcomes.

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Question 2: HER2-targeted Therapy for HER2 Discrepant or Negative Patients
Table 7. Summary study design, treatment, patient characteristics, KQ2

Study	Treatments Compared	Age or Menopause Status	Disease Extent	ER+	PR+	n FISH+ IHC-	n FISH- IHC3+	n FISH- IHC1,2+	n FISH- IHC-
Adjuvant treatment for resected early breast cancer									
NSABP B31 Paik et al., 2007; Paik et al., 2008; Romond et al., 2005	Tx: AC → (P+TRZ) (n=1,019 randomized)	≥50 years 48.4%	>2 cm >3 + nodes 61.4% 42.6%	51.9%	39.0%	56	10	69	82
	Cx: AC → P (n=1,024 randomized)	48.4%	57.1% 43.3%	52.8%	41.4%	69	21	80	92
NCCTG N9831 Perez et al., 2007; Reinholz et al., 2007; Perez et al., 2006; Romond et al., 2005	Tx: AC → (P+TRZ) (n=884 randomized)	≥50 years 50.4%	>2 cm >3 + nodes 61.5% 39.1%	51.2%	39.4%	123	23		59
	Cx: AC → P (n=895 randomized)	48.9%	58.7% 39.1%	52.8%	41.3%	95	30		44
First- or second-line treatment for advanced breast cancer									
CALGB 9840 Seidman et al., 2004, 2008	Tx: P (q wk vs. q3wk)+TRZ (n=115 randomized)	Menopausal status 75% post	≥3 metastatic sites 15%	55%	NR				113
	Cx: P (q wk vs. q3wk) (n=113 randomized)	84% post	11%	49%	NR				115
CALGB 150002 (from 9840) Kaufman et al., 2007	Tx: P (q wk vs. q3wk)+TRZ (n=115 randomized)	75% post	15%	55%	NR	central FISH-, polysomy +: 19 central FISH-, polysomy -: 53			
	Cx: P (q wk vs. q3wk) (n=113 randomized)	84% post	11%	49%	NR	central FISH-, polysomy +: 19 central FISH-, polysomy -: 50			
EGF100151 Cameron et al., 2008; Geyer et al., 2006	Tx: capecitabine (2 g/m^2 days 1-14 q 3wk) + lapatinib (1.25 g q day) (n= 198 randomized)	Median 54 yrs; range 26-80 yrs	≥3 metastatic sites 49%	ER+ &/or PR+ 48%		15	1	14	23
	Cx: capecitabine alone (2.5 g/m^2 days 1-14 q 3wk) (n=201 randomized)	Median 51 yrs; range 28-83 yrs	48%	46%		7	2	14	21

Abbreviations: AC: Adriamycin [doxorubicin]/cyclophosphamide; Cx: control; ER+: estrogen-receptor positive; IHC: immunohistochemistry; FISH: fluorescent in situ hybridization; mos: months; PR+: progesterone-receptor positive; P: paclitaxel; q wk: every week; q3wk: every 3 week; TRZ: trastuzumab; Tx: treatment; yrs: years.

One trial on trastuzumab in adjuvant therapy (NSABP B31) reported data on post-hoc subgroup analyses in a brief published communication (Paik, Kim, and Wolmark, 2008). Another adjuvant trastuzumab trial (NCCTG N9831) compared local, central, and reference laboratory results of HER2 testing in a published article that did not report outcomes (Perez, Suman, Davidson, et al., 2006). Both trials reported subgroup outcomes in meeting abstracts, with slides available online (B31: Paik, Kim, Jeong, et al., 2007; N9831: Perez, Romond, Suman, et al., 2007, and Reinholz, Jenkins, Hillman, et al., 2007). A single, published report provided baseline characteristics and preliminary outcomes data for patients randomized to treatment arms common to B31 and N9831 (Romond, Perez, Bryant, et al., 2005). Data were reported in this publication on each trial separately and both trials combined.

Two trials on patients with advanced or metastatic disease published full reports with subgroup analyses (Seidman, Berry, Cirrincione, et al., 2008; Cameron, Casey, Press, et al., 2008). The EGF100151 trial on chemotherapy with or without lapatinib (Cameron, Casey, Press et al., 2008) also published an earlier report (Geyer, Forster, Lindquist et al., 2006), but without results of repeat HER2 testing by a central or reference laboratory or analyses relevant to Key Question 2. CALGB 9840, the only preplanned analysis relevant to this key question, is on a HER2-negative (i.e., non-overexpressor) subgroup randomized to chemotherapy with or without trastuzumab within a larger trial studying an unrelated question (Seidman, Berry, Cirrincione, et al., 2004, 2008). CALGB 9840 also is the source of all patients in the subgroup analyzed post-hoc in CALGB 150002 (Kaufman, Broadwater, Lezon-Geyda, et al., 2007).

Treatments and Subgroups Compared

Adjuvant therapy. Two trials (NSABP B31, NCCTG N9831) investigated outcomes of adjuvant doxorubicin plus cyclophosphamide (AC; every three weeks for four cycles), followed by paclitaxel (P; every three weeks for four cycles), with versus without trastuzumab (+/-TRZ; weekly for 12 months, beginning concurrently with paclitaxel) in women with fully resected early breast cancer. Outcomes are as-yet unreported for a third arm of N9831, which began trastuzumab therapy after all eight cycles of chemotherapy (AC→P→TRZ). Both B31 and N9831 limited eligibility to HER2-positive patients, defined as FISH-positive/IHC unknown, IHC3+/FISH-unknown, or IHC2+/FISH-positive. Patients were initially evaluated by local laboratory testing, and randomized if classified HER2-positive by these results. They were subsequently re-evaluated by central laboratory testing, but continued with assigned treatments regardless of results. A planned interim analysis at two years' median followup (2.4 years for B31 patients; 1.5 years for N9831 patients) for all patients randomized to the treatment arms common to both trials, pooled patients assigned to the control arms(n=1,679; AC→P) and those assigned to concurrent trastuzumab (n=1,672; AC→P+TRZ) (Romond, Perez, Bryant, et al., 2005). Trastuzumab significantly improved overall survival (OS) at four years: 91.4 percent versus 86.6 percent; hazard ratio (HR) =0.67; 95 percent CI: 0.48–0.93; p=0.015. The B31 (Paik, Kim, and Wolmark, 2008; Paik, Kim, Jeong, et al., 2007) and N9831 (Perez, Romond, Suman, et al., 2007 and Reinholz, Jenkins, Hillman et al., 2007) results included here were unplanned, post-hoc analyses. They compared outcomes of adjuvant AC→(P+/-TRZ) in subgroups found HER2 discordant or negative by central lab results, using data collected for the pooled analysis of Romond, Perez, Bryant, et al. (2005) without longer followup.

Advanced/metastatic disease. A randomized, controlled trial (CALGB 9840) that studied paclitaxel in women receiving first- or second-line therapy for metastatic breast cancer reported outcomes at two meetings (Seidman, Berry, Cirrincione, et al., 2004; Kaufman, Broadwater, Lezon-Geyda, et al., 2007) and in a published article (Seidman, Berry, Cirrincione, et al., 2008). Primary randomization in this trial compared once-weekly to every-third-week paclitaxel dosing regimens. Testing for HER2 status began after enrolling the first 171 patients, and HER2-negative patients (termed "HER2 non-overexpressors" by study authors and defined as 0 or 1+ or IHC 2+/FISH negative by local laboratory tests) were also randomized to treatment with or without trastuzumab. Seidman, Berry, Cirrincione, et al. (2004, 2008) reported outcomes for this second randomization without separating results by paclitaxel treatment frequency. HER2-positive patients (by local laboratory tests) all received trastuzumab and are excluded from the analysis for Key Question 2.

For all patients randomized (n=735), CALGB 9840 investigators first reported that response rate and time to progression (TTP) were better with weekly paclitaxel than with every third week, although the difference in median OS (24 versus 16 months; HR=1.19, p=0.17) was not statistically significant (Seidman, Berry, Cirrincione, et al., 2004). As prespecified in the CALGB 9840 protocol, the final analysis (Seidman, Berry, Cirrincione, et al., 2008) comparing paclitaxel schedules pooled additional patients (n=158) randomized to the identical dose of paclitaxel every third week (all without trastuzumab) in another trial (CALGB 9342; Winer, Berry, Woolf et al., 2004) with those randomized to this schedule in CALGB 9840. In this combined analysis, weekly paclitaxel statistically significantly improved response rate (42 percent versus 29 percent; OR=1.75, p=0.0004), TTP (median, nine versus five months; HR=1.43, p<0.0001), and OS (median, 24 versus 12 months; HR=1.28, p=0.0092), when compared with treatment every third week. Data in Table 8 on HER2 non-overexpressors exclude patients from CALGB 9342.

A post-hoc analysis on HER2 non-overexpressors randomized to paclitaxel with versus without trastuzumab in CALGB 9840 compared outcomes for subsets found FISH negative by central laboratory testing who had or did not have chromosome 17 polysomy (CALGB 150002; Kaufman, Broadwater, Lezon-Geyda, et al., 2007). This analysis was not included in the published final report (Seidman, Berry, Cirrincione, et al., 2008). It also did not include patients from CALGB 9342, none of whom were randomized to paclitaxel with or without trastuzumab.

The EGF100151 trial randomized patients with locally advanced or metastatic breast cancer to capecitabine (1 g/m^2 twice daily for 14 days every three weeks) plus lapatinib (1.25 g/m^2 daily) or to capecitabine alone (1.25 g/m^2 twice daily for 14 days every three weeks). Eligibility required: a T4 primary tumor and stage IIIB or IIIC disease, for those without distant metastasis; a history of progressive disease after one or more regimens that included an anthracycline, a taxane, and trastuzumab (given separately or in combinations); and local laboratory HER2 test results of IHC3+ or IHC2+/FISH positive. An interim analysis on 163 patients randomized to capecitabine plus lapatinib and 161 randomized to capecitabine monotherapy reported median TTP was 8.4 months in the combination arm and 4.4 months in the capecitabine monotherapy arm (HR=0.49; 95 percent CI: 0.34–0.71, p<0.001) (Geyer, Forster, Lindquist, et al., 2006). A second report included more patients (n=198, capecitabine plus lapatinib; n=201, capecitabine monotherapy; Cameron, Casey, Press, et al., 2008). By intent-to-treat analysis, median TTP was 6.2 months in the combined arm and 4.3 months in the monotherapy arm (HR=0.57; 95 percent CI: 0.43–0.77, p<0.001). A second interim analysis for OS found 28 percent had died (median OS, 15.6 months) in the combined therapy arm and 32 percent had died (median OS, 15.3

months) in the capecitabine monotherapy arm (HR=0.78; 95 percent CI: 0.55–1.12; p=0.177); followup for survival continues. Central laboratory IHC and FISH retesting of samples from 300 (75 percent) of the 399 randomized in this trial identified small subgroups with HER2-discordant or -negative results (Table 7).

Study Quality

Only one of four included trials (CALGB 9840) stratified randomization by HER2 status, the most informative evidence level defined in this report's study design hierarchy (see Methods, Table 3). The others are post-hoc analyses of treatment effects in HER2-discordant or -negative subgroups from larger randomized, controlled trials. One trial on adjuvant trastuzumab (NSABP B31) and both trials on patients with metastatic or advanced disease (CALGB 9840 and EGF100151) included multivariate analyses. However, neither CALGB 9840 nor EGF100151 used multivariate analysis to adjust treatment outcomes in HER2 discordant or HER2 negative subgroups. Since these subgroups from each study are small and underpowered, and since results from three of four trials are interim analyses with limited followup, we did not assess study quality using the checklist derived from REMARK and other sources (see "Methods").

Patient Characteristics

Adjuvant therapy. Patients from B31 and N9831 were initially randomized based on positive results of local lab testing, given their assigned regimen, and followed on these randomized, controlled trials. Those in subgroups included here subsequently were reclassified HER2 discordant or HER2 negative by central laboratory results. Baseline patient characteristics and prognostic factors (Table 7) were reported for all patients randomized to each treatment arm in each trial (Romond, Perez, Bryant, et al., 2005), including those classified as HER2 positive by both local and central laboratory results. At the level of initial randomization, baseline characteristics and prognostic factors of the groups treated with versus without trastuzumab were similar. However, data were not reported to separately compare baseline characteristics and prognostic factors by treatment arm for each subgroup of HER2-discordant or -negative patients (by central laboratory results).

Data are available from B31 for two HER2-discordant groups:

- FISH positive/IHC 0, 1+, or 2+: n=56 +TRZ; n=69 -TRZ (data not reported separately for FISH-positive, IHC 0, 1+ subset)
- FISH negative/IHC 3+: n=10 +TRZ; n=21 -TRZ;

and for two (partially overlapping) HER2-negative groups:

- FISH negative/IHC 1+ or 2+: n=69 +TRZ; n=80 -TRZ
- FISH negative/IHC 0, 1+, or 2+: n=82 +TRZ; n=92 -TRZ (13 and 12 patients per arm added to the 69 and 80 in the arms above).

Data are available from N9831 for two HER2-discordant groups:

- FISH positive/IHC 0, 1+, or 2+: n=123 +TRZ; n=95 -TRZ (data not reported separately for FISH-positive, IHC 0, 1+ subset)
- FISH negative/IHC 3+: n= 23 +TRZ; n=30 -TRZ;

and for one HER2-negative group:

- FISH negative/IHC 0, 1+, or 2+: n=59 +TRZ; n=44 -TRZ.

Advanced/metastatic disease. Patients in CALGB 9840 had metastatic disease undergoing first- or second-line therapy. All were randomized to weekly or every third week paclitaxel, and those who were HER2 negative (IHC 2+/FISH negative or IHC 0 or 1+) by local laboratory results were simultaneously randomized to receive (n=113) or not receive (n=115) trastuzumab. The analysis pooled outcomes in the HER2-negative arms for patients given paclitaxel weekly or every third week. Subsequent analyses (CALGB 150002) compared outcomes separately for subgroups from CALGB 9840 who were FISH negative by central laboratory results and had (+/-TRZ, n=19 each arm) or did not have (+TRZ, n=53; -TRZ, n=50) chromosome 17 polysomy.

Patients in EGF100151 had locally advanced or metastatic disease that progressed after one or more regimens with an anthracycline, a taxane, and trastuzumab (given separately or in combinations, as adjuvant therapy or for metastasis). Women (n=399) with local laboratory HER2 test results of IHC3+ or IHC2+/FISH positive were randomized to capecitabine with or without lapatinib. Baseline characteristics and prognostic factors of the groups treated with versus without lapatinib were similar. Subsequent central laboratory reanalysis by FISH and IHC of tumor samples from 300 patients (75 percent of all randomized) identified HER2 discordant or HER2 negative subgroups (Table 7). Data were not reported to separately compare baseline characteristics or prognostic factors by treatment arm for any of these subgroups.

Results, Key Question 2

Adjuvant AC→(P±TRZ). The only available data are from post-hoc subgroup analyses, without stratification for the subgroups' defining characteristics. Neither the B31 nor the N9831 analyses reported subgroup-specific comparisons of baseline characteristics or prognostic factors by treatment arm. Furthermore, one subgroup mixed results for a discordant subgroup (IHC 0, 1+, FISH positive) with results for initially equivocal but ultimately positive (IHC 2+ but amplified by FISH) patients. Finally, data are presently unavailable from studies that classified patients using assay thresholds consistent with current guidelines (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007 see "Results and Conclusions, Key Question 1, Narrative Review").

Neither trial reported median followup durations, or showed numbers per arm at risk over time, for the specific subgroups compared. In each subgroup from each treatment arm, failure events (e.g., death or relapse) occurred in less than 25 percent of patients (range: 5–23 percent) at the time of analysis. Therefore, length of followup was inadequate for reliable estimates of median event-free durations for any outcome reported. The interim analyses for all patients randomized in the larger trials that were sources of these subgroups (Romond, Perez, Bryant, et al., 2005) also lacked sufficient followup for reliable estimates of median overall survival or median disease-free survival (DFS).

For HER2 discrepant patients who were FISH positive and IHC 0, 1+ or 2+ by central laboratory testing, between-arm differences in outcome were not statistically significant in either trial. In B31 (n=56 +TRZ; n=69 -TRZ), the HR for failure in analysis of DFS was 0.30 (95 percent CI: 0.08–1.07; p=0.064) and the HR for failure in analysis of recurrence-free interval (RFI) was 0.35 (95 percent CI: 0.10–1.28; p=0.11). In N9831 (n=123 +TRZ; n=95 -TRZ), the HR for failure in analysis of DFS was 0.98 (95 percent CI: 0.33–2.91; p=0.97).

Question 2: HER2-targeted Therapy for HER2 Discrepant or Negative Patients
Table 8. Summary time to event outcomes, KQ2

Study	Time to Event Outcomes											
HER2 Discordant (all data on adjuvant AC→P +/- TRZ)												
FISH+ IHC 0, 1+, or 2+ by central lab:												
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
NSABP B-31[a]	DFS	Tx	56							Cox prop	0.064	0.30 (0.08-1.07)
		Cx	69							hazards		
NCCTG N9831	DFS	Tx	123							???	0.97	0.98 (0.33-2.91)
			95									
FISH- IHC 3+ by central lab:												
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
NSABP B-31[a]	DFS	Tx	10							Cox prop	0.94	0.91 (0.08-10)
		Cx	21							hazards		
NCCTG N9831	DFS	Tx	23								0.57	0.61 (0.11-3.29)
		Cx	30									

???

Question 2: HER2-targeted Therapy for HER2 Discrepant or Negative Patients
Table 8. Summary time to event outcomes, KQ2 (continued)

Study	Time to Event Outcomes											
HER2 Negative												
adjuvant AC→P +/- TRZ: FISH- IHC 1+, 2+ by central lab:												
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
NSABP B-31[a]	DFS	Tx	69		~98%	~95%	~90%	~90%	~86%	Cox prop	0.02	0.30 (0.11-0.83)
		Cx	80		~90%	~79%	~75%	~70%	~62%	hazards		
adjuvant AC→P +/- TRZ: FISH– IHC 0, 1+, or 2+												
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
NSABP B-31[a]	DFS	Tx	82		~97%	~90%	~87%	~87%	~84%	Cox prop	0.014	0.34 (0.14-0.80)
		Cx	92		~92%	~80%	~76%	~72%	~65%	hazards		
NCCTG N9831	DFS	Tx	59						81.2%	???	p	HR (95%CI)
		Cx	44						60.9%		0.13	0.51 (0.21-1.2)
P +/- TRZ as 1st or 2nd line therapy for metastatic disease												
CALGB 9840	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	
	OS	Tx	113	21.6	~75%	~40%	~25%	20%		K-M	0.65	
IHC2+/FISH- or IHC 0, 1+		Cx	115	21.6	~70%	~40%	~25%	20%		analysis		
	TTP	Tx	113	6.5	~30%	~13%	~7%	~5%		K-M	0.28	
		Cx	115	5.5	~25%	~12%	~12%	~4%		analysis		
CALGB 150002 (from 9840)	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	
	OS	Tx	19	~30	~90%	~65%	~30%			???	0.538	
		Cx	19	~23	~69%	~48%	~30%					
central FISH-, polysomy 17												
capecitabine +/- lapatinib for advanced or metastatic disease progressing after an anthracycline, a taxane, and trastuzumab												
EGF100151	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95% CI)
	PFS	Tx								K-M	0.46	0.77 (0.39-1.54)
Cameron et al., 2008		Cx								analysis		
	(sample include 74 patients not centrally confirmed to meet protocol HER2 eligibility criteria)											

90.2%
82.6%

[a] Subgroup analyses reported from NSABP B31 adjusted each Cox proportional hazards model used to estimate HR for included patients' ER and nodal status; subgroup analyses from NCCTG N9831 are unadjusted.

Abbreviations: AC: Adriamycin [doxorubicin]/cyclophosphamide; CI: confidence interval; Cx: control; DFS: disease-free survival; HR: hazard ratio; IHC: immunohistochemistry; FISH: fluorescent in situ hybridization; K-M: Kaplan-Meyer; Med: median; mos: months; OS: overall survival; P: paclitaxel; prop: proportional; q wk: every week; q3wk: every 3 week; TRZ: trastuzumab; TTP: time to progression; Tx: treatment; yr: year(s)

Question 2: HER2-targeted Therapy for HER2 Discrepant or Negative Patients

Table 9. Summary tumor response, KQ2

Study	Tumor Response (%)										
CALGB 9840	Grp	N	CR	PR	OR (CR+PR; with 95% CI)	SD	PD	NE	Test	p	Comments
Seidman et al., 2004, 2008	+TRZ	112			38% (29%-48%)			variate	multi-variate logistic regression	0.28	OR=1.35 (0.78-2.34)
	-TRZ	114			32% (23%-41%)						
CALGB 150002 (from 9840) Kaufman et al., 2007	Grp	N	CR	PR	OR (CR+PR)	SD	PD	NE	Test	p	Comments
	+TRZ	19			63%			???		0.048	FISH-/polysomy+
	- TRZ	19			26%						
	+TRZ	53			36%			???		NS	FISH-/polysomy-
	- TRZ	50			36%						

Abbreviations: CR: complete response; Grp: group; NE: not evaluable; NS: not significant; OR: overall response; PD: progressive disease; PR: partial response; SD: stable disease; TRZ: trastuzumab;

Few patients were FISH negative and IHC 3+ by central laboratory results (from B31: n=10 +TRZ; n=21 -TRZ; from N9831: n=23 +TRZ; n=30 -TRZ). B31 reported HR for failure was 0.91 for both DFS and RFI (for each outcome, 95 percent CI: 0.08–10.0; p=0.94), and N9831 reported hazard ratio for failure was 0.61 (95 percent CI: 0.11–3.29; p=0.57). Each between-arm subgroup comparison was not statistically significant.

Only B31 analyzed outcomes of patient subgroups that were HER2 negative by FISH but IHC 1+ or 2+ by central laboratory testing [n=69 +TRZ; n=80 -TRZ]). Between-arm differences reported by Paik, Kim, Jeong et al. (2007) were statistically significant for DFS (HR=0.30; 95 percent CI: 0.11–0.83; p=0.02) and RFI (HR=0.31; 95 percent CI: 0.10–0.95; p=0.041), and favored the subgroup given trastuzumab.

Both trials reported on patients who were FISH negative and IHC 0, 1+ or 2+ by central laboratory testing. In B31, this subgroup added FISH-negative/IHC 0 patients (13 and 12 per arm, respectively) to those in the FISH-negative/IHC 1+ or 2+ arms shown above (combined n=82 +TRZ; combined n=92 -TRZ). Between-arm differences were statistically significant for DFS (7 events, +TRZ, 20 events, -TRZ; HR=0.34; 95 percent CI: 0.14–0.80; p=0.014) and RFI (HR=0.36; 95 percent CI: 0.14–0.92; p=0.034), and again favored the subgroup given trastuzumab. One patient died in the trastuzumab arm, while 10 died in the control arm (HR=0.08; 95 percent CI: 0.01–0.64, p=0.017). In N9831 (n=59 +TRZ, n=44 -TRZ), the between-arm difference in DFS (HR=0.51; 95 percent CI: 0.21–1.2; p=0.13) was not statistically significant.

HER2 gene copy number and magnitude of benefit from trastuzumab. Additional unpublished subset analyses from the B31 trial presented at the June 2007 ASCO annual meeting (Paik, Kim, Jeong, et al., 2007), and similar analyses from the N9831 trial (Reinholz, Jenkins, Hillman, et al., 2007) and the HERA trial (McCaskill-Stevens, Proctor, Goodbrand, et al., 2007) presented at the December, 2007 San Antonio Breast Cancer Symposium, investigated the hypothesis that higher HER2 gene copy numbers, or higher HER2/CEP17 FISH ratios, were associated with a larger magnitude of relative benefit from trastuzumab. Data from the N9831 and HERA trials showed that the hazard ratio for DFS did not grow more favorable to the trastuzumab arm as average FISH ratios increased from 2.0 to 15 or greater (N9831), or from 2 to greater than 8 (HERA). Additionally, investigators found the HR for DFS did not increase as average HER2 gene copy number per cell increased from 4 to greater than 18 (HERA), or from 2 to greater than 10 (B31).

Polysomy 17 and adjuvant trastuzumab. An unpublished post-hoc analysis of data from N9831 presented at the December 2007 San Antonio Breast Cancer Symposium evaluated whether polysomy 17 influenced effects of adjuvant trastuzumab (Reinholz, Jenkins, Hillman, et al., 2007). Investigators reported that among patients with amplified HER2 genes, trastuzumab increased DFS whether or not these patients had polysomy 17. Central lab results identified very few patients without HER2 overexpression by IHC or HER2 gene amplification by FISH, but with polysomy 17. DFS was lower (79 percent versus 83 percent at 3 years; 65 percent versus 75 percent at 5 years) among those given trastuzumab than among those not given trastuzumab, although the sample size was small and few events had occurred in either arm (6 of 24 given trastuzumab, 3 of 13 controls). Investigators also analyzed slightly larger patient subsets without HER2 overexpression by IHC, HER2 gene amplification by FISH, or polysomy 17. DFS was substantially higher (94 percent versus 77 percent at 3 years; 84 percent versus 55 percent at 5 years) among those given than among those not given trastuzumab. As in the subset with polysomy 17, few events had occurred in either arm in the subset without polysomy (4 of 34 given trastuzumab, 13 of 33 controls). Additionally, unpublished data from the NSABP B31 trial

showed no impact on prognosis or degree of benefit from trastuzumab (Dr. S. Paik; personal communication, May 2008).

HER2-negative patients with metastatic disease given P±TRZ for first- or second-line therapy. Patients found IHC 2+/FISH negative or IHC 0, 1+ by local laboratory results were randomized in CALGB 9840 to have or not have trastuzumab added to paclitaxel (n=113 +TRZ; n=115 -TRZ). Between-arm differences in OS (median: 21.6 versus 19.6 months, p=0.67), time to progression (TTP; median: 12 versus 6 months, p=0.088), and overall response rate (ORR; 35 percent versus 29 percent, p=0.32) were not statistically significant (Seidman, Berry, Cirrincione, et al., 2008).

CALGB 150002 reported that subgroups from CALGB 9840 found FISH negative by central laboratory results, and also found to have chromosome 17 polysomy (n=19 +TRZ; n=19 -TRZ), showed a statistically significant increase in ORR (63 percent versus 26 percent, p=0.048) among those given trastuzumab plus paclitaxel compared with those given paclitaxel alone (Kaufman, Broadwater, Lezon-Geyda, et al., 2007). In contrast, ORR did not differ between treatment arms (36 percent in each) for centrally FISH-negative patients without chromosome 17 polysomy. The ORR difference between arms for the centrally FISH-negative subgroup with polysomy 17 (+/-TRZ; n=19 each) did not yield statistically significant differences between arms for either OS (p=0.538) or TTP (p=0.88).

HER2-negative patients with advanced or metastatic disease that progressed after an anthracycline, a taxane, and trastuzumab given capecitabine ± lapatinib. Few patients randomized to capecitabine with or without lapatinib in the EGF100151 trial were HER2 discordant (Table 7). Furthermore, outcomes were not reported separately for those found FISH positive but IHC negative by central laboratory testing (with lapatinib, n=15; without lapatinib, n=7), or those found FISH negative but IHC 3+ by central lab results (with lapatinib, n=1; without lapatinib, n=2). Investigators identified a total of 74 patients (23.5 percent of 315 tested in the central laboratory) whose local results were not confirmed by the central lab as meeting HER2 eligibility criteria of IHC 3+ or FISH positive/IHC2+ (Cameron, Casey, Press et al., 2008); distribution between treatment arms was not reported. In an exploratory Kaplan-Meier analysis, investigators found no statistically significant difference between arms (capecitabine with or without lapatinib) in PFS (HR=0.772; 95 percent CI: 0.386–1.543; p=0.46).

Conclusions and Discussion, Key Question 2

Adjuvant trastuzumab. Currently available evidence is inconclusive on outcomes of trastuzumab added to adjuvant chemotherapy for resected HER2-discordant or HER2-negative patients. Evidence on each subgroup may be used to generate hypotheses, but is too weak to test hypotheses, for the following reasons. All available evidence is from post-hoc analyses on subgroups not directly randomized or stratified by the HER2 subgroups of interest. Furthermore, available reports did not show direct comparisons of baseline characteristics and prognostic factors for the specific subgroups compared. Thus, it is uncertain whether the HER2-discordant or HER2-negative subgroups were balanced by treatment arm (i.e., with or without trastuzumab; although treatment arms appeared well-balanced across all patients randomized). Finally, the data used for the two adjuvant studies are from interim analyses, with inadequate followup to estimate median survival for all patients randomized, and inadequate information on median duration of followup in the specific subgroups compared. Thus, although these were large, well-designed and well-conducted randomized, controlled trials, since the overwhelming majority of

patients they randomized were unequivocally HER2-positive, only poor quality evidence is presently available on outcomes of adjuvant trastuzumab in either HER2 discordant or HER2 negative patient subgroups.

Adjuvant trastuzumab in HER2-discordant patients. Evidence is unavailable to evaluate effects of trastuzumab specifically for HER2-discordant patients who are FISH positive but IHC negative (0, 1+) by central lab results. Analyses reported from each trial pooled outcomes for these patients with outcomes for those who tested FISH positive and IHC 2+. The latter subset (initially considered equivocal if tested first by IHC) was classified HER2 positive by each trial protocol, and is ultimately classified HER2 positive by algorithms in current guidelines. A more informative analysis limited to the discordant subgroup might compare outcomes with versus without trastuzumab using data pooled from B31 and N9831 on patients who were FISH positive but IHC 0 or 1+ by central lab tests. Results from a systematic review (see Table 6, Key Question 1) estimates this subgroup as 2.4 percent (95 percent CI: 1–4.3 percent) of all breast cancer patients (Dendukuri, Khetani, McIsaac, et al., 2007).

Sample size is insufficient for conclusions from HER2-discordant B31 (total n=31) and N9831 (total n=53) subgroups that tested FISH negative but IHC 3+ by central lab results. The proportion of FISH-negative, IHC 3+ patients is 2.2 percent across both trials (total randomized: 3,822). Results of the systematic review summarized in Table 6 (Key Question 1) estimate this subgroup as 1.2 percent (95 percent CI: 0.6–2.1 percent) of all breast cancer patients (Dendukuri, Khetani, McIsaac, et al., 2007). Although at least three other randomized trials investigated adjuvant trastuzumab, they confirmed eligibility by central or reference laboratory FISH tests before randomizing patients, and have not reported on either of the HER2 discordant subgroups of interest. Thus, large database or registry analyses may be the only source of better evidence on outcomes of adjuvant trastuzumab for the two HER2 discordant subgroups, which together comprise approximately 4 percent of all breast cancer patients.

Factors influencing discordant results. Discordant results may occur if one assay is correct and the other in error, either due to preanalytic, analytic, or postanalytic factors (see Key Question 1). As with any assay, 100 percent accuracy cannot be expected even from the most careful and proficient laboratories. Proficiency testing and other quality control and quality assurance measures to minimize false-negative and false-positive results are recommended in current practice guidelines (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007). However, concordance of different methods to classify an individual as HER2 positive or negative is at least partly independent from accuracy of performing a specific assay. Even with the most careful and highly accurate laboratory techniques, discordance in classification may occur between a method that detects gene amplification (FISH in these studies, but also true with CISH or SISH) and a method that detects protein overexpression (IHC in these studies, but also true with Western blots).

By current guidelines, clinicians may categorize identical discordant patients differently with respect to HER2 status, depending on the selection and sequence of tests they order. So, for example, FISH-positive and IHC 0 or 1+ patients (1 to 4 percent of cases; see Table 6, Key Question 1) would be classified HER2 positive if tested only by FISH, but would be classified HER2 negative if tested initially by IHC, since reflex FISH would not be performed. Conversely, FISH-negative and IHC 3+ patients (1 to 2 percent of cases; see Table 6) would be considered HER2 negative if tested only by FISH, but HER2 positive if tested initially by IHC. NSABP B31 and NCCTG N9831 report the frequency of these subsets based on careful central laboratory

results for FISH and IHC assays, although results are pooled across some IHC scores (see "Results and Conclusions, Key Question 1"). However, these data do not permit assessment of the subset frequencies independent of tissue fixation artifacts that may have occurred at some local hospitals and laboratories, or the margin of error that might exist even in the most proficient laboratories. Nor can the clinical consequences of such discordances be assessed from the available evidence.

Adjuvant trastuzumab in HER2-negative patients. Scant but intriguing evidence suggests the hypothesis that some patients currently classified as HER2 negative may benefit from adjuvant trastuzumab. Data reported from B31 showed significantly longer DFS and RFI in FISH-negative IHC ≤2+ patients given trastuzumab than in similar patients managed without trastuzumab, whether the analysis did or did not include those who were IHC 0. However, a similar analysis of data from N9831 did not show significant differences. Since both were interim analyses of trials in which fewer than 25 percent of subjects had reached a failure event, neither provides conclusive evidence as yet, and follow up analyses from these trials will be of great interest. Blinded review of IHC and FISH scoring would also be useful for samples from these trials, and from other adjuvant trastuzumab trials that confirmed eligibility by central lab testing before randomizing each patient. Recent guidelines conclude that present evidence does not demonstrate improved outcomes with use of adjuvant trastuzumab for patients who would be classified HER2 negative by protocols of B31, N9831, and similar studies (Wolff, Hammond, Schwartz, et al., 2007a; Carlson, Moench, Hammond, et al., 2006; Hanna, O'Malley, Barnes, et al., 2007).

Importantly, the B31 and N9831 subgroup analyses combine results for HER2-negative patients many now consider to be different: those with the so-called "triple-negative" subtype (i.e., negative for HER2, estrogen receptor, and progesterone receptor), and the luminal subtypes (luminal A or luminal B) that are negative for HER2 but positive for at least one of the hormone receptors. These subtypes were initially defined in studies using microarrays to subdivide breast cancer patients by gene expression patterns (for reviews, see Peppercorn, Perou, and Carey, 2008; Razzak, Lin, and, Winer, 2008; Kang, Martel, and Harris 2008). There is evidence that the triple negative and luminal subsets differ with respect to prognosis, chemotherapy response, and outcomes (Carey, Dees, Sawyer, et al., 2007; Liedtke, Mazouni, Hess, et al., 2008), and they clearly differ with respect to effects of endocrine therapy. Further complexity comes from reports that there is substantial but incomplete overlap between triple negative patients and those classified in the "basal-like" subset by gene expression arrays (Cheang, Voduc, Bajdik, et al., 2008). Notably, new phase III trials have recently opened (and others are planned) specifically for patients with triple negative or "basal-like" breast cancer (Kilburn, 2008). Results from these studies will likely be more conclusive than analyses that pool all HER2-negative patients to determine outcomes for subsets of HER2-negative breast cancer.

Adjuvant trastuzumab in HER2-equivocal patients. Among patients with initially equivocal HER2 test results by current clinical practice guidelines (those scored 2+ if IHC is first, or HER2 gene copy number from 4.0 to 6.0 or HER2/CEP17 ratio from 1.8 to 2.2 if ISH is first), ultimately, most are definitively categorized as HER2 positive or HER2 negative after guideline-recommended followup testing. Data are presently unavailable either to estimate effects of adjuvant trastuzumab on outcomes for the subset with initially equivocal results subsequently classified HER2 positive, or to demonstrate lack of benefit in those subsequently classified HER2 negative. For the minority who remain equivocal after followup testing, the guidelines' treatment recommendation depends on whether the patient would have been included or

excluded from key randomized, controlled trials. For example, patients with HER2/CEP17 ratios 2.0 or greater but less than 2.2 were included and randomized in the adjuvant trastuzumab trials. Therefore, the guidelines consider current evidence insufficient to deny these patients trastuzumab with adjuvant chemotherapy. In contrast, patients with HER2/CEP17 ratios 1.8 or greater but less than 2.0 were excluded from these trials, and the guidelines consider current evidence insufficient to include trastuzumab in their adjuvant therapy regimens. Figures 2 and 3 (see Key Question 1) include information on trial eligibility of patients whose test results are equivocal by each HER2 assay.

Advanced or metastatic disease. No data were reported on patients with advanced or metastatic disease and discordant results from IHC and ISH HER2 testing. Evidence is available from one trial (CALGB 9840; n=226) that randomized metastatic breast cancer patients who were HER2 negative by local laboratory testing to chemotherapy with or without trastuzumab (Seidman, Berry, Cirrincione, et al., 2008). Additionally, a small subset of advanced and metastatic patients randomized to chemotherapy with or without lapatinib in another trial (EGF100151; n=74) were found by central lab confirmatory testing not to meet protocol criteria for HER2 positivity (Cameron, Casey, Press, et al., 2008). Thus, one source of good quality evidence (CALGB 9840) and one source of moderate quality evidence (EGF100151) suggest that HER2-negative patients with advanced or metastatic disease do not benefit from treatments targeting the HER2 molecule. Additional evidence supporting this conclusion comes from an analysis of data pooled from three pivotal trials of trastuzumab for metastatic breast cancer. The analysis showed that among patients found IHC 2+ by the presently unavailable "clinical trial assay," benefit from trastuzumab was limited to those subsequently shown to have amplified HER2 genes by FISH (Mass, Press, Anderson et al., 2005).

CALGB 15002 investigators compared outcomes with versus without trastuzumab for a subgroup of FISH-negative patients who either had (n=38) or did not have (n=103) polysomy 17, (Kaufman, Broadwater, Lezon-Geyda, et al., 2007). Overall response rate was significantly higher with versus without trastuzumab for those with polysomy 17, but was identical with or without trastuzumab for those without polysomy 17. In contrast, the N9831 study on adjuvant therapy (Reinholz, Jenkins, Hillman, et al., 2007) reported no impact of polysomy 17 on benefit from trastuzumab, and unpublished data from a second study (NSABP B31; Dr. S. Paik, personal communication, May 2008)) suggested the same finding. This might be due to different definitions of polysomy 17 for CALGB 15002 (average CEP17 copy number per cell greater than 2.2) and N9831 (more than 3 CEP17 signals in more than 30% of nuclei). It might also reflect differences between adjuvant therapy and treatment for metastatic disease with respect to polysomy 17 as a predictor of benefit from trastuzumab. Note also that studies reviewed for "Results and Conclusions, Key Question 1" report conflicting data on a possible association of polysomy 17 with overexpression of HER2 protein. Thus, presently available evidence leaves unanswered questions with respect to the utility of polysomy 17 to select patients for HER2-targeted therapy.

Key Question 3a

For breast cancer patients, what is the evidence on clinical benefits and harms of using HER2 assay results to guide selection of chemotherapy regimen?

Study Selection

The search strategy for studies on HER2 testing in breast cancer yielded 3,218 citations. Initial review of titles and abstracts selected 219 citations potentially relevant to Key Question 3 for retrieval and review as full articles. Of these, 161 were considered potentially relevant to Key Question 3a (HER2 status to guide choice of chemotherapy regimen) while 62 were considered potentially relevant to Key Question 3b (HER2 status to guide choice of hormonal therapy regimen). Four reports were considered for both question 3a and 3b.

Twenty separate studies met selection criteria and were abstracted for Key Question 3a (Table 10; Appendix Table IIIa-A[*]). Eleven studies investigated adjuvant chemotherapy for resected early stage breast cancer, including nine randomized, controlled trials, an uncontrolled series, and the standard-dose control arm of a randomized, controlled trial of high-dose chemotherapy with autologous stem-cell support (HDC/AuSCS). Six studies investigated neoadjuvant (preoperative) chemotherapy for locally advanced breast cancer; one was a randomized, controlled trial and five were uncontrolled, single-arm series. Three studies investigated first- or second-line therapy for advanced or metastatic breast cancer. Two randomized, controlled trials compared different regimens; the third randomized, controlled trial compared different doses of one drug, but pooled arms for the analysis by HER2 status.

Available Studies

Eleven studies on postsurgical adjuvant chemotherapy. The available evidence included one retrospective analysis of an uncontrolled single-arm series (Yang, Klos, Zhou, et al., 2003), and ten randomized, controlled trials. However, for one of the randomized, controlled trials, (Tanner, Isola, Wiklund, et al., 2006), one arm was excluded, since patients received HDC/AuSCS. Each randomized, controlled trial was designed to compare outcomes of treatment regimens in populations not selected or stratified for HER2 status, and most published earlier reports that compared patients, prognostic factors, and outcomes by treatment arm for all randomized patients. With only one exception (Martin, Pienkowski, Mackey, et al., 2005), reports from randomized, controlled trials included for Key Question 3a were secondary or correlative analyses on patient subgroups with archived tissue samples that permitted HER2 testing. The proportion of originally randomized patients included in the analyses by HER2 status ranged from 34 to 92 percent (see Table 10). A subset of trials compared baseline

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 10. Summary design, treatment, patient characteristics, KQ3a

Study/Design	Treatments	Age or Menopause Status	Extent of Disease	(% of pts analyzed by HER2 status) ER+ PR+	HER2-
Adjuvant chemotherapy for resected early breast cancer					
Yang et al., 2003 series	cyclophosphamide + methotrexate + fluorouracil (CMF; n=94)	≥50 yr: 52.1%	≥3 cm: 67% N+: 62%	NR NR	IHC only: 36% 64% **HER2+**
Gusterson et al., 2003; stratified RCT	perioperative CMF (one cycle)	post: 47% of n=760 of 1275 N- patients randomized	>2 cm: 53%, HER2+ 40%, HER2- 100% N0	of HER2+: 36% 24% of HER2-: 51% 38%	IHC only: 12.8% 87.2%
	no adjuvant therapy				IHC only: 20.8% 79.2%
	Multiple cycles of CMF	post: 45% of n=746 of 1229 N+ patients randomized	T size, NR; 100% node+; ≥4 nodes +: 49%, HER2+ 43%, HER2-	of HER2+: 32% 22% of HER2-: 59% 45%	IHC only: 17.3% 82.7%
	perioperative CMF (one cycle)				IHC only: 21.6% 78.4%
Moliterni et al., 2003; RCT	8 cycles CMF + 4 cycles doxorubicin (CMF→ A; n=248 of 277 randomized)	≥52 yr: 67%	~65%, <2.1 cm 100% N1	only reported for all randomized to each arm	IHC only: 18.1% 81.9%
	12 cycles of CMF alone (n=258 of 275 randomized)	≥52 yr: 69%			IHC only: 19.4% 80.6%
Colozza et al., 2005; RCT	epirubicin(E), weekly for 4 months (n=133 of 166 randomized)	>50 yr: 51%	≤2 cm: 46% 1-3 N+: 52%	63%	IHC only: 40.6% 59.4%
	6 cycles CMF (n=133 of 174 randomized)	>50 yr: 56%	≤2 cm: 45% 1-3 N+: 59%	55% 56% 63%	IHC only: 27.8% 72.2%
Pritchard et al. 2006; RCT	6 cycles of CEF (n=312 of 351 randomized)	100% pre	FISH: pos neg T2 52% 49% 1-3N+ 57% 63%	62% NR	by FISH: 24.0% 76.0%
	6 cycles of CMF (n=316 of 359 randomized)	100% pre		56% NR	by FISH: 27.8% 72.2%
Knoop et al., 2005; RCT	9 cycles of CEF (n=352 of 480 randomized)	post: 31.5%	T≥2.1 cm: 60.7% 1-3 N+: 29.5%	25% NR	IHC 3+ or FISH+ 32.5% 67.5%
	9 cycles of CMF (n=421 of 500 randomized)	post: 30.2%	T≥2.1 cm: 57.6% 1-3 N+: 33.3%	27% NR	IHC 3+ or FISH+ 32.8% 67.2%
Dressler et al., 2005, Thor et al., 1998; 3-arm RCT (CALGB 8541)	4 cycles high-dose CAF (n=179 of 519 randomized)[a] (A=doxorubicin)	mn, 50.1 yrs 42.5% pre	mn T size, 2.91 cm mn # N^+, 4.51	68% 54%	$FISH^+$ 17.3% 82.7% IHC^+ 24.8% 75.2%
	6 cycles moderate-dose CAF (n=167 of 513 randomized)[a]	mn, 51.4 yrs 38.3% pre	mn T size, 2.88 cm mn # N^+, 4.43	71% 65%	$FISH^+$ 20.7% 79.4% IHC^+ 25.7% 74.3%
	4 cycles low-dose CAF (n=178 of 518 randomized)[a]	mn, 50.4 yrs 41.1% pre	mn T size, 3.07 cm mn # N^+, 4.92	66% 58%	$FISH^+$ 18.8% 81.2% IHC^+ 22.9% 77.1%

[a] Data on eligible patients randomized to each arm are from Budman, Berry, Cirrincione, et al., 1998.

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 10. Summary design, treatment, patient characteristics, KQ3a (continued)

Study/Design	Treatments	Age or Menopause Status	Extent of Disease	(% of pts analyzed by HER2 status) ER+ PR+	HER2-
Adjuvant chemotherapy for resected early breast cancer (continued)					
Del Mastro et al. 2004, 2005; RCT (GONO-MIG-1)	up to 9 cycles FEC14 regimen (q2wk; n=370 of ~607 randomized)	median, 54 yrs range, 25-70	T1: 47% N+: 62% T2: 46% N-: 38% T3-4: 5% T? 1%	54% 42%	IHC 3+ CB11 50 (13.5%) 320 (86.5%) HER2+
	6 cycles FEC21 regimen (q3wk; n=361 of ~607 randomized)				IHC 3+ CB11 53 (14.7%) 308 (85.3%)
Tanner et al., 2006; control arm from RCT	9 cycles of FEC (n=180 of 251 randomized; n=211 from HDC/AuSCS arm excluded)	≥50 yr: 42% of all tested	HER2: pos neg T:2-5cm 60% 52% 5-9 N+ 41% 47% ≥10 N+ 59% 53%	only reported pooled data for both study arms	CISH only: 31.1% 68.9%
Hayes et al., 2007; RCT (randomly selected 2 groups of 750 ea)	4 cycles AC → paclitaxel (n=1,570 randomized)	post: 38%	Grp1 Grp2 T>2cm 66% 64% 1-3 N+ 48% 46% 4-9 N+ 40% 43%	Grp1 57% NR Grp2 62% NR	not reported
	4 cycles AC → observation (n=1551 randomized)	post: 38%			not reported
Martin et al., 2005[b]; RCT	6 cycles DAC (n=630 with known HER2 status of 745 randomized) (D=docetaxel)	median, 49 yrs range, 26-70 pre, 56%	T1: 40% 1-3N+: 63% T2: 52% ≥4N+: 37% T3: 8%	ER+ &/or PR+: 76%	155 (24.6 %) 475 (75.4%)
	6 cycles FAC (n=632 with known HER2 status of 746 randomized)	median, 49 yrs range, 23-70 pre, 55%	T1: 43% 1-3N+: 62% T2: 51% ≥4N+: 38% T3: 6%	ER+ &/or PR+: 76%	164 (26.0%) 468 (74.0%)

[b] Except for HER2 status, data shown compare all patients randomized to TAC versus all patients randomized to FAC

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 10. Summary design, treatment, patient characteristics, KQ3a (continued)

Study/Design	Treatments	Age or Menopause Status	Extent of Disease	(% of pts analyzed by HER2 status) ER+ PR+	HER2-
Neoadjuvant (preoperative) chemotherapy for locally advanced breast cancer					
Learn et al., 2005[c]; 3-arm RCT	4 cycles AC ± D (concurrent or after resection) (n=104 of 144 randomized)	mean, 48 yrs median, 47 yrs range, 27-73	T ≤2 cm: 28% N0:61% T 2-5 cm:47% N1:39% T >5 cm: 25% N2: 0	only reported data for n=121 with biopsy specimens	TAB 250 (n=104 classified) **HER2+** IHC 41 (39%) 63 (61%)
Arriola et al., 2006; series	4 cycles of doxorubicin followed by surgery (n=232)	mean, 47 yrs	T3: 70% N1: 40%	52% 67%	IHC + FISH then CISH 18% 82%
Park et al., 2003; series	4 cycles of doxorubicin followed by surgery (n=67)	≥50 yrs, 18%	5-10 cm 91% >10 cm 9% N status NR	46% NR	CISH only: 46% 54%
Zhang et al., 2003; series	3-6 cycles of FAC followed by surgery (n=97)	≥50 yrs, 44%	T2 53% ≥T3 34% N^- 33% N^+ 67%	65% 56%	IHC 3+ or FISH+ 28% 72%
Tulbah et al., 2002; series	3-4 cycles of paclitaxel + cisplatin followed by surgery (n=54)	$HER2^+$ $HER2^-$ ≤50 91% 84% pre 91% 78%	$HER2^+$ $HER2^-$ ≥T3 86% 78% N0 36% 28% N1 55% 56% N2 9% 16%	of $HER2^+$: 55% 50% of $HER2^-$: 50% 34%	IHC 3+ 41% 59%
Tinari et al., 2006; series	median 4 (range, 3-6) cycles FEC, q3wk followed by surgery (n=77)	median, 46 yrs range, 25-74	T 2-5 cm: 75% T >5 cm: 25%	62% 45%	IHC 3+ or 2+ & FISH+ 20 (26%) 57 (74%)

[c] Except for ER, PR and HER2 status, data shown pool evaluable patients (n=142) randomized to AC, AC+D, or AC→adjuvant D

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 10. Summary design, treatment, patient characteristics, KQ3a (continued)

Study/Design	Treatments	Age or Menopause Status	Extent of Disease	(% of pts analyzed by HER2 status) ER+ PR+	HER2+	HER2-
First- or second-line chemotherapy for advanced or metastatic breast cancer						
Harris et al., 2006; RCT	paclitaxel (n=165 of 474 randomized to 3 dose arms, but pooled for HER2 analysis)	median: 54.9 yr	# metastatic sites: median, 1	ER^+ &/or PR^+: 58%	FISH 26% CB11 20% Hercep. 3^+ 21%	74% 80% 79%
Di Leo et al., 2004; RCT	doxorubicin (A; n=91 of 165 randomized)	54 yr	≥3 sites: 46% visceral: 79%	NR	IHC+ ≥1% & FISH+:	69%
	docetaxel (T; n=85 of 161 randomized)	51 yr	≥3 sites: 51% visceral: 76%	NR	IHC+ ≥1% & FISH+:	59%
Konecny et al., 2004; RCT	epirubicin + cyclophosphamide (EC; n=137 of 254 randomized)	mean: 55 yr (31-74)	1-2 sites: 57% ≥3 sites: 42%	52.6% 48.9%	FISH only 36% 16%	64%
	epirubicin + paclitaxel (ET; n=138 of 262 randomized)	mean: 55 yr (29-75)	1-2 sites: 53% ≥3 sites: 42%	60.9% 49.3%	FISH only 35% 25%	65%

Abbreviations: Please refer to the text or list of abbreviations at the end of the report for definition of specific chemotherapy regimens/agents.
Grp: group; IHC: immunohistochemistry; FISH: fluorescent in situ hybridization; mn: mean; q wk: every week; q3wk: every 3 weeks;

characteristics and known prognostic factors between the subgroups with known HER2 status and those with undetermined HER2 status, and a smaller subset also compared outcomes. None of these studies used trastuzumab for HER2-positive patients; studies addressing the use of trastuzumab are included in the discussion of Key Question 2.

Studies on the CMF regimen. The uncontrolled series (Yang, Klos, Zhou, et al., 2003; n=94) and one comparative randomized, controlled trial (Gusterson, Gelber, Goldhirsch, et al., 2003; n=2,504 randomized) studied the cyclophosphamide plus methotrexate plus fluorouracil (CMF) regimen. The Gusterson and co-workers trial separately randomized groups of node-negative and node-positive patients. Tissue blocks for determining HER2 status were unavailable for 515 (40 percent) of 1,275 randomized node-negative patients and for 483 (39 percent) of 1,229 randomized node-positive patients. Node-negative patients were randomized to one perioperative cycle of adjuvant CMF or to observation. Node-positive patients were randomized to multiple cycles of adjuvant CMF or to one perioperative cycle of adjuvant CMF. The relevance of these findings for current practice may be limited as taxane-based regimens have largely replaced CMF when anthracyclines are not used, particularly for hormone-receptor-negative patients.

Studies on anthracycline-based regimens. Four randomized, controlled trials (Moliterni, Menard, Valagussa, et al., 2003; Colozza, Sidoni, Mosconi, et al., 2005; Pritchard, Shepherd, O'Malley, et al., 2006; Knoop, Knudsen, Balslev, et al., 2005) compared CMF versus anthracycline-based regimens, and a fifth randomized, controlled trial compared an anthracycline-based regimen without autologous stem-cell support (AuSCS) versus a higher-dose regimen with AuSCS (Tanner, Isola, Wiklund, et al., 2006). Only the non-AuSCS arm of the Tanner and co-workers study met selection criteria for data abstraction. Moliterni, Menard, Valagussa, et al. (2003) compared CMF followed by doxorubicin (CMF→A) versus CMF alone, and included 92 percent of originally randomized patients. Colozza, Sidoni, Mosconi, et al. (2005) compared epirubicin (E) alone versus CMF, and included 76 percent of originally randomized patients. Pritchard, Shepherd, O'Malley, et al. (2006) and Knoop, Knudsen, Balslev, et al. (2005) compared cyclophosphamide plus epirubicin plus fluorouracil (CEF) versus CMF, although the Pritchard and co-workers study gave 6 cycles while the Knoop and co-workers study gave 9 cycles. Pritchard and co-workers included 89 percent of originally randomized patients while Knoop and co-workers included 79 percent. Tanner, Isola, Wiklund, et al. (2006) also gave 9 cycles of CEF in the non-AuSCS arm of their trial, although the doses administered were higher than those in the Pritchard and Knoop trials. Outcomes by HER2 status for 72 percent of those randomized to the non-AuSCS arm are considered a single-arm study in this review.

Two randomized, controlled trials with two reports each compared different doses (Dressler, Berry, Broadwater, et al., 2005; Thor, Berry, Budman, et al., 1998) or dose intensities and schedules (Del Mastro, Bruzzi, Nicolo, et al., 2005; Del Mastro, Bruzzi, Venturini, et al., 2004) for anthracycline-based regimens. The Dressler and co-workers study investigated interaction of HER2 status with dose in 524 patients from the Cancer and Leukemia Group B (CALGB) trial 8541. This trial randomized 1,549 patients to high-dose (600/60/600 mg/m^2 every four weeks for 16 weeks), moderate-dose (400/40/400 mg/m^2 every four weeks for 24 weeks) or low-dose (300/30/300 mg/m^2 every four weeks for 16 weeks) regimens of cyclophosphamide, doxorubicin and fluorouracil (CAF) (Budman, Berry, Cirrincione, et al., 1998). Although earlier reports (Thor, Berry, Budman, et al., 1998; Muss, Thor, Berry, et al., 1994) included different proportions of randomized patients tested for HER2 status by IHC and/or PCR, Dressler and

colleagues compared outcomes separately by assay method (IHC, FISH, or PCR) for HER2 status subgroups from each dose arm (n=524, 33.8 percent of originally randomized patients).

In the GONO-MIG-1 study, Del Mastro and colleagues (2004, 2005) randomized 1,214 patients to either six cycles of CEF every three weeks (FEC21) or up to nine cycles at the same dose (600/60/600 mg/m^2) every two weeks (FEC14). The analysis by HER2 status included 731 (60 percent) of originally randomized patients.

Studies on regimens with a taxane. Two randomized, controlled trials investigated effects of HER2 status on outcomes of regimens with versus without a taxane (Hayes, Thor, Dressler, et al., 2007; Martin, Pienkowski, Mackey, et al., 2005). Hayes and colleagues (2007; CALGB trial 9344) randomized 3,121 patients to doxorubicin plus cyclophosphamide (AC) followed by paclitaxel or observation. The trial used a 3 x 2 factorial design to compare three doses of doxorubicin in AC, each followed or not by paclitaxel. Since outcomes were not statistically significantly different across doxorubicin doses, the analysis of outcomes with versus without paclitaxel by HER2 status pooled patients from all three doxorubicin doses. Two groups of 750 patients each were randomly selected for this correlative analysis, but tissue blocks were available and analyzed for only 1,322 (42 percent of those originally randomized).

Martin, Pienkowski, Mackey, et al. (2005) stratified patients (n=1,491) by number of involved axillary lymph nodes and randomized them to six three-week cycles of docetaxel plus doxorubicin plus cyclophosphamide (TAC) or fluorouracil plus doxorubicin plus cyclophosphamide (FAC). The preplanned analysis by HER2 status included 1,262 (85 percent) of originally randomized patients. Patients were not stratified by HER2 status. In the TAC group, 20.8 percent were HER2 positive and 15.4 percent lacked tumor specimens for measuring HER2; in the FAC group, 22 percent were HER2 positive and 15.3 percent lacked tumor specimens. The study does not report the distribution of other prognostic factors by treatment group and HER2 status combined, which would be useful in ensuring balance in this subset of trial patients with known HER2 status.

Evidence hierarchy. The first section of Table 11 categorizes available studies on HER2 status and outcomes of adjuvant chemotherapy according to the evidence hierarchy used in this evidence report (see “Methods”). No trials stratified patients by HER2 status or randomized patients to therapy guided or not guided by HER2 status, the highest category of evidence. Only one randomized, controlled trial that compared TAC versus FAC, reported a preplanned multivariate subgroup analysis (Martin, Pienkowski, Mackey, et al., 2005). Eight randomized, controlled trials, one that compared CMF versus no or minimal CMF, four that compared CMF versus an anthracycline-based regimen, two that compared different doses or schedules of anthracycline-based regimens, and one that compared AC alone versus followed by paclitaxel, reported post-hoc multivariate subgroup analyses. Finally, single-arm data from two reports provided univariate analyses by HER2 status.

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen

Table 11. Hierarchy of evidence, KQ3a

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
Adjuvant chemotherapy for resected early breast cancer						
HER2 stratified or HER2-guided RCT						
RCT prespecified MV SGA	Martin 2005	1262	adjuvant	TAC vs. FAC	DFS	Cox regression treatment by FISH HER2 interaction, p=NS
						FISH+ TAC > FAC, FISH- TAC > FAC
RCT post-hoc MV SGA	Gusterson 2003	1506	adjuvant	LN-: no tx vs. CMF	OS	LN- adjusted Cox regression IHC HER2+, tx < cx p=NS
				LN+: periop CMF vs. prolonged CMF		LN- adjusted Cox regression IHC HER2-, tx ≈ cx p=NS
						LN+ adjusted Cox regression IHC HER2+, tx < cx p=NS
						LN+ adjusted Cox regression IHC HER2-, tx > cx p<0.05
					DFS	LN- adjusted Cox regression IHC HER2+, tx < cx p=NS
						LN- adjusted Cox regression IHC HER2-, tx > cx p=NS
						LN+ adjusted Cox regression IHC HER2+, tx > cx p=NS
						LN+ adjusted Cox regression IHC HER2-, tx > cx p<0.05
	Moliterni 2003	506	adjuvant	CMF→A vs. CMF	OS	Cox regression treatment by IHC HER2 interaction p=0.052
						HER2+ tx > cx p= NS, HER2- tx < cx p=NS
					RFS	Cox regression treatment by IHC HER2 interaction p=NS
						HER2+ tx > cx p= NS, HER2- tx < cx p=NS
	Colozza 2005	266	adjuvant	CMF vs. epirub	OS	Cox regression treatment by IHC HER2 interaction p=NS
						cx HER2+ < HER2- p=0.024, tx HER2+ < HER2- p=NS
					RFS	Cox regression treatment by IHC HER2 interaction p=NS
						cx HER2+ ≈ HER2- p=NS, tx HER2+ < HER2-p=NS
	Pritchard 2006	628	adjuvant	CMF vs. CEF	OS	Cox regression treatment by FISH HER2 interaction p=0.02

				HER2+ tx > cx p=0.06, HER2- tx ≈ cx p=NS
			RFS	Cox regression treatment by FISH HER2 interaction p=0.02
				HER2+ tx > cx p=0.003, HER2- tx ≈ cx p=NS
	Knoop 2005	805 adjuvant	CMF vs. CEF	OS	Cox regression HER2+ tx > cx p=0.09, HER2- tx > cx p=0.23
				RFS	Cox regression HER2+ tx > cx p=0.10, HER2- tx > cx p=0.10
	Dressler 2005	521 adjuvant	CAF: high vs. mode-	DFS	Cox regression FISH HER2 by CAF dose interaction, p=0.033
			rate vs. low dose		Cox regression IHC HER2 by CAF dose interaction, p=0.0003
					Cox regression PCR HER2 by CAF dose interaction, p=0.043
					FISH+/PCR+/IHC+ high > moderate ≈ low dose
					FISH-/PCR-/IHC- high ≈ moderate ≈ low dose
	Del Mastro 2004	731 adjuvant	FEC q2wk vs. q3wk	DFS	Cox regression IHC HER2 by Tx schedule interaction, p=0.12
					FEC q2wk HER2 + ≈ HER2-, FEC q3wk HER2+ < HER2-
				OS	Cox regression IHC HER2 by Tx schedule interaction, p=0.38
					FEC q2wk HER2 + ≈ HER2-, FEC q3wk HER2+ < HER2-
	Hayes 2007	1500 adjuvant	AC vs. AC→P	OS	Cox regression treatment by FISH HER2 interaction p=0.01
					HER2+ tx > cx, HER2- tx ≈ cx
				DFS	Cox regression treatment by FISH HER2 interaction p=0.01
					HER2+ tx > cx, HER2- tx ≈ cx

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 11. Hierarchy of evidence, KQ3a (continued)

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
Adjuvant chemotherapy for resected early breast cancer (continued)						
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis						
1-arm UV analysis	Yang 2003	94	adjuvant	CMF	DFS	IHC HER2+↓ vs. HER2- p=0.002
	Tanner 2006	180	adjuvant	FEC	OS	CISH HER2+ < HER2- but not statistical tests described
					RFS	CISH HER2+ < HER2- but not statistical tests described
Neoadjuvant (preoperative) chemotherapy for locally advanced breast cancer						
HER2 stratified or HER2-guided RCT						
RCT prespecified MV SGA						
RCT post-hoc MV SGA	Learn 2005	104	neoadjuvant	AC vs. AC+D	pCR	IHC HER2+, AC vs. AC+D, p=NS
						IHC HER2-, AC vs. AC+D, p=NS
					cORR (CR+PR)	IHC HER2+, AC vs. AC+D, p=NS
						IHC HER2-, AC vs. AC+ D, p<0.05
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis	Park 2003	67	neoadjuvant	doxorub	pResp	CISH HER2+ > HER2- p=0.013
	Zhang 2003	97	neoadjuvant	FAC	DFS	CISH HER2+ ≈ HER2- p=NS
					ORR	IHC HER2+ > HER2- p=NS
					pResp	IHC HER2+ > HER2- p=NS
1-arm UV analysis	Arriola 2006	229	neoadjuvant	doxorub	pResp	CISH HER2+ > HER2- p=0.03
	Tulbah 2002	52	neoadjuvant	paclit+cispl	pResp	IHC HER2+ ≈ HER2- p=NS
					OS	IHC HER2+ (3+) ≈ HER2- p=NS
					OS	IHC HER2+ (2+/3+) ≈ HER2- p=0.051
					DFS	IHC HER2+ (3+) ≈ HER2- p=NS
					DFS	IHC HER2+ (2+/3+) ≈ HER2- p=0.09
	Tinari 2006	77	neoadjuvant	FEC	pResp (pCR+MRD)	IHC 3+ or IHC2+/FISH+ HER2+ vs. HER2-, p=0.008

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 11. Hierarchy of evidence, KQ3a (continued)

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
First- or second-line chemotherapy for advanced or metastatic breast cancer						
HER2 stratified or HER2-guided RCT						
RCT prespecified MV SGA						
RCT post-hoc MV SGA	Di Leo 2004	149	metastatic	doxorub vs. docetax	OS	Cox regression treatment by IHC HER2 interaction p=.10 IHC/FISH HER2+ tx < cx p=NS, HER2- tx > cx p=.07
					TTP	Cox regression treatment by IHC HER2 interaction p=NS IHC/FISH HER2+ tx > cx p=NS, HER2- tx > cx p=NS
					Resp	logistic regression treatment by IHC HER2 interaction p=.01 IHC/FISH HER2+ tx > cx p=.04, HER2- tx > cx p=NS, HER2? tx ≈ cx p=NS
	Konecny 2004	275	metastatic	epirub+cyclophosph vs. epirub+paclitaxel	OS	Cox regression treatment by IHC HER2 interaction p=NS FISH HER2+ tx > cx p=.059, HER2- tx ≈ cx p=NS
					PFS	Cox regression treatment by IHC HER2 interaction p=.109 FISH HER2+ tx > cx p=.062, HER2- tx ≈ cx p=NS
					ORR	logistic regression treatment by IHC HER2 interaction p=NS FISH HER2+ tx > cx p=.005, HER2- tx > cx p=.046
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis						
1-arm UV analysis	Harris 2006	156	metastatic	paclitaxel	OS	IHC CB11 HER2+ < HER2- p=NS
					OS	FISH HER2+ < HER2- p=NS
					OS	IHC HercepTest HER2+ ≈ HER2- p=NS
					ORR	IHC CB11 HER2+ ≈ HER2- p=NS
					ORR	FISH HER2+ ≈ HER2- p=NS
					ORR	IHC HercepTest HER2+ > HER2- p=.026

Abbreviations: Please refer to the text or list of abbreviations at the end of the report for definition of specific chemotherapy regimens/agents.
cx: control; DFS: disease-free survival; HR: hazard ratio; MV: multivariate; ORR: overall response rate; OS: overall survival; q2wk: every 2 weeks; q3wk: every 3 weeks; RCT: randomized, controlled trial; RFS: recurrence-free survival; SGA: subgroup analysis; TTP: time to progression; tx: treatment; UV: univariate analysis;

Study quality assessment. The first section of Table 12 shows that, of nine studies that analyzed the relationship of HER2 status to outcome differences in previously completed randomized, controlled trials on adjuvant chemotherapy, each was prospectively designed; included a large, well-defined and representative study population; and treated patients in each study arm homogeneously, or used rule-based selection for non-study therapies. However, only two reports (Dressler, Berry, Broadwater, et al., 2005; Martin, Pienkowski, Mackey, et al., 2005) included a prespecified hypothesis on the relationship of HER2 status to differences between regimens in treatment outcome. Each study adequately described the assays and thresholds they used to for classify patients' HER2 status, but only five (Colozza, Sidoni, Mosconi, et al., 2005; Dressler, Berry, Broadwater, et al., 2005; Del Mastro, Bruzzi, Nicolo, et al., 2005; Tanner, Isola, Wiklund, et al., 2006; Hayes, Thor, Dressler, et al., 2007) reported that individuals who assessed HER2 status were blinded to patient and tumor factors and to treatment outcomes. Only three studies from randomized, controlled trials (Moliterni, Menard, Valagussa, et al., 2003; Pritchard, Shepherd, O'Malley, et al., 2006; Martin, Pienkowski, Mackey, et al., 2005) included ≥85 percent of originally randomized patients. However, a fourth (Hayes, Thor, Dressler, et al., 2007) randomly selected two large subsets (n=750 each) and separately analyzed more than 85 percent of patients in each. Six studies from randomized, controlled trials (Moliterni, Menard, Valagussa, et al., 2003; Colozza, Sidoni, Mosconi, et al., 2005; Pritchard, Shepherd, O'Malley, et al., 2006; Knoop, Knudsen, Balslev, et al., 2005; Dressler, Berry, Broadwater, et al., 2005; Hayes, Thor, Dressler, et al., 2007) had 9 or more years' median follow-up, but in only one of these (Moliterni, Menard, Valagussa, et al., 2003) was median follow-up ~15 years. Reporting of methodologic details for multivariate analyses was inadequate in all studies.

Six studies on preoperative neoadjuvant chemotherapy. Six studies, including one randomized, controlled trial and five uncontrolled series, compared outcomes by HER2 status for patients undergoing neoadjuvant (preoperative) chemotherapy. The randomized, controlled trial (Learn, Yeh, McNutt, et al., 2005) randomized patients (n=144) to one of three arms: doxorubicin plus cyclophosphamide (AC), AC plus docetaxel (AC+D), or AC followed by docetaxel after resection (AC→D). Analysis of pathologic outcomes at resection pooled patients from the AC and AC→D arms and compared these versus the AC+D arm. The secondary, unplanned analysis by HER2 status included 104 (72 percent) of originally randomized patients.

Two uncontrolled series, one prospective (n=232, Arriola, Moreno, Varela, et al., 2006) and the other retrospective (n=67, Park, Kim, Lim, et al., 2003) reported on patients given doxorubicin alone. One uncontrolled retrospective series (n=97, Zhang, Yang, Smith, et al., 2003) reported on patients given three to six cycles of fluorouracil plus doxorubicin plus cyclophosphamide (FAC). A similar uncontrolled, retrospective series (n=77; Tinari, Lattanzio, Natoli, et al., 2006) reported on patients given three to six cycles of fluorouracil plus epirubicin plus cyclophosphamide. Finally, one uncontrolled retrospective series (n=54, Tulbah, Ibrahim, Ezzat, et al., 2002) reported on patients given three or four cycles of paclitaxel plus cisplatin. Each series reported outcomes by HER2 status for all patients (n=232 for the Arriola and co-workers series; n<100 for each of the others).

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 12. Study quality ratings, KQ3a

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation
									1) 2) 3) 4) 5) 6)
Adjuvant Chemotherapy									
Yang et al., 2003	N	N	N	Y	?	Y	Y	?	NA
Gusterson et al., 2003	Y	N	Y	Y	?	Y	N	med: 6 yrs	? ? ? Y ? N
Moliterni et al., 2003	Y	N	Y	Y	?	Y	Y	med: 14.8 yrs	? ? Y Y ? Y
Colozza et al., 2005	Y	N	Y	Y	Y	Y	N	min 8 yrs	? N ? Y ? N
Pritchard et al., 2006	Y	N	Y	Y	?	Y	Y	med: 10 yrs	? ? ? Y ? N
Knoop et al., 2005	Y	N	Y	Y	?	Y	N	med: 10 yrs	? N Y ? ? N
Dressler et al., 2005; Thor et al., 1998	Y	Y	Y	Y	Y	Y	N	med: 9 yrs	Y ? ? Y ? Y
Del Mastro et al., 2004, 2005;	Y	N	Y	Y	Y	Y	N	med: 6.7 yrs	Y N ? N ? N

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 12. Study quality ratings, KQ3a (continued)

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, representative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation					
									1)	2)	3)	4)	5)	6)
Adjuvant Chemotherapy (continued)														
Tanner et al., 2006	Y	N	Y	Y	Y	Y	N	?	NA					
Hayes et al., 2007	Y	N	Y	Y	Y	Y	N	med: ~10 yrs	Y	Y	?	Y	?	Y
Martin et al., 2005	Y	Y	Y	N	?	Y	Y	med: 4.6 yrs	Y	Y	?	Y	?	N
Neoadjuvant (Preoperative) Chemotherapy														
Learn et al., 2005	Y	N	N	N	?	Y	N	pCR at resection	?	?	NA	?	?	N
Arriola et al., 2006	Y	Y	Y	Y	?	Y	Y	pCR at resection	?	N	NA	N	?	N
Park et al., 2003	N	N	N	Y	?	Y	Y	pCR at resection	NA					
Zhang et al., 2003	N	N	N	N	?	Y	Y	pCR at resection	NA					
Tulbah et al., 2002	N	N	N	Y	Y	Y	Y	pCR at resection	NA					
Tinari et al., 2006	N	N	N	Y	Y	Y	Y	pCR at resection	?	?	NA	Y	?	?

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 12. Study quality ratings, KQ3a (continued)

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation					
									1)	2)	3)	4)	5)	6)
Chemotherapy for Advanced or Metastatic Disease														
Harris et al., 2006	Y	N	Y	Y	Y	Y	N	med: 8.3 yrs	?	?	?	Y	?	N
Di Leo et al., 2004	Y	N	Y	Y	Y	Y	N	med: 23 months	?	N	?	N	?	N
Konecny et al., 2004	Y	N	Y	Y	?	Y	N	?	?	N	Y	?	?	N

Evidence hierarchy. As shown in Section 2 of Table 11, no studies on neoadjuvant chemotherapy reported either of the two highest evidence categories. The only study from a randomized, controlled trial on neoadjuvant chemotherapy (Learn, Yeh, McNutt, et al., 2005) reported a post-hoc multivariate subgroup analysis. Two series (Park, Kim, Lim, et al., 2003; Zhang, Yang, Smith, et al., 2003) reported post-hoc multivariate subgroup analyses, while three series reported univariate analyses only.

Study quality assessment. Section 2 of Table 12 shows that only two studies (one a randomized, controlled trial) on neoadjuvant chemotherapy were prospectively designed (Learn, Yeh, McNutt, et al., 2005; Arriola, Moreno, Varela, et al., 2006), and only one reported a prespecified hypothesis for the relationship of HER2 status to outcome of neoadjuvant chemotherapy (Arriola, Moreno, Varela, et al., 2006). Only one study (Arriola, Moreno, Varela, et al., 2006) included ≥100 patients. Four of six (Arriola, Moreno, Varela, et al., 2006; Park, Kim, Lim, et al., 2003; Tulbah, Ibrahim, Ezzat, et al., 2002; Tinari, Lattanzio, Natoli, et al., 2006; but not Learn, Yeh, McNutt, et al., 2005) adequately described the assays and thresholds used to classify patients' HER2 status, but only two (Tulbah, Ibrahim, Ezzat, et al., 2002; Tinari, Lattanzio, Natoli, et al., 2006) reported HER2 assays were scored by assessors blinded to patient and tumor characteristics and treatment outcomes. Patients in each study were treated homogeneously, and each series, but not the randomized, controlled trial (Learn, Yeh, McNutt, et al., 2005), reported on all enrolled patients. Follow-up was not an issue for any study on neoadjuvant therapy, since the outcome of interest was pathologic responses at resection. Reporting of methodologic details for multivariate analyses was inadequate in all studies.

Three studies on chemotherapy for advanced or metastatic breast cancer. Each was a secondary analysis from a randomized, controlled trial designed to compare outcomes of treatment regimens in populations not selected or stratified for HER2 status, and each published earlier reports comparing outcomes by treatment arm for all randomized patients. One randomized, controlled trial (n=474, Harris, Broadwater, Lin, et al., 2006; CALGB 9342) randomized patients with stage IV or inoperable disease undergoing first- or second-line therapy to three different doses of paclitaxel. The analysis of outcomes by HER2 status included 35 percent of originally randomized patients, and pooled data across all three doses. Thus, Harris and co-workers (2006) was considered a single-arm study in this systematic review.

A second randomized, controlled trial (n=326, Di Leo, Chan, Paesmans. et al., 2004) randomized patients to doxorubicin alone (A) or docetaxel alone (T). Eligibility required patients to have metastatic disease and to have failed prior CMF (either as adjuvant therapy or for metastasis), but no prior exposure to either of the randomized drug therapies. The analysis by HER2 status included 54 percent of originally randomized patients. The third randomized, controlled trial (n=516, Konecny, Thomssen, Luck, et al., 2004) randomized patients to first-line therapy for metastatic disease with either epirubicin plus cyclophosphamide (EC) or epirubicin plus paclitaxel (ET). Up to one prior hormonal therapy for metastasis was permitted, with patients stratified by prior hormonal therapy. The analysis by HER2 status included 53 percent of originally randomized patients.

Evidence hierarchy. As shown in Section 3 of Table 11, no studies on advanced or metastatic disease reported evidence of the two highest categories. Two randomized, controlled trials (Di Leo, Chan, Paesmans. et al., 2004; Konecny, Thomssen, Luck, et al., 2004) reported post-hoc multivariate subgroup analyses. The third study, a pooled analysis across trial treatment arms (Harris, Broadwater, Lin, et al., 2006) only reported a univariate analysis.

Study quality assessment. Section 3 of Table 12 shows that each of the three included studies on HER2 status as a predictor of chemotherapy outcomes for advanced or metastatic breast cancer was designed prospectively, but none reported a prespecified hypothesis for the effect of HER2 status on outcomes. Each study included a large, well defined, and representative study population, adequately described the HER2 assays and thresholds they used to classify patients' HER2 status, and treated patients in each study arm homogeneously. Only two of three (Harris, Broadwater, Lin, et al., 2006; Di Leo, Chan, Paesmans. et al., 2004) reported blinding HER2 assessors to patient and tumor characteristics and to treatment outcomes. Each omitted 15 percent or more of enrolled patients from the analysis of outcomes by HER2 status, and each omitted key methodologic details on their multivariate analyses from the published reports. Long-term follow-up was available in only one study (Harris, Broadwater, Lin, et al., 2006), and one did not report the median duration of follow-up (Konecny, Thomssen, Luck, et al., 2004).

Patient Characteristics

Eleven studies on postsurgical adjuvant chemotherapy. Although all investigated adjuvant chemotherapy, the eleven studies varied with respect to their patient groups' distributions of baseline characteristics and risk factors for recurrent disease (Appendix Tables IIIa-B and IIIa-C[*], Table 10). Only a subset of these studies compared the HER2 positive and negative subgroups for baseline characteristics and risk factors. Also, only a subset of the nine randomized, controlled trials compared patients included in the analysis by HER2 status with those excluded because tissue blocks were missing or unsuitable.

Studies on CMF. Of the two CMF studies, the retrospective series by Yang, Klos, Zhou, et al. (2003) pooled data for node-negative and node-positive patients, groups that Gusterson, Gelber, Goldhirsch, et al. (2003) randomized separately to different treatment arm pairs. Yang, Klos, Zhou, et al. (2003) only reported baseline characteristics and risk factors for all patients analyzed. Gusterson, Gelber, Goldhirsch, et al. (2003) compared HER2-positive versus HER2-negative patients separately for the node-positive and node-negative groups, but did not compare those with known HER2 status versus those lacking tissue blocks for HER2 assays. In node-negative patients, HER2 positivity was statistically significantly associated with larger tumor size, hormone-receptor negativity, and higher tumor grade. In node-positive patients, HER2 positivity was statistically significantly associated with menopausal status, hormone-receptor negativity, and higher tumor grade.

Studies on regimens with versus without an anthracycline. Three (Colozza, Sidoni, Mosconi, et al., 2005; Pritchard, Shepherd, O'Malley, et al., 2006; Tanner, Isola, Wiklund, et al., 2006) of five studies comparing adjuvant regimens with versus without an anthracycline compared baseline characteristics of HER2 positive and negative subgroups. Three (Colozza, Sidoni, Mosconi, et al., 2005; Knoop, Knudsen, Balslev, et al., 2005; Tanner, Isola, Wiklund, et al., 2006) explored whether subgroups tested for HER2 status were similar to the total study population or the subgroup not tested. Two trials (Moliterni, Menard, Valagussa, et al., 2003; Pritchard, Shepherd, O'Malley, et al., 2006) determined HER2 status on 92 percent or 89 percent, respectively, of the patients originally randomized and did not report comparisons to all or omitted patients. Each trial's full treatment arms were well balanced for baseline characteristics and prognostic factors.

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Moliterni, Menard, Valagussa, et al. (2003) did not report data comparing baseline factors by HER2 status. All patients in this trial had one to three positive nodes, and approximately 65 percent had tumors smaller than 2.1 cm in diameter. Colozza, Sidoni, Mosconi, et al. (2005) reported that treatment arms were well balanced, whether comparing all patients randomized or only those tested for HER2 status. However, significantly more patients randomized to epirubicin than to CMF were HER2 positive (41 percent versus 28 percent, p=.03). Progesterone receptor positivity was the only factor statistically significantly associated with HER2 positivity. This trial included node-positive and node-negative patients (4 or more positive nodes in less than 25 percent), and approximately 45 percent with tumors 2 cm or smaller in diameter.

Pritchard, Shepherd, O'Malley, et al. (2006) reported baseline characteristics of patients tested for HER2 status were similar to those of all randomized patients, but did not show data for this comparison. They showed data comparing FISH-positive and FISH-negative subgroups; except for a shift toward younger age in the FISH-positive subgroup, there were no significant differences. Just over half the patients in this trial had T2 or T3 tumors, all had positive lymph nodes, with four or more positive nodes in 37 percent and 43 percent of the FISH-negative and FISH-positive groups, respectively. Knoop and co-workers (2005) reported that among all patients tested for HER2 status, treatment arms were well balanced for prognostic factors. However, they did not report comparing the HER2-positive versus HER2-negative patients, either by treatment arms or across treatments. Tumors were larger than 2 cm diameter in approximately 60 percent of patients, and approximately 30 percent had four or more positive nodes. Tanner, Isola, Wiklund, et al. (2006) reported (but did not show data) that baseline characteristics of all patients tested for HER2 status did not differ from those of the entire trial cohort. They showed that baseline characteristics were similar for HER2-tested subgroups from each arm. However, the AuSCS arm was excluded from this review, and data were not reported comparing baseline characteristics of HER2-positive versus HER2-negative patients from the FEC arm.

Studies on dose or dose intensity of anthracycline-based regimens. Studies from randomized, controlled trials that compared dose (Dressler, Berry, Broadwater, et al., 2005) or dose intensity (Del Mastro, Bruzzi, Nicolo, et al., 2005) of anthracycline-based regimens reported baseline characteristics and prognostic factors of patients with known HER2 status were similar to those of patients omitted from the analyses, since HER2 status was unknown. Dressler and co-workers (2005) did not report data comparing baseline characteristics or prognostic factors of HER2-positive versus HER2-negative patients. Del Mastro and co-workers (2005) found a greater proportion of HER2-positive than HER2-negative patients lacking expression of both estrogen and progesterone receptors (62 percent versus 32.5 percent). Other baseline characteristics and prognostic factors were similar between subgroups by HER2 status and between treatment arms.

Studies on regimens with versus without a taxane. One of two studies from randomized, controlled trials on regimens with versus without a taxane compared baseline characteristics and prognostic factors of patient with known HER2 status versus those of patients with unknown HER2 status. The trial comparing paclitaxel versus observation after AC (Hayes, Thor, Dressler, et al., 2007) showed similar baseline characteristics, prognostic factors and overall survival in the two subgroups they randomly selected and tested for HER2 status (n=643 and 679, respectively). These subgroups were also similar to all treated patients (n=3,121), and to all non-tested patients (n=1,799). Tumor diameter was 2 cm or smaller in approximately 35 percent, and approximately 54 percent had 4 or more positive nodes. The randomized, controlled trial that compared TAC versus FAC (Martin, Pienkowski, Mackey, et al., 2005) only compared patient characteristics

and prognostic factors by treatment arm for all patients randomized. Neither study compared HER2-positive versus HER2-negative patients, either pooled across treatments or by treatment arm.

Six studies on preoperative neoadjuvant chemotherapy. The randomized, controlled trial on neoadjuvant therapy (Learn, Yeh, McNutt, et al., 2005) did not compare treatment arms or patient subgroups by HER2 status (neither known versus unknown nor positive versus negative) with respect to baseline characteristics or prognostic factors. This study only reported patient and tumor characteristics for all randomized patients

Only one (Tulbah, Ibrahim, Ezzat, et al., 2002) of the five included series compared baseline characteristics and prognostic factors for HER2-positive and HER2-negative subgroups. Across all five studies, approximately 55 percent to 65 percent of included patients were positive for estrogen receptors, and 45 percent to 55 percent were positive for progesterone receptors. However, their study samples varied somewhat with respect to tumor size and number of positive nodes. The series reported by Arriola, Moreno, Varela, et al. (2006) included 30 percent T2 and 70 percent T3 tumors, with 60 percent of patients node negative and 40 percent N1. Most patients (91 percent) in the series reported by Park, Kim, Lim, et al. (2003) had tumors between 5 and 10 cm in diameter. However, they did not report nodal status. Zhang, Yang, Smith, et al. (2003) include a few patients (13 percent) with T1 tumors, and approximately 33 percent node-negative patients. Most patients in the Tulbah, Ibrahim, Ezzat, et al. (2002) series had T3 or larger tumors, and approximately 55 percent had N1 disease. They reported generally well-balanced HER2-positive and HER2-negative subgroups. Finally, 75 percent of patients in the Tinari, Lattanzio, Natoli, et al. (2006) series had tumors with diameters between 2 and 5 cm; number of positive nodes was not reported.

Three studies on chemotherapy for advanced or metastatic breast cancer. Each of three included randomized, controlled trials reported that baseline characteristics and prognostic factors for the subgroup tested for HER2 status were similar to those of patients not tested. However, none compared HER2-positive versus HER2-negative subgroups, either separately by treatment arm or across arms.

Harris, Broadwater, Lin, et al. (2006) reported the only statistically significant difference between patients tested for HER2 (and other biomarkers) and those not tested was a shorter disease-free interval among those tested (19 versus 31 months, p=.0003). Investigators attributed this difference to discarding of tissue blocks after 10 years, thus a shorter interval from diagnosis to metastasis for those with blocks remaining. Hormone-receptor status (positive in 58 percent) and median number of metastatic sites (one) were the only prognostic factors reported among those tested for HER2 status. The analysis by HER2 status pooled patients across three trial arms randomized to different paclitaxel doses.

Di Leo, Chan, Paesmans, et al. (2004) showed the subgroups tested for HER2 status from each treatment arm were similar to each other and to the untested patients. Approximately half the included patients had three or more sites of disease, and more than three fourths had visceral involvement. They did not report hormone receptor status.

Konecny, Thomssen, Luck, et al. (2004) reported no statistically significant differences in baseline characteristics or prognostic factors between groups tested for HER2 and those not tested from each treatment arm compared separately. However, the HER2-positive and HER2-negative groups were not directly compared, either separately by treatment arm or pooled across arms.

Results, Key Question 3a

Eleven studies on postsurgical adjuvant chemotherapy.

Studies on CMF. Both studies on CMF reported superior outcomes in HER2-negative compared with HER2-positive patients (see Tables 13 and 14). The Gusterson, Gelber, Goldhirsch, et al. (2003) trial used proportional hazards models to compare hazard ratios (HR) for disease-free (DFS) and overall survival (OS) after no or one cycle of CMF in node-negative patients; each HR was not statistically significant. They also compared multiple versus single cycles of CMF in node-positive patients. Results favored multiple cycles for the HER2-negative subgroup and were statistically significant, but were not significant for HER2-positive patients:

- OS, $HER2^-$ (n=406 multiple; n=200, one): HR=0.69, 95 percent CI: 0.52–0.92; p=.01
- OS, $HER2^+$ (n=85 multiple; n=55, one): HR=1.15, 95 percent CI: 0.62–1.54; p, NS
- DFS, $HER2^-$ (n=406 multiple; n=200, one): HR=0.57, 95 percent CI: 0.46–0.72; p<.0001
- DFS, $HER2^+$ (n= 85 multiple; n=55, one): HR=0.77, 95 percent CI: 0.51–1.16; p, NS

The Yang, Klos, Zhou, et al. (2003) uncontrolled series (n=94) reported that at 5 years, DFS in the HER2-negative subgroup was superior to DFS in the HER2-positive subgroup (n=60, 86 percent versus n=34, 53 percent; log rank p<.1; stratified log rank, p=.002 after adjustment for nodal status).

Studies on regimens with versus without an anthracycline. Only one (Pritchard, Shepherd, O'Malley, et al., 2006) of four included randomized, controlled trials comparing regimens with versus without an anthracycline reported superior outcomes with the anthracycline regimen that reached statistical significance for HER2-positive but not HER2-negative patients. Pritchard, Shepherd, O'Malley, et al. (2006) used multivariate analysis (MVA) to test for an interaction of comparative treatment effect with HER2 status. The study compared CEF versus CMF and reported the following results for OS and relapse-free survival (RFS):

- OS, $HER2^-$ (n=237, CEF; n=228, CMF): HR=1.06, 95 percent CI: 0.83–1.44; p, NS
- OS, $HER2^+$ (n=75, CEF; n=88, CMF): HR=0.65, 95 percent CI: 0.42–1.02; p=.06
- OS, treatment by HER2 interaction from MVA: HR=2.04, 95 percent CI: 1.14–3.65, p=.02
- RFS, $HER2^-$ (n=237, CEF; n=228, CMF): HR=0.91, 95 percent CI: 0.71–1.18; p, NS
- RFS, $HER2^+$ (n 75, CEF; n 88, CMF): HR=0.52, 95 percent CI: 0.34–0.80; p=.003
- RFS, treatment by HER2 interaction from MVA: HR=1.96, 95 percent CI: 1.15–3.65; p=0.01

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 13. Summary time to event outcomes, KQ3a

Study	Time to Event Outcomes												
Adjuvant chemotherapy for resected early breast cancer													
Yang et al., 2003	Outcome	Grp	N	Med (mos)	1 yr	2.5 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
CMF; single-arm series	DFS	HER2+	34	6-7 years		~60%			53%	log rank	<.01		p=.002 in stratified log rank that adjusted for nodal status
		HER2-	60	not reached		~90%			86%				
Gusterson et al., 2003	Outcome	Grp	N	Med (mos)	2 yr	3 yr	4 yr	5 yr	6 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	64	not reached					76±5	Cox	NS	1.15 (0.54-2.46)	unadjusted univariate analyses; adjusted results also NS
		Cx	54	not reached					79±6	prop hazards			
760 node-neg pts randomized to periop CMF (Tx) or no adj Tx (Cx)	OS (HER2-)	Tx	436	not reached					85±2	Cox	NS	1.04 (0.68-1.61)	
		Cx	206	not reached					87±2	prop hazards			
	DFS(HER2+)	Tx	64	not reached	~84%	~68%	~65%	~62%	61±6	Cox	NS	1.22 (0.66-2.25)	unadjusted univariate analyses; adjusted results also NS
		Cx	54	not reached	~86%	~75%	~73%	~70%	68±7	prop hazards			
	DFS (HER2-)	Tx	436	not reached	~90%	~85%	~80%	~77%	71±2	Cox	NS	0.82 (0.61-1.09)	
		Cx	206	not reached	~85%	~77%	~72%	~70%	68±3	prop hazards			
Gusterson et al., 2003	Outcome	Grp	N	Med (mos)	2 yr	3 yr	4 yr	5 yr	6 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	85	not reported					46±6	Cox	NS	1.15 (0.62-1.54)	unadjusted univariate analyses; adjusted results also NS
		Cx	55	not reported					40±7	prop hazards			
746 node-pos pts randomized to prolonged (Tx) or periop (Cx) CMF	OS (HER2-)	Tx	406	not reached					71±2	Cox	.01	0.69 (0.52-0.92)	analyses gave similar results
		Cx	200	not reached					61±4	prop hazards			
	DFS(HER2+)	Tx	85	~36	~60%	~50%	~43%	~40%	38±5	Cox	NS	0.77 (0.51-1.16)	unadjusted univariate analyses; adjusted results also NS
		Cx	55	~24	~50%	~42%	~35%	~30%	29±6	prop hazards			
	DFS (HER2-)	Tx	406	>72	~80%	~70%	~63%	~57%	52±3	Cox	<.0001	0.57 (0.46-0.72)	analyses gave similar results
		Cx	200	~40	~63%	~55%	~45%	~40%	36±4	prop hazards			
Moliterni et al., 2003	Outcome	Grp	N	Med (mos)	2 yr	4 yr	6 yr	8 yr	10 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	45	>192	~92%	~83%	~73%	~68%	64%	Cox		0.61 (0.32-1.16)	HR=0.48, p=.052 for treatment x HER2 interaction term
		Cx	50	~170	~90%	~80%	~63%	~57%	54%	model			
RCT; CMF→A (Tx) vs CMF (Cx)	OS (HER2-)	Tx	203	>192	~97%	~90%	~86%	~83%	76%	Cox		1.26 (0.89-1.79)	
		Cx	208	>192	~97%	~94%	~90%	~83%	77%	model			
	RFS(HER2+)	Tx	45	>192	~85%	~75%	~62%	~58%	55%	Cox		0.83 (0.46-1.49)	HR=0.68, p not signif. for treatment x HER2 interaction term
		Cx	50	~102	~85%	~65%	~62%	~52%	46%	model			
	RFS (HER2-)	Tx	203	~162	~90%	~80%	~65%	~60%	56%	Cox		1.22 (0.91-1.64)	
		Cx	208	>192	~90%	~80%	~74%	~65%	59%	model			
Colozza et al., 2005	Outcome	Grp	N	Med (mos)		4 yr	6 yr	% at 8 yr±SD		Test	Comments:		
	OS (HER2+)	Tx	54	not reached		~89%	~80%	75.8±5.8		log	CMF HER2+ versus CMF HER2-, p=.024;		
		Cx	37	not reached		~77%	~70%	67.6±7.7		rank	all other comparisons not statistically significant		
RCT; epirubicin (Tx) vs. CMF (Cx); n=133 each group tested for HER2 status	OS (HER2-)	Tx	79	not reached		~90%	~87%	84.5±4.1		log	including epirubicin HER2+ versus epirubicin		
		Cx	96	not reached		~93%	~90%	87.4±3.4		rank	HER2-, p=0.24.		
	RFS(HER2+)	Tx	54					60.1±6.9		log	Interaction terms by Cox MVA:		
		Cx	37					68.6±7.2		rank	for OS: HR=1.61, CI: 0.64-4.01, p not signif.		
	RFS (HER2-)	Tx	79					65.9±5.4		log	for RFS: HR=1.02, CI: 0.40-2.58, p not signif.		
		Cx	96					70.3±4.7		rank			

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 13. Summary time to event outcomes, KQ3a (continued)

Study	Time to Event Outcomes												
Adjuvant chemotherapy for resected early breast cancer (continued)													
Pritchard et al., 2006 RCT; CEF (Tx) vs. CMF (Cx); HER2 status by FISH results	Outcome	Grp	N	Med (yrs)	2 yr	4 yr	6 yr	8 yr	10 yr	Test	p	HR (95%CI)	Comments
	OS (HER2$^+$)	Tx	75	not reached	~93%	~70%	~62%	~58%	~57%	log	.06	0.65 (0.42-1.02)	HR=2.04, CI: 1.14-3.65, p=.02 for treatment by
		Cx	88	~5.3	~92%	~62%	~47%	~46%	~45%	rank			
	OS (HER2$^-$)	Tx	237	not reached	~93%	~83%	~75%	~67%	~63%	log	NS	1.06 (0.83-1.44)	HER2 interaction in Cox MVA
		Cx	228	not reached	~93%	~80%	~75%	~67%	~62%	rank			
	RFS (HER2$^+$)	Tx	75	not reached	~77%	~67%	~58%	~57%	~56%	log	.003	0.52 (0.34-0.80)	HR=1.96, CI: 1.15-3.65, p=.01 for treatment by
		Cx	88	~2.5	~63%	~43%	~42%	~34%	~31%	rank			
	RFS (HER2$^-$)	Tx	237	~10	~81%	~67%	~60%	~54%	~50%	log	NS	0.91 (0.71-1.18)	HER2 interaction in Cox MVA
		Cx	228	~10	~81%	~64%	~58%	~54%	~50%	rank			
Knoop et al., 2005 RCT (n=805); CEF (Tx) vs. CMF (Cx)	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	120							Cox	.09	0.73 (0.50-1.05)	HRs and 95% CIs all adjusted by multivariate analysis for T size, nodal & menopausal status; stratified for grade, ER and TOP2A status
		Cx	143					proportional hazards					
	OS (HER2-)	Tx	249							Cox	.23	0.82 (0.59-1.13)	
		Cx	293					proportional hazards					
	RFS (HER2$^+$)	Tx	120							Cox	.10	0.75 (0.53-1.06)	
		Cx	143					proportional hazards					
	RFS (HER2$^-$)	Tx	249							Cox	.10	0.79 (0.60-1.05)	
		Cx	293					proportional hazards					
Dressler et al., 2005; Thor et al., 1998; separate survival curves show similar results for HER2 status by IHC, FISH, and PCR; only abstracted data for HER2 by IHC	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments:
	OS HER2$^+$ by IHC	high	44	>108		~97%		~97%	93% (86-100)				HR and p data are for interaction of CAF dose with HER 2 status in model for DFS
		mod	43	~87		~93%		~66%	58% (47-75)				
		low	40	~96		~90%		~66%	63% (49-80)				
	OS HER2$^-$ by IHC	high	134	~100		~93%		~80%	74% (67-81)				
		mod	124	>108		~96%		~86%	78% (80-92)				
		low	138	~100		~93%		~80%	74% (67-81)				
	DFS HER2$^+$ by IHC	high	44	>108		~97%		~90%	87% (74-96)	multi-variate	.0003	0.42 (0.19-0.93)	HER2 by IHC
		mod	43	~36		~60%		~47%	47% (34-64)				
		low	40	~66		~65%		~58%	53% (39-71)	propor-tional	.033	0.92 (0.81-1.04)	HER2 by FISH
	DFS HER2$^-$ by IHC	high	134	>108		~83%		~70%	64% (56-73)				
		mod	124	>108		~83%		~70%	65% (57-74)	hazards	.043	0.58 (0.25-1.35)	HER2 by PCR
		low	138	~90		~78%		~63%	59% (51-68)				
Del Mastro et al. 2004, 2005; Tx = FEC_{14} Cx = FEC_{21}	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	50	>72	~100%	~100%	~96%	~92%	89.9%	prop	.22	0.59 (0.26-1.37)	for all FEC_{14}, $HER2^+$ vs. $HER2^-$: EFS, HR=1.21 (0.65-2.24) p=.54; OS, HR=1.85 (0.88-3.89), p=.103
		Cx	53	>72	~98%	~89%	~85%	~81%	75.1%	hazards			
	OS (HER2-)	Tx	320	>84	~100%	~99%	~96%	~95%	91.9%	prop	.34	0.79 (0.49-1.28)	
		Cx	308	>84	~100%	~99%	~96%	~94%	90.7%	hazards			
	EFS ($HER2^+$)	Tx	50	>72	~100%	~98%	~85%	79%	77.7%	prop	.092	0.54 (0.27-1.11)	for all FEC_{21}, $HER2^+$ vs. $HER2^-$: EFS, HR=2.07 (1.27-3.38), p=.003; OS, HR=2.47 (1.34-4.57), p=.004
		Cx	53	>72	~91%	~82%	~68%	~67%	62.5%	hazards			
	EFS ($HER2^-$)	Tx	320	>84	~100%	~93%	~90%	~85%	81.5%	prop	.57	0.91 (0.65-1.27)	
		Cx	308	>84	~98%	~93%	~87%	~83%	80.9%	hazards			

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 13. Summary time to event outcomes, KQ3a (continued)

Study	Time to Event Outcomes												
Adjuvant chemotherapy for resected early breast cancer (continued)													
Tanner et al., 2006 FEC arm only	Outcome OS RFS 	Grp HER2+ HER2- HER2+ HER2-	N 56 124 56 124	Med (mos) ~54 >84 ~48 >84	2 yr ~79% ~94 ~68% ~84%	3 yr ~64% ~83% ~62% ~74%	4 yr ~58% ~74% ~50% ~67%	5 yr ~46% ~68% ~46% ~66%	6 yr ~41% ~64% ~46% ~65%	Test not reported not reported	p	HR (95%CI) HDC/AuSCS, not HER2+ vs. HER2- in same arm	Comments only reported statistical comparisons of FEC vs.
Hayes et al., 2007 AC→P (Tx) vs. AC alone (Cx) HER2 status based on CB11 IHC test results;	Outcome OS HER2+ OS HER2- DFS HER2+ DFS HER2- 	Grp Tx Cx Tx Cx Tx Cx Tx Cx	N	Med (mos) not reached ~60-96 not reached not reached not reached ~48-60 not reached ~120-132	3 yr ~87-92% ~70-75% ~87-92% ~85-87% ~80-87% ~53-60% ~83-87% ~80-85%	6 yr ~75-78% ~52-62% ~76-80% ~74-77% ~69-72% ~45-50% ~70-75% ~65-67%	9 yr ~70-78% ~47-49% ~68-70% ~63-66% ~62-67% ~45-48% ~65-69% ~55-60%	Test Cox multi-variate regression Cox multi-variate regression	p .01 .01	HR (95%CI) 0.57 0.59	Comments: total n=1322; HR & p for interaction of of HER2+ status and effect of adding paclitaxel HR & p, as for OS
Martin et al., 2005 Tx = DAC Cx = FAC	Outcome DFS HER2+ DFS HER2- DFS HER2 Unknown	Grp Tx Cx Tx Cx Tx Cx	N 155 164 475 468 115 114	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test Cox prop hzrds models	p	HR (95%CI) 0.60 (0.41-0.88) 0.76 (0.59-1.00) 0.72 (0.45-1.17)	Comments K-M DFS curves not shown separately by HER2 status; p-values not reported
Neoadjuvant (preoperative) chemotherapy for locally advanced breast cancer													
Learn et al., 2005	did not report time-to-event outcome												
Arriola et al., 2006	did not report time-to-event outcomes												
Park et al., 2003	did not report time-to-event outcomes												
Zhang et al., 2003; FAC, n=97 (n=78 also given post-op chemoTx)	Outcome DFS	Grp HER2+ HER2-	N 28 69	Med (mos) 48 (for all patients)	1 yr ~90% ~90%	2 yr ~83% ~80%	3 yr ~60% ~70%	4 yr ~45% ~60%	5 yr	Test not specified	p NS	HR (95%CI) not reported	Comments
Tulbah et al., 2002;	Outcome OS DFS HER2-	Grp HER2+ HER2- HER2+	N 22 32 21 31	Med (mos) not reached not reached 34.5±7.8 (all 52 pts)	1 yr ~95% ~97% ~88% ~92%	2 yr ~79% ~97% ~75% ~83%	3 yr ~66% ~72% ~75% ~52%	4 yr ~66% ~72% ~52%	5 yr	Test log rank log rank	p .31 .43	HR (95%CI)	Comments if HER2+ = IHC 2+/3+, OS favored HER2- 90% vs. 79% p=.051 if HER2+ = IHC 2+/3+ DFS still not statistically significant (p=.09)
Tinari et al., 2006;	did not report time-to-event outcome by HER2 status												

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Table 13. Summary time to event outcomes, KQ3a (continued)

Study	Time to Event Outcomes													
First- or second-line chemotherapy for advanced or metastatic breast cancer														
Harris et al., 2006	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	10yr	Test	p	HR (95%CI)	Comments
	OS	CB11+	30	11.3							Log rank	.14		
		CB11-	126	13.1										
		FISH+	37	10.9							Log rank	.26		
		FISH-	109	13.1										
	HercepTest	2+/3+	46	11.5							Log rank	.84		
	HercepTest	0/1+	105	13.2										
Di Leo et al., 2004	Outcome	Grp	N	Med (mos)	6 mos	1 yr	1.5 yr	2 yr	2.5 yr	Test	p	HR (95%CI)		Comments
	OS HER2+	1	15	10.8	~.85	~.3	No line	No line	No line	Cox regression	.33	1.47(0.68-3.15)		In full TAX 303 trial, no statistically significant differences between Tx arms with respect to OS or TTP
		2	21	14.4	~.95	~.6	~.46	No line	No line					
Grp 1: A	OS HER2-	1	63	16.9	~.8	~.72	~.5	~.3	No line	Cox regression	.07	0.64(0.40-1.03)		
Grp 2: T		2	50	12.6	~.8	~.6	~.32	~.28	0					
					3 mos	6 mos	9 mos	12 mos	15 mos	18 mos				
	TTP HER2+	1	15	4.7	~.75	~.4	~.25	~.15	~.15	0	Cox regression	.73	0.88(0.43-1.82)	
		2	21	7.0	~.75	~.6	~.15	~.1	~0	No line				
	TTP HER2-	1	63	5.9	~.74	~.5	~.35	~.25	~.15	<.1	Cox regression	.22	0.77(0.52-1.16)	
		2	50	5.0	~.74	~.45	~.2	~.1	<.1	No line				
	PFS	Tx												
		Cx												
Konecny et al., 2004	Outcome	Grp	N	Med (mos) (95%CI)		1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	1												
	HER2+		49	16.4(12.1-20.1)		~.65	~.3	~.25	~.25		log rank	.010		
Grp 1: EC	HER2-		88	33.1(20.9-50.6)		~.78	~.57	~.45	~.4					
Grp 2: ET		2												
	HER2+		48	21.4(15.3-27.3)		~.74	~.45	~.25	~.1		log rank	.463		
	HER2-		90	27.5(17.1-35.2)		~.7	~.55	~.35	~.2					
	HER2+	1	49	16.4(12.1-20.1)		~.6	~.3	~.25	~.25		log rank	.319		
		2	48	21.4(15.3-27.3)		~.7	~.4	~.25	~.1					
	HER2-	1	88	33.1(20.9-50.6)		~.78	~.58	~.43	~.4		log rank	.292		
		2	90	27.5(17.1-35.2)		~.7	~.55	~.35	~.15					
	PFS	1												
	HER2+		49	7.1(4.1-9.3)		~.2	~.08	~.08			log rank	.010		
	HER2-		88	10.4(6.9-14.9)		~.54	~.22	~.12						
		2												
	HER2+		48	10.5(8.1-11.9)		~.35	~.1	~.05			log rank	.584		
	HER2-		90	9.6(7.5-11.3)		~.35	~.15	~.08						
	HER2+	1	49	7.1(4.1-9.3)		~.2	~.08	~.08			log rank	.116		
		2	48	10.5(8.1-11.9)		~.35	~.1	~.05						
	HER2-	1	88	10.4(6.9-14.9)		~.47	~.25	~.1			log rank	.350		
		2	90	9.6(7.5-11.3)		~.52	~.13	~.08						

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen

Table 14. Summary tumor response, KQ3a

Study	Tumor Response (%)
Adjuvant chemotherapy for resected early breast cancer	
Yang et al., 2003	not reported
Gusterson et al., 2003	not reported
Moliterni et al., 2003	not reported
Colozza et al., 2005	not reported
Pritchard et al., 2006	not reported
Knoop et al., 2005	not reported
Dressler et al., 2005	not reported
Del Mastro et al 2004, 2005	not reported
Tanner et al., 2006	not reported
Hayes et al., 2007	not reported
Martin et al., 2005	not reported
Neoadjuvant (preoperative) chemotherapy for locally advanced breast cancer	
Learn et al., 2005; n=104 classified for HER2 status	Grp N pCR ORR (cCR+cPR) Test p Comments: for ORR data, by multi-variate analysis: $HER2^+$, AC 32 22% 75% logistic NS AC, $HER2^+$ vs. $HER2^-$, p=0.06; $HER2^+$, AC+D 9 22% 78% $HER2^-$, AC 37 24% 51% <.05 HER^-, AC+D 26 24% 81% AC+D, $HER2^+$ vs. $HER2^-$, p=0.99
Arriola et al., 2006	Grp N pCR PR SD PD NE (regression) Test p Comments all 229 27 Whitney .03 "association of $HER2^+$ with pCR"
Park et al., 2003	Grp N pCR PR OR (CR+PR) NR (PD+NE) Test p Comments $HER2^+$ 31 5 (16%) 22 (71%) 27 (87%) 4 (13%) Fisher's exact .013 $HER2^-$ 36 0 17 (47%) 17 (47%) 19 (53%)
Zhang et al., 2003	Grp N cCR+cPR cNR p RR 95%CI pCR+MRD ERD p RR 95%CI tests $HER2^+$ 28 93% 7% 0.14 1.2 1.1-1.4 18% 82% .53 1.4 0.54-3.67 Fisher's exact & asymptotic $HER2^-$ 69 78% 22% 13% 87%
Tulbah et al., 2002	Grp N pCR PR SD PD NE Test p Comments $HER2^+$ 21 6 (29%) Mann-Whitney NS also NS if IHC 2+ and 3+ considered HER2+ $HER2^-$ 31 7 (23%)
Tinari et al., 2006	Grp N TR OR SD PD NE Test p OR Comments all 77 23.4% 72.7% 3.9% $HER2^+$ 20 univariate logistic regression .008 5.28 (1.57-19.6) $HER2^-$ 57

Abbreviations: ERD: extensive residual disease; MRD: minimal residual disease; NE: not evaluable; NS: not significant; OR: overall response (cPR + minimal residual disease + PR); ORR: overall response rate; PD: progressive disease; RR: relative risk; SD: stable disease; TR: tumor response (cPR + minimal residual disease);

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen

Table 14. Summary tumor response, KQ3a (continued)

Study	Tumor Response (%)							
First- or second-line chemotherapy for advanced or metastatic breast cancer								
Harris et al., 2006			N	CR+PR (%)	p	Comments:		
	HER2 by CB11				0.96	did not report logistic regression analysis		
	Pos		30	23				
	Neg	126	24					
	HER2 FISH							
	Pos		37	22				
	Neg	109	25					
	HercepTest							
	Pos (2-3)		46	35				
	Neg (0-1)		105	18				
	HercepTest							
	Pos (3)		30	23				
	Neg (0-2)		121	23				
Di Leo et al., 2004	Grp1	N	%(CR+PR)		A versus T OR (95%CI)	p	Comments:	
	HER2+	15	27		HER2+ 5.50(1.28-23.69)	.04	By MV logistic regression,	
Grp 1: A	HER2-	63	35	0.026	HER2-1.24(0.58-2.68)	.70	treatment x HER2 status	
Grp 2: T	HER2 unk	13	31		HER2 unk 1.25(0.25-6.24)	1.00	OR=3.64, CI: 1.39-9.54	
	All	91	33		All 1.72(0.94-3.18)	.09	p=0.01; remains SS after	
			0.98				adjusting for visceral &	
							Tx x visceral interaction	
	HER2+	21	67		(In full TAX 303 trial, response rates were 48% with			
	HER2-	50	40		docetaxel (n=161), 33% with doxorubicin (n=165),			
	HER2 unk	14	36		p=0.008)			
	All	85	46					
Konecny et al., 2004	Grp	N	CR+PR(95%CI)	SD	PD	NE	Test	p	Comments
	HER2+	97	60(51-70)			chi sq	.004		by MV logistic regression, adjusted
Grp1: EC	HER2-	178	41(34-49)					Tx*HER2 interaction: p=0.256	
Grp 2: ET	Grp 1								
	HER2+	49	45(32-60)				chi sq	.130	
	HER2-	88	33(22-43)						
	Grp 2								
	HER2+	48	76(63-88)				chi sq	.005	
	HER2-	90	50(39-61)						
	HER2+								
	Grp1	49	45(32-60)				chi sq	.004	by MV logistic regression, OR=3.64
	Grp2	48	76(63-88)						CI: 1.48-8.92, p=0.005
	HER2-								
	Grp1	88	33(22-43)				chi sq	.002	by MV logistic regression, OR=1.92
	Grp2	90	50(39-61)						CI: 1.01-3.64, p=0.046

The other trials reported no statistically significant differences for any subgroups they compared. Moliterni, Menard, Valagussa, et al. (2003) compared CMF alone versus CMF followed by doxorubicin (CMF→A) in HER2-positive (n=50, CMF; n=45, CMF→A) and HER2-negative (n=208, CMF; n=203, CMF→A) subgroups. Confidence intervals spanned 1.00 and HRs were not statistically significant for either outcome (OS, RFS) in either subgroup. With Cox MVA, treatment by HER2 interaction terms were:

- OS: HR=0.48, p=.052
- RFS: HR=0.68, p, NS

Colozza, Sidoni, Mosconi, et al. (2005) compared CMF versus epirubicin alone (E), in HER2-positive (n=37, CMF; n=54, E) and HER2-negative (n=96, CMF; n=79, E) subgroups. Log rank analyses of Kaplan-Meier survival curves showed a statistically significant difference in OS at 8 years after CMF favoring HER2-negative over HER2-positive patients: (87.4 +/- 3.4) percent versus (67.6 +/- 7.7) percent, p=.024. All other subgroup comparisons were not statistically significant, and Cox MVA interaction terms for treatment effect by HER2 status also were not statistically significant.

Knoop, Knudsen, Balslev, et al. (2005) compared CMF versus CEF in HER2-positive (n=143, CMF; n=120, CEF) and HER2-negative (n=293, CMF; n=249, CEF) subgroups. For both OS and RFS, hazard ratios from Cox multivariate analyses (stratified by tumor grade, estrogen receptor and TOP2A status; and adjusted for tumor size, nodal and menopausal status) uniformly spanned 1.00 and were not statistically significant for either HER2-positive or HER2-negative subgroups.

The Tanner, Isola, Wiklund, et al. (2006) study showed separate Kaplan-Meier curves for HER2-positive (n=56) and HER2-negative (n=124) subgroups from the tailored FEC arm for both OS and RFS. However, they did not report statistical significance of differences between these HER2 status subgroups (although they reported statistical significance of differences between HER2 status subgroups treated by HDC/AuSCS versus subgroups treated with tailored FEC).

Studies on dose or dose intensity of anthracycline-based regimens. In one of two included studies, multivariate proportional hazards analysis showed statistically significant interaction of anthracycline-based regimen dose or dose-intensity with HER2 status to predict outcome. Dressler, Berry, Broadwater, et al. (2005) compared DFS after high-, moderate-, or low-dose CAF regimens in HER2-positive and HER2-negative subgroups. They reported separate MVAs using FISH, IHC, or PCR to classify patients' HER2 status. Results for DFS at five years comparing high-dose versus low-dose plus moderate-dose CAF subgroups were:

- HER2/FISH (n=91, $HER2^+$; n=433, $HER2^-$): HR=0.822 (95 percent CI: 0.553–1.220)
- HER2/IHC (n=127, $HER2^+$; n=396, $HER2^-$): HR=0.834 (95 percent CI: 0.590–1.181)
- HER2/PCR (n=91, $HER2^+$; n=400, $HER2^-$): HR=0.732 (95 percent CI: 0.507–1.056)
- HER2/FISH, interaction CAF dose by HER2: HR=0.919 (95 percent CI: 0.814-1.038); p=.033
- HER2/IHC, interaction CAF dose by HER2: HR=0.418 (95 percent CI: 0.188-0.930); p=.0003
- HER2/PCR, interaction CAF dose by HER2: HR=0.585 (95 percent CI: 0.253-1.352); p=.043

Investigators stated (but did not report HRs, CIs, or p values) that MVA yielded similar results for statistically significant interaction of CAF dose with HER2 status to predict OS.

Del Mastro, Bruzzi, Nicolo, et al. (2005) compared outcomes after identical doses of FEC administered every 14 days (FEC14) or every 21 days (FEC21). Multivariate proportional hazards analysis showed that interaction terms for HER2 status by randomly assigned treatment (dose intensity or treatment frequency) were not statistically significant for EFS (HR=0.53; p=.12) or OS (HR=0.646; p= .379). HER2 status (HER2-positive, n=103; HER2-negative, n=628) was statistically significant to predict EFS (HR=2.04, p=.005) and OS (HR=2.41, p=.006), while randomly assigned treatment (FEC14, n=370; FEC21, n=361) was not statistically significant to predict either outcome (EFS, HR=0.85, p=.335; OS, HR=0.72, p=.379).

Studies on regimens with versus without a taxane. One of two included studies reported statistically significant interaction of HER2 status with added paclitaxel to predict treatment outcome. Hayes, Thor, Dressler, et al. (2007) compared outcomes with versus without paclitaxel (following AC) in HER2-negative and HER2-positive subgroups, separately for each of two groups they randomly selected for HER2 testing. For each group, OS and DFS for HER2-positive patients given paclitaxel were superior to the same outcomes in HER2-positive patients not given paclitaxel. In contrast, OS and DFS for HER2-negative patients given paclitaxel appeared similar to the same outcomes for HER2-negative patients not given paclitaxel. They used Cox multivariate analyses, separately in each randomly selected group, and in the two groups combined, to test the statistical significance of an interaction term for HER2 positivity and paclitaxel treatment. Results for Group 2 and for Groups 1 and 2 pooled showed a statistically significant interaction favoring paclitaxel treatment in HER2-positive patients:

- Group 1, n=643: recurrence, HR=0.63, p=.15; death, HR=0.61, p=.17
- Group 2, n=679: recurrence, HR=0.52, p=.03; death, HR=0.52, p=.03
- Groups 1+2, n=1,322: recurrence, HR=0.59, p=.01; death, HR=0.57, p=.01

Hayes, Thor, Dressler, et al. (2007) also investigated whether patients' estrogen-receptor status modified the impact of HER2 status on outcomes of paclitaxel. The researchers reported results of an exploratory analysis suggesting that, among HER2-positive patients, paclitaxel improved DFS whether patients were estrogen-receptor negative or positive. However, among HER2-negative patients, paclitaxel apparently improved DFS for ER-negative patients but not for ER-positive patients. HER2-negative, ER-positive patients comprised more than 50 percent of the patients in this study. However, the authors caution that additional prospective studies are needed to validate this finding before clinical practice changes and HER2-negative, ER-positive patients are no longer offered taxanes.

Martin, Pienkowski, Mackey, et al. (2005) compared DFS in patients randomized to AC plus docetaxel (TAC, n=745; HER2 positive, 155; HER2 negative, 475; HER2 unknown, 115) versus AC plus fluorouracil (FAC, n=746; HER2 positive, 164; HER2 negative, 468; HER2 unknown, 114). Subgroup analyses using a Cox proportional hazards model adjusted for age, tumor size and other prognostic factors showed superior outcomes with TAC compared to FAC for all subgroups, including by known HER2 status. A test for interaction of HER2 status with treatment effect, using the ratio of hazard ratios, was not statistically significant (ratio of HRs=0.85; p=.41).

Six studies on preoperative neoadjuvant chemotherapy. The primary outcome of interest for studies on neoadjuvant (preoperative) therapy is pathologic complete (pCR) and partial (PR) response rates, although clinical responses (cCR, cPR) also are considered. One randomized, controlled trial compared responses after neoadjuvant chemotherapy regimens (AC) with versus without added docetaxel (AC+D) (Learn, Yeh, McNutt, et al., 2005). Rates of cPR were similar with each regimen for HER2-positive (22 percent of each subgroup; AC, n=32; AC+D, n=9) and HER2-negative (24 percent of each subgroup; AC, n=37; AC+D, n=26) patients. Multivariate logistic regression analysis of overall clinical responses (ORR = cCR+cPR) showed a statistically significant increase with added docetaxel in HER2-negative patients (AC, ORR=51 percent; AC+D, ORR=81 percent; p<.05) but not in HER2-positive patients (AC, ORR=75 percent; AC+D, ORR=78 percent; p, NS). However, investigators did not report inclusion of an interaction term in their analysis.

Although two (Zhang, Yang, Smith, et al., 2003; Tulbah, Ibrahim, Ezzat, et al., 2002) of five uncontrolled series did report OS and/or DFS outcomes, these may have been influenced by postsurgical treatments that were not identical for all patients. Three of five series reported statistically significantly higher likelihood of response in the HER2-positive subgroups. Arriola, Moreno, Varela, et al. (2006) evaluated clinical and pathologic responses after preoperative treatment with doxorubicin alone. Although they did not report response rates for the HER2-positive (n=43) and HER2-negative (n=180) subgroups, a Mann-Whitney U test showed p=.03 for association of HER2 positivity with pCR. Park, Kim, Lim, et al. (2003) also investigated preoperative therapy with doxorubicin alone. They reported statistically significantly higher pCR (16 percent versus 0) and PR (71 percent versus 47 percent) in the HER2-positive (n=31) than the HER2-negative (n=36) subgroups, p=.013 by Fisher's exact test.

The study reported by Tinari, Lattanzio, Natoli, et al. (2006)compared marker assay results in paired core biopsy specimens (pre-chemotherapy) and resected tumors (post-chemotherapy), and focused primarily on changes induced by anthracycline-based neoadjuvant chemotherapy in HER2 and topoisomerase IIα (TopIIα) expression. However, they also used multivariate logistic regression analysis to compare pathologic tumor responses (TR, defined as either a pCR or minimal residual disease) in HER2 subgroups by core biopsy assays. Tinari and colleagues (2006) reported a 5.28-fold increase (95 percent CI: 1.57-19.6; p=.008) in the likelihood of achieving TR in HER2-positive than in HER2-negative patients.

Zhang, Yang, Smith, et al. (2003) investigated preoperative FAC in HER2-positive (n=28) and HER2-negative (n=69) patients. While overall clinical response rate was higher for the HER2-positive than the HER2-negative subgroup (CR+PR: 93 percent versus 78 percent), the risk ratio for response was not statistically significant (RR=1.2, 95 percent CI: 1.1–1.4, p=.14, Fisher's exact test). Overall pathologic response rates (pCR plus minimal residual disease, MRD) showed an even smaller difference between HER2-positive and HER2-negative subgroups that also was not statistically significant (18 percent versus 13 percent, RR=1.4, 95 percent CI: 0.54–3.67, p=.53, Fisher's exact test). Tulbah, Ibrahim, Ezzat, et al. (2002) investigated preoperative paclitaxel plus cisplatin in HER2-positive (n=21) and HER2-negative (n=31) subgroups. Pathologic complete response rates did not differ significantly between the groups (29 percent versus 23 percent; p=NS).

Three studies on chemotherapy for advanced or metastatic breast cancer. One of three studies did not compare different regimens and pooled data across arms randomized to different paclitaxel doses (Harris, Broadwater, Lin, et al., 2006); one compared monotherapy with doxorubicin (A) versus monotherapy with docetaxel (T) (Di Leo, Chan, Paesmans. et al., 2004);

and one compared epirubicin plus cyclophosphamide (EC) versus epirubicin plus paclitaxel (ET) (Konecny, Thomssen, Luck, et al., 2004).

Harris, Broadwater, Lin, et al. (2006) used log rank analysis to compare Kaplan-Meier curves for OS between HER2-positive and HER2-negative patients, separately for test results by three different HER2 assays: CB11 IHC, the HercepTest™ IHC, and FISH. Differences between the curves were not statistically significant for any comparison. They also compared overall response rates (ORR=CR+PR) for subgroups defined by each HER2 assay. Results were statistically significant (HER2-positive, n=46, ORR=35 percent; HER2-negative, n=105, ORR=18 percent; p=.026) only with the HercepTest™ assay, and only when both 2+ and 3+ scores were considered HER2 positive.

Di Leo, Chan, Paesmans, et al. (2004) compared OS and time to progression (TTP) in patients randomized to A or T in HER2-positive (A, n=15; T, n=21) and HER2-negative (A, n=63; T, n=50) subgroups. There were no statistically significant differences between treatment arms for either outcome in either HER2 status subgroup. In contrast, ORR statistically significantly favored T over A in the HER2-positive subgroup (T, n=21, ORR=67 percent versus A, n=15, ORR=27 percent; OR=5.50, 95 percent CI: 1.28–23.69; p=.04). However, the difference was not statistically significantly different for the HER2-negative subgroup (T, n=50, ORR=40 percent versus A, n=63, ORR=35 percent; OR=1.24, 95 percent CI: 0.58–2.68; p=.70).

Konecny, Thomssen, Luck, et al. (2004) compared HER2-positive (EC, n=49; ET, n=48) and HER2-negative (EC, n=88; ET, n=90) subgroups randomized to EC or ET for OS and PFS. With the EC regimen, OS (median, 33.1 versus 16.4 months, log rank p=.01) and PFS (median, 10.4 versus 7.1 months, log rank p=.01) were significantly greater among HER2-positive than among HER2-negative patients. In each other comparison (OS or PFS; for the ET regimen by HER2 status, or for EC versus ET separately in subgroups by HER2 status) the difference was not statistically significant. Univariate chi square tests suggested each ORR difference was statistically significant (between all HER2-positive versus all HER2-negative patients, and separately by treatment arm and HER2 status subgroups; excluding those randomized to EC by HER2 subgroups). However, the interaction of treatment effect with HER2 status was not statistically significant (p=.256) by multivariate logistic regression.

Conclusions and Discussion, Key Question 3a

Across all three treatment settings (adjuvant, neoadjuvant, advanced/metastatic), currently available evidence comparing chemotherapy outcomes in HER2-positive and HER2-negative patient subgroups may be used to generate hypotheses, but is too weak to test hypotheses. Only one study (on adjuvant therapy; Martin, Pienkowski, Mackey, et al., 2005) is from a randomized, controlled trial that prespecified a multivariate subgroup analysis by HER2 status. Investigators reported the interaction of assigned treatment (with versus without paclitaxel) with HER2 status to predict outcome was not statistically significant (ratio of HRs=0.85; p=.41).

All other evidence is from post-hoc analyses on subgroups not directly randomized, selected, or stratified by HER2 status. All other reports from randomized, controlled trials were secondary or correlative analysis on patient subgroups with archived tissue samples available for HER2 testing. Many compared baseline characteristics and prognostic factors of patients with known versus unknown HER2 status, sometimes separately by treatment arm, but more often pooled across treatment arms. However, since few directly compared baseline characteristics and prognostic factors for HER2-positive and HER2-negative subgroups separately from each arm, it is uncertain whether these subgroups were well balanced. A minority of studies reported

multivariate analyses that tested the statistical significance of interactions between treatment effects of different regimens and HER2 status.

Evidence on adjuvant CMF chemotherapy. Evidence from two studies (one randomized, controlled trial and one series) suggests HER2-positive patients may derive quantitatively smaller benefit from CMF (smaller improvements in OS and DFS) than experienced by HER2-negative patients. However, such evidence cannot prove that CMF provides no benefit to HER2-positive patients.

Evidence on adjuvant anthracycline therapy. An analysis from one of four randomized, controlled trials reports a statistically significant interaction between use of a regimen that includes an anthracycline and HER2 status as outcome predictors. Data from this study suggest HER2-positive patients (but not HER2-negative patients) experience a statistically significant improvement in outcome from inclusion of an anthracycline in their treatment regimen. Again, this does not prove that HER2-negative patients do not benefit from anthracycline therapy. Given the highly statistically significant result favoring anthracycline therapy for the large population of breast cancer patients included in the Early Breast Cancer Trialists' Collaborative Group (EBCTCG 2005) overview analysis, a more complete test of this hypothesis is needed before one can conclude that omitting anthracyclines from adjuvant chemotherapy regimens does not worsen outcome in HER2-negative patients. The absence of a statistically significant interaction in three other randomized, controlled trials is not informative, given the differences in specific treatment regimens, populations studied, and small numbers in the HER2-positive subgroups.

Two trials compared different doses or dose intensities (frequencies) of anthracycline-based regimens. One (Dressler, Berry, Broadwater, et al., 2005) reported a statistically significant interaction of CAF dose with HER2 status to predict treatment outcome, whether HER2 status was based on FISH, IHC, or PCR assays. Data from this study suggested the highest of three CAF doses (now considered by many oncologists the standard dose for all patients) improved outcomes for HER2-positive patients, but suggested no benefit from the highest dose for HER2-negative patients. In contrast, the interaction of dose intensity (frequency) with HER2 status to predict treatment outcome was not statistically significant in a second randomized, controlled trial (Del Mastro, Bruzzi, Nicolo, et al., 2005). Available data are too weak to conclude that HER2-positive patients clearly experience better outcomes with the higher-dose or dose-intensity anthracycline-based regimens.

Evidence on adding paclitaxel to adjuvant AC chemotherapy. A correlative analysis from one randomized, controlled trial (Hayes, Thor, Dressler, et al., 2007) provides evidence that adding paclitaxel after AC improves OS and DFS for HER2-positive patients, but may not improve these outcomes for HER2-negative patients. Here again, these strongly suggestive data are too weak by themselves to conclude that use of paclitaxel in adjuvant regimens is not beneficial in HER2-negative patients. Additionally, the only trial with a prespecified multivariate subgroup analysis (Martin, Pienkowski, Mackey, et al., 2005) reported that the interaction of concurrently added paclitaxel with HER2 status was not statistically significant.

The potential interaction between HER2 status, estrogen receptor status, and progesterone receptor status as predictors of chemotherapy efficacy is receiving increasing attention. The Hayes, Thor, Dressler, et al. (2007) article is the only included study on chemotherapy for breast cancer that addresses this issue, although the analysis only includes HER2 status and ER status. In an exploratory analysis, the authors found that adding paclitaxel improved survival for all HER2-positive patients and for HER2-negative/ER-negative patients, but not for HER2-negative/ER-positive patients. As discussed in the Conclusions and Discussion for Chapter 2,

many researchers are investigating breast cancer subtypes identified by different combinations of ER, PR, and HER2, including the so-called "triple-negative" subtype (i.e., negative for HER2, estrogen receptor, and progesterone receptor), and the luminal subtypes (luminal A or luminal B) that are negative for HER2 but positive for at least one of the hormone receptors. There is evidence that the triple negative and luminal subsets differ with respect to prognosis, chemotherapy response, and outcomes (Carey, Dees, Sawyer et al., 2007; Liedtke, Mazouni, Hess et al., 2008), and they clearly differ with respect to effects of endocrine therapy. New phase III trials for patients with triple negative or "basal-like" breast cancer (Kilburn, 2008) should provide more insight in the future.

Systematic reviews on adjuvant chemotherapy. Recent systematic reviews and meta-analyses on HER2 status to predict chemotherapy outcomes were reported by Gennari and colleagues (Gennari, Sormani, Pronzato, et al., 2008) and by Pritchard and colleagues (Pritchard, Messersmith, Elavathil, et al., 2008; Dhesy-Thind, Pritchard, Messersmith, et al., 2008). Gennari and co-workers (2008) pooled data from eight randomized trials that compared adjuvant regimens with versus without an anthracycline (four of which did not meet selection criteria for this review). Two (NSABP B11, Paik, Bryant, Park, et al., 1998; NSABP B15, Paik, Bryant, Tan-Chiu, et al., 2000) considered patients HER2-positive if membranes of any tumor cells showed antibody staining by IHC, a threshold for HER2 positivity inconsistent with the ASCO/CAP and NCCN guidelines. Substantial numbers of patients from these early (but otherwise well done) randomized, controlled trials may have been classified as HER2 positive who would now be classified as HER2 negative using the currently recommended thresholds. Thus, pooling data from these analyses with later analyses that used current IHC scoring criteria to classify patients may potentially bias the outcome comparisons. We excluded a third study included by Gennari and colleagues (2008) since it was only published as an abstract, without slides available on the web (De Laurentiis, Caputo, Massarelli, et al., 2001). We excluded a fourth study they included (Di Leo, Gancberg, Larsimont, et al., 2002), since patients were not treated identically within each arm and patients with unknown hormone receptor status were given tamoxifen. We replicated the results of the Gennari, Sormani, Pronzato, et al., (2008) meta-analysis including the same studies the authors did and reached the same results. Then we redid the analysis including only the studies meeting criteria for the current review, which meant excluding the four studies mentioned above. Removing these studies widened the confidence intervals, but did not alter the overall conclusions.

The systematic reviews and meta-analyses reported by Pritchard and colleagues (Pritchard, Messersmith, Elavathil, et al., 2008; Dhesy-Thind, Pritchard, Messersmith, et al., 2008) also included randomized, controlled trials that did not meet selection criteria for this review. In addition to the four discussed above, we excluded three trials on anthracycline-based regimens that were reported only as meeting abstracts but without slides, audio or video available on the web to provide full access to presented data (Petruzelka, Pribylova, Vedralova, et al., 2000; Vera, Albanell, Lirola, et al., 1999; Arnould, Fargeot, Bonneterre, et al., 2003; Bonneterre, Roche, Kerbrat, et al., 2003). We also excluded one fully published study in which patients were not treated identically within each arm (Di Leo, Larsimont, Gancberg, et al., 2001) and a second fully published study on high-dose chemotherapy with autologous stem-cell transplant that did not report data by HER2 status separately for the conventional-dose arm (Rodenhuis, Bontenbal, van Hoesel, et al., 2006).

The Gennari and co-workers (2008) meta-analysis reports statistically significant improvement in DFS (six trials included) and OS (seven trials included) of HER2-positive

patients given an anthracycline compared to the same outcomes for HER2-positive patients not given an anthracycline (HR for relapse=0.71, 95 percent CI: 0.61–0.83; p<.001; HR for death =0.73, 95 percent CI: 0.62–0.85; p<.001). In contrast, including an anthracycline apparently did not statistically significantly improve DFS or OS for patients with HER2-negative disease (HR for relapse=1.00, 95 percent CI: 0.90–1.11; p=.75; HR for death=1.03, 95 percent CI: 0.92–1.16; p=.60). The meta-analysis reported by Pritchard and co-workers (2008) included the same six trials for DFS and the same seven trials for OS, and reported identical pooled results (hazard ratios, confidence intervals) as those reported by Gennari and co-workers (2007). These analyses support the need for more definitive tests of the hypothesis that the balance of potential benefit versus harm of anthracyclines in HER2-negative patients may not justify their use. Furthermore, as discussed in Key Question 2 and in this section, future analyses and new studies should probably subdivide the HER2 negative group, and analyze subsets who are triple-negative (or "basal-like") separately from those who are positive for one or both hormone receptors (luminal A or B).

Pritchard, Messersmith, Elavathil, et al. (2008) also reported a meta-analysis on DFS that included three randomized, controlled trials comparing higher-dose or intensity versus lower-dose or intensity anthracycline regimens: two are included here (Dressler, Berry, Broadwater, et al., 2005; Del Mastro, Bruzzi, Nicolo, et al., 2005), and one we excluded (Di Leo, Larsimont, Gancberg, et al., 2001). They found significant improvement of DFS at higher doses for HER2-positive patients (HR=0.54; 95 percent CI: 0.38-0.79) but not for HER2-negative patients (HR=0.98; 95 percent CI: 0.78-1.22). However, a test for the interaction of anthracycline regimen dose or dose intensity with HER2 status to predict DFS was not statistically significant. Thus, present evidence is too weak to support conclusions about HER2 status as a sole predictor of differences in outcome between higher- and lower-dose anthracycline-based regimens. Longer-term data on potential toxicities (particularly decreased ejection fraction and congestive heart failure) of the higher doses are also needed.

Pritchard, Messersmith, Elavathil, et al. (2008) reported on a final meta-analysis that pooled results on DFS from two randomized, controlled trials on adjuvant therapy (Hayes, Thor, Dressler, et al., 2007; Martin, Pienkowski, Mackey, et al., 2005) and one on neoadjuvant therapy (Learn, Yeh, McNutt, et al., 2005) that compared taxane-containing versus non-taxane-containing regimens. While all three trials were included in this systematic review, the validity of pooling them for meta-analysis seems uncertain. Postsurgical therapy in the Learn, Yeh, McNutt, et al. (2005) trial may have affected DFS and may not have been uniform in all three arms. The meta-analytic results suggest the magnitude of benefit from including a taxane in the regimen may be greater for HER2-positive patients (HR=0.60; 95 percent CI: 0.46–0.78) than for HER2-negative patients (HR=0.83; 95 percent CI: 0.71–0.98). However, these results also show statistically significant evidence of benefit for each group from including a taxane in the regimen. Thus, the evidence is presently too weak to support conclusions on HER2 status as a sole predictor of whether or not any subgroup of breast cancer patients benefits from paclitaxel therapy.

These meta-analyses were thorough and used appropriate methodologies. The difference in the trials included in the meta-analyses versus the current systematic review is due to varying prespecified inclusion and exclusion criteria, which are a matter of opinion. The main concern regarding the meta-analyses is their relevance to current practice. The current ASCO/CAP guidelines recommend a different approach to measuring HER2 status than used in the trials incorporated into the meta-analyses, which is why we chose not to perform a formal meta-

analysis. Whether and how the change in measurement of HER2 status alters the results of the trials and meta-analyses is unknown since necessary data are unavailable.

Evidence on neoadjuvant chemotherapy. Available evidence on whether HER2 status affects rates of complete pathologic response (pCR) to neoadjuvant chemotherapy is limited to four uncontrolled series (retrospective analysis in three). Although two of four reported statistically significantly higher pCR rates in HER2-positive than HER2-negative patients, these data are too weak to conclude that the regimens tested are of no benefit to HER2-negative patients. Furthermore, data are lacking to directly compare any neoadjuvant regimens. Since a number of trials have already compared different neoadjuvant therapies, correlative studies using archived tissue samples may be useful. However, it is also possible that conclusions on relative benefits of different regimens from studies in the adjuvant setting may generalize to the neoadjuvant setting.

Evidence on chemotherapy for advanced disease. Evidence also is limited on differences by HER2 status for outcomes of chemotherapy for advanced or metastatic disease. Three randomized, controlled trials investigated different treatments: one studied paclitaxel alone (at different doses), one studied an anthracycline alone versus a taxane alone, and one studied an anthracycline plus cyclophosphamide versus an anthracycline plus a taxane. Small patient groups limited statistical power.

In summary, although present evidence is suggestive, it is too weak to determine in either the adjuvant, neoadjuvant, or metastatic disease settings, whether a more favorable balance of benefit versus risk from chemotherapy can be achieved by selecting patients for anthracycline- or taxane-based regimens based on HER2 status.

Research needs. Future trials that compare adjuvant chemotherapy regimens with versus without an anthracycline, or with versus without a taxane, could determine HER2 status at the time of diagnosis, and stratify randomization by HER2 assay results. This approach might provide more definitive tests for the hypotheses that neither an anthracycline nor a taxane improves outcomes of HER2-negative patients. Another possibility is for the EBCTCG to collect individual patient data on HER2 status using current scoring thresholds from all trials that compared adjuvant regimens with versus without an anthracycline, or with versus without a taxane. If sufficient tumor samples are available, this might be a more efficient and more definitive approach for testing hypotheses on the interaction of HER2 status with assigned treatment to predict outcome. Future analyses should also obtain more complete information on estrogen and progesterone receptor status of all patients. This would enable investigators to further subdivide the HER2-negative subset, so that triple-negatives (or those with “basal-like” breast cancer if gene array data were obtained) can be analyzed separately from the luminal A and B subtypes.

Key Question 3b

For breast cancer patients, what is the evidence on clinical benefits and harms of using HER2 assay results to guide selection of hormonal therapy?

Study Selection

Of the 219 articles retrieved for Question 3, 66 were assessed for potential relevance to Question 3b. Only six articles met the selection criteria. The primary reasons for article exclusion are as follows: not reporting outcomes identified in selection criteria; not reporting outcomes by HER2 status, nonidentical treatment of patients, measurement of HER2 status inconsistent with current specialty society recommendations; lack of primary data; or inclusion of only HER2-positive patients, only HER2-negative patients, or fewer than 20 HER2-positive cases.

Two of the studies that did not meet the selection criteria were by Berry, Muss, Thor, et al. (2000) and by Ellis, Coop, and Singh, et al. (2001). The first uses data from the CALBG 8541 trial, and data from this trial are included in the previous section on chemotherapy for breast cancer. It is excluded here because while the chemotherapy regimens were randomized across patients, the use of tamoxifen was not. Rather, tamoxifen was prescribed based on clinician preferences. Its use increased over time after recommendations for its use in ER-positive, postmenopausal women were released during the course of the trial and as the percentage of postmenopausal women recruited also rose. Although the study by Ellis, Coop, and Singh, et al. (2001) on the neoadjuvant use of letrozole versus tamoxifen reportedly affected clinical practice, it is excluded from this systematic review for two reasons: It reported on clinical response (breast palpation) rather than the more definitive pathological response, and it used a broader definition of HER2 positivity (IHC scores of 2+ and 3+ were designated as positive, without any further evaluation of IHC 2+ scores using FISH).

Four of the six studies that met selection criteria investigated outcomes of tamoxifen; while two others compared an aromatase inhibitor (letrozole or anastrozole) to tamoxifen (Tables 15 and 16). No studies on selective estrogen receptor modulators met selection criteria. Five of the studies were secondary analyses by HER2 status of randomized, controlled trials, while the sixth was a prospective, uncontrolled series. One of the secondary analyses addressed neoadjuvant therapy; four focused on adjuvant therapy; and the uncontrolled series reported on metastatic disease. None of these studies used trastuzumab for HER2-positive patients; studies addressing the use of trastuzumab were reviewed in Chapter 2.

The neoadjuvant study (von Minckwitz, Sinn, Raab, et al., 2007) was a secondary analysis of a randomized trial comparing a chemotherapy regimen (doxorubicin and docetaxel) with or without the addition of tamoxifen. The four secondary analyses of randomized trials of adjuvant therapy included comparisons of (1) letrozole versus tamoxifen (Rasmussen, Regan, Lykkesfeldt, et al., 2008; Mauriac, Keshaviah, Debled, et al., 2007); (2) anastrozole versus tamoxifen (Dowsett, Allread, Knox, et al., 2008); (3) tamoxifen plus radiotherapy versus radiotherapy alone (Knoop, Bentzen, Nielsen, et al., 2001); (4) tamoxifen versus no tamoxifen following mastectomy or breast-conserving surgery plus radiotherapy (Ryden, Jirstrom, Bendahl, et al., 2005). The study of metastatic disease (Arpino, Green, Allred, et al., 2004) was a prospective, uncontrolled series of HER2-positive or HER2-negative patients given tamoxifen.

Study hierarchy, quality assessment, summary descriptions, and results are summarized in Tables 15–19; detailed abstraction data can be found in Appendix Tables IIIb-A–IIIb-K[*].

Patient Characteristics

Patients in the von Minckwitz, Sinn, Raab, et al. (2007) neoadjuvant trial had unilateral primary breast carcinoma at least 3 cm in largest diameter with no distant metastases or inflammatory disease. They comprised 194 of the 250 patients in the GEPARDO [German Preoperative Adriamycin-Docetaxel] trial. The average age was 48 years and 51 percent (control [Cx] group) to 57 percent (tamoxifen [TAM] group) were premenopausal. Forty-seven percent (Cx) to 53 percent (TAM) had clinically positive lymph nodes, and all had a Karnofsky score of at least 70 percent. For hormone-receptor status, 53 percent (TAM) to 59 percent (Cx) were ER-positive, while 35 percent (TAM) to 44 percent (Cx) were PR positive. HER2 status was measured centrally using IHC, and a HercepTest™ score of 3+ was considered positive. About 24 percent of the participants were HER2 positive.

Patients in the Rasmussen, Regan, Lykkesfeldt, et al. (2008) study comprised 3,533 of the 4.922 patients in the monotherapy arms of the BIG 1-98 trial. They were postmenopausal with early stage invasive cancer. The median age was around 60 years, and about 37 percent (HER2-negative patients) to 45 percent (HER2-positive patients) had tumors larger than 2 cm. Fewer than half had positive lymph nodes (42 percent for HER2-negative pts; 47 percent for HER2-positive patients). The median estrogen receptor level was 85 for HER2-positive patients and 90 for HER2-negative patients ($p<0.0001$), while the median progesterone receptor level was 10 in HER2-positive patients and 70 in HER2-negative patients ($p<0.0001$). HER2 positivity was defined as amplification by FISH or HercepTest™ 3+ by IHC (in 0.5 percent of patients with no FISH result). Seven percent of the patient population was HER2-positive.

Patients in the Dowsett, Allread, Knox, et al. (2008) study comprised 1,782 of the 5,880 patients in the monotherapy arms of the ATAC trial; most were from the United Kingdom. Sixty-seven percent of the patients had prior radiotherapy; 9 percent, prior chemotherapy; and 3 percent, tamoxifen prior to surgery. The median age was 63 years; and all of the women were postmenopausal. Sixty-seven percent had tumors that were no larger than 2 cm; 66 percent had negative lymph nodes; and all were hormone receptor positive (78 percent were PR+). HER2-positivity was defined by a score of 3+ on IHC or 2+ on IHC plus FISH amplification. Ten percent of the patients in the study were HER2-positive.

Patients in the Knoop, Bentzen, Nielsen, et al. (2001) adjuvant study were postmenopausal with a median age of 66 years. They had a high risk of recurrence, defined as having positive axillary lymph node(s), tumor larger than 5 cm diameter, or skin/deep fascial involvement. Sixty-six percent of the patients were estrogen-receptor (ER) positive, and 43 percent, progesterone-receptor (PR) positive.

In the original randomized, controlled trial, the Danish Breast Cancer Cooperative Group's 77c protocol, patients were randomized to receive tamoxifen three times daily for a year or to observation. All patients were also treated with mastectomy, lower axillary lymph node dissection, and radiotherapy. In the secondary analysis, data on HER2 status were available on a subset (n=1,515, 88 percent) of those in the original trial. Eighteen percent of these patients

[*] Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection

Table 15. Hierarchy of evidence, KQ3b

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA						
RCT post-hoc MV SGA	Von Minckwitz 2007	194	Neoadjuvant tumor≥3cm, age 18-70	doxorubicin+ docetaxel + tamoxifen (TAM)	pCR	Univariate: Not reported; Log reg: IHC HER2 as predictor of pCR p=0.126; HER2*TAM not reported
	Rasmussen 2008	3533	Adjuvant postmen HR+	tamoxifen vs. letrozole	DFS TTR	Univariate: FISH/IHC HER2+ vs. HER2- p<.0001 Cox[a]: FISH/IHC HER2*Tx, p=.60 Cox: FISH/IHC HER2*Tx NS
	Dowsett 2008	1782	Adjuvant, postmen, HR+	tamoxifen vs. anastrozole (ANA) for 5 yrs	TETR	Univariate: FISH/IHC HER2 – vs. + ANA:p<0.0001, TAM:p=.002 Cox: FISH/IHC HER2 – vs. + ANA:p<0.001, TAM:p=.014 "no indication" of greater differential benefit of ANA vs. TAM but no statistics provided and only 44 HER2+ pts so CIs wide
	Ryden 2005, 2007	470	Adjuvant, Stage II, Premen or <50 years	tamoxifen vs. observation	RFS	Univariate: IHC HER2- Tx vs. Cx p=.07 (ER+) IHC HER2+ Tx vs. Cx p=.2 (ER+) FISH HER2- or HER2+ Tx vs. Cx p=.14 (ER+) Cox regression: IHC HER2*TAM p=.4 (ER+); p=.3 (ER+/PR+) FISH/IHC HER2*TAM p=.95 (unclear if ER+ only)
	Knoop 2001	1515	Adjuvant, High risk, Postmen	tamoxifen vs. observation	DFS	Univariate: IHC HER2 lo+ or - Tx vs. Cx p=.0001 (HR+) IHC HER2 hi+ Tx vs. Cx p=.5 (HR+) Cox regression: IHC HER2 and HER2*TAM not significant (HR+)
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis	Arpino 2004	136	Metastatic, 1st line Tx	tamoxifen	ORR TTF OS	No stat signif diff FISH HER2 + vs. - in CR+PR+SD univariate: FISH HER2 - vs. + p=.007 Cox regression: HER2+ as predictor TTF p=.54 univariate: FISH HER2 - vs. + p=.07 Cox regression: HER2+ as predictor OS p=.97
1-arm UV analysis						

[a] Stratified for randomization group and chemotherapy

Abbreviations: DFS: disease-free survival; HR: hazard ratio; MV: multivariate; ORR: overall response rate; OS: overall survival; pCR: pathologic complete response; RCT: randomized, controlled trial; RFS: recurrence-free survival; SGA: subgroup analysis; TETR: time to early tumor recurrence; TTF: time to treatment failure; TTR: time to tumor recurrence; Tx: treatment; UV: univariate analysis;

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 16. Summary study quality assessment, KQ3b

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤15%)	Sufficiently long followup	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation					
									1)	2)	3)	4)	5)	6)
von Minckwitz et al., 2007	?	N	Y	Y	?	Y	N	Y	Y	N	?	?	?	N
Rasmussen et al., 2008, Mauriac et al., 2007	N	Y	Y	Y	Not reported	Y	N	51 mos/ 24 mos	?	?	?	Y	?	N
Dowsett et al., 2008	N	N	Y	Y	?	Y	N	Y	Y	?	?	?	?	N
Ryden et al., 2005, 2007	Y	?	?	N	?	Y	N	Med=14 yrs if no breast event	N	N	Y	?	N	?
Knoop et al., 2001	Y	N	Y	N	?	Y	Y	?	N	N	?	Y	N	?
Arpino et al., 2004	Y	N	Y	Y	?	Y	N	?	Y	?	?	N	N	?

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 17. Summary design, enrollment and treatment, KQ3b

Study	Therapeutic Setting	Treatment(s)	Age	Extent of Disease	Performance Status Scale	Index	Result	Hormone Receptor Status (%)
Neoadjuvant Hormonal Therapy								
von Minckwitz et al., 2007, Germany, multicenter RCT, secondary analysis	Primary breast carcinoma ≥ 3 cm largest diameter; no distant metastases, age 18-70	Doxorubicin + docetaxel ± tamoxifen (TAM); followed by surgery within 14-28 days	Median=48 Range=27-67 Premen: 51%(TAM-) 57%(TAM+)	Positive nodes: 47% (TAM-) 53% (TAM+)	Karnofsky score ≥70% ≥90%	 100% 96.3%		ER+ 59.2 (TAM-) 53.1 (TAM+) PR+ 43.9 (TAM-) 34.7 (TAM+)
Adjuvant Hormonal Therapy								
Rasmussen et al., 2008, Mauriac et al., 2007, international, multicenter RCT, secondary analysis	Postmenopausal women with HR+, early invasive breast cancer, in monotherapy arms of BIG 1-95 trial	Letrozole (LET) vs. TAM; 44%-54% had mastectomy; 21% -32% had chemotherapy	Median=~60	Positive lymph nodes: 42–47%	Not reported			Median ER=85-90 Median Pr=10-70
Dowsett et al., 2008, international, multicenter RCT, secondary analysis	Postmenopausal women with operable, invasive breast cancer HR+, in monotherapy arms of ATAC trial. Most from UK.	Anastrozole (ANA) vs. TAM for 5 years; mastectomy, 41%; chemotherapy, 9%; TAM presurgery, 3%	Median=63	Positive lymph nodes: 30%	Not reported			Pr+, 78%

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 17. Summary design, enrollment and treatment, KQ3b (continued)

Study	Therapeutic Setting	Treatment(s)	Age	Extent of Disease	Performance Status Scale Index Result	Hormone Receptor Status (%)
Adjuvant Hormonal Therapy (continued)						
Ryden et al., 2005, multicenter RCT, secondary analysis	Stage II, premenopausal/ <50 yrs	TAM for 2 yrs vs. control; mastectomy or breast-conserving surgery + radiotherapy; <2% pts received adjuvant chemotherapy	Median=45 Range=2-75	~70% are node positive; tumor 25 in TAM group vs. 22 in control (p=0.03)	Not reported	TAM Cx ER- PR- 30 26 ER- PR+ 8 10 ER+ PR- 4 5 ER+ PR+ 54 57 P=0.6 Not done 4 2
Knoop et al., 2001, Denmark, multicenter RCT, secondary analysis	Postmenopausal, "high risk"	Grp 1: TAM 10 mg 3x/day for 1 year (n=868) + radiotherapy Grp 2: Radiotherapy (n=848)	Median=66, Range=45-88	High risk=positive axillary lymph nodes, tumor >5 cm, or tumor invaded skin or deep fascia	Not reported	ER+ 66% (11% HER2+) PR+ 43% (7% HER2+)
Metastatic Hormonal Therapy						
Arpino et al., 2004, multicenter, US? PRO	First line, ER+	TAM 2x/day, 10 mg (n=56) or 10 mg/m^2 (n=149).	HER2+: 66%<65 yo; 16% premen HER2-: 57%<65yo; 12% premen	Not reported	Not reported	Her2+ Her2- ER+ 100 100 PR+ 78 96

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 18. Summary time to event outcomes, KQ3b

Study	Time to Event Outcomes												
Neoadjuvant Hormonal Therapy													
von Minckwitz et al., 2007	Not reported												
Adjuvant Hormonal Therapy													
Rasmussen et al., 2008, Mauriac et al., 2007	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	Test	p	HR (95%CI)		Comments
	DFS	HER2+									LET vs. TAM		HER2+ vs. HER2- (any Tx): HR=2.09(1.59-2.76) p<0.0001.
		All	239		~0.95	~0.87	~0.82	~0.75			HER2+		
		LET	134		~0.97	~0.90	~0.86	~0.79			0.62(0.37-1.03)		
		TAM	105		~0.94	~0.84	~0.75	~0.70					
		HER2-											
		All	3,294		~0.98	~0.96	~0.91	~0.88			HER2-		
		LET	1,648		~0.98	~0.97	~0.95	~0.90			0.72(0.59-0.87)		
		TAM	1,646		~0.98	~0.95	~0.90	~0.86					
Dowsett et al., 2008	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTR TAM		837										"[N]o indication of a greater differential benefit of anastrozole over tamoxifen in the HER-2-positive patients. However, there were only 44 events in the HER-2-positive group, so the CIs are wide."
		HER2-			~0.01	~0.04	~0.06	~0.08	~0.09		0.0018	2.25	
		HER2+			~0.01	~0.06	~0.10	~0.15	~0.25				
	ANA		875										
		HER2-			~0.01	~0.02	~0.04	~0.05	~0.06		<0.0001	3.27	
		HER2+			~0.01	~0.08	~0.12	~0.16	~0.20				
Ryden et al., 2005, 2007 ER+ pts	Outcome	Grp	N	Med (mos)	5 yr	10yr	15 yr	Test	p	HR (95%CI)	Comments		
	RFS	HER2+ (IHC 3+)											
		Tx	8		~0.7	~0.7	~0.7	LR	0.2	0.38 (0.08-1.79)	No stat diff in RFS between HER2+ and HER2- pts (measured by IHC or FISH) among untreated pts.		
		Cx	13		~0.4	~0.4	~0.4						
	RFS	HER2- (IHC 0-2+)											
		Tx	115		~0.75	~0.7	~0.65	LR	0.07	0.69(0.46-1.03)	VEGFR2 status was predictive of TAM efficacy. Using the combined HER2 measure, there was a TAM effect in the ER+/HER2- group (n=275; HR=0.64, 95%CI: 0.44-0.93, p=0.02), but not in the ER+/HER2+ cohort (n=24; HR=0.71, 95%CI: 0.23-2.20, p=0.6).		
		Cx	124		~0.7	~0.6	~0.55						
	RFS	HER2+ (FISH)											
		Tx			Data not reported			LR	0.14	0.21 (0.03-1.67)			
		Cx			Data not reported								
	RFS	HER2- (FISH)											
		Tx			Data not reported			LR	0.14	0.73(0.47-1.12)			
		Cx			Data not reported								

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 18. Summary time to event outcomes, KQ3b (continued)

Study	Time to Event Outcomes													
Adjuvant Hormonal Therapy (continued)														
Knoop et al., 2001 ER+ or PR+ pts only	Outcome DFS: HER2 - & low + (n=1,005) HER2 hi + (n=52)	Grp TAM Cx TAM Cx	N Not reported Not reported	Med (mos)	5 yr 57(2) 43(2) 63(11) 41(9)	10 yr 34(2) 26(2) 37(12) 35(8)	Test LR LR Cox MV Cox	p .0001 .5 HER2+ (n=54): RR TAM vs. Cx=0.89 (95%CI:0.63-1.27) HER2- (n=998): RR TAM vs. Cx=0.86 (95%CI:0.78-0.93) HER2 and HER2*TAM: Not significant (p values not reported)	HR (95%CI)	Comments Bonferroni p=.0006 Bonferroni p=.5			
	NOTE: Analysis limited to steroid-receptor positive pts. Standard errors in parentheses. LR=log-rank test of differences in DFS probabilities for pts with the variables in question when treated with TAM or not.													
Metastatic Hormonal Therapy														
Arpino et al., 2004	Outcome TTF OS HER2+ HER2+ as predictor of TTF HER2+ as predictor of survival	Grp HER2- HER2- HER2+	N 104 32 104 32	Med (mos) 7 5 31 25	1 yr ~.35 ~.20 ~.85 ~.90	2 yr ~.18 ~.03 ~.60 ~.52	3 yr ~.08 No line ~.50 ~.30	4 yr ~.08 No line ~.25 ~.20	5 yr 0 No line ~.20 ~.08	10yr 0 ~.05 ~.05	Test LR LR MV Cox MV Cox	p HR (95%CI) .007 .07 0.54 1.15 0.97 0.99		Comments HER2+ pts had lower median ER levels, even when all pts ER+ adjusted adjusted

Abbreviations: ANA: anastrozole; Cx: control; DFS: disease-free survival; HR: hazard ratio; LET: letrozole; LR: log rank; MV: multivariate; OS: overall survival; RR: relative risk; TAM: tamoxifen; TTF: time to treatment failure; TTR: time to tumor recurrence; Tx: treatment;

Question 3b: HER2 Results to Guide Hormonal Treatment Regimen Selection
Table 19. Summary tumor response and quality of life, KQ3b

Study	Tumor Response (%)							
Neoadjuvant Hormonal Therapy								
von Minckwitz et al., 2007		ALL (pCR)		ER+ (pCR)		ER- (pCR)		
		HER2+	HER2-	HER2+	HER2-	HER2+	HER2-	
	TAM+	0%	10.7%	0%	0%	0%	24.2%	
	TAM-	8.7%	9.6%	9.1%	2.2%	8.3%	21.4%	
Adjuvant Hormonal Therapy								
Rasmussen et al., 2008, Mauriac et al., 2007	Not reported							
Dowsett et al., 2008	Not reported							
Ryden et al., 2005	Not reported							
Knoop et al., 2001	Not reported							
Metastatic Hormonal Therapy								
Arpino et al., 2004	Grp	N	cCR+cPR+cSD	PD	NE	Test	p	Comments
	HER2-	104	56%	44%		χ^2	NS	
	HER2+	32	47%	53%				

Abbreviations: cCR: clinical complete response; cPR: clinical partial response; ER: cSD: clinical stable disease; estrogen-receptor; NE: not evaluable; NS: not significant; PD: progressive disease; SD: stable disease;

were HER2 positive by IHC, but approximately 11 percent had IHC results roughly comparable to a 3+ score by HercepTest™*. However, the proportions of HER2-positive patients differed between the arms of the trial: 8 percent of patients in the tamoxifen arm were HER2 positive, while 14 percent of those in the control arm were HER2 positive (p=0.001).

Patients in the Ryden, Jirstrom, Bendahl, et al. (2005) and Ryden, Landberg, Stal, et al. (2007) adjuvant trial had Stage II invasive cancer and included 470 or the 564 patients in the original trial. The median age was 45 years, and all were premenopausal or younger than 50 years old. The median tumor size ranged from 22 in the control group to 25 in the tamoxifen group. Both hormone-receptor-positive and hormone-receptor-negative patients were included. Fifty-four percent of patients in the tamoxifen group and 57 percent of patients in the control group were ER positive and PR positive, respectively; 30 percent and 26 percent, were ER negative and PR negative, respectively; the remainder were either ER negative/PR positive or ER positive/PR negative. Approximately 70 percent of the patients had positive lymph nodes. Patients were randomized to tamoxifen for two years versus no tamoxifen. Patients also underwent mastectomy or breast-conserving surgery plus radiotherapy. Less than 2 percent of patients, evenly distributed across arms in the original trial, received additional chemotherapy (n=8) or goserelin (n=1).

Data on HER2 status were available on 428 patients, or 76 percent of the original trial participants. The authors reported that baseline prognostic factors were similar in the groups with and without archived pathological specimens available for the secondary analysis. HER2 status was measured by FISH, using a cutoff of six signals/tumor cell (13 percent of patients were HER2 positive) and by IHC using a cutoff of 3+ on the HercepTest™ (15 percent were HER2 positive). The correlation between IHC 3+ and FISH amplification was r=0.82 (p<0.001); κ=0.84.

Patients with metastatic disease in the Arpino, Green, Allred, et al. (2004) single-arm study were drawn from the Southwest Oncology Group's (SWOG) protocol 8228 and ancillary study 9314. Approximately 60 percent of the patients were younger than 65 years old, and approximately 14 percent were premenopausal. All patients were ER positive; 78 percent of the HER2-positive and 96 percent of the HER2-negative patients were PR positive. Patients received tamoxifen twice daily as first-line therapy until disease progression.

Data on HER2 status were available on 136 patients, or about 39 percent of the original study participants. HER2 status was measured by FISH with a cutoff of HER2/CEP17 ratio of 2 or more (24 percent of patients were HER2 positive) and by IHC with a cutoff of complete membrane staining in 10 percent or more of tumor cells (21 percent of patients were HER2 positive), but only the FISH results were used in this analysis.

Outcomes Reported and Followup

The outcome for the neoadjuvant study (von Minckwitz, Sinn, Raab, et al., 2007) was pathological complete response, and surgery was performed within 14–28 days after chemotherapy was completed. In the two studies on the BIG 1-98 trial, Mauriac, Keshaviah,

* The description of the criteria used to designate HER2 status is unclear, but it appears that cases were considered positive if at least 50% of the cancer cells had membrane staining (roughly comparable to HercepTest™ 3+ score). Cases with membrane staining of less than 50% of the tumor cells were designated low HER2 positive. Overall HER2-positive proportions include high and low positive scores, but in the analysis, patients were grouped into negative/low positive findings vs. high positive findings.

Debled, et al. (2007) assessed time to early tumor recurrence (TETR), defined as a recurrence within 2 years, which was also the median followup; while Rasmussen, Regan, Lykkesfeldt, et al. (2008) reported on disease-free survival with a median followup of 51 months. In the comparison of anastrozole versus tamoxifen from the ATAC trial, Dowsett, Allread, Knox, et al. (2008) examined time to recurrence; the duration of followup was unclear, possibly 68 months. The only outcome reported in the Knoop, Bentzen, Nielsen, et al. (2001) adjuvant study was disease-free survival (DFS); the duration of followup was not reported, but the tables included estimates of DFS at 10 years. The Ryden, Jirstrom, Bendahl, et al. (2005) adjuvant trial only reported recurrence-free survival (RFS) and had 14 years; median followup for patients without a breast cancer event. The Arpino, Green, Allred, et al. (2004) uncontrolled study on metastatic disease reported overall response rates (ORR; sum of complete plus partial responses), time to failure (TTF), and overall survival (OS). “Nearly all” of the tumor blocks were more than 10 years old; some were more than 20 years old.

Results by Hierarchy Level, Study Quality Assessment

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Randomized trial, prespecified multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, post-hoc multivariate subgroup analysis. Five of the six studies that met the selection criteria were post-hoc analyses of randomized controlled trials. The only neoadjuvant study compared pathological tumor response in patients receiving doxorubicin and docetaxel with or without tamoxifen (von Minckwitz, Sinn, Raab, et al., 2007). The pCR rate among ER-positive and HER2-positive patients was 0 percent for those receiving tamoxifen versus 9 percent for those not receiving it; among HER2-negative patients the corresponding numbers were 24 percent and 21 percent. The numbers were small, however. There were only 25 ER-positive and HER2-positive patients, with 1 pCR, while there were 61 ER-positive but HER2-negative patients, with 14 pCRs. In a multivariate logistic regression model including menopausal status, tumor size, grade, and nodal status, the odds ratio for HER2 was 3.66 (95 percent CI: 0.69–19.30, p=.126). Analysis of the interaction term between HER2 status and treatment group was not reported. Consequently, the study confirms that the prognosis is poorer in HER2-positive patients, but it does not indicate whether or not tamoxifen is more or less effective in HER2-positive versus HER2-negative patients.

Two studies compared the use of an aromatase inhibitor versus tamoxifen. In secondary analyses of the BIG 1-98 trial, disease-free survival and time to early tumor recurrence were examined. Rasmussen, Regan, Lykkesfeldt, et al. (2008) reported a hazard ratio of letrozole versus tamoxifen among HER2-positive patients of 0.62 (95 percent CI: 0.37–1.03) and among HER2-negative patients of 0.72 (95 percent CI: 0.59–0.87). While the numerical values of the hazard ratios are similar, the result for HER2-negative patients is statistically significant, while that for HER2-positive patients is not. The number of HER2-positive patients is 239, much smaller than the 3,294 HER2-negative patients. Mauriac, Keshaviah, Debled, et al. (2007) report that the time to early tumor recurrence does not appear to be statistically significantly different by treatment group in either HER2-positive or HER2-negative patients, and the HER2 status/treatment group interaction term in a multivariate analysis is not statistically significant. Consequently, this study suggests that letrozole increases disease-free survival among HER2-

negative patients relative to tamoxifen, but it does not provide evidence on a greater effect among HER2-positive patients.

In the secondary analysis of the ATAC trial, Dowsett, Allread, Knox, et al. (2008) compare the effect of anastrozole and tamoxifen by HER2 status. They examine time to treatment recurrence by HER2 status and report hazard ratios of HER2-negative versus HER2-positive patients of 2.25 (p=.0018) for anastrozole and 3.27 (p<.0001) for tamoxifen. These results demonstrate that HER2-positive patients have a poorer prognosis than HER2-negative patients but do not compare the effectiveness of each treatment within each HER2 group. The authors report that there is "no indication of a greater differential of anastrozole over tamoxifen in the HER-2-positive patients. However, there were only 44 events in the HER-2-positive group, so the CIs are wide." No further details of the analysis are provided. In the multivariate analysis, no analysis of an interaction term between HER2 status and treatment group is reported.

Two studies compared patients treated with tamoxifen versus a control group; they both included a multivariate analysis. Table 20 summarizes results reported by Knoop, Bentzen, Nielsen, et al. (2001) from their secondary analysis on outcomes of adjuvant tamoxifen by HER2 status in hormone-receptor-positive patients. The results showed that patients who were HER2 negative or low HER2 positive had statistically significantly longer disease-free survival when they were treated with tamoxifen; the difference in survival (with versus without tamoxifen) was not statistically significant for patients that were high HER2 positive.

Table 20. Summary results for DFS in Knoop, Bentzen, Nielsen, et al. (2001)

HER2 Status	Treatment group	5-year DFS (% ± SE)	10-year DFS (% ± SE)	Log rank p value	p value with Bonferroni correction
Negative/low-positive (n=1,005)	Tamoxifen	57% (±2)	34% (±2)	.0001	.0006
	Control	43% (±2)	26% (±2)		
High-positive (n=52)	Tamoxifen	63% (±11)	37% (±12)	.5	.5
	Control	41% (±9)	35% (±8)		

Abbreviations: DFS: disease-free survival; SE: standard error;

A multivariate Cox model was constructed that included tumor size, proportion node positive, histologic grade, p53 value, EGFR, HER2, tamoxifen, and interactions between tamoxifen and p53, HER2 and EGFR. The coefficients for HER2 and for the interaction term for HER2 and tamoxifen were not statistically significant (specific p values and coefficients not reported for these variables). Node positive proportion (RR=1.011), grade (RR=1.103), p53 (1.54), and tamoxifen (RR=0.73) were statistically significant at p<.01. In other words, after controlling for other variables, HER2 was not a statistically significant predictor for outcomes of treatment with tamoxifen in this study.

The results of the secondary analysis of the adjuvant trial by Ryden, Jirstrom, Bendahl, et al. (2005) are summarized in Table 21. All patients were ER positive. No result was statistically significant.

Table 21. Summary results for RFS in Ryden, Jirstrom, Bendahl, et al. (2005)

HER2 Status	IHC		FISH	
	Log rank p value	Hazard ratio TAM vs. Cx (95% CI)	Log rank p value	Hazard ratio TAM vs. Cx (95% CI)
HER2- (n=239)[a]	.07	0.69 (0.46-1.03)	.14	0.73 (0.47-1.12)
HER2+ (n=21)[b]	.2	0.38 (0.08-1.79)	.14	0.21 (0.03-1.67)

[a]IHC 0-2+ or FISH nonamplified
[b]IHC 3+ or FISH amplified
Abbreviations: Cx: control; TAM: tamoxifen

The authors also reported that among untreated patients, the difference in outcome between HER2-positive and HER2-negative patients (measured with either IHC or FISH; in both univariate and multivariate Cox proportional hazard models) was not statistically significant. In contrast, the marker VEGFR2 was a statistically significant predictor of outcome of tamoxifen treatment. In a univariate analysis among ER-positive/PR-positive patients with HER2 status measured using IHC, the duration of RFS was longer among tamoxifen-treated patients than controls in the HER2-negative subgroups (p=.03) but not among HER2-positive (p=.3) patients.

In a multivariate Cox model, the interaction term between treatment (tamoxifen versus control) and HER2 status was not statistically significant when the model was run for ER-positive patients (p=.4) or ER-positive/PR-positive patients (p=.3). The covariates in the model were not clearly listed but probably included age, tumor size, nodal status, Nottingham histologic grade, tamoxifen, and the interaction term.

Randomized trial, treatment by HER2 subgroup analysis. No studies of this type were identified.

Single-arm study, prespecified multivariate analysis. No studies of this type were identified.

Single-arm study, post-hoc multivariate analysis. The prospective but uncontrolled study on use of tamoxifen for metastatic disease by Arpino, Green, Allred, et al. (2004) compared outcomes for HER2-positive versus HER2-negative patients. ORR was 56 percent for HER2-negative patients and 47 percent for HER2-positive patients (χ^2 test, p=NS). Median TTF was 7 months for HER2-negative patients versus 5 months for HER2-positive patients (log rank p=.007). Finally, median OS was 31 months for HER2-negative patients versus 25 months for HER2-positive patients (log rank p=.07). While all of the patients were ER positive, median ER levels were lower in HER2-positive than in HER2-negative patients.

Multivariate, partially nonparametric Cox models for TTF and OS included menopausal status, disease-free interval, ER and PR levels, HER1 status, and HER2 status. HER2-positive status was not a statistically significant predictor of either TTF or overall survival. HER1 status, premenopausal status, and disease-free interval before recurrence were statistically significant predictors of TTF, while ER and PR levels and disease-free interval prior to recurrence were significant predictors of OS. The hazard ratios for HER2-positive versus HER2-negative subgroups were 1.15 (p=.54) for TTF and 0.99 (p=.97) for OS. Therefore, after controlling for other factors, this study provided no evidence of a difference in outcomes after treatment with tamoxifen between HER2-positive and HER2-negative patients.

Single-arm study, univariate analysis. No studies of this type were identified.

Conclusions, Key Question 3b

The evidence on use of HER2 status to predict outcomes of hormonal therapy is weak and inconclusive. Four studies reviewed here addressed use of tamoxifen in different breast cancer patient populations; two compared tamoxifen with aromatase inhibitors. Evidence is lacking from the most informative types of studies, trials in which randomization is stratified by HER2 status or randomization to therapy directed by HER2 results or not. Less-informative designs were used, including post-hoc multivariate analyses in five randomized trials and one post-hoc multivariate analysis in a single-arm study. In comparing tamoxifen with aromatase inhibitors in a secondary analysis of randomized, controlled trial results, the most persuasive finding would be a significant interaction term between HER2 status and treatment group, after controlling for other important prognostic factors.

In the two comparison studies included, one had an insignificant interaction term (suggesting that there is no differential in the impact of the two treatments based on a patient's HER2 status), and the other did not report an interaction term although they included a qualitative statement that there was no evidence that one treatment was more effective than the other in HER2 positive patients. Some results suggest that tamoxifen may be more effective among HER2-negative patients, but a conclusion is undermined by the paucity of studies and inconsistent findings. Importantly, data demonstrating a difference in magnitude of benefit by HER2 status would not by themselves be sufficient to conclude there is no benefit in HER2-positive patients also positive for hormone receptors. Studying the differential impact of hormonal therapy by HER2 status is hindered by the inverse relationship between HER2 status and hormone receptor status, which leads to relatively small numbers of HR-positive and HER2-positive patients on which to base the results.

Key Question 4

What is the evidence that monitoring serum or plasma concentrations of HER2 extracellular domain in patients with HER2-positive breast cancer predicts response to therapy, or detects tumor progression or recurrence, and if so, what is the evidence that decisions based on serum or plasma HER2 assay results improve patient management and outcomes?

Study Selection

Studies were included for Key Question 4 if they were:

- randomized trials, prospective single-arm studies, or retrospective series of identically treated patients; that
- measure serum or plasma HER2 concentrations in breast cancer patients, either at baseline or at multiple time points; and either:
    a. associate baseline values or changes in HER2 concentration with one or more outcomes of interest (primary or secondary); or
    b. compare outcomes of treatment decisions based on assay results with outcomes of decisions made in absence of assay results.

Of 15 studies meeting selection criteria, five were randomized trials and 10 single-arm designs. One of the randomized trials compared three different doses of a single selective

estrogen receptor modulator, droloxifene (Yamauchi, O'Neill, Gelman, et al., 1997). Since the range of doses assessed in the trial do not produce different results, the data pooled across dosing groups will be treated as a single-arm design, therefore, four randomized trials and 11 single-arm designs are presented in separate summary tables; detailed abstraction data can be found in Appendix Tables IV-A–IV-K*. All but one study meeting study selection criteria addressed subgroup analyses of baseline sHER2 measurements to predict outcomes after treatment. The study reported by Fornier, Seidman, Schwartz, et al. (2005) was the only one that focused on changes in serial measurements. No studies meeting selection criteria addressed whether serial sHER2 measurements confer lead time compared with other monitoring techniques.

Patient Characteristics

Randomized trials. Two of the four trials (Table 22) selected patients with metastatic breast cancer undergoing first-line systemic therapy. The comparisons in these two trials were paclitaxel with or without trastuzumab, and epirubicin with either paclitaxel or cyclophosphamide. The third trial included postmenopausal patients with locally advanced (stage IIIB), locoregionally recurrent or metastatic breast cancer randomized to either letrozole or tamoxifen. The fourth trial selected patients with locally advanced or metastatic breast cancer given capecitabine with or without lapatinib as second-line treatment after progression following treatment with an anthracycline, a taxane and trastuzumab. A total of 1,153 patients were included in these trials, with individual samples sizes ranging from 101 to 562.

Two of the randomized trials selected patients for being positive on tissue (t) HER2 testing. Gasparini, Gion, Mariani, et al. (2007) selected patients with 2+ or 3+ scores on the IHC HercepTest™. Cameron, Casey, Press, et al. (2008) included patients who were 3+ on IHC or 2+ with a positive FISH result. Muller, Witzel, Luck, et al. (2004) performed tissue testing on only 29 of 103 patients and only nine patients had 3+ results by Dako-style scoring of an IHC assay using the CB11 mAb. No tHER2 results were reported for Lipton, Ali, Leitzel, et al. (2003).

Patient characteristics were reported in various ways. Only age was reported by all four studies. Baseline data in the two treatment groups in the Muller, Witzel, Luck, et al. (2004) trial were combined; median age was 48 years. In the Gasparini, Gion, Mariani, et al. (2007) and Cameron, Casey, Press, et al. (2008) trials, median ages by treatment group were in the low and mid-50s and in the Lipton, Ali, Leitzel, et al. (2003) study median ages were in the mid-60s.

The proportion of patients with three or more disease sites was 27 percent in the Gasparini, Gion, Mariani, et al. (2007) study, 49 percent in the Cameron, Casey, Press, et al. (2008) trial and 10 percent and 11 percent of the two treatment groups studied by Lipton, Ali, Leitzel, et al. (2003).

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 22. Randomized trials, design, treatment, patient characteristics, KQ4

Study	Therapeutic Setting	Treatments Compared	Age Mean, range	Number of Disease Sites (%) 1	2	≥3	Performance Status Scale	Index	Result	E+&P+	E+/P+
Cameron et al., 2008, multicenter international	tHER2+ LABC/MBC, 2nd-line	Grp 1: capecitabine (n=201) Grp 2: lapitinib + capecitabine (n=198)	51, 28-83 54, 26-80	22 20	30 31	48 49	ECOG %1	%0	59 41 62 38		
Gasparini et al., 2007, Italy, multicenter; 12/00 – 09/04 Phase II RCT	First-line, untreated MBC, t-IHC 2+/3+	Grp 1: paclitaxel (n=61) Grp 2: paclitaxel + trastuzumab (n=63)	54.27, 30-71 56.02, 32-72	33 40	40 33	27 27	ECOG %0 %1	% 0, 1-2:	82, 18 81, 19	37 37	27 10
Muller et al., 2004, Germany, multicenter RCT	First-line tx for MBC	Grp1: epirubicin + paclitaxel (ET, n=54, 65% sHER2+); Grp2:epirubicin+cyclophosphamide (EC, n=47, 62% sHER2+)	Grp1+Grp2: 48, 31-63							61 (E+)	
Lipton et al., 2003, multinational, multicenter RCT	First-line, postmeno-pausal locally advanced (stage IIIB), loco-regionally recurrent BC, MBC, ER+/PR? and/or PR+/ER?	Grp1: letrozole (n=283, 31% sHER2+) Grp2: tamoxifen (n=279, 28% sHER2+)	65, 42-94 63, 31-90	53 55	37 34	10 11	KPS rng md rng	md	90 50-100 90 50-100	38 40	28 27

Abbreviations: BC: breast cancer; E+&P+: estrogen and progesterone receptor positive; E+/P+: estrogen and/or progesterone receptor positive; ECOG: Eastern Cooperative Oncology Group; ER: estrogen receptor; Grp: group; KPS: Karnofsky performance score; LABC: locally advanced breast cancer; MBC: metastatic breast cancer; md: median; PR: progesterone receptor; RCT: randomized, controlled trial; s: serum; rng: range; t: tissue; tx: treatment;

Gasparini, Gion, Mariani, et al. (2007) used the ECOG performance status scale, finding that 82 percent and 81 percent had the highest level (0). Cameron, Casey, Press, et al. (2008) reported that 62 percent and 59 percent were at ECOG level 0. Median Karnofsky Performance Scale values were 90 in both groups included by Lipton, Ali, Leitzel, et al. (2003).

In the study by Gasparini and co-workers, 37 percent were both estrogen and progesterone-receptor positive, while the proportions for the twp groups from Lipton and co-workers' study was 38 percent and 40 percent, respectively. Muller, Witzel, Luck, et al. (2004) only noted that 61 percent were estrogen-receptor positive. Cameron, Casey, Press, et al. (2008) reported the proportions of patients in the two groups who were either positive on one or both receptors: 48 percent and 46 percent.

Single-Arm Designs. All 11 studies selected patients with metastatic breast cancer (Summary Table 23). The total number of patients across studies is 706; individual sample sizes ranged from 35 to 94. Treatments were first-line systemic therapy in six studies, second-line in one study, second- or third-line in one study, second-line or higher in one study and a mix of first- and second-line or higher in two studies. Regimens in six studies were taxane-based (two with anthracyclines, two with trastuzumab); one study combined trastuzumab with vinorelbine, one study used the aromatase inhibitor letrozole, one study used the selective estrogen receptor modulator droloxifene, and three studies used other chemotherapy regimens.

Two studies selected patients who were tHER2 3+ on IHC or positive on FISH. Five studies included mixed patient populations that were positive and negative on HER2 tissue testing (Colomer, Llombart-Cussac, Lloveras, et al., 2007; Colomer, Montero, Lluch, et al., 2000; Im, Kim, Lee, et al., 2005; Fornier, Seidman, Schwartz, et al., 2005; Sandri, Johansson, Colleoni, et al., 2004). The remaining four studies did not provide data on tissue HER2 testing (Yamauchi, O'Neill, Gelman, et al., 1997; Colomer, Llombart-Cussac, Lluch, et al., 2004; Luftner, Henschke, Flath, et al., 2004; Colomer, Llombart-Cussac, Tusquets, et al., 2006).

Regarding age, one study had a median age of 48 years, another had a median of 49 years. One study had 53 percent at age 64 or older, another had a median age of 64 years and a third had mean ages in sHER2 positive and negative groups of 63 and 64 years. The other 6 studies had median ages in the 50s.

Nine studies gave the distribution of patients by number of disease sites and one study gave the number of involved organs (43 percent had three or more involved organs). In seven studies, the percentage of patients with three or more disease sites ranged from 18 percent to 43 percent; in another study all patients had two or fewer disease sites. Four studies provided average number of disease sites: the medians were two in two studies and three in two studies.

Four studies provided ECOG performance status data: the percentages in categories 0 or 1 (better performance status) were 75, 98, 98, and 88 percent. Two studies used the Karnofsky Performance Scale: in one study the mean value was 90 percent and in the other 83 percent were at 80 percent or 90 percent on the scale.

Seven studies gave baseline information on hormone receptor status, 4 of which reported the proportion of patients those estrogen positive, ranging from 49 percent to 67.3 percent. One study gave the proportion progesterone positive (34 percent). Two studies gave percentages of different combinations of hormone receptor status: the proportions who were both estrogen and progesterone positive were 17 percent and 37 percent; the proportions who were either estrogen or progesterone positive were 34 percent or 18 percent.

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 23. Single-arm studies, design, enrollment and treatment, KQ4

Study	Therapeutic Setting	Treatments Compared	Age Mean, range	Number of Disease Sites (%) 1	2	≥3	Performance Status Scale	Index	Result	ER+&PR+
Im et al., 2005, Korea, multicenter	MBC (1st-line)	Epirubicin+paclitaxel (n=40, 14.8% sHER2+)	49, 35-70	44	31	26	ECOG	%0 %1 %2	21 54 26	
Colomer et al., 2000, Spain, 1 center	MBC, no previous CHT for met dz (1st-line)	Doxorubicin+ paclitaxel (n=55, 43.6% sHER2+)	35% pre-menopausal	55	45					67 (ER+)
Fornier et al., 2005, USA, 1 center	MBC, tHER2 +/-	Paclitaxel+trastuzumab (n=55 of 95, 69% sHER2+)	51, 33-67	med 2, rng 1-4			KPS	mn	90 70-100	
Esteva et al., 2002, US, 1 center	MBC tHER2+, +/- previous tx for met dz	Trastuzumab+docataxel (n=30, 70% sHER2+)	45, 33-78	16	40	42	KPS rng	%90	63 20 16	
Colomer et al., 2004, Spain, 7 centers	progressive advanced BC (1st-line)	Paclitaxel+gemcitabine (n=42, 29.3% sHER2+)	53, 29-72	med 3, rng 1-6						49 (ER+)
Luftner et al., 2004, Germany, 1 center	stage IV BC, 1 or 2 previous CHT	Dose-intensified paclitaxel (n=35; 1st-line 6%, 2nd-line 60%, 3rd-line 34%, 63% sHER2+)	48, 31-63	26	31	43 (# involved organs)				34
Burstein et al., 2003, US, 17 centers	stage IV BC, tHER2+	Trastuzumab+vinorelbine (n=43)	55, 29-82	md 3, rng 1-6			ECOG	%0 %1 %2	70 28 2	37 18
Colomer et al., 2007	MBC (2nd-line)	Letrozole (n=226, 25% sHER2+)	~63/64	36	31	33	ECOG	%0 %1	51 49	62
Yamauchi et al., 1997, US, ? centers	MBC (1st-line)	3 doses of droloxifene (n=94 of 369, 34% sHER2+)	47% < 64 53% ≥ 64	45	32	18		%3 %1-2	17	55 (ER+) 34 (PR+)
Sandri et al., 2004, Italy, 1 center	stage IV BC, ≥ 1 prev CHT for met dz (2nd-line+)	Cyclophosphamide + methotrexate (n=39)	56, 36-81	26	39	36				
Colomer et al., 2006, Spain, 6 centers	advanced BC (1st-line)	IV vinorelbine+ IV gemcitabine (n=47, 29.8% sHER2+)	64, 34-81	med 2, rng 1-4			ECOG	%0 %1 %2	41 47 12	67 (ER+)

Abbreviations: BC: breast cancer; CHT: chemohormonal therapy; dz: disease; ECOG: Eastern Cooperative Oncology Group; ER+: estrogen-receptor positive; IV: intravenous; MBC: metastatic breast cancer; med: median; met: metastatic; PR: progesterone-receptor positive; rng: range;

Evidence Hierarchy and Quality Assessment

No studies conducted stratified randomization on sHER2 status or randomized patients to whether sHER2 guided treatment (Tables 24 and 25) and only one performed prespecified subgroup analyses (Gasparini, Gion, Mariani, et al., 2007). Three randomized trials reported results from post-hoc treatment by sHER2 subgroup analyses (Cameron, Casey, Press, et al. 2008; Muller, Witzel, Luck, et al., 2004; Lipton, Ali, Leitzel, et al., 2003). Two single-arm studies included multivariate analyses (Colomer, Montero, Lluch, et al., 2000; Yamauchi, O'Neill, Gelman, et al., 1997). Overall, the bulk of studies (7 of 13) belonged to lowest category of the hierarchy.

Results by Hierarchy Level

Multivariate analysis was performed in only three studies: one randomized trial (Gasparini, Gion, Mariani, et al., 2007) and two single-arm designs (Colomer, Montero, Lluch, et al., 2000; Yamauchi, O'Neill, Gelman, et al., 1997). Summary study descriptions and results are arrayed in Tables 26–29.

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Randomized trial, prespecified multivariate subgroup analysis. The only trial that performed a prespecified multivariate subgroup analyses was Gasparini, Gion, Mariani, et al. (2007, n=123 patients given first-line treatment by paclitaxel with or without trastuzumab for metastatic breast cancer). One quality concern was uncertainty over whether sHER2 results were scored blindly to outcome. Also, this study addressed 11 predictor variables plus treatment interaction terms in logistic and Cox regression analyses, however there appeared to be too few events in terms of response and progression to support models with so many variables. Thus, the study was not large enough for the type of modeling used. Overall, it is unclear whether the multivariate analysis was well-conducted. It is unclear how candidate variables were selected, what model-building strategy was used, whether assumptions were tested, whether the standard metastatic breast cancer prognostic factors were included in final models, how continuous variables were categorized; also, the model did not appear to go through validation.

For time-to-progression, the Cox regression treatment by sHER2 interaction was nearly statistically significant (p=0.0538). Among patients with elevated sHER2 values, results significantly favored paclitaxel plus trastuzumab, while in those with normal sHER2, results nonsignificantly favored paclitaxel alone. Logistic regression analysis of overall response rate showed no significant treatment by sHER2 interaction (p=.6044); in both groups, combination treatment was favored, but not significantly.

Randomized trial, post-hoc multivariate subgroup analysis. No studies of this type were identified.

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection

Table 24. Hierarchy of evidence, KQ4

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA	Gasparini 2007	123	MBC 1st, t+	paclit vs. paclit+trastuz	TTP ORR	Cox regression sHER2 by treatment interaction p=.0538 logistic regression sHER2 by treatment interaction p=.6044
RCT post-hoc MV SGA						
RCT treatment by HER2 SGA	Cameron 2008	367	LABC/ MBC 2nd, t+	capecit (Cp)+/- lapatinib (Lp)	PFS	cont sHER2/highest vs. other quartiles Cp p<.001, Cp+Lp0.12 Cp vs. Cp+Lp↑ highest quartile sHER2+ p<.001, other quartiles p=.002
	Muller 2004	101	MBC 1st, t+/-	epirub+paclit (ET) vs. epirub+cycloph (EC)	OS PFS ORR	ET sHER2+↓ vs. - p=.092, EC sHER2+ vs. - p=NS sHER2- EC vs. ET p=NS, sHER2+ EC↓ vs. ET p=.0341 ET sHER2+ vs. - p=NS, EC sHER2+↓ vs. - p=.059
	Lipton 2003	562	locally advanced, recurrent, MBC 1st, t?	letrozole (LET) vs. tamoxifen (TAM)	TTP TTF ORR CB	sHER2+ LET↑ vs. TAM p=.0596, sHER2- LET↑ vs. TAM p=.0019 sHER2+ LET↑ vs. TAM, p=.0418, sHER2- LET↑ vs. TAM p=.0066 sHER2+ LET vs. TAM, p=.4507, sHER2- LET↑ vs. TAM p=.0078 sHER2+ LET vs. TAM, p=.3057, sHER2- LET↑ vs. TAM p=.0162
1-arm prespecified MV analysis	Colomer 2007	226	MBC 2nd	letrozole (LET)	ORR TTP OS	univariate sHER2+ ↓ vs. sHER2- p=.036 univariate sHER2+ ↓ vs. sHER2- p=.004 Cox regression sHER2+ ↓ vs. sHER2- p<.001 univariate sHER2+ ↓ vs. sHER2- p<.0005
	Colomer 2000	55	MBC 1st, t+/-	doxorub+paclit	RD RD ORR ORR	univariate sHER2+↓ vs. sHER2- p=.035 Cox regression sHER2+↓ vs. sHER2- p=.04 univariate sHER2+↓ vs. sHER2- p=.01 logistic regression sHER2↓ + vs. sHER2- p=.03
1-arm post-hoc MV analysis	Yamauchi 1997	94	MBC 1st, t?	3 doses droloxif	TTP OS ORR ORR	Cox regression sHER2+↓ vs. sHER2- p=.0003 Cox regression sHER2+↓ vs. sHER2- p=.003 univariate sHER2+↓ vs. sHER2- p=.00001 logistic regression sHER2+↓ vs. sHER2- p=.0001

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 24. Hierarchy of evidence, KQ4 (continued)

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
1-arm UV analysis	Im 2005	38	MBC 1st, t+/-	epirub+paclit	RD	sHER2+↓ vs. sHER2- p=<0.001
					TTP	sHER2+↓ vs. sHER2- p=<0.001
					OS	sHER2+↓ vs. sHER2- p=<0.076
					Resp	sHER2+ vs. sHER2- p=0.45
	Fornier 2005	55	MBC, t+/-	paclit+trastuz	ORR	sHER2+ vs. sHER2- p=1.0, sHER2 Δ<15 vs. Δ≥15 p=0.005
					ORR	sHER2 ≥15% vs. < 15% p=0.015
	Esteva 2002	30	MBC 2nd+, t+	trastuz+docet	ORR	sHER2+↑ vs. sHER2- p=0.04
	Colomer 2004	42	MBC 1st, t?	paclit+gemcitab	RD	sHER2+↓ vs. sHER2- p=0.04
					Resp	sHER2+↓ vs. sHER2- p=0.02
	Luftner 2004	35	MBC 2nd+, t?	dose intense paclit	RD	sHER2+↓ vs. sHER2- p=0.042
					PFS	sHER2+↓ vs. sHER2- p=0.098
					ORR	sHER2+ vs. sHER2- p=0.40
	Sandri 2004	39	MBC 2nd+,	cycloph+methotrex	TTP	sHER2+↓ vs. sHER2- p=0.007
					OS	sHER2+↓ vs. sHER2- p=<0.001
	Burstein 2003	43	MBC, t+	trastuz+vinorelb	Progr	no ↓ in sHER2 predicted progression; baseline, Δ did not predict
	Colomer 2006	47	MBC 1st, t?	IVvinorelb+IVgemcit	ORR	sHER2+ vs. sHER2- p=0.9

Abbreviations: cycloph: cyclophosphamide; DFS: disease-free survival; droloxif: droloxifene; epirub: epirubicin; gemcit: gemcitabine; HR: hazard ratio; MV: multivariate; ORR: overall response rate; OS: overall survival; paclit: paclitaxel; pCR: pathologic complete response; PFS: progression-free survival; RCT: randomized, controlled trial; RD: residual disease; RFS: recurrence-free survival; SGA: subgroup analysis; TETR: time to early tumor recurrence; trastuz: trastuzumab; TTF: time to treatment failure; TTR: time to tumor recurrence; Tx: treatment; UV: univariate analysis; vinorelb: vinorelbine;

t+/-

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 25. Study quality assessment, KQ4

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Cameron et al., 2008	Y	N	Y	N	?	Y	Y	≥ 6 wk	treatment x HER2 SGA
Gasparini et al., 2007	Y	Y	N	Y	?	Y	Y	med: 16.6 mos	? ? ? ? ? N
Muller et al., 2004	Y	N	N	Y	?	Y	N	med 8.9 mo (0.5-36)	treatment x HER2 SGA
Lipton et al., 2003	Y	N	N	Y	Y	Y	N	3 mos	treatment x HER2 SGA
Colomer et al., 2000	Y	Y	N	Y	?	Y	N	med 23 mos	? ? ? ? ? N
Yamauchi et al., 1997	Y	N	N	Y	?	Y	N	?	? N ? ? ? N
Colomer et al., 2007	Y	Y	Y	Y	?	Y	Y	≥ 4 wk	? ? ? ? ? N
Im et al., 2005	Y	Y	N	Y	?	Y	Y	med 22.5 mos	NA
Fornier et al., 2005	Y	N	N	Y	?	Y	N	≥ 4 wk	NA
Esteva et al., 2002	Y	Y	N	Y	Y	Y	Y	≥ 8 wk	NA
Colomer et al., 2004	Y	Y	N	Y	?	Y	Y	26 mos	NA
Luftner et al., 2004	Y	Y	N	Y	?	Y	Y	≥ 4 wk	NA

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 25. Study quality assessment, KQ4 (continued)

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Burstein et al., 2003	Y	Y	N	Y	?	Y	Y	8 wk	NA
Sandri et al., 2004	Y	N	N	Y	?	Y	N	2 mo	NA
Colomer et al., 2006	Y	Y	N	Y	?	Y	Y	med 79 mo	NA

Abbreviations: mos: months; NA: not applicable; SGA: subgroup analysis; wks: weeks;

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 26. Randomized trials, summary time to event outcomes, KQ4

Study	**Time to Event Outcomes**												
Cameron et al., 2008	Outcome	Grp	N	Med (mos)	6 mos	1 yr				Test	p	HR (95%CI)	
	PFS	sHER2+,Cp								Cox	<.001	sHER2 as continuous variable	
capecitabine (Cp) +/- lapatinib (Lp)		sHER2-,Cp											
		sHER2+,CpLp								Cox	.12		
		sHER2-,CpLp											
		sHER2+,Cp		2.6						Cox	<.001	2.3 (1.5, 3.6) highest sHER2 quartile vs. other quartiles	
		sHER2-,Cp		4.8									
		sHER2+,CpLp		6.0						Cox	.12	1.5 (0.9, 2.4)	
		sHER2-,CpLp		6.7									
		sHER2+,Cp		~3	~17					Cox	<.001	0.320 (0.181, 0.567) highest sHER2 quartile	
		sHER2+,CpLp		~6	~50								
		sHER2-,Cp		~20	~43	~15				Cox	.002	0.561 (0.389, 0.81) other quartiles	
		sHER2-,CpLp		~30	~52	~28							
Gasparini et al., 2007	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP												Cox regression p value for sHER2 by treatment interaction: .0538
paclitaxel vs. paclitaxel + trastuzumab													
Muller et al., 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	sHER2+/EC	19	~8.4	~50	~15	~0			LR	.092		
epirubicin + paclitaxel vs. epirubicin+ cyclophosphamide (ET vs. EC)		sHER2-/EC	35	~22	~77	~40	~15						
		sHER2+/ET	18	~16	~60	~10	~0			LR	NS		
		sHER2-/ET	29	~14	~65	~10	~0						
	PFS	sHER2-/EC	35	~7	~30	~0	~0			LR	NS		
		sHER2-/ET	29	~9	~21	~0	~0						
		sHER2+/EC	19	~12	~21	~0	~0			LR	.0341		
		sHER2+/ET	18	~9	~28	~0	~0						

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection

Table 26. Randomized trials, summary time to event outcomes, KQ4 (continued)

Study	Time to Event Outcomes											
Lipton et al., 2003	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	sHER2+	164										
letrozole vs.		letrozole	87	6.1	~28	~7	~6	~4		Cox	.0596	0.73 (0.53,1.01)	
tamoxifen		tamoxifen	77	3.3	~17	~5	~3						
		sHER2-	398										
		letrozole	196	12.2	~53	~29	~20	~14		Cox	.0019	0.70 (0.56,0.88)	
		tamoxifen	202	8.5	~38	~20	~10	~8					
	TTF	sHER2+	164										
		letrozole	87	6.0						Cox	.0418		
		tamoxifen	77	3.2									
		sHER2-	398										
		letrozole	196	11.6						Cox	.0066		
		tamoxifen	202	6.2									

Abbreviations: Cox: Cox proportional hazards; HR: hazard ratio; LR: log rank; med: median; mos: months; TTF: time to treatment failure; TTP: time to progression; yr: years;

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection

Table 27. Randomized trials, summary tumor response, KQ4

Study	Tumor Response (%)								
Cameron et al., 2008 capecitabine (Cp) +/- lapatinib (Lp)	Not reported								
Gasparini et al., 2007 paclitaxel vs. paclitaxel + trastuzumab	Grp	N	CR	PR	SD	PD	Test	p	Comments WHO criteria logistic regression p value for sHER2 by treatment interaction: 0.6044
Muller et al., 2004 epirubicin + paclitaxel vs. epirubicin+ cyclophosphamide (ET vs. EC)	Grp	N	CR+PR		SD	PD	Test	p	Comments
	sHER2+/ET	18	50.0		33.3	16.7	Chi sq	NS	UICC criteria
	sHER2-/ET	26	46.2		38.5	15.4			
	sHER2+/EC	17	29.4		35.3	35.3	Chi sq	.059	
	sHER2-/EC	31	41.9		35.5	22..6			
Lipton et al., 2003 letrozole vs. tamoxifen	Grp	N	CR+PR				Test	p	Comments
	sHER2+	164							UICC criteria
	letrozole		17				log regr	.4507	
	tamoxifen		13	SD+PD	87				
	sHER2-								
	letrozole		39	83			log regr	.0078	
	tamoxifen	398	26		74				
	Grp	N	CR+PR	61 SD	PD		Test	p	Comments
	sHER2+	164							UICC criteria
	letrozole		33				log regr	.3057	
	tamoxifen		26		74				
	sHER2-								
	letrozole		57	67			log regr	.0162	
	tamoxifen	398	45		55				

Abbreviations: Chi sq: Chi square; CR: complete response; Grp: group; log reg: logistic regression; NS: not significant; PR: partial response; UICC: International Union against Cancer; WHO: World Health Organization;

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 28. Single-arm studies, summary time to event outcomes, KQ4

Study	Time to Event Outcomes											
Im et al., 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
epirubicin+paclitaxel (n=40)	TTP	sHER2+	4	2.8							<.001	
		sHER2-	19	8.3								
	RD	sHER2+	3	1.5	0						<.001	
		sHER2-	13	6.7	~43	~33						
	OS	sHER2+	4	12.4	~50	~26					.076	
		sHER2-	23	not reached	~72	~56						
Colomer et al., 2000	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
doxorubicin+ paclitaxel	Resp Dur	sHER2+	15	7.5	~26						.035	
(n=55)		sHER2-	24	11	~50	~35				MV Cox	.04	
Colomer et al., 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
paclitaxel+gemcitabine	Resp Dur	sHER2+	5	7.9	~40	~0					.04	
(n=42)		sHER2-	24	14.4	~55	~37						
Luftner et al., 2004	Outcome	Grp	N	Mn (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
dose-intensified paclitaxel	Resp Dur	sHER2+	9	6.0	~0		LR				.042	
(n=35)		sHER2-	5	2	~60							
	PFS	sHER2+	22	3	~3	LR					.098	
		sHER2-	13	4	~10							
Colomer et al., 2007	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
Letrozole (n=226)	TTP	sHER2+	42	4	~36	~12	~7			LR	.004	
		sHER2-	184	14	~57	~34	~12					
						LR	?				<.001	
	OS	sHER2+	42	~22	~82	44					<0.0005	
		sHER2-	184		~91	75	~63					
Yamauchi et al., 1997	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
3 doses of droloxifene	TTP	sHER2+	32	~3	~13	~13				MV Cox	0.0003	0.36 (0.21, 0.63)
(n=94 of 369)		sHER2-	62	~8	~43	~28						(adjusted)
	OS	sHER2+	32	~28	~74	~54				MV Cox	0.003	0.35 (0.17, 0.70)
		sHER2-	62		~92	LR ~63						(adjusted)
Sandri et al., 2004	Outcome	Grp	N	Mn (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)
cyclophosphamide+	TTP	sHER2+	?	2	~0	LR 0	~0			LR	0.007	
methotrexate (n=39)		sHER2-	?	8	~34	~12	~7					
	OS	sHER2+	?	11	~47	~0	LR 0			LR	<0.001	
		sHER2-	?	16	~84	~49	~42					

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Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 29. Single-arm studies, summary tumor response, KQ4

Study	**Tumor Response (%)**								
Im et al., 2005	Grp	N	CR	PR	SD	PD	Test	p	Comments
epirubicin+paclitaxel	sHER2+	4	0	75	25		Chi sq	0.45	WHO criteria
(n=40)	sHER2-	23	13.0	43.4	26.1	17.4			
Colomer et al., 2000	Grp	N	CR	PR	No response		Test	p	Comments
doxorubicin+paclitaxel	sHER2+	24	0	62	37		Chi sq	0.021	WHO criteria
(n=55)	sHER2-	31	26	52	23				MV logistic regression for ORR
	IHC+	11	9	55	36		Chi sq	0.219	sHER2 p value: 0.03
	IHC-	28	18	64	18				
Fornier et al., 2005	Grp	N	Response		No response		Test	p	Comments
paclitaxel+trastuzumab	sHER2+	38	50		50		FE	1.0	Response= CR+PR
(n=55)	sHER2-	17	47		43				criteria described
	Δ<15	25	68		32		FE	0.005	
	Δ≥15	13	15		85				
	Δ≥55%	25	68		32		FE	0.015	OR 4.25, 95% CI: 1.37-13.19
	Δ<55%	30	33		67				
Esteva et al., 2002	Grp	N	CR+PR		SD+PD		Test	p	Comments
trastuzumab+docataxel	sHER2+	21	76		24		FE	0.04	ECOG criteria
(n=30)	sHER2-		33		67				
	IHC 3+	19	63		37		FE	0.99	
	IHC 0-2+	9	5		60	40			
	FISH+	24	67		33		FE	0.60	
	FISH-	4	50		50				
Colomer et al., 2004	Grp	N	Response		No response		Test	p	Comments
paclitaxel+gemcitabine	sHER2+	15	42		58		FE	0.02	WHO criteria
(n=42)	sHER2-		83		17				
Luftner et al., 2004	Grp	N	CR+PR		SD	PD	Test	p	Comments
dose-intensified	sHER2+	26	22	40.9	36.4	22.7	MH	0.40	mean duration 25.7 wks
paclitaxel (n=35)	sHER2-	13	38.5		30.8	30.8			mean duration 65.2 wks (p=0.042)
									internationally accepted criteria (referenced)
Burstein et al., 2003	Grp	N	No progression			Progression			Comments
trastuzumab+	sHER2+	?							AU ROC=0.8947, baseline or Δ in sHER2 do not
vinorelbine (n=43)									predict response, but no ↓ in sHER2 predicted
	sHER2-	?							progression
									RECIST criteria

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Table 29. Single-arm studies, summary tumor response, KQ4 (continued)

Study	**Tumor Response (%)**						
Colomer et al., 2007	Grp	N	CR+PR	No response	Test	p	Comments
letrozole (n=226)	sHER2+	42	14	86		.036	
	sHER2-		31	70			
Yamauchi et al., 1997	Grp	N	Response	No response	Test	p	Comments
3 doses of droloxifene	sHER2+	184	32	9	FE	.00001	criteria?
(n=94 of 369)	sHER2-		62	56	44		MV logistic regression for response
							sHER2 p value: .0001
Colomer et al., 2006	Grp	N	CR+PR	91	No response	Test	p	Comments
IV vinorelbine+	sHER2+	14	50		50		.9	WHO criteria
IV gemcitabine (n=47)	sHER2-		48.5		51.5			

Abbreviations: AU: area under; Chi-sq: Chi square; CR: complete response; FE: fixed effects; Grp: group; MV: multivariate; ORR: overall response rate; PR: partial response; RECIST: Response Evaluation Criteria in Solid Tumors; ROC: receiver operating characteristic; s: serum; t: tissue; WHO: World Health Organization;

33

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Randomized trial, treatment by HER2 subgroup analysis. Among three randomized trials that described treatment by sHER2 subgroup analyses, Muller, Witzel, Luck, et al. (2004) reported on a subset of 101 patients with serum available, out of 597 patients (17 percent) randomized to epirubicin plus either paclitaxel (ET) or cyclophosphamide (EC). This study was a retrospective analysis of previously reported randomized trial. These authors found within the ET group a trend for worse overall survival for sHER2 positive patients (p=.092), but no significant difference between sHER2 groups receiving EC. Regarding progression-free survival, outcomes for the two treatments did not differ among the sHER2 negative, but results were significantly worse for EC among those sHER2 positive. For overall response rate, sHER2 groups did not differ among those receiving ET, but those getting EC had worse results when sHER2 was positive. No test for treatment by sHER2 interaction was reported.

These results should be viewed cautiously because the analyzed subset comprised less than 20 percent of those originally randomized and multivariate analysis was not used to adjust for any imbalances between treatments by sHER2 subgroups. Additionally, it is unclear sHER2 results were scored blindly with respect to outcome.

Lipton, Ali, Leitzel, et al. (2003) addressed 562 postmenopausal women given either letrozole or tamoxifen as first-line therapy for advanced breast cancer. This retrospective analysis included 62 percent of all patients randomized in the trial; however, this is the only randomized trial that used blinded assessment of sHER2 in relation to outcome. Results were better in terms of time-to-progression and time to treatment failure for those receiving letrozole, regardless of sHER2 status. For overall response rate and rate of clinical benefit (overall response plus stable disease), letrozole was significantly better than tamoxifen for sHER2 negative patients, but not for those sHER2 positive. No tests of treatment by sHER2 interaction were reported.

Cameron, Casey, Press, et al. (2008) randomized 399 patients with tissue HER2 positive locally advanced or metastatic breast cancer to receive capecitabine with or without lapatinib. Exploratory analyses of the relation between sHER2 status and progression-free survival were conducted in 92 percent of those randomized. When sHER2 was divided into the highest quartile versus other quartiles, both sHER2 subgroups had significantly better progression-free survival when treated with capecitabine plus lapatinib compared to capecitabine alone. This study did not describe the sHER2 assay methods clearly, did not report that sHER2 was scored blind to outcome and used an uncommon threshold for sHER2 positivity. No test of treatment by sHER2 status was reported.

Randomized trial results summary. The methodologic quality of these randomized trials is generally poor. Only one randomized trial was conducted with a prespecified plan to assess the relation of sHER2 to outcome. The same trial was the only one that conducted multivariate analyses, however it appeared to have too few events to support the large number of predictor and interaction terms used and the modeling techniques were overall poorly described. The other three trials performed retrospective treatment by sHER2 subgroup analyses of 17 percent, 62 percent and 93 percent of patients originally enrolled. Only one study used blinded assessment of sHER2 in relation to outcome.

These four randomized trials each addressed a different comparison of treatments. The only study that tested treatment by sHER2 status interactions found them to be nonsignificant for TTP and ORR in a comparison of paclitaxel with and without trastuzumab. A comparison of epirubicin either with paclitaxel or cyclophosphamide did not consistently find sHER2 to be related to different treatment outcomes (OS, PFS, ORR). A trial comparing letrozole and

tamoxifen found sHER2 to be a more consistent predictor of treatment outcome for TTP and TTF, less so for ORR and clinical benefit. A trial of capecitabine with or without lapatinib found better PFS for those receiving combination treatment for both those in the highest quartile and lower quartiles of sHER2 values. Only the Gasparini, Gion, Mariani, et al. (2007) trial, which analyzed nearly all patients randomized, used multivariate methods, while the other two trials used univariate analyses of much smaller subsets of those randomized.

Single-arm study, multivariate analysis. Among three single-arm studies that conducted multivariate analysis, Colomer, Llombart-Cussac, Lloveras, et al. (2007) included 226 patients with metastatic breast cancer who received letrozole. The authors prespecified their interest in assessing the relation between sHER2 status and treatment outcomes; however they provided inadequate detail in describing Cox regression methods such as selection of candidate variables, model-building strategy, testing of assumptions, forcing of standard prognostic variables and handling of continuous variables. It is unclear if sHER2 results were scored blind to outcomes and validation of the final model was not mentioned. The multivariate analysis found sHER2 and ECOG performance status to be significant independent predictors of time to progression.

Colomer, Montero, Lluch, et al. (2000) included 55 patients with metastatic disease who were receiving first-line doxorubicin and paclitaxel. Of the 77 patients originally enrolled in this Phase II study, 75 percent had evaluable serum samples. The plan to assess the relation between sHER2 and outcome was prespecified in this study; however, the multivariate logistic and Cox regression techniques were poorly described. It is unclear how candidate variables were selected, what model-building strategy was used, whether assumptions were tested, whether final models included all standard prognostic variables and whether continuous variables were well handled. Furthermore, models did not appear to be validated and it is unclear if sHER2 was scored blindly to outcome. In the logistic regression of response, there were only 39 events, but six variables entered into the multivariate model (more than the recommended one variable per greater than 10 events). A similar problem existed for the Cox regression of response duration. These authors found elevated sHER2 to be significantly associated with poorer results on response duration and overall response rate, in both univariate and multivariate analyses.

The study by Yamauchi, O'Neill, Gelman, et al. (1997) was originally a randomized comparison of three doses of droloxifene as first-line hormonal therapy. Of the 369 patients randomized, 94 were included in this retrospective analysis (25 percent). Logistic regression of overall response and Cox regression of time-to-progression and overall survival all used the stepwise model building strategy, a method with major weaknesses. The description of modeling methods was poor, lacking details on: candidate variable selection, whether assumptions were tested, whether final models included standard prognostic variables and whether continuous variables were well handled. The article did not make clear whether sHER2 results were scored blindly to outcome. Multivariate analyses entered dose into models but was not retained, suggesting similar results by different doses and dose groups were pooled. After adjustment for other variables, this study found consistently worse results for sHER2 positive patients on time to progression, overall survival and overall response rate.

Single-arm study, univariate analysis. These studies reported on 55 patients or fewer. With the exception of the study by Esteva, Valero, Booser, et al. (2002), positive sHER2 results were associated with worse outcomes. The lack of multivariate analyses in these studies makes these findings of limited use for guiding treatment decisions. These studies could be described as exploratory, hypothesis-generating investigations that might inform future, more sophisticated studies.

Single-arm study results summary. This body of evidence is quite heterogeneous with respect to treatment regimens, outcomes assessed, and definitions of elevated sHER2. Only three of 11 studies conducted multivariate analyses, but the modeling methods were poorly described. Evidence from single-arm series more often shows that sHER2 status predicts outcomes among patients treated, however, there were several instances in which it was nonpredictive and one study found better response among those with elevated sHER2 in conflict with all other studies.

Conclusions, Key Question 4

The evidence is weak on whether sHER2 predicts outcome after treatment with any regimens in any setting. Evidence primarily focused on first-line or second- and subsequent-line treatment of metastatic disease using variety of regimens. Furthermore these studies used different thresholds for a positive sHER2 result and varied on whether patient selection required positive tissue HER2 status. There were only four randomized trials and only one used multivariate analysis, while three single-arm studies performed multivariate analysis. The quality of reporting on multivariate analyses lacked sufficient detail. Univariate analyses provide very limited information value, suggesting candidate variables for future multivariate analyses. These studies do not support clear conclusions for whether sHER2 predicts disease progression, treatment response, or outcomes of any specific treatment regimen.

Key Question 5

In patients with ovarian, lung, prostate, or head and neck cancers, what is the evidence that:

a. testing tumor tissue for HER2; or

b. monitoring serum or plasma concentrations of HER2;

either predicts response to therapy, or detects tumor progression or recurrence; and if so, what is the evidence that decisions based on her2 assay results improve patient management and outcomes?

Study Selection

Studies were included for Key Question 5 if they were:

- randomized trials, prospective single-arm studies, or retrospective series of identically treated patients; that
- measured HER2 in tumor tissue, serum, or plasma from patients with ovarian, lung, prostate, or head and neck cancers, and either:
    a. associated HER 2 status from tissue assays, or baseline values or changes in serum or plasma HER2 concentration, with one or more outcomes of interest (primary or secondary; see above); or
    b. compared outcomes of treatment decisions based on tumor HER2 status, or serum or plasma assay results, with outcomes of decisions made in absence of test results.

Part I. Lung Cancer

Overview. A total of 13 studies met study selection criteria (total N=1,500 patients). The study by Krug, Miller, Patel, et al. (2005) was originally a randomized comparison of trastuzumab plus either docetaxel or paclitaxel, but which combined the two treatment arms. Thus, the Krug and co-workers study is treated as a single-arm design and is presented with 12 other single-arm studies. Study hierarchy, quality assessment, summary descriptions, and results are summarized in Tables 30–34; detailed abstraction data for all parts of Key Question 5 can be found in Appendix Tables V-A–V-RR*.

Study populations. All studies were single-arm designs that included patients with non-small cell lung cancer (NSCLC). Of the 13 studies, 5 addressed the use of surgery without adjuvant treatments. Four of these studies included early stage (I or II) patients (Koukourakis, Giatromanolaki, Guddo, et al., 2000; Koukourakis, Giatromanolaki, O'Byrne, et al., 1999; Saad, Liu, Han, et al., 2004; Pelosi, Del Curto, Dell'Orto, et al., 2005) and the fifth study included a range of patients across stages I–IV (Pfeiffer, Clausen, Andersen, et al., 1996). The eight studies of systemic or multimodality treatments included patients with locally advanced, recurrent or late stage (III-IV) disease. Eight studies report summary age data; all average age values (means or medians) were in the 50s and 60s. Five studies examined outcomes of treatment with gefitinib for advanced NSCLC (Cappuzzo, Ligorio, Janne, et al., 2007; Daniele, Macri, Schena, et al., 2007; Cappuzzo, Gregorc, Rossi, et al., 2003; Hirsch et al., 2005; Cappuzzo, Varella-Garcia, Shigematsu, et al., 2005). Two studies gave combination chemotherapy regimens that included trastuzumab and a taxane to patients with advanced NSCLC (Krug, Miller, Patel, et al., 2005; Langer, Stephenson, Thor, et al., 2004). The remaining study offered multi-modality therapy (chemotherapy, surgery and radiotherapy) to patients with stage IIIA NSCLC (Graziano, Kern, Herndon, et al., 1998).

Results by hierarchy level, study quality assessment.

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Randomized trial, prespecified multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, post-hoc multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, treatment by HER2 subgroup analysis. No studies of this type were identified.

Single-arm study, prespecified multivariate analysis. No studies of this type were identified.

* Appendixes cited in this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/her2/her2.pdf.

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 30. Hierarchy of evidence, KQ5, lung cancer

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA						
RCT post-hoc MV SGA						
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis	Koukourakis 1999	189	NSCLC T1-2, N0-1	surgery	OS	univariate: IHC HER2 not associated with OS Cox regression: IHC HER2 not entered in model
	Cappuzzo 2005	101	locally advanced, metastatic NSCLC	gefitinib	ORR	univariate: IHC HER2+↑ vs. – p=.001
					ORR	Cox regression IHC HER2+↑ vs. – p=.08
					OS	univariate IHC HER2+↑ vs. – p=.056 (discrepancies)
					TTP	univariate IHC HER2+↑ vs. – p=.02 (discrepancies)
	Hirsch 2005	56	stage IIIB/IV BAC. BAC-like AC	gefitinib	OS	univariate FISH HER2+ vs. – p=.80
					OS	Cox regression FISH HER2 not entered in model
					ORR	univariate FISH HER2+ vs. – p>.05
	Saad 2004	100	stage I AC/BAC	surgery	OS	univariate AC IHC HER2+↓ vs. – p=signif
					OS	Cox regression AC IHC HER2+↓ vs. – signif independent predictor
					OS	univariate BAC IHC HER2+↓ vs. – p=signif
					OS	Cox regression BAC IHC HER2+↓ vs. – signif independent predictor

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 30. Hierarchy of evidence, KQ5, lung cancer (continued)

Level of Evidence	Study	n	Setting	Treatments		Outcome	Results
1-arm UV analysis	Cappuzzo 2007	42	stage III/IV NSCLC	gefitinib	Resp	FISH HER2+↑ vs. – p=.007	
					TTP	FISH HER2+ vs. – p=.2	
					OS	FISH HER2+ vs. – p=.1	
	Daniele 2007	42	stage III/IV NSCLC	gefitinib	Resp	FISH/CISH+↑ vs. – p=.0005	
	Krug 2005	65	stage IIIB/IV NSCLC	docet/paclit +trastuz	OS	IHC HER2+ vs. – p=NS	
	Pelosi 2005	345	stage I NSCLC	surgery	OS	FISH HER2+ vs. – p=NS	
					DFS	FISH HER2+ vs. – p=NS	
	Langer 2004	56	stage IIIB/IV recurrent NSCLC	trastuz+ paclit+ carbopl	OS	IHC HER2 3+ vs. 2+ vs. 1+ p=.77	
					PFS	IHC HER2 3+ vs. 2+ vs. 1+ p=.34	
	Cappuzzo 2003	63	stage IIIB/IV NSCLC	gefitinib	TTP	IHC HER2+ vs. – p=NS	
					OS	IHC HER2+ vs. – p=NS	
					ORR	IHC HER2+ vs. – p=.126	
	Koukourakis 2000	112	T1-2, N0-1 NSCLC	surgery	OS	IHC HER2+ vs. – p=NS	
	Graziano 1998	66	stage IIIA NSCLC	cispl+etop (PE), surgery,PE, RT	OS	IHC HER2+ vs. – p=.617	
					ORR	IHC HER2+ vs. – p=.999	
	Pfeiffer 1996	186	stage I-IV NSCLC	surgery	OS	IHC HER2 none vs. low vs. high p=NS	

Abbreviations: carbopl: carboplatin; cispl: cisplatin; DFS: disease-free survival; etop: etoposide; HR: hazard ratio; MV: multivariate; ORR: overall response rate; OS: overall survival; paclit: paclitaxel; pCR: pathologic complete response; PFS: progression-free survival; RCT: randomized, controlled trial; RFS: recurrence-free survival; SGA: subgroup analysis; trastuz: trastuzumab; TTP: time to progression; Tx: treatment; UV: univariate;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer
Table 31. Study quality assessment, KQ5, lung cancer

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long followup	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation					
									1)	2)	3)	4)	5)	6)
Koukourakis et al., 1999	N	N	N	Y	?	Y	Y	?	?	?	?	?	?	N
Cappuzzo et al., 2005	Y	N	?	Y	?	Y	N	?	?	?	?	?	?	N
Hirsch et al., 2005	Y	N	?	Y	?	Y	N	?	?	N	?	?	?	N
Saad et al., 2004	N	N	N	Y	Y	Y	Y	AC: 52 ± 20 mo; BAC: 40 ± 17 mo	?	?	?	?	?	N
Cappuzzo et al., 2007	Y	Y	N	Y	?	Y	Y	≥ 4 wk	NA					
Daniele et al., 2007	N	N	N	Y	?	Y	Y	med 14.8 mos	NA					
Krug et al., 2005	Y	N	N	N	?	Y	Y	?	NA					
Pelosi et al., 2005	N	N	Y	Y	?	Y	Y	NET/NSCLC 53.3 ± 53.6 mo/: 72.6 ± 49.3 mos	NA					
Langer et al., 2004	Y	N	N	N	?	Y	Y	med 34 mos	NA					
Cappuzzo et al., 2003	Y	Y	N	Y	?	Y	Y	?	NA					
Koukourakis et al., 2000	N	N	N	Y	?	Y	Y	med 46 mos	NA					
Graziano et al., 1998	Y	Y	N	N	?	Y	N	?	NA					
Pfeiffer et al., 1996	N	N	N	Y	?	Y	Y	66 mos (40-119)	NA					

Abbreviations: AC: adenocarcinoma; BAC: bronchoalveolar carcinoma; NSCLC: non-small cell lung cancer

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 32. Single-arm studies: summary design, treatment, patient characteristics, KQ5, lung cancer

Study	Therapeutic Setting	Treatments	Age	Stage (%)	Performance Status (%)	Other Prognostic Factors (%)
Cappuzzo et al., 2007	Stage IIIb/IV NSCLC, never smoked or EGFR FISH+/Akt+	Gefitinib (n=42) 1st/2nd+-line	md 60.9 rng 43-80	IIIB 7 IV 93	ECOG 0 71 1 19 2 10	Never smoker 86 Former 10 Current 5
Daniele et al., 2007	Stage III/IV NSCLC	Gefitinib (n=42) 1st+-line	Resp md 60 rng 37-77 No Resp md 63 rng 37-77	III 5 IV 95		
Krug et al., 2005	Stage IIIB or IV NSCLC	Docetaxel+trastuzumab (Grp1, n=31) or paclitaxel+trastuzumab (Grp2, n=34)		Grp1 Grp2 IIIB 10 18 IV 18 82	KPS Grp1 Grp2 90 35 26 80 52 62 70 13 12	Grp1 Grp2 Bone mets 37 21 Wt loss >5% 10 18
Cappuzzo et al., 2005	Locally advanced/ metastatic NSCLC, progressed after CHT/ medical contraindications to CHT	Gefitinib (n=101)				
Hirsch et al., 2005	Stage IIIB or IV bronchioalveolar adenocarcinoma (BAC) or adenocarcinoma with BAC features	Gefitinib (n=56), 1st-line, 2nd+-line	md 68 rng 34-88	IIIB or IV	SWOG 0 46 1 43 2 11	Smoker 71 Never smoker 29
Pelosi et al., 2005	Stage I NSCLC	Surgery with no (neo)adjuvant tx (n=345)	Males mn 63.4 md 64 rng 35-82 sd 8.1 Females mn 61.6 md 62 rng 41-80 sd 8.8	I		

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 32. Single-arm studies: summary design, treatment, patient characteristics, KQ5, lung cancer (continued)

Study	Therapeutic Setting	Treatments	Age	Stage (%)	Performance Status (%)	Other Prognostic Factors (%)
Saad et al., 2004	stage I conventional AC (n=50) and BAC (n=50)	complete surgical resection, no CHT or RT	AC mn 64 sd 11 BAC mn 57 sd 7	I		
Langer et al., 2004	Recurrent, stage IV, or stage IIIB NSCLC, HercepTest 1+/2+/3+	Trastuzumab, paclitaxel and carboplatin (n=56)	md 59 rng 31-77	IIIB 9.4 IV 81.2 recurrent 9.4	fully active: 52.8 ambulatory, light work: 47.2	
Cappuzzo et al., 2003	Stage IIIB/IV NSCLC, pretreated with 1st-line platinum-based CHT and RT	2nd+-line gefitinib (n=63)	mn 58.5 rng 31-79	IIIB 17.5 IV 82.5	ECOG 0 25 1 64 2 11	
Koukourakis et al., 1999	Surgically treated NSCLC	Surgery alone (n=189)	<60 26% >60 74%	T1 29.2 T2 70.8 N0 56.5 N1 43.5		
Koukourakis et al., 2000	Operable NSCLC T1,2-N0,1	Surgery alone without RT or CHT (n=112)	md 63 rng 45-76	T1 37 T2 63 N0 37.5 N1 62.5		
Graziano et al., 1998	Stage IIIA NSCLC with ipsilateral mediastinal node involvement	cisplatin-etoposide (PE), surgery, PE, RT (n=66)				
Pfeiffer et al., 1996	NSCLC	Surgery without adjuvant RT (2 pts had adjuvant CHT; n=186)	mn 61 rng 42-79 IIIA	Ia 46.2 II 25.8 IIIa 22.0 IIIb 1.6 IV 4.3		

Abbreviations: AC: adenocarcinoma; BAC: bronchoalveolar carcinoma;CHT: chemohormonal therapy; Grp: group; KPS: Karnofsky performance score; md: median; mn: mean; NSCLC: non-small cell lung cancer; SWOG: Southwest Oncology Group; rng: range; RT: radiation therapy; sd: standard deviation;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 33. Single-arm studies, summary time to event outcomes, KQ5, lung cancer

Study	Time to Event Outcomes												
Cappuzzo et al., 2007; gefitinib	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	FISH+	20	6.4									
		FISH-	16	3.1									
	OS	FISH+	20	not reached									
		FISH-	16	10.4									
Krug et al., 2005 docetaxel/ paclitaxel + trastuzumab	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC+	20	~14	~65	~30	~20	~20			NS		IHC 2+/3+
		IHC-	45	~16	~59	~33	~16	~10					
Cappuzzo et al., 2005 gefitinib	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	23	20.8	60.9	~38					0.056		discrep-
		FISH-	78	8.4	37.2	~15							ancies
	TTP	FISH+	23	9.05	34.8						0.02		discrep-
		FISH-	78	2.7	9.0	~5							ancies
Hirsch et al., 2005 gefitinib	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	17	16	~64	~26					0.80		Cox HER2
		FISH-	39	13	~61	~35							not entered
Pelosi et al., 2005 surgery	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	5		100	~80	~60	~60	~60	LR	NS		
		FISH-	340		94	~88`	~80	~72	~70				
	DFS	FISH+	5		~80	~60	~60	~60	~60	LR	NS		
		FISH-	340	~13 yrs	~86	~77`	~74	~70	~65				
Saad et al., 2004 surgery	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	AC-IHC+	19	~24	~81	~50	~18	~0		?	signif		Cox: HER2
		AC-IHC-	31	~43	~96	~75	~54	~41					independent
		BAC-IHC+	9	~39	~91	~63	~27	~0	~0	?	signif		Cox: HER2
		BAC-IHC-	41	~30	~100	~100	~50	~30	~19				independent
Langer et al., 2004 trastuzumab, paclitaxel and carboplatin	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC3+	8	10.9	37.5	25					0.77		
		IHC2+	23	8.6	26.1	13.5							
		IHC1+	22	14.3	59.1	11.4							
	PFS	IHC3+	8	2.7	-						0.34		
		IHC2+	23	3.8	~9								
		IHC1+	22	3.9	~6								

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 33. Single-arm studies, summary time to event outcomes, KQ5, lung cancer (continued)

Study	Time to Event Outcomes												
Cappuzzo et al., 2003 gefitinib	Outcome TTP OS	Grp IHC 2+/3+ IHC 0 /1+ IHC 2+/3+ IHC 0 /1+	N 15 28 15 28	Med (mos) 3.5 3.7 5.7 6.8	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p NS NS	HR (95%CI)	Comments
Koukourakis et al., 1999; surgery	Outcome OS	Grp IHC+ IHC-	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p 0.51	HR (95%CI)	Comments Cox HER2 not entered
Koukourakis et al., 2000 surgery	Outcome OS	Grp IHC+ IHC-	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p NS	HR (95%CI)	Comments
Graziano et al., 1998 cisplatin-etoposide (PE), surgery, PE, RT	Outcome OS	Grp IHC+ IHC-	N 10 37	Med (mos) 10.5 17.5	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p 0.617	HR (95%CI)	Comments
Pfeiffer et al., 1996 surgery	Outcome OS	Grp IHC-none IHC-low IHC-high	N 29 108 49	Med (mos) ~34 ~24 ~24	1 yr ~75 ~58 ~75	2 yr ~66 ~50 ~50	3 yr ~42 ~38 ~40	4 yr ~32 ~33 ~30	5 yr ~20 ~25 ~25	Test LR	p NS	HR (95%CI)	Comments

Abbreviations: AC: adenocarcinoma; BAC: bronchoalveolar carcinoma; DFS: disease-free survival; Grp: group; LR: log rank; NS: not significant; OS: overall survival; RT: radiation therapy; TTP: time to progression;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 34. Single-arm studies, summary tumor response, KQ5, lung cancer

Study	Tumor Response (%)								
Cappuzzo et al., 2007	Grp	N	Response		No Response		Test	p	Comments
	FISH+	20	70					.007	
	FISH-	16	25						
Daneile et al., 2007	Grp	N	Response		No Response		Test	p	Comments
	FISH/ CISH+	13	69					.0005	
	FISH/ CISH-	29	10						
Krug et al., 2005 docetaxel/ paclitaxel + trastuzumab	Grp	N	CR	PR	SD	PD	Test	p	Comments
Cappuzzo et al., 2005 gefitinib	Grp	N	CR+PR		SD	PD	Test	p	Comments
	FISH+	23	34.8				Chi sq	.001	
	FISH-	78	6.4					.08	Cox MV adjusted for EGFR mutation HR 0.22 (95% CI: 0.04, 1.21)
	Grp	N	CR+PR+SD			PD	Test	p	Comments
	FISH+	23	56.5				Chi sq	.04	
	FISH-	78	33.3						
Hirsch et al., 2005 gefitinib	Grp	N	CR+PR		SD	PD	Test	p	Comments
	FISH+	11	36%					>.05	
	FISH-	28	46%						
Pelosi et al., 2005 surgery	Grp	N	CR	PR	SD	PD	Test	p	Comments
Saad et al., 2004 surgery	Grp	N	CR	PR	SD	PD	Test	p	Comments
Langer et al., 2004 trastuzumab, paclitaxel and carboplatin	Grp	N	CR	PR	SD	PD	Test	p	Comments
					?				
Cappuzzo et al., 2003 gefitinib	Grp	N	CR	PR	SD	PD	Test	p	Comments
	IHC 2+/3+	15		13.3	26.7			.126	
	IHC 0/1+	28		14.3	50.0				
Koukourakis et al., 1999 surgery	Grp	N	CR	PR	SD	PD	Test	p	Comments
						ChiSq			

Question 5: HER2 Results to Guide Treatment Regimen Selection, Lung Cancer

Table 34. Single-arm studies, summary tumor response, KQ5, lung cancer (continued)

Study	**Tumor Response (%)**								
Koukourakis et al., 2000 surgery	Grp	N	CR	PR	SD	PD	Test	p	Comments
Graziano et al., 1998 cisplatin-etoposide (PE), surgery, PE, RT	Grp HER2+ HER2-	N 10	Response 30 33				Test FE	p .999	Comments
Pfeiffer et al., 1996 surgery	Grp	N	CR	PR	SD	PD	Test	p	Comments

Abbreviations: Chi sq: Chi square; CR: complete response; EGFR: epidermal growth factor receptor; FE: fixed effects; Grp: group; PD: progressive disease; PR: partial response; RT: radiation therapy; SD: stable disease;

Single-arm study, post-hoc multivariate analysis. Of the 13 studies, four conducted multivariate analyses, none of which was prespecified. Two multivariate analyses addressed surgery for early stage NSCLC (Koukourakis, Giatromanolaki, O'Byrne, et al., 1999; Saad, Liu, Han, et al., 2004) and two that offered gefitinib to patients with advanced NSCLC (Cappuzzo, Varella-Garcia, Shigematsu, et al., 2005; Hirsch, Varella-Garcia, McCoy, et al., 2005). Among the surgical series, the retrospective series by Koukourakis and co-workers found that HER2 in univariate analysis was not associated with overall survival and was, therefore, not entered into a multivariate model. This multivariate analysis was generally poorly described. Details were lacking about how candidate variables were selected, how models were constructed, whether assumptions were tested, whether standard prognostic factors were include in final models and whether continuous variables were well handled. In addition, no mention was made of model validation. Saad and co-workers analyzed two separate retrospective groups of 50 surgical patients with adenocarcinoma (AC) and bronchioalveolar carcinoma (BAC) of the lung. For both subgroups, HER2 was significant univariate and multivariate predictor of overall survival, however very few details were provided for the multivariate analyses. The Saad, Liu, Han, et al. (2004) study is the only multivariate analysis which clearly used an assessor of HER2 results who was blinded to outcome.

Among prospective gefitinib series, Hirsch, Varella-Garcia, McCoy, et al. (2005) found that HER2 was not a significant univariate predictor of overall survival and was not entered in the multivariate model; HER2 was also not associated with response. These authors used a stepwise multivariate model selection procedure, a weak method, and otherwise provided few details about analytic techniques. Hirsch and co-workers also found that FISH-negative patients had a higher response rate than FISH-positive patients in univariate analysis. The Cappuzzo, Varella-Garcia, Shigematsu, et al. (2005) study of gefitinib reported a significant univariate association with overall response rate that nearly achieved statistical significance in multivariate analysis. Univariate analyses by Cappuzzo and co-workers (2005) of overall survival and time to progression appeared significant, but the article was flawed with discrepancies in reporting of results, and poor reporting of multivariate analysis methods.

Single-arm study, univariate analysis. Nine single-arm studies conducted univariate analyses of the association between HER2 status and outcomes; five were prospective designs and 4 were retrospective. Three studies addressed use of surgery alone as treatment (Pelosi, Del Curto, Dell'Orto, et al., 2005; Koukourakis, Giatromanolaki, Guddo, et al., 2000; Pfeiffer, Clausen, Andersen, et al., 1996), 3 studies gave patients gefitinib (Cappuzzo, Ligorio, Janne, et al., 2007; Daniele, Macri, Schena, et al., 2007; Cappuzzo, Gregorc, Rossi, et al., 2003), two studies involved trastuzumab-based combination regimens (Krug, Miller, Patel, et al., 2005; Langer, Stephenson, Thor, et al., 2004) and one study used multimodality therapy entailing chemotherapy, surgery and radiotherapy (Pfeiffer, Clausen, Andersen, et al., 1996). The Krug, Miller, Patel, et al. (2005) study was originally a randomized trial of trastuzumab plus either docetaxel or paclitaxel. Since no difference in efficacy was seen between the two taxane groups, Krug and co-workers combined arms to assess the relation between HER2 and outcome; thus, this study is treated for purposes of this analysis as a single-arm design. None of these seven studies reported significant associations between HER2 and overall survival, disease-free survival, progression-free survival, time-to-progression or overall response rate.

Evidence summary–lung cancer. Overall, the evidence on the relation between HER2 and outcome for treatment of lung cancer is weak and heterogeneous. No randomized studies have analyzed whether there are HER2 by treatment effect interactions. Of 13 single-arm studies, only

4 were multivariate analyses. All four multivariate analyses were poorly described, and none were prespecified, thus it is unclear if they were well-conducted. The two multivariate studies of surgery for early stage NSCLC found conflicting results (one study suggesting HER2 is predictive, one study did not). Similar results were found in the two multivariate studies of gefitinib. Seven studies were univariate analyses of single-arm studies. Univariate analyses provide very limited information value, at best suggesting candidate variables for future multivariate analyses. Future research should place studies at higher levels in the evidence hierarchy. The body of evidence is not promising, with mixed results among post-hoc multivariate analyses and lack of significant findings among univariate studies.

Part II. Ovarian Cancer

Overview. A total of seven studies met study selection criteria (578 patients). The study by Camilleri-Broet, Hardy-Bessard, Le Tourneau, et al. (2004) was originally a randomized trial comparing cisplatin, epirubicin and one of two doses of cyclophosphamide. Efficacy results did not differ between cyclophosphamide dose groups, so results of the two arms were combined in this retrospective analysis. This study is, therefore, treated as a single-arm design using a retrospective (post-hoc) multivariate analysis. A randomized trial by Malamou-Mitsi, Crikoni, Timotheadou, et al. (2007) is similarly treated as a single-arm design, in which two groups treated with paclitaxel and platinum compounds are pooled in a retrospective multivariate analysis. The third multivariate analysis of a single-arm study was prespecified by Di Leo, Bajetta, Biganzoli, et al. (1995). The remaining four studies were single-arm studies that presented univariate analyses. Study hierarchy, quality assessment, summary descriptions, and results are arrayed in Tables 35–39.

Study populations. All seven studies included patients with ovarian cancer that was either advanced or relapsed/refractory. Six studies focused on chemotherapy regimens, including one study using cisplatin, epirubicin and cyclophosphamide (Camilleri-Broet, Hardy-Bessard, Le Tourneau, et al., 2004), one that used paclitaxel and a platinum compound (Malamou-Mitsi, Crikoni, Timotheadou, et al., 2007); one that used a platinum compound and cyclophosphamide (Hengstler, Lange, Kett, et al., 1999), one that used liposomal doxorubicin (Campos, Penson, Mays, et al., 2001) and one that combined mitoxantrone and ifosfamide (Di Leo, Bajetta, Biganzoli, et al., 1995). One study gave the hormonal agent, letrozole (Bowman, Gabra, Langdon, et al., 2002) and one study offered patients trastuzumab (Bookman, Darcy, Clarke-Pearson, et al., 2003). Median age values of five studies reporting them were in the 50s in four studies and in the 60s in two studies. Distributions of tumor grade in five studies placed the majority of patients in moderately or poorly differentiated categories.

Results by hierarchy level, study quality assessment.

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Question 5: HER2 Results to Guide Treatment Regimen Selection, Ovarian Cancer

Table 35. Hierarchy of evidence, KQ5, ovarian cancer

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA						
RCT post-hoc MV SGA						
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis	Di Leo 1995	72	relapsed/ refractory stage III-IV OC	mitoxant+ ifosfam	Resp TTF OS	univariate IHC HER2+ vs. – p=NS Cox regression IHC HER2 + vs. – p=NS Cox regression IHC HER2 + vs. – p=NS
1-arm post-hoc MV analysis	Malamou-Mitsi 2007	95	stage IIc-IV OC	pacl+carb/ cispl	TTP OS	Cox regression IHC HER2 + vs. – p=NS Cox regression IHC HER2 + vs. – p=NS
	Camilleri-Broet 2004	117	advanced OC	cispl+ epirub + cycloph	PFS PFS OS OS	univariate IHC HER2+ vs. – RR 2.13 (95% CI: 1.13, 4.01) Cox regression IHC HER2+ vs. – RR 2.08 (95% CI: 1.11, 3.91) univariate IHC HER2+ vs. – RR 2.07 (95% CI: 1.03, 4.17) Cox regression IHC HER2+ vs. – RR 2.07 (95% CI: 1.03, 4.15)
1-arm UV analysis	Bookman 2003	41	recurrent/ persistent OC primary peritoneal carcinoma tHER2 2+/3+	trastuz	PFS OS Resp Tox	no relationship between IHC HER2 and PFS no relationship between IHC HER2 and OS no relationship between IHC HER2 and clinical response cycle 1 toxicity IHC HER2 2+↓ vs. 3+ p=0.023
	Bowman 2002	50	relapsed OC	letrozole	Progr	IHC HER2 high vs. low p=0.026, CA125 progression
	Campos 2001	70	relapsed/ refractory OC	liposomal doxorub	Resp	IHC HER2+ vs. – p=0.579, CA125 response
	Hengstler 1999	44	OC	standard carbopl/cispl +cycloph	OS	RNA PCR HER2 high vs. low p=0.0003

Abbreviations: carbopl: carboplatin; cispl: cisplatin; DFS: disease-free survival; etop: etoposide; HR: hazard ratio; ifosfam: ifosfamide; mitoxant: mitoxantrone; MV: multivariate; OC: ovarian cancer; OS: overall survival; pacl: paclitaxel; PFS: progression-free survival; Prog: progression; Resp: response; RFS: recurrence-free survival; RR: relative risk; SGA: subgroup analysis; Tox: toxicity; trastuz: trastuzumab; TTF: time to treatment failure; TTP: time to progression; Tx: treatment; UV: univariate;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Ovarian Cancer
Table 36. Study quality assessment, KQ5, ovarian cancer

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long followup	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation
									1) 2) 3) 4) 5) 6)
Malamou-Mitsi et al., 2007	Y	N	N	Y	?	Y	Y	?	Y N ? Y ? N
Di Leo et al., 1995	Y	Y	N	N	Y	Y	Y	?	? ? ? ? ? N
Camilleri-Broet et al., 2004	Y	N	N	Y	?	Y	N	median 68 months	? ? ? ? ? N
Bookman et al., 2003	Y	N	N	Y	Y	Y	Y	8 weeks	NA
Bowman et al., 2002	Y	N	N	N	?	Y	N	?	NA
Campos et al., 2001	N	N	N	Y	?	Y	Y	≥ 30 days	NA
Hengstler et al., 1999	N	N	N	Y	?	Y	Y	?	NA

Question 5: HER2 Results to Guide Treatment Regimen Selection, Ovarian Cancer
Table 37. Single-arm studies, design, enrollment and treatment, KQ5, ovarian cancer

Study	Therapeutic Setting	Treatments	Age	Stage (%)	Performance Status (%)	Tumor Grade (%)
Malamou-Mitsi et al., 2007	stage IIc-IV epithelial ovarian cancer	paclitaxel+carboplatin /cisplatin, single regimen vs. alternating regimens (n=95)	Grp1 not alternating md 61 rng 27-77.5 Grp2 alternating md 64 rng 34.5-76	Grp1 Grp2 IIc 7 12 III 82 70 IV 11 18	Scale? Grp1 Grp2 0 51 58 1 42 30 2 7 12	Grp1 Grp2 I 7 14 II 38 28 III 53 58 ? 2 0
Camilleri-Broet et al., 2004	advanced ovarian carcinoma	cisplatin + epirubicin + cyclophosphamide (n=117)	md 59 rng 23-70	IIIa 4 IIIb 25 IIIc 65 IV 22 (1 missing)	WHO 0 32 1 70 2 9 (6 missing)	well 0 mod w/o nucl atyp 15 mod w/ nucl atyp 47 poor/un w/ nucl atyp 34 (21 missing)
Bookman et al., 2003	recurrent or persistent ovarian or primary peritoneal carcinoma, tIHC 2+/3+	trastuzumab (n=41)	md 59 rng 44-82		0 25 1 16	well 1 mod 9 poor 31
Bowman et al., 2002	previously treated relapsed ovarian carcinoma	letrozole (n=50)	md 65 rng 43-83		WHO 0-2	well 3 mod 13 poor 39 not documented 5
Campos et al., 2001	previously treated relapsed and refractory ovarian carcinoma	liposomal doxorubicin (n=70)	md 57 rng 31-77	GOG Ia 4 IIIa 52 IV 16	Scale? 0 18 1 20 2 13 3 2 Unk 19	Poor 42
Hengstler et al., 1999	primary epithelial ovarian carcinoma	standard carboplatin or cisplatin + cyclophosphamide (n=44)		20 III/IV 50		
Di Leo et al., 1995	previously treated relapsed and refractory stage III-IV ovarian carcinoma	mitoxantrone + ifosfamide (n=72)	md 57 rng 39-74 I/II		0 29 1 35 2 8	well 11 mod 20 poor 27 not documented 14

Abbreviations: ECOG: Eastern Cooperative Oncology Group; GOG: Gynecologic Oncology Group; Grp: group; md: median; rng: range; Unk: unknown; w/o: without;

ECOG

Question 5: HER2 Results to Guide Treatment Regimen Selection, Ovarian Cancer
Table 38. Single-arm studies, summary time to event outcomes, KQ5, ovarian cancer

Study	**Time to Event Outcomes**												
Malamou-Mitsi et al., 2007	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	IHC+	17	~16	~59	~33	~29	~29	~29	LR	0.96		
		IHC-	78	~18	~67	~41	~38	~25	~22				
	OS	IHC+	17	~32	~78	~65	~41	~41	~33	LR	0.60		
		IHC-	78	~39	~85	~69	~53	~36	~36				
Camilleri-Broet et al., 2004 cisplatin + epirubicin + cyclophospha-mide	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	RR (95%CI)	Comments
	PFS	HER2+ pts	15	12	~58	~8	0			Cox	0.02	2.13 (1.13-4.01)	UV
		HER2- pts	102	15	~70	~30	~20	~9	~6	Cox	0.02	2.08 (1.11-3.91)	MV
	OS	HER2+ pts	15	25	~86	~46	~20	0		Cox	0.02	2.07 (1.03-4.17)	UV
		HER2- pts	102	35	~92	~52	~46	~36	~28	Cox	0.04	2.07 (1.03-4.15)	MV
Bookman et al., 2003 trastuzumab	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	PFS		41										no relation between IHC expression level and PFS/OS
	OS		41										
Bowman et al., 2002 letrozole	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Campos et al., 2001 liposomal doxorubicin	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS												
Hengstler et al., 1999 carboplatin or cisplatin + cyclophospha-mide	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	low HER2		101	~90	~86	~86	~65	~65	LR	0.0003		
		mod HER2		99	~100	~92	~92	~68	~59				
		high HER2		17	~82	~18	~18	~9	~9				
Di Leo et al., 1995 mitoxantrone + ifosfamide	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments

Abbreviations: Grp: group; HR: hazard ratio; LR: log rank; Med: median; mod: moderate; mos: months; MV: multivariate; OS: overall survival; PFS: progression-free survival; TTP: time to progression; UV: univariate; yr: year(s);

Question 5: HER2 Results to Guide Treatment Regimen Selection, Ovarian Cancer

Table 39. Single-arm studies, summary tumor response, KQ5, ovarian cancer

Study	Tumor Response (%)									
Malamou-Mitsi et al., 2007	Not reported									
Camilleri-Broet et al., 2004 cisplatin + epirubicin + cyclophospha-mide	Grp	N	CR	PR	SD	PD		Test	p	Comments no relation between HER2 and expression and response
Bookman et al., 2003 trastuzumab	Grp	N	CR	PR	SD	PD		Test	p .023	Comments no relation between tHER2 expression level and response; IHC3+ more likely to experience cycle 1 toxicity
Bowman et al., 2002 letrozole	Grp	N	CR	PR	SD	PD		Test	p .026	Comments high HER2 (not defined) associated with CA125 progression
Campos et al., 2001 liposomal doxorubicin	Grp IHC+ IHC- IHC?	N 4 30 24	CR+PR 50 30 17		SD 11 29	PD 57 50	? 2 4	Test FE	p .579	Comments ≥50%↓ in CA125
Hengstler et al., 1999 carboplatin or cisplatin + cyclophospha-mide	Grp	N	CR	PR	50	PD		Test	p	Comments
Di Leo et al., 1995 mitoxantrone + ifosfamide	Grp IHC+ IHC-	N 8 14	CR+PR 25 14		SD+PD 75 86			Test FE	p .602	Comments

Abbreviations: CR: complete response; FE: fixed effects; Grp: group; PD: progressive disease; PR: partial response; SD: stable disease;

Randomized trial, prespecified multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, post-hoc multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, treatment by HER2 subgroup analysis. No studies of this type were identified.

Single-arm study, prespecified multivariate analysis. The study by Di Leo, Bajetta, Biganzoli, et al. (1995) conducted the only prespecified multivariate analysis. In this Phase II study, 72 patients received mitoxantrone plus ifosfamide for persistent or relapsed ovarian cancer. Observers evaluating HER2 were blinded to outcome data. Cox regression and recursive partitioning was carried out, but the report provides poor details about selection of candidate variables, model-building strategies, testing of assumptions, whether standard prognostic factors were included in final models and whether continuous variables were handled well. The article does not mention validation of models. The candidate variables that were tested included tumor imaging, tumor grade, residual tumor volume, number of disease sites, tumor responsiveness, p53 marker values and HER2 values. The only significant variable on univariate or multivariate analyses of time-to-treatment-failure and overall survival was clinically or radiologically detectable disease on study entry. HER2 was not predictive for these outcomes, nor did it predict response on univariate analysis.

Single-arm study, post-hoc multivariate analysis. Two studies of this type are available (Camilleri-Broet, Hardy-Bessard, Le Tourneau, et al., 2004; Malamou-Mitsi, Crikoni, Timotheadou, et al., 2007). Authors of the former study gave cisplatin, epirubicin and cyclophosphamide to 117 of 164 patients with advanced ovarian cancer. Analyses were performed in a both mixed group of marker data taken from primary and metastatic lesions and a subset (of unspecified size) with primary tumor specimens. Focusing on the primary tumor subset, both univariate and multivariate analyses found HER2 and the presence of ascites to be significant predictors of progression-free and overall survival. Multivariate analyses are poorly described in this article. The study by Malamou-Mitsi and colleagues (2007) entailed giving 95 patients with stage IIc-IV epithelial ovarian cancer paclitaxel plus carboplatin or alternating regimens of paclitaxel plus either carboplatin or cisplatin. In a retrospective multivariate analysis, standard prognostic variables were entered into the Cox regression models for overall survival and time-to-progression, but investigators used an inappropriate stepwise selection method for building the final model. Furthermore, it is unclear if validation was conduction. IHC HER2 was not found to be a significant predictor of either outcome.

Single-arm study, univariate analysis. Of four studies with sample sizes between 41 and 70 patients, three found significant relationships between HER2 and at least one outcome (Bookman, Darcy, Clarke-Pearson, et al., 2003; Bowman, Gabra, Langdon, et al., 2002; Hengstler, Lange, Kett, et al., 1999). The study by Bookman, Darcy, Clarke-Pearson, et al. (2003) addressed progression-free survival, overall survival response and toxicity, finding a significant relation only between HER2 and cycle one trastuzumab toxicity. Hengstler, Lange, Kett, et al. (1999) found that HER2 results on a RNA PCR assay were significantly related to overall survival among 44 patients treated with a platinum compound plus cyclophosphamide. Bowman, Gabra, Langdon, et al. (2002) gave letrozole to 50 patients, finding that IHC HER2 results were related to CA125 progression. Campos, Penson, Mays, et al. (2001) showed that

IHC HER2 status was not associated with CA125 response among 70 patients treated with liposomal doxorubicin.

Evidence summary–ovarian cancer. The evidence on the relation between HER2 results and outcome comes from six ovarian cancer studies that each addressed a different treatment. Three generally poorly reported multivariate analyses of single-arm series using different chemotherapy regimens are available, the prespecified analysis found HER2 not to be predictive, while among the two post-hoc analyses, it was a significant independent predictor in one and not the other. No randomized studies are available to address potential treatment by HER2 interactions. Four univariate analyses provide are capable of only suggesting candidate variables for future multivariate analyses, showing mixed results with respect to whether HER2 is associated with outcome. Future research should place studies at higher levels in the hierarchy. This weak body of evidence does not support conclusions about whether HER2 predicts treatment outcomes.

Part III. Prostate Cancer

Overview/study populations. Only four studies met selection criteria (total N=147). One study focused on neoadjuvant therapy for high-risk nonmetastatic prostate cancer (Prayer-Galetti, Sacco, Pagano, et al., 2007). Two studies addressed hormonal therapy for advanced prostate cancer (Nishio, Yamada, Kokubo, et al., 2006; Arai, Yoshiki, Yoshida, et al., 1997) and one study managed patients with stage A1 disease expectantly (Fox, Persad, Coleman, et al., 1994). Three studies used tissue HER2 testing (Prayer-Galetti, Sacco, Pagano, et al., 2007; Nishio, Yamada, Kokubo, et al., 2006; Fox, Persad, Coleman, et al., 1994) and the fourth study used serum testing (Arai, Yoshiki, Yoshida, et al., 1997). Study hierarchy, quality assessment, summary descriptions, and results are arrayed in Tables 40–43.

Results by hierarchy level, study quality assessment.

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Randomized trial, prespecified multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, post-hoc multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, treatment by HER2 subgroup analysis. No studies of this type were identified.

Single-arm study, prespecified multivariate analysis. No studies of this type were identified.

Question 5: HER2 Results to Guide Treatment Regimen Selection, Prostate Cancer

Table 40. Hierarchy of evidence, KQ5, prostate cancer

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA						
RCT post-hoc MV SGA						
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis						
1-arm UV analysis	Prayer-Galetti 2007	22	non-meta-static high risk PC	neoadjuvant triptorelin+ estramustine+ docetaxel	Path Resp DFS	IHC HER2+ vs. – p=NS IHC HER2+ vs. – p=NS
	Nishio 2006	47	bone metastases PC	maximal androgen blockade	DSS RFS	IHC HER2+ vs. – p=.00084 IHC HER2+ vs. – p=.0485
	Arai 1997	33	stage D2 PC	antiandrogen tx	PFS	sHER2+ vs. – p=.05
	Fox 1994	45	stage A1 PC	expectant	OS	IHC HER2+ vs. – p=.0316

Abbreviations: DFS: disease-free survival; DSS: disease-specific survival; MV: multivariate; Path Resp: pathologic response; PC: prostate cancer; PFS: progression-free survival; Prog: progression; Resp: response; RFS: recurrence-free survival; RR: relative risk; SGA: subgroup analysis; Tx: treatment; UV: univariate;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Prostate Cancer
Table 41. Study quality assessment, KQ5, prostate cancer

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long followup	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Prayer-Galetti et al., 2007	Y	N	N	N	?	Y	Y	median 53 months (30-64)	NA
Nishio et al., 2006	N	N	N	Y	?	Y	Y	48.7 months (6.9-79	NA
Arai et al., 1997	N	N	N	Y	?	Y	Y	> 36 months	NA
Fox et al., 1994	N	N	N	Y	?	Y	Y	3-216 months	NA

Question 5: HER2 Results to Guide Treatment Regimen Selection, Prostate Cancer

Table 42. Single-arm studies, design, enrollment and treatment, KQ5, prostate cancer

Study	Therapeutic Setting	Treatments	Age	Stage (%)	PSA	Gleason Score (%)
Prayer-Galetti et al., 2007	non-metastatic high-risk prostate cancer	neoadjuvant triptorelin+ estramustine+ docetaxel (n=22)	Md 63 rng 55-73	T2a 5 T2b 10 T3 86 N+ 24 N0 76	≤ 4 10 4.1-10.0 0 10.1-20.0 14 ≥ 20.1 76	5 5 6 10 7 33 8 33 9 10 10 10
Nishio et al., 2006	Bone metastatic prostate cancer Grp1: HER2+ (n=21) Grp2: HER2- (n=28)	maximal androgen blockade: antiandrogens + LH-RH agonists, antiandrogens + bilateral orchiectomy (n = 47)	Grp1 Grp2 mn 74.4 73.0 md 72 72 rng 63-85 61-91 sd 6.5 7.9	Grp1 Grp2 T1c 4.8 3.6 T2a 4.8 14.3 T2b 23.8 25.0 T3a 0 0 T3b 9.5 10.7 T4 52.4 46.4 Tx 4.8 0 N0 52.4 67.9 N1 42.9 32.1 N2 4.8 0	Grp1 Grp2 mn 798.5 1076.2 md 426 270.4 rng 34-3780 37-10060 sd 1076.2 2323.6	Grp1 Grp2 7 4.8 10.7 8 42.9 28.6 9 38.1 60.7 10 14.3 0
Arai et al., 1997	histologically diagnosed, untreated prostate cancer	antiandrogen tx for stage D2 disease, including bilateral orchiectomy, leuporelin acetate, or DES (n=33)		6 C 19 D1 6 D2 40		
Fox et al., 1994	histologically diagnosed, untreated prostate cancer	expectant (n=45)	mn 65 rng 54-75	A1 100 B		<4 100

Abbreviations: DES: diethylstilbestrol; Grp: group; LH-RH: luteinizing-hormone-releasing hormone; md: median; mn: mean; PSA: prostate-specific antigen; rng: range: sd: standard deviation;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Prostate Cancer
Table 43. Single-arm studies, summary time to event outcomes, KQ5, prostate cancer

Study													
Prayer-Galetti et al., 2007	Outcome DFS	Grp HER2+ HER2-	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments no correlation
Nishio et al., 2006 maximal androgen blockade	Outcome CSS RFS	Grp IHC+ IHC- IHC+ IHC-	N 21 28 21 28	Med (mos) ~ 32 NR ~ 9 ~ 19	1 yr 100 ~ 92 ~ 42 ~ 73	2 yr ~ 60 ~ 80 ~ 32 ~ 45	3 yr ~ 41 ~ 70 ~ 22 ~ 35	4 yr ~ 39 ~ 60 ~ 15 ~ 28	5 yr ~ 10 ~ 60 0 ~ 23	Test LR LR	p .0084 .0485	HR (95%CI)	Comments
Arai et al., 1997 antiandrogen tx for stage D2	Outcome PFS	Grp sHER2+ sHER2-	N 11 22	Med (mos) ~ 9 ~ 15	1 yr ~ 30 ~ 60	2 yr ~ 10 ~ 38	3 yr ~ 10 ~ 38	4 yr 0 ~ 38	5 yr 0	Test LR	p .05	HR (95%CI)	Comments
Fox et al., 1994 expectant tx	Outcome OS	Grp IHC+ IHC-	N 16 29	Med (mos) ~ 35 ~ 162	1 yr ~ 87 ~ 93	2 yr ~ 67 ~ 88	3 yr ~ 47 ~ 84	4 yr ~ 38 ~ 84	5 yr ~ 38 ~ 84	Test W-G	p .0316	HR (95%CI)	Comments

Abbreviations: Grp: group; LR: log rank; NR: not reported; OS: overall survival; PFS: progression-free survival; RFS: recurrence-free survival; tx: treatment; W-G: Wilcoxon-Gehan;

Single-arm study, post-hoc multivariate analysis. No studies of this type were identified.

Single-arm study, univariate analysis. All four studies involved univariate analyses, three of which were retrospective case series and 1 was a prospective phase II study. The phase II study (Prayer-Galetti, Sacco, Pagano, et al., 2007) selected 22 patients with nonmetastatic high risk prostate cancer, giving the chemohormonal therapy prior to surgery. IHC HER2 status was not found to be related to either pathologic response or disease-free survival. Nishio, Yamada, Kokubo, et al. (2006) included 47 patients treated with maximal androgen blockade for advanced disease manifested by bone metastases. Tissue IHC HER2 was found to be associated with disease-specific survival and prostate-specific antigen (PSA) relapse-free survival. Arai, Yoshiki, Yoshida, et al. (1997) selected 33 patients with advanced (stage D2) patients treated with antiandrogen monotherapy and found that serum HER2 was associated with progression-free survival. Fox, Persad, Coleman, et al. (1994) reported on 45 patients with stage A1 disease treated expectantly and observed an association between IHC HER2 and overall survival.

Evidence summary–prostate cancer. This small body of evidence is too weak to show whether HER2 predicts outcomes after treatment for prostate cancer. No randomized studies or multivariate analyses of single-arm studies are available. The only studies meeting selection criteria were three small retrospective case series and one phase II study, all using univariate analyses. Two studies found tissue IHC HER2 to predict outcomes, one study found IHC HER2 did not predict outcomes, and one study found serum HER2 to be predictive. These exploratory studies would need to be confirmed by large studies higher in the evidence hierarchy.

Part IV. Head and Neck Cancer

Overview/study populations. Two studies met selection criteria (total n=113). One study examined surgery alone for patients with malignant salivary tumors (Nagler, Kerner, Ben-Eliezer, et al., 2003). The other study (Khan, King, Smith, et al., 2002) gave surgery and external beam radiotherapy (EBRT) to patients with squamous cell carcinoma of the oral cavity or oropharynx. Study hierarchy, quality assessment, summary descriptions, and results are arrayed in Tables 44–47.

Results by hierarchy level, study quality assessment.

Randomization stratified on HER2/randomized to whether treatment was guided by HER2. No studies of this type were identified.

Randomized trial, prespecified multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, post-hoc multivariate subgroup analysis. No studies of this type were identified.

Randomized trial, treatment by HER2 subgroup analysis. No studies of this type were identified.

Question 5: HER2 Results to Guide Treatment Regimen Selection, Head and Neck Cancer

Table 44. Hierarchy of evidence, KQ5, head and neck cancer

Level of Evidence	Study	n	Setting	Treatments	Outcome	Results
RCT stratified on HER2 status/ HER2-guided vs. non-HER2-guided						
RCT prespecified MV SGA						
RCT post-hoc MV SGA						
RCT treatment by HER2 SGA						
1-arm prespecified MV analysis						
1-arm post-hoc MV analysis						
1-arm UV analysis	Nagler 2003	36	malignant salivary tumors	surgery	OS	IHC HER2+ vs. – p=.0004
	Khan 2002	77	SCC or oral cavity or oropharynx	surgery + EBRT	DFS	IHC HER2+ vs. – RR=0.83 (95% CI: 0.29–2.4)
					OS	IHC HER2+ vs. – RR=1.4 (95% CI: 0.62–3.3)
					OS	FISH HER2+ vs. – p=.15

Abbreviations: DFS: disease-free survival; EBRT: external-beam radiation therapy; MV: multivariate; OS: overall survival; SCC: squamous-cell carcinoma; SGA: subgroup analysis; UV: univariate;

Question 5: HER2 Results to Guide Treatment Regimen Selection, Head and Neck Cancer
Table 45. Study quality assessment, KQ5, head and neck cancer

Study	Prospective design	Prespecified hypotheses about relation of marker to outcome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long followup	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Nagler et al., 2003	N	N	N	Y	?	Y	Y	?	NA
Khan et al., 2002	N	N	N	Y	Y	Y	N	?	NA

Question 5: HER2 Results to Guide Treatment Regimen Selection, Head and Neck Cancer
Table 46. Single-arm studies, design, enrollment and treatment, KQ5, head and neck cancer

Study	Therapeutic Setting	Treatments	Age	Stage (%)	Performance Status (%)	Tumor Grade (%)
Nagler et al., 2003	Malignant salivary tumors	Surgery, no adjuvant therapy (n=36)	mn 56 rng 15-79 sd 4 >60 45%			
Khan et al., 2002	SCC of oral cavity (57%) or oropharynx (43%)	Primary surgical excision and EBRT with curative intent (n=77)	41-56 25% 56-59 59-66 25% 66-79 25%	T1-2 34 T3-4 66 N0 18 N1 43 N2-3 39 II 9 III 28 IV 63		20 mod 39 mod well 20 well-diff 21

Abbreviations: diff: differentiated; EBRT: external-beam radiation therapy; mn: mean; mod: moderate; rng: range; SCC: squamous cell carcinoma; sd: standard deviation
poor-mod

Question 5: HER2 Results to Guide Treatment Regimen Selection, Head and Neck Cancer
Table 47. Single-arm studies, time to event outcomes, KQ5, head and neck cancer

Study	Time to Event Outcomes												
Nagler et al., 2003 surgery	Outcome OS	Grp IHC+ IHC-	N 10 26	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test LR	p 0.0004	HR (95%CI)	Comments
Khan et al., 2002 surgery+EBRT	Outcome DFS OS OS	Grp IHC+ vs. - IHC+ vs. - FISH disom/- FISH polysom FISH+	N ? ? 47 5 4	Med (mos) 5.8 3.1 2.2	1 yr	2 yr	3 yr	4 yr	5 yr	Test UV Cox UV Cox LR	p 0.15	RR (95%CI) 0.83 (0.29, 2.4) 1.4 (0.62, 3.3)	Comments LR test for combined di-somic, non-overexpressed + polysomic

Abbreviations: DFS: disease-free survival; LR: log rank; OS: overall survival; UV: univariate;
30
72

43
40
0

Single-arm study, prespecified multivariate analysis. No studies of this type were identified.

Single-arm study, post-hoc multivariate analysis. No studies of this type were identified.

Single-arm study, univariate analysis. The study of surgery for malignant salivary gland tumors (Nagler, Kerner, Ben-Eliezer, et al., 2003) found HER2 to be a significant predictor of overall survival. In contrast, the study by Khan, King, Smith, et al. (2002) of surgery plus external-beam radiation therapy for squamous cell carcinoma of the oral cavity and oropharynx found that IHC HER2 was not a significant predictor of disease-free survival or overall survival. Khan and co-workers also reported that FISH HER2 was not significantly associated with overall survival.

Evidence summary–head and neck cancer. The evidence on whether HER2 predicts outcomes after treatment for head and neck cancer is weak. No randomized studies or single-arm designs using multivariate analyses met study selection criteria. Two studies were univariate analyses of single-arm studies. Additional studies are needed that are placed at higher levels in the evidence hierarchy.

Conclusions, Key Question 5

This systematic review found only weak evidence on how well serum or tissue HER2 testing predicts outcomes after treatment for malignancies in any of these sites: lung, ovary, head and neck, or prostate. Overall, the evidence is heterogeneous with respect to treatment regimens and thresholds for positive HER2 test results. Of 22 studies addressed for the four types of malignancies, there were no randomized trials that could have analyzed HER2 by treatment effect interactions. Six multivariate analyses in single-arm designs were performed, all of which were poorly described, so it is unclear if they were well conducted. Data from these exploratory analyses did not consistently find that HER2 status predicts treatment results. Univariate analyses provide very limited information value, at best suggesting candidate variables for future multivariate analyses.

Chapter 4. Discussion and Future Research

The human epidermal growth factor (EGF) receptor-2 (HER2; also referred to as HER2/*neu* and as ERBB2) gene is amplified and the HER2 protein overexpressed in approximately 18–20 percent of breast cancer cases. Evidence from multiple randomized trials demonstrates that adding trastuzumab, a therapeutic monoclonal antibody that targets HER2, to adjuvant chemotherapy regimens for HER2-positive breast cancer improves patient outcomes. HER2 also is overexpressed in varying proportions of other epithelial malignancies such as ovarian, thyroid, lung, salivary gland/head and neck, stomach, colon, and prostate cancers. This evidence report is a systematic review of the evidence on novel applications of HER2 testing to the management of cancer patients including: potential for response to trastuzumab among breast cancer patients who have negative, equivocal, or discordant HER2 assay results; use of HER2 assay results to guide selection of breast cancer treatments other than trastuzumab (i.e., chemotherapy regimen or hormonal therapy regimen); the use of serum HER2 to monitor treatment response or disease progression in breast cancer patients; and use of HER2 testing to manage patients with ovarian, lung, prostate, or head and neck tumors.

HER2 assay results are influenced by multiple biologic, technical and performance factors. Since many aspects of HER2 assays have not been standardized until very recently (Wolff, Hammond, Schwartz, et al., 2007), the effects of these disparate influences cannot be isolated in the existing literature that compares results of different methods. Discordances between IHC and FISH results might arise in one of three ways. They may be artifacts of one accurate or two inaccurate tests. Alternatively, they may reflect a threshold issue, either related to changes in threshold definitions over time, or an inherent problem of using a continuous measure to classify patients dichotomously. Finally, discordant test results might accurately reflect a small number of different patients with respect to the biologic mechanisms that can increase membrane levels of the HER2 protein. This clearly affects the interpretation of evidence on the use of "HER2 status" to predict treatment or disease outcomes, which presumes accurate classification by tissue assays. Future studies reporting outcomes as a function of HER2 status should report separately on patients with concordant, equivocal, and discordant assay results.

To assess the quality of the available evidence on using HER2 status to guide treatment decisions, we took a two-fold approach. First, we applied a hierarchical framework to evaluate how informative various designs and analytic strategies would be to predict outcomes according to HER2 status. The most informative would be a trial in which randomized assignment to treatment groups was stratified by HER2 status or patients were randomized to receive treatment guided by HER2 results or not. Prespecified subgroup analyses guard against the problems of data dredging. In contrast, post-hoc subgroup analyses may generate hypotheses, but do not support strong inferences about effectiveness. The least-informative situation would be a single-arm study that presents univariate comparisons of HER2 groups. To further assess the quality of predictive studies, we adapted the "Reporting Recommendations for Tumor Marker Prognostic Studies" (REMARK) statement (McShane, Altman, Sauerbrei, et al., 2005). Good quality characteristics of predictive studies include: prospective design; prespecified hypotheses about relation of marker to outcome; large, well-defined, representative study population; marker assay methods well-described; blinded assessment of marker in relation to outcome; homogeneous treatment(s), either randomized or rule-based selection; low rate of missing data ($\leq$15 percent); and a well-described, well-conducted multivariate analysis of outcomes.

Overall, few trials included in this evidence report were intended or designed to investigate the key questions of the systematic review. With exception of two trials (Seidman, Berry, Cirrincione, et al., 2004; Martin, Pienkowski, Mackey, et al., 2005), evidence for this review consisted mostly of post-hoc analyses on subgroups not directly randomized, selected, or stratified by HER2 status. Nearly all were secondary or correlative analysis on patient subgroups with archived tissue samples available for HER2 testing. Direct comparison of baseline and prognostic factors for HER2-positive and HER2-negative subgroups were infrequently reported, so it is uncertain whether these subgroups were well balanced in such studies.

Going forward, cancer therapy trial protocols should incorporate elements to facilitate robust analyses of the potential of HER2 to improve treatment management by providing predictive information on disease progression and response to treatment. These elements include:

- Detailed reporting of how HER2 status was ascertained, including assay methods, thresholds, validation, and quality assurance measures.
- Since HER2 status is now routinely ascertained for all newly diagnosed breast cancer patients, relevant data should be recorded for all participants, and accessible to other researchers, to permit subgroup analyses of outcomes by HER2 status. Investigators of ongoing and completed trials should similarly contribute data or tissue samples to large international collaborations for patient-level meta-analysis.
- Perform stratified randomization by HER2 status or prospectively specify HER2 subgroup analysis of outcomes.
- Report on and, where sample size permits, prospectively specify subgroup analyses on participants with equivocal or discordant HER2-positive assay results (e.g., IHC 2+ and FISH positive). Of particular importance is recording, tracking and making accessible to researchers assay results and outcomes data on patients with equivocal results of initial assays, as defined in the ASCO/CAP guideline.
- Future studies should report more completely and statistically compare subgroups from each treatment arm by HER2 status for known prognostic factors and baseline characteristics of patients and their tumors.

The case for these measures is strongest for breast cancer therapy trials, as the relation of HER2 status to outcomes of treatments other than trastuzumab has been hypothesized, but not confirmed; and as many therapeutic agents, classes, and regimens have been and will be tested. The case for incorporating these measures into therapeutic trials for other cancers is less compelling in that the relationship of HER2 status to outcome of any therapy has not been established. However, the argument in favor of doing so is that it would provide an efficient approach to screening for such relationships. This approach can be generalized to promising biomarkers other than HER2. Because existing evidence is scant on serum HER2 (sHER2) results to predict disease progression or treatment outcome, serial collection of serum samples at standard intervals to be assayed subsequently for sHER2 levels in trials planned for other purposes offers an opportunity to examine potential utility.

For Key Question 2, potential for response to trastuzumab among breast cancer patients who have equivocal, discordant, or negative HER2 assay results, evidence is scant, but intriguing. Future research should address whether there are other markers that might predict for these subgroups response to therapy that targets HER2. For example, studies will address patients with triple-negative or “basal-like” breast cancer and not merely patients who are negative on HER2 tests. In addition, patients with HER2/CEP17 ratios either ≥ 1.8 but <2.0 or ≥ 2.0 but <2.2 deserve

attention. Post-hoc analysis from the only trial found to address this question in HER2-negative patients reported an association of benefit from trastuzumab with presence of polysomy 17. It is likely most efficient to evaluate markers with the potential to identify trastuzumab-responsive patients in samples and patients pooled across the large adjuvant trastuzumab trials that have already been completed.

For Key Question 3a, use of HER2 results to guide selection of breast cancer treatments other than trastuzumab (i.e., chemotherapy regimen or hormonal therapy regimen), there are suggestions that HER2 status predicts clinical benefit from certain regimens. Future trials that compare adjuvant chemotherapy regimens with versus without an anthracycline, or with versus without a taxane, could determine HER2 status at the time of diagnosis, and stratify randomization by HER2 assay results. This approach might provide more definitive tests for hypotheses about whether an anthracycline or a taxane improves outcomes of HER2-negative patients. For emerging targeted therapies in the adjuvant setting, and for all therapies in the neoadjuvant and advanced disease settings, future trials should prospectively collect data on HER2 status and prospectively define hypotheses they will test on treatment outcomes in HER2 subgroups.

The most attractive and pragmatic approach currently available is work that could be done using the individual patient data of the Early Breast Cancer Trialists Collaborative Group (EBCTCG).

- EBCTCG has published an individual patient-level meta-analysis (17 trials, N=14,000; minimum 5 years followup) of randomized trials that compared CMF versus an anthracycline-based regimen for adjuvant chemotherapy of breast cancer.
- The absolute difference in rates of relapse or death favored anthracyclines by 3 percent at 5 years and 4 percent (SE: 1) at 10 years.
- The opportunity is to access as many archived tumor specimens as possible of participants in these trials and determine HER2 status using IHC and ISH with current scoring thresholds.
- If sufficient tumor samples can be obtained and tested, this would permit a rigorous assessment of the benefit from anthracyclines to HER2-positive and HER2-negative patients.

In the ASCO 2007 update of recommendations for the use of tumor markers in breast cancer (Harris, Fritsche, Mennel, et al., 2007), the expert panel continued to recommend that HER2 status should not be used to withhold endocrine therapy from HER2-positive patients, nor should it be used to select a specific endocrine therapy. They summarized conflicting results on these issues, especially data addressing the hypothesis that aromatase inhibitors may be more effective than tamoxifen in HER2-positive patients. In our evidence review, the same data supplemented by recent studies still does not support conclusions about how well HER2 status predicts relative outcomes of different endocrine therapies in patients with hormone-receptor-positive breast cancer. Research is ongoing to compare tamoxifen and newer hormonal agents (i.e., aromatase inhibitors or selective estrogen receptor modulators) in hormone-receptor-positive patients. Implications of HER2 status should be prospectively investigated in ongoing and future trials that compare hormonal therapies. In addition, retrospective analyses of HER2 status should be conducted for additional completed trials comparing hormonal therapies, an attractive approach because long-term followup of outcomes is already available. Of particular importance, given the inverse relationship between hormone-receptor and HER2 status, is accumulating a large

enough sample of patients who are both hormone-receptor positive and HER2 positive to evaluate the use of hormonal therapy in HER2-positive patients.

For Key Question 4, current evidence does not support conclusions on sHER2 as a predictor of outcomes after treatment by any regimens in any setting of breast cancer treatment. Evidence primarily focused on first-line or second- and subsequent-line treatment of metastatic disease using variety of regimens. Furthermore, these studies used different thresholds for a positive sHER2 result and varied on whether patient selection required positive tissue HER2 status. There were only three randomized trials and only one used multivariate analysis, while two single-arm studies performed multivariate analysis. The quality of reporting on multivariate analyses was poor. Univariate analyses provide very limited information value, suggesting candidate variables for future multivariate analyses. These studies do not support clear conclusions for whether sHER2 predicts disease progression, treatment response, or outcomes of any specific treatment regimen. A potentially useful approach to filling the evidence gap is to identify one or more completed randomized, controlled trials with banked serial serum specimens and either known tissue HER2 status or banked tissue from all, or nearly all, randomized patients. Once identified, an appropriate multivariate analysis could assess the relation between sHER2 changes and treatment outcomes. In addition, future trials should prospectively collect serial sHER2 samples.

For Key Question 5, this systematic review did not find evidence to support conclusions on serum or tissue HER2 testing to predict treatment outcomes for malignancies in any of these sites: lung, ovary, head and neck, or prostate. Overall, the evidence is weak and heterogeneous with respect to treatment regimens and thresholds for positive HER2 test results. Of 22 studies addressed for the four types of malignancies, there were no randomized trials that could have analyzed HER2 by treatment effect interactions. Six multivariate analyses in single-arm designs were performed, all of which were poorly described, so it is unclear if they were well conducted. Data from these exploratory analyses did not consistently find that HER2 status predicts treatment results. Univariate analyses provide very limited information value, at best suggesting candidate variables for future multivariate analyses. Future studies of nonbreast malignancies should prospectively collect and test serum and tissue specimens for HER2 status and perform planned multivariate analyses, preferably in randomized trials.

Given the human and financial cost of cancer therapy trials, the limited resources available, and the long duration of followup needed to assess outcomes particularly for early stage or slowly growing cancers, it is imperative that tumor tissue blocks be collected, optimally fixed, saved, and made available for correlative tumor marker studies from all randomized patients. Agreement to share blocks with investigators should be made a condition for institutions seeking to participate in cooperative group trials.

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List of Acronyms/Abbreviations

-	negative; without
?	unknown; unclear
+	positive; with
+/-	with or without
→	followed by
↓	Decrease
Δ	Change
A	doxorubicin (Adriamycin®)
AACC	American Association for Clinical Chemistry
AC	doxorubicin (Adriamycin®)/cyclophosphamide
AI	aromatase inhibitor
ANA	Anastrozole
ASCO/CAP	American Society of Clinical Oncology
BAC	bronchioalveolar adenocarcinoma
CAF	cyclophosphamide, doxorubicin (Adriamycin®) and fluorouracil
CALGB	Cancer and Leukemia Group B
CAP	College of American Pathologists
cCR	clinical complete response
CEF	cyclophosphamide plus epirubicin plus fluorouracil
CEP17	chromosome 17 centromere
ChiSq	chi square
CHT	Chemotherapy
CI	confidence interval
CISH	chromogenic in-situ hybridization
Cisplat	Cisplatin
CMF	cyclophosphamide plus methotrexate plus fluorouracil
cPR	clinical partial response
CR	complete response
cSD	clinical stable disease
Cx	Control
D	Docetaxel
DAC	docetaxel plus doxorubicin (Adriamycin®)/cyclophosphamide
DES	Diethylstilbestrol
DFS	disease-free survival
DNA	deoxyribonucleic acid
Disom	Disomy
Docetax	Docetaxel
Doxorub	Doxorubicin
E	Epirubicin
Ea	Each
EBRT	external-beam radiation therapy
EC	epirubicin/cyclophosphamide
ECD	extracellular domain
ECOG	Eastern Cooperative Oncology Group
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
Epirub	Epirubicin
ER	estrogen receptor
ERD	extensive residual disease
ET	epirubicin/paclitaxel (Taxol®)
FAC	fluorouracil plus doxorubicin (Adriamycin®)/cyclophosphamide
FDA	U.S. Food and Drug Administration
FE	fixed effects
FEC14	cyclophosphamide plus epirubicin plus fluorouracil every 14 days
FEC21	cyclophosphamide plus epirubicin plus fluorouracil every 21 days
FISH	fluorescence in-situ hybridization
Grp	Group

HDC/AuSCS	high-dose chemotherapy with autologous stem-cell support
HER	human epidermal growth factor receptor
HR	hazard ratio
IAUC	International Union Against Cancer
IHC	Immunohistochemistry
ISH	in-situ hybridization
IV	Intravenous
KPS	Karnofsky Performance Score
K-M	Kaplan-Meyer
KQ	Key Question
LABC	locally advanced breast cancer
LET	Letrozole
LH-RH	luteinizing hormone releasing hormone
LN	lymph node
log regr	log regression
LR	log-rank; likelihood ratio
MAb	monoclonal antibody
MBC	metastatic breast cancer
Med	Median
MH	Mantel-Hantzel
Mn	Mean
Mos	Months
MRD	minimal residual disease
MTX	Methotrexate
MV	Multivariate
N	Number
N	Node
N	No
NA	not applicable
NCCN	National Comprehensive Cancer Network
NCCTG	North Central Cancer Treatment Group
NE	not evaluable
Neg	Negative
NR	not reported
NS	not significant
NSABP	National Surgical Adjuvant Breast and Bowel Project
NSCLC	non-small cell lung cancer
OC	ovarian cancer
ORR	overall response rate
OS	overall survival
P	Paclitaxel
Paclit	Paclitaxel
PCR	polymerase chain reaction
PD	progressive disease
PE	cisplatin/etoposide
Polysom	Polysomy
Pos	Positive
Post	Postmenopausal
Postmen	Postmenopausal
PR	progesterone receptor; partial response
Pre	Premenopausal
Premen	Premenopausal
PRO	Prospective
Progr	Progression
Prop	proportional; proportion
pt(s)	patient(s)
q wk	Weekly
q2wk	every 2 weeks
q3wk	every 3 weeks

RCT	randomized controlled trial
Resp	Response
RFI	recurrence-free interval
RFS	recurrence-free survival
Rng	Range
RR	relative risk
RT	radiation therapy
S	Serum
SABCS	San Antonio Breast Cancer Symposium
SCC	squamous cell carcinoma
sd, SD	standard deviation
SD	stable disease
SE	standard error
SERM	selective estrogen receptor modulators
SGA	subgroup analysis
sHER2	serum HER2
SISH	silver in-situ hybridization
SWOG	Southwest Oncology Group
T, t	tumor, tissue
TAC	paclitaxel (Taxol®) plus doxorubicin (Adriamycin®)/cyclophosphamide
TAM	Tamoxifen
tHER2	tissue HER2
TRZ	Trastuzumab
TTF	time to failure
TTP	time to progression
Tx	Treatment
Unk	Unknown
US	United States
UV	Univariate
W-G	Wilcoxon-Gehan test
WHO	World Health Organization
Y	Yes
Yo	years old
yr(s)	year(s)

Appendix A. Exact Search Strings

MEDLINE search (performed through 2/23/07)

EMBASE search (performed through 2/23/07)

Cochrane Controlled Clinical Trials Register (performed through 2/23/07)

Database Search Strategies:

- Genes, erbB-2[MeSH] OR Receptor, erbB-2[MeSH] OR
- "Her-2*"[tiab] OR "Her2*"[tiab] OR "erbB-2"[tiab] OR "erbB2"[tiab] OR
- "epidermal growth factor receptor-2"[tiab] OR
- "epidermal growth factor receptor 2"[tiab] OR
- receptor, epidermal growth factor[mh] OR
- epidermal growth factor receptor-neu receptor[nm])

NOT
- (animals [mh] NOT humans[mh])

For key questions 1 and 2 the results of the above search were combined with the results of a search using:

- Immunohistochemistry[MeSH] OR immunohistochemistry[tiab] OR
- immunocytochemistry[tiab] OR "IHC"[tiab] OR
- In Situ Hybridization, Fluorescence[MeSH] OR
- "fluorescence in situ hybridization"[tiab] OR
- "fluorescence in-situ hybridization"[tiab] OR "FISH"[tiab] OR
- (chromogenic[tiab] AND hybridization[tiab]) OR "CISH"[tiab] OR
- ((gold-facilitated[tiab] OR autometallographic[tiab] OR "bright field"[tiab] OR bright-field[tiab]) AND hybridization[tiab]) OR
- "GOLDFISH"[tiab]

AND

- Breast neoplasms[MeSH] OR "breast neoplasm*"[tiab] OR
- "breast cancer*"[tiab] OR "breast tumor*"[tiab] OR "breast tumour*"[tiab]

For key question 3, the results of the first search above were combined with the results of a search using:

- serum[tiab] OR blood[tiab] OR circulating[tiab]

AND

- Breast neoplasms[MeSH] OR "breast neoplasm*"[tiab] OR

- “breast cancer*”[tiab] OR “breast tumor*”[tiab] OR “breast tumour*”[tiab]

For key question 4, the results of the first search above were combined with the results of a search using:

- “Ovarian Neoplasms"[MeSH] OR
- "Lung Neoplasms"[MeSH] OR
- "Prostatic Neoplasms"[MeSH] OR
- "Colorectal Neoplasms"[MeSH] OR
- "Stomach Neoplasms"[MeSH] OR
- "Esophageal Neoplasms"[MeSH] OR
- "Urinary Bladder Neoplasms"[MeSH] OR
- "Uterine Neoplasms"[MeSH] OR
- "Uterine Cervical Neoplasms"[MeSH] OR
- "Head and Neck Neoplasms"[MeSH] OR
- "Thyroid Neoplasms"[MeSH] OR
- ((neoplasm*[tiab] OR cancer[tiab] OR cancers[tiab] OR tumor[tiab] OR tumors[tiab] OR tumour*[tiab]) NOT breast[ti])

The results of all of the above searches were limited to citations also identified by the Cochrane Handbook search strategy for controlled trials (Alderson et al. 2004):

- randomized controlled trial [pt] OR
- controlled clinical trial [pt] OR
- randomized controlled trials [mh] OR
- random allocation [mh] OR
- double-blind method [mh] OR
- single-blind method [mh] OR
- clinical trial [pt] OR
- clinical trials [mh] OR
- "clinical trial" [tw] OR
- ((singl* [tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw]) AND (mask* [tw] OR blind* [tw])) OR
- placebos [mh] OR
- placebo* [tw] OR
- random* [tw] OR
- research design [mh:noexp] OR
- comparative study [mh] OR
- evaluation studies [mh] OR
- follow-up studies [mh] OR
- prospective studies [mh] OR
- control* [tw] OR
- prospectiv* [tw] OR
- volunteer* [tw])

Appendix B. Listing of Excluded Studies

Exclusion Codes

Retrieval Code (field 12)
GET retrieve full copy
DNG do not retrieve full copy
UNC uncertain; needs check by second reviewer

Selection Decision Code (field 12)
INC include
EXC exclude (with codes for exclusion reasons)

Full Review Codes (field 42)

I. Key Question Codes

Q1 breast tissue HER2, accuracy/concordance,
Q2A breast tissue HER2 discrepant, trastuzumab therapy
Q2B breast tissue HER2 negative, trastuzumab therapy
Q2AB either/both Q2A and Q2B
Q3A breast tissue HER2 status, chemotherapy
Q3B breast tissue HER2 status, hormonal therapy
Q3AB either/both Q3A and Q3B
Q4 breast serum HER2
Q5S serum HER2, non-breast solid tumors
Q5T tissue HER2, non-breast solid tumors
Q5ST serum & tissue HER2, non-breast solid tumors
NRQ not relevant question (note if NDE, INV, ANM, HNM, NRD, NRO, NRT, NRS, NIT)
Q#? unclear if relevant to any key question

II. Study Design Codes

RCT randomized controlled trial
PRO prospective single-arm study
PI phase I trial
PII phase II trial
QEX quasi-experimental study (nonrandomized comparative)
DAC diagnostic accuracy/concordance study
ADS assay development study
RET retrospective study
CR case report (n≤5)
CS case series
PRG prognostic study
ADB administrative database
REG registry
SR systematic review
NPD no primary data
NRA narrative review article (GET only if helps for Intro; DNG if 2002 or earlier)
MA meta-analysis
CEA cost-effectiveness analysis
D? design unclear/possibly relevant
LTR letter
EDT editorial
NDE not relevant design
ICT incomplete cross-tabluation of assay results
HNM HER-2 not measured
INV in vitro study
ANM animal model study

III. Sample Size Code (single-arm only)

FEW n < 25
N25 25 ≤ n ≤ 49
N50 50 ≤ n ≤ 99
N100 n ≥ 100
N? n unclear

IV. Disease/Outcome/Intervention Codes

BC breast cancer
OST other solid tumor
NST non-solid tumor
DS? disease unclear
NRD not relevant disease
O? outcome unclear
NRO not relevant outcome (or no follow-up)
NRS no relevant subgroup analysis for outcomes
FU? follow-up uncertain
TRZ trastuzumab-based treatment
CHT chemotherapy
HT hormonal therapy
T? treatment unclear
NRT not relevant treatment
NIT not identically treated
IT? uncertain whether identically treated
PC prostate cancer
LC lung cancer
HNC head & neck cancer
OC ovarian cancer

V. HER2 Assay Method Codes

IHC immunohistochemistry, one antibody or antiserum
WBL Western blot (measures HER2 protein)
MAB IHC comparing ≥2 antibodies or antisera
FIS fluorescence in-situ hybridization
CIS chromogenic in-situ hybridization
PCR polymerase chain reaction
RNA dot-blot or other methods for mRNA assay (including rt-PCR)
OTH any other HER2 assay methods
TMA tissue micro-array methods
MNV assay method not adequately validated, quality assured, etc.
MV? uncertain assay method validation, quality assurance
OBF other body fluid (not serum or plasma)
HM? HER2 assay method unclear
ELS ELISA or other enzyme assay
CTC circulating tumor cells
SBL Southern blot

OSI Pharmaceuticals, Genentech and Roche announce data from clinical studies of Tarceva. Expert Rev Anticancer Ther 2001; 1(1):4-5.
Notes: Q? D? DS?

Temporary treatment protocol trastuzumab (Herceptin(registered trademark)) for back-up: PROTOCOLE TEMPORAIRE DE TRAITEMENT TRASTUZUMAB (HERCEPTIN(registered trademark)) EN SITUATION ADJUVANTE. Oncologie 2005; 7(SUPPL. 2):s86-s99.
Notes: Q2A? NRA (practice guideline)

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Notes: Q5 CS N50 OC

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Notes: Q5 CS N50 HNC

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Notes: Q3A? RET N100 BC IT? IHC

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Notes: Q5? RET CS N50 LC IT?

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Notes: Q3AB? RCT N100 BC CHT HT IHC

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Notes: Q5 CS N25 HNC CHT RT

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Notes: Q5 CS? N? HNC IT?

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Notes: Q5 RET CS N100 HNC NRS?

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Notes: Q3A? D? N50 BC IT? HM?

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Notes: Q5 PRO CS N25 OC IT?

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Notes: Q5? CS N100 OC IT? NRS?

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Notes: Q4? RCT N100 BC HT ELS

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Notes: Q5? CS N25 IT? LC

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Notes: Q3A RCT N100 BC IHC
Q3? IT?

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Notes: Q5 CS N100 HNC

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Notes: Q5 CS N100 HNC IT?

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Notes: Q3B?

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Notes: Q5? PI OC GT

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Notes: Q4 RET N100 BC T? ELS

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Notes: Q5 NRA NPD LC

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Notes: Q3B? RET N50 IT? IHC

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Notes: Q3A? PRO N50 BC CHT IT? IHC

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Notes: Q3A? PRO N25 O? IT? CHT IHC FIS

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Notes: Q3A RET N100 BC CHT CIS TMA

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Notes: Q5 PII FEW LC PCR

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Notes: Q4?

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from abstract: Q3AB? IT?

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Notes: Q5 CS N25 PC

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Notes: Q4?

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Notes: Q1? Q2? PII N100 BC TRZ IHC FIS

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Notes: Q2? PRO N25 BC TRZ IHC

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Notes: Q4? N50 BC T? HM?

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Notes: Q3B? RET N100 BC HT IHC

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Notes: Q3AB QEX RCT N100 BC CHT HT IHC FIS PCR

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Notes: Q4 PRO N100 CHT TRZ ELS?

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Notes: Q4 PRO N25 TRZ ELS?

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Notes: Q2? NRA?

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Notes: Q3A? Q4? N50 BC CHT ELS

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Notes: Q4? RET? N25 BC CHT ELS

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Notes: Q3A? Q4? RET? N50 BC CHT ELS

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Notes: Q4? PRO N25 O? T? ELS OBF (saliva)

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Notes: Q3A? PRO? N25 BC CHT HDC HM?

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Notes: Q1? Q3A? RET N100 BC CHT HDC IHC FIS

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Notes: Q3A? RCT N100 BC CHT IHC

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Notes: Q5 RET CS N50 LC

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Notes: Q3A? RET N100 BC CHT FIS

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Notes: Q4? QEX? N25 BC T? O? ELS?

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Notes: Q4?

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Notes: Q4? D? N50 BC T? O? ELS?

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Notes: Q5 CS N50 LC IHC

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Notes: Q2? Q3A? RET N100 BC TRZ CHT FIS

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Notes: Q5 CS N25 OC IHC

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Notes: Q3A? RCT BC N100 CHT IHC FIS

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Notes: Q5 CS N25 OC

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Notes: Q2A? Q3A? PII N25 BC TRZ CHT IHC only

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Notes: Q1? Q3A? Q4? PII N50 BC TRZ CHT IHC FIS ELS?

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Notes: Q2? Q3A? PRO N25 BC TRZ CHT IHC only

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Notes: Q5 PII N100 OC IHC

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Notes: Q5 RET CS LC IHC

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Notes: Q5 D? LC

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Notes: Q5 CS N50 LC IHC

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Notes: Q3A? RET N50 BC CHT FIS

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Notes: Q3B? RET? RCT? N100 BC HT IHC

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Notes: Q1? Q2? Q3? NRA? N? BC TRZ CHT IHC FIS

CORPORATE NAME: NCCN HER2 Testing in Breast Cancer Task Force

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Notes: Q4? NRA? N? BC T? O? HM?

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Notes: Q4? NRA?

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Notes: Q4? SR N? BC O? T? ELS

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Notes: Q5 CS N100 OC HM?

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Notes: Q5 CS N50 OC HM?

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Notes: Q3 CCS N? BC

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Notes: Q1 Q2? Q3? DAC N100 CHT (anthracycline if IHC 2+ or 3+) IHC CIS

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Notes: Q4 D? N50 BC T? ELS

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Notes: Q5 CS N50 HNC ELS

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Notes: Q5 RCT N50 LC HM?

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Notes: Q4? D? N25 BC CHT ELS?

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Notes: Q5 CS N50 OC ELS

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Notes: Q5 CS N25 HNC HM?

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Notes: Q2? PRO N25 BC TRZ CHT IHC

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Notes: Q3B? D? N? BC HT O? HM?

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Notes: Q4? PRO? N50 BC HT CHT ELS?

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Notes: Q3B? RET N100 BC HT IHC (HER2+ if $\geq$5% of cells had membrane staining)

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Notes: Q2? PRO N100 BC TRZ IHC

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Notes: Q3A PRO? N100 BC CHT IHC

Colleoni M, Viale G, Zahrieh D et al. Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment . Clin Cancer Res 2004; 10(19):6622-8.
Notes: Q3A RET N100 BC CHT IHC

Concin N, Hefler L, van Bavel J et al. Biological markers in pT1 and pT2 ovarian cancer with lymph node metastases. Gynecol Oncol 2003; 89(1):9-15.
Notes: Q5 RET CS OC IHC

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Notes: Q4? D? N100 BC T? ELS

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Notes: Q1? Q3A? D? N? BC CHT OTH (ria)

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Notes: Q1? Q3A? RET? N25 BC CHT IHC FIS

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Notes: Q1? Q3A? Q3B? RET N100 BC CHT HT IHC ELS (cytosol extract)

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Notes: Q5 CS N50 HNC IHC

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Notes: Q5 CS N50 OC IHC

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Notes: Q3A RCT N100 BC CHT FIS

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Notes: Q3A RCT N100 BC CHT MAB

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Notes: Q5 CS N50 LC ELS

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Notes: Q4 PRO? N50 BC TRZ ELS

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Notes: Q5? NRA NPD LC

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Notes: Q3B PRO D? N100 BC HT O? IHC FIS

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Notes: Q5 CS N50 HNC IHC

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Notes: Q3A? PRO N25 BC CHT IHC

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Notes: Q1? Q2? CEA N? BC TRZ CHT IHC FIS

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Notes: Q3B PRO N100 BC HT IHC

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Notes: Q3B RCT N? BC HT IHC

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Notes: Q3B RCT N100 BC HT IHC

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Notes: Q1? Q3B? PRO? N100 BC HT IHC FIS

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Notes: Q4? PRO N100 BC T? O? HM?

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Notes: Q3B RET N50 BC HT IHC

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Notes: Q4 RET? N100 BC TRZ ELS

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Notes: Q2? Q4? PII N25 BC TRZ CHT FIS ELS

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Notes: Q3A?

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Notes: Q5? NRA NPD LC

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Notes: Q4 RET N50 BC T? ELS

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Notes: Q4 RET N100 BC T? ELS

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Notes: Q4 D? N100 BC T? O? ELS?

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Notes: Q4 RET PRG? N50 BC CHT ELS

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Notes: Q4 RET QEX N100 BC CHT ELS

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Notes: Q5 CS N25 HNC HM?

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Notes: Q3A? RET N100 BC CHT IT? IHC

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Notes: Q3A RET N25 BC CHT IHC (only 3+ considered HER2+)

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Notes: Q5 CS FEW LC HM?

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Notes: Q5 CS N50 HNC IHC

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Notes: Q5 CS N50 LC HM?

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Notes: Q5 CS N100 LC IHC

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Notes: Q4? D? FEW BC T? O? RNA CTC

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Notes: Q5 CS N100 PC IHC

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Notes: Q4 RET N100 BC T? O? HM? ELS?

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Notes: Q3A? PRO N25 BC CHT (RTx) IHC

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Notes: Q1? Q3A PRO N25 BC CHT (+RTx) IHC PCR

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Notes: Q1? Q2? NRA?

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Notes: Q2? Q3A? PRO N25 BC TRZ CHT IHC

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Notes: Q5 CS N25 HNC IHC

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Notes: Q1? Q3B? RET N100 BC HT MAB

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Notes: Q5 RET CS HNC IT?

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Notes: Q5 CS N25 HNC IHC

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Notes: Q5 CS N25 HNC IHC NB

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Notes: Q5 PI N? OC

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Notes: Q3A? RET N100 BC CHT IHC

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Notes: Q5 CS N50 OC IHC

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Notes: Q5 CS N25 OC IHC

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Notes: Q3B PRO N50 BC HT (adjuvant) HM?

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Notes: Q5 CS N25 HNC IHC

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Notes: Q4? PRO FEW BC CHT HM? (ELS?)

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Notes: Q3B? RET PRG N? BC HT HM?

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Notes: Q3A D? N50 BC CHT HM?

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Notes: Q3? RCT N100 BC CHT HT IHC only

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Notes: Q5 CS N25 OC IHC

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Notes: Q5 CS N50 HNC HM?

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Notes: Q5 CS N50 HNC HM?

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Notes: Q5 CS N100 HNC IHC

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Notes: Q5 CS FEW OC IHC

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Notes: Q5 CS N100 BC

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Notes: Q3A? D? N50 BC CHT FIS

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Notes: Q5 CS N100 OC ELS

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Notes: Q5? D? LC

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Notes: Q5 PII N50 OC HM?

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Notes: Q5 RCT N100 OC IHC

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Notes: Q3A? RCT N100 BC CHT HT IHC

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Notes: Q3A? HDC RET N25 BC CHT IHC

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Notes: Q5 PRG N25 HNC

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Notes: Q5 CS N25 LC IHC

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Notes: Q3B? RET? N25 BC HT HM?

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Notes: Q5 PII FEW HNC

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Notes: Q4? RET N100 BC CHT HT ELS

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Notes: Q5 CS N25 OC IHC

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Notes: Q3AB Q4 Q5 SR BC

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Notes: Q3A? RCT? N100 BC CHT IHC

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Notes: Q3A? RCT? N100 BC CHT IHC?

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Notes: Q5 CS N100 LC IHC

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Notes: Q5 CS N100 LC IHC

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Notes: NRA? Q4? D? N? BC T? O? ELS?

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Notes: Q1? Q3A? Q4? PRO? N100 BC CHT IHC FIS ELS

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Notes: Q3A? PRO? N100 BC CHT IHC

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Notes: Q4?

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Notes: Q4? RET N100 BC CHT HT ELS

CORPORATE NAME: Cancer and Leukemia Group B

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Notes: Q5? NRA NPD HNC

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Notes: Q5? NRA NPD HNC

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Notes: Q5 CS N100 PC IHC

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Notes: Q3A? RET N100 BC CHT? FIS

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Notes: Q5? PI N? D?

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Notes: Q5 CS N50 LC IHC

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Notes: Q5 CS FEW LC HM?

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Notes: Q5 PRO CS N100 OC HM?

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Notes: Q4? RET FEW BC TRZ ELS

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Notes: Q3B? RET N100 BC HT (adjuvant) IHC

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Notes: Q3B? RET N100 BC HT IHC

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Notes: Q5 CS N100 OC HM?

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Notes: Q4? D? FEW BC T? O? ELS

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Notes: Q3B? RET N100 BC HT IHC

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Notes: Q5 CS N100 OC PCR

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Notes: Q3? RET PRG? N50 BC T? IHC

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Notes: Q3B?

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Notes: Q2? NRA

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Notes: Q2? Q4? PRO? N50 BC TRZ ELS IHC FIS

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Notes: Q2? RET N25 BC TRZ IHC

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Notes: Q4? PRO? N25 BC TRZ ELS

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Notes: Q2? NRA?

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Notes: Q5 PI N50 PC HM?

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Notes: Q1? Q2? Q3A? PRO N25 BC TRZ CHT IHC FIS

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Notes: Q4? NRA

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Notes: Q5 CS FEW HNC IHC

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Notes: Q4? PRO N100 BC T? ELS

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Notes: Q4? PRO? N100 BC T? ELS IHC

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Notes: Q5 CS FEW HNC

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Notes: Q5 CS N100 LC ELS

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Notes: Q3A RET N50 BC CHT IHC

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Notes: Q2? Q3A? PII N25 BC TRZ CHT IHC FIS (on 25 of 39 tested by IHC); no information on kits/reagents etc. for IHC or FISH

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Notes: Q2? PRO? FEW BC TRZ CHT IHC

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Notes: Q3A? PRO? N50 BC CHT HM?

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Notes: Q3A? RET N50 BC CHT? SBL

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Notes: Q3A? NRA?

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Notes: Q4? PRO? N100 BC CHT ELS

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Notes: Q5 CS N? HNC HM?

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Notes: Q3A? Q3B? RET N100 BC CHT? HT? PCR IHC SBL

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Notes: Q4? PRO? N50 BC T? ELS IHC?

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Notes: Q5 CS N25 LC IHC

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Notes: Q3B? PRO? N25 BC CHT IHC?

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Notes: Q4? PRO? N25 BC CHT ELS

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Notes: Q4? PRO? N100 BC CHT? ELS

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Notes: Q4? PRO? N100 BC T? ELS

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Notes: Q4 PRO? N100 BC T? O? ELS IHC

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Notes: Q5 CS N25 OC FIS

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Notes: Q5 CS N100 BC

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Notes: Q5 CS N25 LC HM?

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Notes: Q5 CS N50 HNC HM?

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Notes: Q1? Q2? RET N100 BC T? O? IHC FIS

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Notes: Q4? PRO N25 BC CHT ELS

Kim YC, Park KO, Kern JA et al. The interactive effect of Ras, HER2, P53 and Bcl-2 expression in predicting the survival of non-small cell lung cancer patients. Lung Cancer 1998; 22(3):181-90.
Notes: Q5 CS N100 LC IHC

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Notes: Q3A? RET N50 BC CHT (HDC) IHC

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Notes: Q2? Q3A? PRO? N25 BC TRZ CHT IHC FIS

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Notes: Q3A? (for intro/bkgd or discussion?) INV NRO

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Notes: Q3B? NRO?

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Notes: Q2?

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Notes: Q3A?

Kong SY, Kang JH, Kwon Y et al. Serum HER-2 concentration in patients with primary breast cancer. J Clin Pathol 2006; 59(4):373-6.
Notes: Q1? Q4? DAC? PRO? N50 BC T? O? IHC FIS ELS

Kong SY, Nam BH, Lee KS et al. Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. Clin Chem 2006; 52(8):1510-5.
Notes: Q1? Q4? DAC? N100 BC T? O? IHC FIS ELS

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Notes: Q2? PRO N100 BC TRZ HM?

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Notes: Q2? PRO? N50 BC TRZ HM?

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Notes: Q4 PRO N50 BC TRZ ELS

Kostler WJ, Steger GG, Soleiman A et al. Monitoring of serum Her-2/neu predicts histopathological response to neoadjuvant trastuzumab-based therapy for breast cancer. Anticancer Res 2004; 24(2C):1127-30.
Notes: Q4 PRO FEW BC TRZ ELS

Kostopoulos I, Arapantoni-Dadioti P, Gogas H et al. Evaluation of the prognostic value of HER-2 and VEGF in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy. Breast Cancer Res Treat 2006; 96(3):251-61.
Notes: Q3A? RCT N100 BC CHT IHC FIS

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Notes: Q5? RCT? LC

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Notes: Q4? PRO? N50 BC T? O? ELS

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Notes: Q5 CS N100 HNC IHC

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Notes: Q5 CS N100 HNC IHC

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Notes: Q5? D? HNC

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Notes: Q3A? RCT N100 BC CHT (HDC) HM?

Kronblad A, Jirstrom K, Ryden L, Nordenskjold B, Landberg G. Hypoxia inducible factor-1alpha is a prognostic marker in premenopausal patients with intermediate to highly differentiated breast cancer but not a predictive marker for tamoxifen response. Int J Cancer 2006; 118(10):2609-16.
Notes: Q3B? RCT N100 BC HT HM?

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Notes: Q5 CS N50 PC IHC

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Notes: Q3A? RCT N100 BC CHT (HDC) IHC

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Notes: Q5 CS N100 OC IHC

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Notes: Q4? PRO? N50 BC T? O? ELS

Lal P, Tan LK, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas. Am J Clin Pathol 2005; 123(4):541-6.
Notes: Q1? Q3B? DAC RET N100 T? O? IHC FIS

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Notes: Q5 CS N25 HNC RNA

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Notes: Q5 CS N? OC CIS IHC

Lassus H, Sihto H, Leminen A et al. Gene amplification, mutation, and protein expression of EGFR and mutations of ERBB2 in serous ovarian carcinoma. J. Mol. Med. 2006; 84(8):671-81.
Notes: Q5 CS N100 OC CIS

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Notes: Q5? PII LC

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Notes: Q5? D? OC

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Notes: Q4? RCT N100 BC HT ELS

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Notes: Q4? PRO? N100 BC T? O? ELS

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Notes: Q5 CS N25 OC IHC

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Notes: Q2? SR (but no Methods section) N? BC TRZ HM?

Lewis JE, Olsen KD, Sebo TJ. Carcinoma ex pleomorphic adenoma: pathologic analysis of 73 cases. Hum Pathol 2001; 32(6):596-604.
Notes: Q5 CS N50 HNC IHC

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Notes: Q2? PRO N25 BC TRZ + CHT IHC (+FIS for some)

Li L, Zhong YP, Zhang W, Zhang JQ, Yao ZQ. [Relationship of expression of C-erbB2, C-erbB3, and C-erbB4 with ovarian carcinoma]. Ai Zheng 2004; 23(5):568-72.
Notes: Q5 CS N25 PC IHC

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Notes: Q3B? RET N100 BC HT HM? TMA?

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Notes: Q4? RCT N100 BC HT ELS

Lipton A, Leitzel K, Ali SM et al. Serum HER-2/neu conversion to positive at the time of disease progression in patients with breast carcinoma on hormone therapy. Cancer 2005; 104(2):257-63.
Notes: Q4? PRO? N100 BC HT ELS

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Notes: Q5 CS N25 HNC IHC

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Notes: Q5 CS N? OC HM?

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Notes: Q5? CS HNC IT?

Love RR, Duc NB, Havighurst TC et al. Her-2/neu overexpression and response to oophorectomy plus tamoxifen adjuvant therapy in estrogen receptor-positive premenopausal women with operable breast cancer. J Clin Oncol 2003; 21(3):453-7.
Notes: Q3B? RCT N100 BC HT IHC

Ludovini V, Sidoni A, Pistola L et al. Evaluation of the prognostic role of vascular endothelial growth factor and microvessel density in stages I and II breast cancer patients . Breast Cancer Res Treat 2003; 81(2):159-68.
Notes: Q3A? RCT N100 BC CHT HM?

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Notes: Q4? PRO N50 BC T? ELS

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Notes: Q1? Q4? DAC? N25 BC T? O? IHC FIS ELS

Luftner D, Jung A, Schmid P et al. Upregulation of HER-2/neu by ovarian ablation: results of a randomized trial comparing leuprorelin to CMF as adjuvant therapy in node-positive breast cancer patients. Breast Cancer Res Treat 2003; 80(3):245-55.
Notes: Q4? RCT N100 BC HT ELS

CORPORATE NAME: Takeda Adjuvant Breast Cancer Study with Leuprorelin Study Group

Luftner D, Schnabel S, Possinger K. c-erbB-2 in serum of patients receiving fractionated paclitaxel chemotherapy. Int J Biol Markers 1999; 14 (2):55-9.
Notes: Q4 PRO N25 BC CHT ELS

Lumachi F, Basso SM. Serum tumor markers in patients with breast cancer. Expert Rev Anticancer Ther 2004; 4(5):921-31.
Notes: Q4? NRA?

Ma BB, Poon TC, To KF et al. Prognostic significance of tumor angiogenesis, Ki 67, p53 oncoprotein, epidermal growth factor receptor and HER2 receptor protein expression in undifferentiated nasopharyngeal carcinoma--a prospective study. Head Neck 2003; 25(10):864-72.
Notes: Q5 CS N50 HNC IHC

MacGrogan G , Mauriac L, Durand M et al. Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. Br J Cancer 1996; 74(9):1458-65.
Notes: Q3A? RET PRG N100 BC CHT IHC

Manna Edel F, Teixeira LC, Alvarenga M. Association between immunohistochemical expression of topoisomerase IIalpha, HER2 and hormone receptors and response to primary chemotherapy in breast cancer. Tumori 2006; 92(3):222-9.
Notes: Q3A? RET N100 BC CHT IHC (no reflex FISH & IHC 2+ considered HER2-)

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Notes: Q4? PRO? N100 BC T? ELS

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Notes: Q2 (n=6 IHC 2+ FISH-; response data only) Q3B? PRO N25 BC TRZ HT IHC FIS

Mark HF, Feldman D, Das S et al. Fluorescence in situ hybridization study of HER-2/neu oncogene amplification in prostate cancer. Exp Mol Pathol 1999; 66(2):170-8.
Notes: Q5 CS N50 PC NRO FIS

Martin-Richard M, Munoz M, Albanell J et al. Serial topoisomerase II expression in primary breast cancer and response to neoadjuvant anthracycline-based chemotherapy. Oncology 2004; 66(5):388-94.
Notes: Q3A? PRO? N25 BC CHT IHC

Mass RD, Press MF, Anderson S et al. Evaluation of clinical outcomes according to HER2 detection by fluorescence in situ hybridization in women with metastatic breast cancer treated with trastuzumab. Clin Breast Cancer 2005; 6(3):240-6.
Notes: Q2A? RET N100 BC TRZ IHC FIS

Mayr D, Kanitz V, Amann G et al. HER-2/neu gene amplification in ovarian tumours: A comprehensive immunohistochemical and FISH analysis on tissue microarrays. Histopathology 2006; 48(2):149-56.
Notes: Q5 CS N100 OC IHC FIS

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Notes: Q3A? RCT N100 BC CHT IHC

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Notes: Q5 CS N? HNC

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Notes: Q4? D? FEW BC T? WBL?

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Notes: Q3A? RET PRG N100 BC CHT IHC

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Notes: Q3A? RET? N100 BC CHT IHC

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Notes: Q5 CS N25 HNC

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Notes: Q2? Q3A? RCT N100 BC TRZ CHT FIS

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Notes: Q5 MA LC

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Notes: Q5 RET CS N100 LC

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Notes: Q5 CS N100 OC IHC

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Notes: NRA Q4

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Notes: Q5 CS N? LC HM?

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Notes: Q5 CS N100 PC HM?

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Notes: Q5 CS N50 LC HM?

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Notes: Q5 CS N100 LC IHC

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Notes: Q3B? PRO? N50 BC HT IHC

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Notes: Q3A RCT N100 BC CHT IHC

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Notes: Q3A RCT N100 BC CHT IHC

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Notes: Q3AB CS N100 BC HM?

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Notes: Q3A D? N50 BC CHT IHC

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Notes: Q5 CS N50 HNC IHC

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Notes: Q5 CS N50 LC IHC

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Notes: Q5 CS N25 LC IHC

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Notes: Q4? PII RCT N25 BC CHT ELS

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Notes: Q4? RET N100 BC T? O? ELS

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Notes: Q5 CS N100 OC HM?

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Notes: Q5 CS N50 LC IHC

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Notes: NRA Q2?

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Notes: Q5 CS N100 LC IHC

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Notes: Q5 RET CS N100 LC

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Notes: Q5? NRA NPD LC

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Notes: Q5? NRA NPD LC

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Notes: Q4? D? N? BC T? O? ELS

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Notes: Q4? PRO? N100 BC T? IHC ELS

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Notes: Q3A? QEX? N50 BC CHT IHC

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Notes: Q2 PII BC

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Notes: Q5 CS N100 HNC IHC

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Notes: Q4? PRO N25 BC T? IHC (primary versus lymph node versus mets) ELS

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Notes: Q5 CS N100 LC PCR

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Notes: Q4? D? N25 BC CHT ELS

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Notes: Q5 CS N? OC IHC

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Notes: Q2? RET? N25 BC TRZ FIS

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Notes: Q2? RET N100 BC TRZ HM?

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conventional-dose arm may be useful if combine data on HER2- from this paper with data on HER2+ from same study in rec #480; also see original NEJM paper from Rodenhuis et al.: NEJM 2003; 349(1):7-16.

CORPORATE NAME: Netherlands Working Party on Autologous Transplantation in Solid Tumours

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Notes: Q5 CS N50 HNC PCR

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Notes: Q1? Q3A? PRO N50 BC CHT IHC FIS? OTH (gene expression profile)

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Notes: NRA Q1? Q2? Q3A? Q3B?

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Notes: Q5 CS N50 OC

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Notes: Q5 RET CS N25 HNC IHC

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Notes: Q3A RET N100 BC CHT IHC

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Notes: Q5 CS N100 OC IHC

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Notes: Q5 PI N25 HNC

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Notes: Q4? RET N100 BC T? ELS

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Notes: Q5 CS N50 OC HM?

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Notes: Q4?

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Notes: Q5? D? LC

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Notes: Q4? PRO N100 BC CHT ELS

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Notes: Q2? Q3A? PRO N25 BC TRZ CHT IHC FIS confirmation for IHC2+

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Notes: Q2? RET N50 BC TRZ IHC

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Notes: Q5 CS N50 OC IHC?

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Notes: Q5 CS N50 OC IHC

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Notes: Q3A? D? N100 BC CHT IHC?

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Notes: Q1? Q3A? D? N50 BC CHT IHC FIS

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Notes: Q4? PRO? N100 BC T? ELS

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Notes: Q3A N25 PRO? BC CHT IHC

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Notes: Q3 PI PII N50 BC

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Notes: Q3A? RET N50 BC CHT IHC

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Notes: Q3A? RET N25 BC CHT IHC

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Notes: Q5 CS N100 LC IHC

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Notes: Q5 CS N100 BC

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Notes: NRA Q4?

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Notes: Q4? D? N? BC T? ELS

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Notes: Q1? Q2? Q3A? PII N50 BC TRZ CHT MAB FIS

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Notes: Q5 CS N25 OC HM?

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Notes: Q5 CS N100 LC IHC

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Notes: Q3A? RET? N25 BC CHT IHC

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Notes: Q3A? RET N50 BC CHT IHC (core biopsy versus surgical specimen; response to pre-operative chemoTx)

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Notes: Q5 CS N50 LC IHC

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Notes: Q5? PI FEW HNC NRS (HOMOG HER2)

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Notes: Q5 CS N50 HNC IHC

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Notes: Q5 CS N100 HNC IHC

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Notes: Q4? D? N? T? O? ELS

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Notes: Q5 CS N50 OC HM?

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Notes: Q3B? RET N? BC HT IHC

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Notes: Q3A? RCT N100 BC CHT IHC (no reflex FISH and IHC 2+ considered HER2+)

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Notes: Q5 RET CS N100 OC IHC

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Notes: Q2? RCT N100 BC TRZ CHT IHC only

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Notes: Q5 CS N50 LC IHC

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Notes: Q3B? RET N100 BC HT IHC

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Notes: Q3B? RET? N50 BC HT IHC

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Notes: Q4? RCT N100 BC HT ELS

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Notes: Q3A? RCT? N100 BC CHT HM?
CORPORATE NAME: International Breast Cancer Study Group

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Notes: Q2? PRO FEW BC TRZ (lapatinib) HM?

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Notes: Q3A? RCT N25 BC CHT IHC

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Notes: NRA Q2? Q3A? TRZ CHT

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Notes: Q4? PRO? N25 BC T? ELS

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Notes: Q4? D? N100 BC T? O? ELS

Sugano K, Kawai T, Ishii M et al. [Clinical study of serum ErbB-2 protein using sandwich radioimmunometric assay (ErbB-2 IRMA 'Eiken')]. Gan To Kagaku Ryoho 1994; 21(8):1245-53.
Notes: Q4? D? N100 BC T? O? ELS

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Notes: Q4? RET? N100 BC T? O? IHC ELS

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Notes: Q5 CS N50 OC IHC

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Notes: Q5 CS N25 LC HM?

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Notes: Q5 CS N50 LC HM?

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Notes: Q3A? PRO? N50 BC CHT IHC

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Notes: Q5 CS N25 LC PCR

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Notes: Q5 CS N100 LC IHC

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Notes: Q5 CS N100 OC IHC

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Notes: Q5 CS N25 HNC

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Notes: Q1? Q2? Q3A? PRO N25 BC TRZ CHT IHC FIS

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Notes: Q3A? Q3B? RET N100 BC CHT HT IHC

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Notes: Q3A? RET N100 BC CHT IHC

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Notes: Q3A? RCT? BC N100 CHT IHC

Thuerigen O , Schneeweiss A, Toedt G et al. Gene expression signature predicting pathologic complete response with gemcitabine, epirubicin, and docetaxel in primary breast cancer. J Clin Oncol 2006; 24(12):1839-45.
Notes: Q3A? PI PII (pooled) N100 BC CHT HM? (no reflex FISH & IHC 2+ considered HER2-)

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Notes: Q5 NRA NPD LC

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Notes: Q3B? RET N25 BC HT HM?

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Notes: Q3B? RET N100 BC HT HM?

Tokunaga E, Kimura Y, Oki E et al. Akt is frequently activated in HER2/neu-positive breast cancers and associated with poor prognosis among hormone-treated patients. Int J Cancer 2006; 118(2):284-9.
Notes: Q3B? RET N100 BC HT HM?

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Notes: Q5 CS N50 OC IHC

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Notes: Q5 PRO CS N50 IHC OC

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Notes: Q3AB CS N25 CHT HT BC

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Notes: Q3B? RET N100 BC HT IHC TMA

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Notes: Q1? Q4? RET N50 BC T? O? IHC FIS PCR ELS

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Notes: Q3A? Q3B? RET PRG N100 BC CHT HT IHC

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Notes: Q5 CS N50 LC IHC

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Notes: Q5? D? LC

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Notes: Q5 CS N50 OC IHC

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Notes: Q5 CS N100 OC IHC

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Notes: Q3A? RET N100 BC CHT MAB

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Notes: Q3A? PRO N50 BC CHT IHC

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Notes: Q5 RET CS N100 OC OTH

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Notes: Q3A? RET N50 BC CHT CIS

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Notes: Q3A? PRO? N50 BC CHT IHC

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Notes: Q3A? PRO? N100 BC CHT IHC

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Notes: Q4? RET? N50 BC T? O? ELS?

Vogel CL, Cobleigh MA, Tripathy D et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 2002; 20(3):719-26.
Notes: Q2? RCT N100 BC TRZ IHC FIS

Vogel CL, Cobleigh MA, Tripathy D et al. First-line Herceptin monotherapy in metastatic breast cancer. Oncology 2001; 61 Suppl 2:37-42.
Notes: Q2? RCT N100 BC TRZ IHC FIS

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Notes: Q4? PRO N100 BC HT ELS

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Notes: Q5 CS N100 LC IHC

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Notes: Q4?

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Notes: Q2?

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Notes: Q3A? D? N50 BC CHT HM?

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Notes: Q5 CS N? LC

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Notes: Q5 CS N100 OC HM?

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Notes: Q5 CS N50 LC IHC

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Notes: Q5 CS N25 OC FIS

Watanabe N, Miyamoto M, Tokuda Y et al. Serum c-erbB-2 in breast cancer patients. Acta Oncol 1994; 33 (8):901-4.
Notes: Q4? D? N100 BC T? O? els

Weed DT, Gomez-Fernandez C, Pacheco J et al. MUC4 and ERBB2 expression in major and minor salivary gland mucoepidermoid carcinoma. Head Neck 2004; 26(4):353-64.
Notes: Q5 RET CS N25 HNC HNC IHC

Weed DT, Gomez-Fernandez C, Yasin M et al. MUC4 and ErbB2 expression in squamous cell carcinoma of the upper aerodigestive tract: correlation with clinical outcomes. Laryngoscope 2004; 114(8 Pt 2 Suppl 101):1-32.
Notes: Q5 RET CS N100 HNC IHC

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Notes: Q5 CS N100 HNC IHC

Weiss J, Friedrich MG. [Prostate carcinoma: higher COX-2 contents in advanced tumors]. Aktuelle Urol 2004; 35(5):359-61.
Notes: Q5? D? PC

Wild PJ, Reichle A, Andreesen R et al. Microsatellite instability predicts poor short-term survival in patients with advanced breast cancer after high-dose chemotherapy and autologous stem-cell transplantation. Clin Cancer Res 2004; 10(2):556-64.
Notes: Q3A? RET N25 BC CHT (HDC) IHC

Willems A, Gauger K, Henrichs C, Harbeck N. Antibody therapy for breast cancer. Anticancer Res 2005; 25(3A):1483-9.
Notes: Q2? NRA

Willsher PC , Beaver J, Pinder S et al. Prognostic significance of serum c-erbB-2 protein in breast cancer patients. Breast Cancer Res Treat 1996; 40(3):251-5.
Notes: Q4? RET? N100 BC HT ELS (+IHC)

Willsher PC , Pinder SE, Gee JMW et al. c-erbB2 expression predicts response to preoperative chemotherapy for locally advanced breast cancer. Anticancer Res. 1998; 18(5 B):3695-8.
Notes: Q3A? RET? N50 BC CHT IHC

Witzel I, Thomssen C, Krenkel S et al. Clinical utility of determination of HER-2/neu and EGFR fragments in serum of patients with metastatic breast cancer. Int J Biol Markers 2006; 21(3):131-40.
Notes: Q4? D? N50 BC T? IHC ELS

Wolf D, Wolf AM, Rumpold H et al. The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. Clin Cancer Res 2005; 11(23):8326-31.
Notes: Q5 CS N50 OC RNA

Wolf M. [Prognostic factors and therapeutic strategy in non-small-cell bronchial carcinoma]. Schweiz Rundsch Med Prax 1997; 86(42):1640-6.
Notes: Q5 CS N? LC HM?

Wright C, Nicholson S, Angus B et al. Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer. Br J Cancer 1992; 65(1):118-21.
Notes: Q3B? RET N50 BC HT HM?

Wu JT, Astill ME, Gagon SD, Bryson L. Measurement of c-erbB-2 proteins in sera from patients with carcinomas and in breast tumor tissue cytosols: correlation with serum tumor markers and membrane-bound oncoprotein. J Clin Lab Anal 1995; 9(3):151-65.
Notes: Q4? Q5? D? N? BC PC OVC T? ELS (serum and tissue cytosol)

Wu Y, Khan H, Chillar R, Vadgama JV. Prognostic value of plasma HER-2/neu in African American and Hispanic women with breast cancer. Int J Oncol 1999; 14(6):1021-37.
Notes: Q4? D? N? BC T? ELS

Xia W, Lau YK, Zhang HZ et al. Strong correlation between c-erbB-2 overexpression and overall survival of patients with oral squamous cell carcinoma. Clin Cancer Res 1997; 3(1):3-9.
Notes: Q5 CS N50 HNC HM?

Xu Y, Yao L, Ouyang T et al. p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005; 11(20):7328-33.
Notes: Q3A? D? N100 BC CHT (neoadj) IHC (HER2+ = IHC 2+ or 3+, without reflex FISH for 2+)

Yamashita H , Toyama T, Nishio M et al. p53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. Breast Cancer Res 2006; 8(4): R48.
Notes: Q3A? RET N50 BC HT IHC

Yan J, Fang Y, Huang B-J, Liang Q-W, Wu QL, Zeng Y-X. Absence of evidence for HER2 amplification in nasopharyngeal carcinoma. Cancer Genet. Cytogenet. 2002; 132(2):116-9.
Notes: Q5 CS N25 HNC

Yan Y, Guo Q, Nan P. [Relationship between c-erbB-2 oncoprotein expression and prognosis in salivary gland malignant pleomorphic adenoma]. Zhonghua Kou Qiang Yi Xue Za Zhi 1995; 30(6):326-8, 383.
Notes: Q5 CS N50 HNC IHC

Yang H, Zhang G, Xu K. [c-erbB2 gene amplification in human primary epithelial ovarian cancer and its clinical significance]. Zhonghua Zhong Liu Za Zhi 1998; 20(5):367-70.
Notes: Q5 CS N100 OC SB

Yano S, Muguruma H, Sone S. [Molecular targetting therapy for non-small-cell lung neoplasms with EGF-R inhibitors]. Nippon Naika Gakkai Zasshi 2003; 92(2):318-23.
Notes: Q5? D? LC

Yasasever V , Dincer M, Camlica H, Duranyildiz D, Dalay N. Serum c-erb B2 oncoprotein levels are elevated in recurrent and metastatic breast cancer. Clin Biochem 2000; 33(4):315-7.
Notes: Q4?

Yasui W, Nishiyama M, Tsuruo T, Tahara E. Molecular targeting therapy for cancer: the Twelfth International Symposium of the Hiroshima Cancer Seminar, November 2002. Cancer Sci 2003; 94(2):221-3.
Notes: Q5? D? LC

Yoo GH, Hung MC, Lopez-Berestein G et al. Phase I trial of intratumoral liposome E1A gene therapy in patients with recurrent breast and head and neck cancer. Clin Cancer Res 2001; 7(5):1237-45.
Notes: Q5 PI FEW BC

Yu CJ, Shun CT, Yang PC et al. Sialomucin expression is associated with erbB-2 oncoprotein overexpression, early recurrence, and cancer death in non-small-cell lung cancer. Am J Respir Crit Care Med 1997; 155(4):1419-27.
Notes: Q5 CS N100 LC IHC

Yu FZ, Sugano K, Ohkura H, Mori S. [Development of sandwich radioimmunometric assay for serum c-erbB-2 oncogene product and its significance in diagnosing breast carcinoma]. Rinsho Byori 1991; 39(10):1087-92.
Notes: Q4? D? N? BC T? O? ELS

Yuan P, Xu BH, Chu DT. Correlation between serum HER-2 oncoprotein and patients with breast cancer. Chin Med Sci J 2004; 19(3):212-5.
Notes: Q4? RET? N50 T? ELS

Yuan P, Xu BH, Zhang C, Qi J. [Serum her-2/neu level and related factors in patients with breast cancer]. Zhonghua Zhong Liu Za Zhi 2003; 25(6):573-4.
Notes: Q4? RET? N50 T? ELS

Zhu L, Chow LW, Loo WT, Guan XY, Toi M. Her2/neu expression predicts the response to antiaromatase neoadjuvant therapy in primary breast cancer: subgroup analysis from celecoxib antiaromatase neoadjuvant trial. Clin Cancer Res 2004; 10(14):4639-44.
Notes: Q3B? D? N25 BC HT IHC FIS

Zidan J, Dashkovsky I, Stayerman C, Basher W, Cozacov C, Hadary A. Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. Br J Cancer 2005; 93(5):552-6.
Notes: Q1? Q2? PRO N50 BC TRZ IHC FIS (primary tumor vesus mets)

Zinner RG, Glisson BS, Fossella FV et al. Trastuzumab in combination with cisplatin and gemcitabine in patients with Her2-overexpressing, untreated, advanced non-small cell lung cancer: report of a phase II trial and findings regarding optimal identification of patients with Her2-overexpressing disease. Lung Cancer 2004; 44(1):99-110.
Notes: Q5 PII FEW LC ELS

Appendix C. Evidence Data Abstraction Tables

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-A: Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (n per group)	n, Evaluated	n, withdrawn or lost to F/U	Treatment Regimen (Agents)
HER2 Discrepant						
Paik et al. 2007; Kim et al, in preparation; Romond et al. 2005	RCT NSABP-B31	adjuvant therapy	2043 (1024, 1019)	1829 w tumor blocks; 1795 w baseline and F/U data	248	AC→ (P ± trastuzumab)
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	RCT NCCTG N9831	adjuvant therapy	1842	1779 (895, 884)	63	AC→ (P ± trastuzumab)
HER2 Negative						
Seidman et al. 2004	RCT CALGB 9840	inoperable or metastatic disease, stratified by 1st or 2nd line therapy	735	228 (HER2-) (113, 115)	0 (507 HER2+ or UNK given TRZ)	4 arm trial: P (weekly vs. q3w) stratified by HER2 status; HER2- randomized to ± TRZ, all HER2+ given TRZ
Kaufman et al. 2007	RCT CALGB 150002	metastatic, 1st or 2nd line; companion study on CALGB 9840 pts	585	303 (samples available for central testing)	282	4 arm trial: P (weekly vs. q3w) stratified by HER2 status; HER2- randomized to ± TRZ, all HER2+ given TRZ

Data Abstraction Table II-B: Patient Characteristics

Study	Age/Menopausal Status	Race (%)	Disease Stage	Disease Stage (%)	Performance Status	Hormone Receptor Status (%)
HER2 Discrepant						
NSABP B-31 Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005; Tan-Chiu et al. 2005	-TRZ +TRZ ≤39 16.7% 16.2% 40-9 34.9% 35.4% 50-9 33.7% 32.4% ≥60 14.7% 16.0% pre NR intra NR post NR	of n=1664 evaluable in Tan-Chiu et al. B 8 W 84 H A O 8	-TRZ +TRZ I IIa NR IIb NR IIIa NR IIIb NR IV 0	-TRZ1 +TRZ ≤2 cm 37.3 2-4 cm 40.7 42.7 44.7 ≥4.1 cm 14.4 16.7 unknown 2.2 1.4 pN0 0 0 pN1 pN2 56.7 57.4 pN3 29.0 29.1 14.3 13.5	Scale Grp1 Grp2 not reported (likely not relevant in adjuvant setting)	-TRZ +TRZ ER+ 52.8 51.9 PR+ 41.4 39.0
NCCTG N9831 Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	-TRZ +TRZ ≤39 17.1% 16.0% 40-9 34.0% 33.7% 50-9 33.7% 32.3% ≥60 15.2% 18.1% pre NR intra NR post NR	B W H not A reported O	-TRZ +TRZ I IIa NR IIb NR IIIa NR IIIb NR IV 0	-TRZ1 +TRZ ≤2 cm 38.0 2-4 cm 40.4 46.1 47.3 ≥4.1 cm 12.6 14.2 unknown 1.2 0.5 pN0 12.6 11.0 pN1 pN2 47.8 49.9 pN3 25.2 25.4 13.9 13.7	Scale Grp1 Grp2 not reported (likely not relevant in adjuvant setting)	-TRZ +TRZ ER+ 52.8 51.2 PR+ 41.3 39.4
HER2 Negative						
CALGB 9840 Seidman et al. 2004 (no separate data for HER2- group randomized to ±TRZ)	mn md rng sd pre 19% intra post	B W H not A reported O	IIa IIb IIIa IIIb IV 100%	Mn T T1 T2 T3 not T4 N0 reported N1 N2	Scale 92% not PS 0-1 stated	ER+ 51% PR+ not reported
CALGB 150002 Kaufman et al. 2007	not reported	not reported	IIa IIb IIIa IIIb IV 100%	not reported	not reported	not reported

Data Abstraction Table II-C: HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
HER2 Discrepant				
Paik et al, 2007; Kim et al, in preparation;	FISH PathVysion™ IHC HercepTest™ (performed by NSABP Division of Pathology Central Laboratory; all considered HER2+ by local laboratories)	FISH: scored as per FDA labeling IHC: scored as per FDA labeling	Pos 1588 (88.5) Equiv 0 Neg 207 (11.5) 3+ 1488 (82.9) 2+ 146 (8.1) 1+ 119 (6.6) 0 (1.9)	reports on discrepant and Her2- subsets by central lab results central IHC results missing due to assay failure for 8 patients (0.5% of total, n=1795)
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	FISH PathVysion™ IHC HercepTest™ (performed by Mayo Medical Labs and a reference laboratory; all considered HER2+ by local laboratories) (data from Prez et al. 2007, slide 18)	FISH: scored as per FDA labeling IHC: scored as per FDA labeling	Pos 1823 (91.2) Equiv 0 Neg 156 (8.8) 3+ 1458 (82.0) <3+ 321 (18.0)	reports on discrepant and HER2- subsets by central lab results
HER2 Negative				
CALGB 9840 Seidman et al. 2004	IHC, with FISH if 2+; reported reported based on local assessment only	IHC 3+ or 2+/FISH+	Pos 507 (69.0) Equiv Neg 228 (31.0)	
CALGB 150002 Kaufman et al. 2007	FISH PathVysion™, with CEP17 to detect polysomy IHC HercepTest™	polysomy = >2.2 copies of centromere 17 despite FISH ratio <2.0	n=265, central FISH+ or no polysomy n=38, polysomy and central FISH- (19% of central FISH-) (n=3, IHC 3+; n=35, IHC<3+)	

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-D: Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
HER2 Discrepant					
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	disease-free survival	overall survival; time to distant recurrence; breast cancer specific survival;	not relevant (adjuvant therapy)	not relevant	baseline, 3 months, then every 6 months to 5 years, and yearly thereafter (for cardiac toxicity); median F/U duration: 2.4 years
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	disease-free survival	overall survival; time to distant recurrence; breast cancer specific survival (but none reported for HER2- or discrepant subgroups)	not relevant (adjuvant therapy)	not relevant	baseline, 3 months, then every 6 months to 5 years, and yearly thereafter (for cardiac toxicity); median F/U duration: 1.5 years
HER2 Negative					
CALGB 9840 Seidman et al. 2004	tumor response rate	time to progression, overall survival	not reported	not reported	not reported
CALGB 150002 Kaufman et al. 2007	tumor response rate	overall survival, time to progression	not reported	not reported	not reported

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-E: Time to Event Outcomes

Study	Time to Event Outcomes												
HER2 Discrepant, Adjuvant Therapy													
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
Paik et al, 2007;		Cx											
Kim et al, in	RFI*	Tx	56							Cox prop	0.11	0.35 (0.10-1.28)	adjusted for ER and
preparation;		Cx	69							hazards			nodal status
Romond et al.	PFS	Tx											
2005		Cx											
	RFS	Tx											
FISH+ IHC-		Cx											
(0, 1+, 2+) by	DFS	Tx	56							Cox prop	0.064	0.30 (0.08-1.07)	adjusted for ER and
central lab		Cx	69							hazards			nodal status
	DSS	Tx											
		Cx											
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
Paik et al, 2007;	RFI*	Tx	10							Cox prop	0.94	0.91 (0.08-10)	adjusted for ER and
Kim et al, in		Cx	21							hazards			nodal status
preparation;	PFS	Tx											
Romond et al.		Cx											
2005	RFS	Tx											
		Cx											
FISH- IHC 3+	DFS	Tx	10							Cox prop	0.94	0.91 (0.08-10)	adjusted for ER and
by central lab		Cx	21							hazards			nodal status
	DSS	Tx											
		Cx											
	DFS	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Perez et al. 2007;	FISH+	Tx	123							???	0.97	0.98 (0.33-2.91)	pools IHC 2+ with 0, 1+
Perez et al. 2006;	IHC<3+	Cx	95										
Romond et al.													
2005	FISH-	Tx	23							0.57	0.61 (0.11-3.29)		
	IHC3+	Cx	30										

???

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-E (continued): Time to Event Outcomes

Study	Time to Event Outcomes												
HER2 Negative, Adjuvant Therapy													
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	RFI*	Tx	69							Cox prop hazards	0.041	0.31(0.10-0.95)	adjusted for ER and nodal status
		Cx	80										
	PFS	Tx											
		Cx											
	RFS	Tx											
		Cx											
FISH- IHC 1+, 2+ by central lab	DFS	Tx	69		~98%	~95%	~90%	~90%	~86%	Cox prop hazards	0.02	0.30 (0.11-0.83)	adjusted for ER and nodal status
		Cx	80		~90%	~79%	~75%	~70%	~62%				
	DSS	Tx											
		Cx											
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	RFI*	Tx	82							Cox prop hazards	0.034	0.36 (0.14-0.92	adjusted for ER and nodal status
		Cx	92										
	PFS	Tx											
		Cx											
	RFS	Tx											
		Cx											
FISH- IHC- (0, 1+, 2+) by central lab	DFS	Tx	82		~97%	~90%	~87%	~87%	~84%	Cox prop hazards	0.014	0.34 (0.14-0.80)	adjusted for ER and nodal status
		Cx	92		~92%	~80%	~76%	~72%	~65%				
	DSS	Tx											
		Cx											
	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS	Tx											
		Cx											
FISH- IHC- (0, 1+, 2+) by central lab	DFS	Tx	59							???	0.13	0.51 (0.21-1.2)	
		Cx	44										
	DSS	Tx											
		Cx											

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-E (continued): Time to Event Outcomes

Study	**Time to Event Outcomes**												
HER2 Negative, Therapy for Metastasis													
CALGB 9840	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Seidman et al.	OS	Tx	113	21.6	~75%	~40%	~25%			???	0.67		
2004		Cx	115	19.6	~70%	~37%	~22%						
	TTP	Tx	113	7.3	~30%	~18%	~10%			???	0.088		
IHC2+/FISH- or		Cx	115	5.5	~25%	~10%	~10%						
IHC 0, 1+	PFS	Tx											
		Cx											
	RFS	Tx											
		Cx											
	DFS	Tx											
		Cx											
	DSS	Tx											
		Cx											
CALGB 150002	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Kaufman et al.	OS	Tx	19	~30	~90%	~65%	~30%			???	0.538		
2007		Cx	19	~23	~69%	~48%	~30%						
	TTP	Tx	19	~12	~50%	~15%					???	0.888	
central FISH-		Cx	19	~6	~29%	~23%	θ17%						
with polysomy 17	PFS	Tx											
from CALGB		Cx											
9840	RFS	Tx											
		Cx											
	DFS	Tx											
		Cx											
	DSS	Tx											
		Cx											

*RFI = recurrence-free interval

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients

Data Abstraction Table II-F: Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value) Selected	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
HER2 Discrepant								
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	study used a Cox proportional hazards model to estimate HR and adjust for two covariates (estrogen receptor status and nodal status); however, Methods section of draft article provided insufficient detail and lacked table(s) on other candidate predictors, univariate results, etc.							
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	insufficient information provided in slides and abstract							
HER2 Negative								
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	study used a Cox proportional hazards model to estimate HR and adjust for two covariates (estrogen receptor status and nodal status); however, Methods section of draft article provided insufficient detail and lacked table(s) on other candidate predictors, univariate results, etc.							
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	insufficient information provided in slides and abstract							
CALGB 9840 Seidman et al. 2004	not reported							
CALGB 150002 Kaufman et al. 2007	not reported							

Data Abstraction Table II-G: Tumor Response and Quality of Life

Study	**Tumor Response**										**Quality of Life**					
HER2 Discrepant (IHC 2+/FISH+)																
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	Grp	N	CR	PR	SD	PD	NE	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
	not reported										not reported					
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005	Grp	N	CR	PR	SD	PD	NE	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
	not reported										not reported					
HER2 Negative																
CALGB 9840 Seidman et al. 2004	Grp	N	CR	PR	SD	PD	NE	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
	+TRZ	112		35% (CR+PR)				???	0.34	p=0.32 by multivariate			not reported			
	-TRZ	111		29% (CR+PR)						logistic regression						
CALGB 150002 Kaufman et al. 2007 central FISH- from CALGB 9840	Grp	N	CR	PR	SD	PD	NE	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
	+TRZ	19		63% (CR+PR)				???	0.048	FISH-/polysomy+			not reported			
	- TRZ	19		26% (CR+PR)												
	+TRZ	53		36% (CR+PR)				???	NS	FISH-/polysomy-						
	- TRZ	50		36% (CR+PR)												

Question 2: HER2-targeted Therapy for HER2 Discrepant/Negative Patients
Data Abstraction Table II-H: Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
HER2 Discrepant								
Paik et al, 2007; Kim et al, in preparation; Romond et al. 2005	**Predictors/**		**Predictors/**					
Perez et al. 2007; Perez et al. 2006; Romond et al. 2005		**Selected**						
HER2 Negative								
CALGB 9840 Seidman et al. 2004	RCT ORR multivariate regression			insufficient detail in abstract or slides				
CALGB 150002 Kaufman et al. 2007					not reported			

Data Abstraction Table II-I: Adverse Events

Toxicity Type	Study	Severity or Grade	Results
HER2 Discrepant (IHC 2+/FISH+)			
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Data Abstraction Table II-I (continued): Adverse Events

Toxicity Type	Study	Severity or Grade	Results
HER2 Negative			
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Adjuvant Chemotherapy						
Yang et al. 2003 rec. # 8840	single arm retrospective series	adjuvant therapy post mastectomy	94 (identically treated; 13 of 107 in series not given adj. chemo)	94 (outcomes reported separately)	0	cyclophosphamide + methotrexate + fluorouracil (CMF)
Gusterson et al. 2003; rec. # 43690	RCT; separate randomization by nodal status	adjuvant therapy: none versus one cycle peri-op versus prolonged	1275 node-neg 1229 node-pos	760 node-neg 746 node-pos	515 node-neg 483 node-pos (no samples)	node-neg: peri-op CMF versus no adj therapy; node-pos: peri-op versus continuous CMF
Moliterni et. al. 2003; rec. # 10210	RCT retrospective analysis by HER2 status	adjuvant therapy post mastectomy or quadrant-ectomy with axillary dissect. (1-3 nodes+)	552	506	46 (HER2 status unknown)	CMF alone (12 cycles) versus CMF for 8 cycles then doxorubicin for 4 cycles (CMF→A)
Colozza et al. 2005; rec. # 3820	RCT retrospective analysis by HER2 status	post-operative adjuvant therapy; node- if ER/PR neg or node+ with ≤9 nodes involved	348	266	82 (no tumor samples)	CMF for 6 cycles versus epirubicin weekly for 4 months
Pritchard et al. 2006; rec. # 1760	RCT retrospective analysis by HER2 status	adjuvant therapy post mastectomy or lumpec-tomy with axillary dissec-tion; all node+	710	634 (by IHC) 628 (by FISH)	71 (no tumor samples) 5 (IHC & FISH failed)	CMF (Cx) versus CEF (Tx); each given for 6 cycles; no endocrine therapy after adjuvant chemoTx
Knoop et al. 2005; rec. # 3450	RCT (2 x 2) retrospective analysis by HER2 status	adjuvant therapy post mastectomy or lumpectomy with axillary dissection	1,195 (980 Danes eligible)	773 (805 tested for HER2 status)	CMF: 79 of 500 CEF: 128 of 480	CMF (Cx) versus CEF (Tx); each given for 9 cycles ± pamidronate, daily for 4 years; no adjuvant tamoxifen
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880 CALGB trial 8541 & lab companion study 8869	3-arm RCT retrospective analysis by HER2 status	adjuvant therapy post mastectomy or lumpec-tomy with axillary dissec-tion; all node+	1,549 (in CALGB 8541)	524 (of 993 in CALGB 8869)	1,025 (556 not in 8869 study + 469 not in Dressler et al.)	4 cycles high dose CAF (600/60/600 mg/m^2) q4wk versus 6 cycles moderate dose CAF (400/40/400 mg/m^2) q4wk versus 4 cycles low dose CAF (300/30/300 mg/m^2) q4wk; similar proportions in each arm given 5 years of twice daily tamoxifen (41%, 40%, 34%) for ER+, post-menopausal disease
Del Mastro et al. 2004, 2005; rec. # 48020 GONO-MIG-1 trial	RCT retrospective analysis by HER2 status	adjuvant therapy for node- high-risk or node+ patients	1,214	731	483 (specimens unavailable for HER2 testing)	6 cycles FEC21 regimen q3wk versus up to 9 cycles FEC14 regimen q2wk (same drug doses in each regimen; ER+ & PR+ patients in each arm received tamoxifen qd for 5 years

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Adjuvant Chemotherapy (continued)						
Tanner et al. 2006; rec. # 1820	STD-dose arm of RCT; retrospective analysis by HER2 status	adjuvant therapy post mastectomy or lumpectomy with axillary dissection	525 (251 to STD-dose arm)	391 (180 for STD-dose arm)	274 (71 from STD-dose arm; no samples)	FEC (9 cycles; individualized doses based on hematological toxicity) versus HDC/AuSCS using CTCb after 3-4 cycles of FEC (did not abstract data from HDC/AuSCS arm); loco-regional RTx + 5 years of tamoxifen for all patients
Hayes et al. 2007; rec. # 47610 CALGB 9344	subset from 3 X 2 RCT; retrospective analysis by HER2 status	adjuvant therapy for node+ patients after surgery with negative margins	1500 (2 groups, 750 each, randomly selected from 3121 in RCT)	1322	178 (no tumor specimens; 1621 RCT patients not analyzed by HER2 status)	4 cycles of AC (randomized to 1 of 3 doxorubicin doses) followed by 4 cycles of paclitaxel or observation (a second; separately reported doxoru-bicin dose did not change outcomes
Martin et al. 2005; rec # 47650	RCT pre-planned subgroups; 2nd interim analysis of ongoing trial	adjuvant therapy for node+ patients after surgery with negative margins	1491	1262	229 (no tumor specimens	6 cycles (3 wks each) of docetaxel + doxorubicin + cyclophosphamide (DAC) versus flluorouracil + doxorubicin + cyclophosphamide (FAC); equal proportion (ER or PR)+ patients, each arm took qd tamoxifen for 5 years
Neoadjuvant (Pre-operative) Chemotherapy						
Learn et al. 2005; rec. # 47640	3 arm RCT; retrospective analysis by HER2 status	pre-operative chemo-therapy for operable breast cancer (T1-3, N0-1, M0)	144	104	40 (no tumor specimen, 23; HER2 status unknown, 17)	4 cycles AC ± docetaxel (D) q3wk, followed by surgery; 3rd arm given AC + post-surgery D (pooled with AC alone controls for analysis by HER2 status); all patients given 5 yrs of TAM qd
Arriola et al. 2006; rec # 950	prospective single-arm series	primary chemotherapy for T2-3 N0-1 operable breast cancer	232	232	0	doxorubicin (75 mg/m^2) 4 cycles, q3wk, then lumpectomy or mastectomy + 3-level axillary dissect.
Park et al. 2003; rec # 9960	retrospective single-arm series	pre-operative chemo-therapy for locally-advanced disease	67	67	0	doxorubicin (50 mg/m^2) 4 cycles, q3wk, prior to breast conservation or mastectomy
Zhang et al. 2003; rec # 9820	retrospective single-arm series	pre-operative chemo-therapy for operable breast cancer	97	97	0	FAC q3wk (6 cycles for 7 patients, 5 cycles for 1, 4 cycles for 81, and 3 cycles for 8)
Tulbah et al. 2002; rec # 11560	retrospective single-arm series	pre-operative chemo-therapy for locally-advanced, non-inflammatory breast cancer	54	54	0	paclitaxel + cisplatin, q3wk, for 3 or 4 cycles

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Neoadjuvant (Pre-operative) Chemotherapy						
Tinari et al. 2006; rec # 2300	retrospective single-arm series	pre-operative chemo-therapy for operable breast cancer	77 (selected; 16 ineligible of 93 consecutive)	77	0	FEC q3wk (median 4 cycles; range 3-6 cycles)
Chemotherapy for Advanced or Metastatic Disease						
Harris et al 2006[10]; rec. # 390, no data on no. of sites, 1994-?	RCT/RET; CALGB 9342	Advanced (Stage IV or inoperable); first or second line Tx. No concurrent hormonal therapy	474	165 (of n=175 w adequate tumor blocks; n= 10, all bio-marker tests unsuccessful)	299 (n=273, no blocks; n=26, blocks inade-quate); similar characteristics & outcomes, w/wo blocks, except DFS	Paclitaxel; compared 3 doses—175, 210, or 250 mg/m^2 q3wk to failure (progression or intolerable toxocity)—but data combined for this analysis)
Di Leo et al 2004; rec. # 5970; 29 of 41 sites in original trial, 7/94-1/97[11]	Phase III RCT (not blinded); TAX 303 trial; secondary analysis	Metastatic disease; first or second line therapy; prior CMF required (adj or for mets); prior anthracic-lines or taxanes excluded	326	176	150 (n=74, Grp1; n=76, Grp 2)	Grp 1: doxorubicin (75 mg/m^2) (A; n=91) vs Grp 2: docetaxel (100 mg/m^2) (T; n=85) every 3 wks; max 7 cycles absent progression or toxicity. No stat sig differences between populations with versus without specimens for HER2 analysis.
Konecny et al 2004; rec. # 6740; ~71 sites, Germany, 10/96-12/99	RCT; secondary analysis	Metastatic; no prior chemo for metastatic disease, no metastasis to CNS or to bone only. Stratified by 0 vs 1 prior hormonal Tx for metastatic disease.	579 enrolled; 516 eligible were randomized & treated	275	241 (n=219, no block; n=17, technically inadequate; n=5, no inva-sive cancer; no SS diffs between pts w/ wo known HER2 status.	Grp 1: epirubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) (EC, n=137); Grp 2: epirubicin (60 mg/m^2) and paclitaxel (175 mg/m^2)(ET, n=138). Chemo given q3 wks for max of 10 cycles; median=6 cycles.

[10] Some data from**:** Winer EP. Berry DA. Woolf S. Duggan D. Kornblith A. Harris LN. Michaelson RA. Kirshner JA. Fleming GF. Perry MC. Graham ML. Sharp SA. Keresztes R. Henderson IC. Hudis C. Muss H. Norton L. Failure of higher-dose paclitaxel to improve outcome in patients with metastatic breast cancer: cancer and leukemia group B trial 9342. J Clin Oncol *22(11):2061-8, 2004 Jun 1.*

[11] Some data from: Chan S, Friedrichs K, Noel D et al. Prospective randomized trial of docetaxel versus doxorubicin in patients with metastatic breast cancer. J Clin Oncol 1999;17(8):2341-54.

Data Abstraction Table IIIa-B: Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Hormone Receptor Status
Adjuvant Chemotherapy						
Yang et al. 2003 rec. # 8840 (of n=107 tested for expression of various markers)	mn md 51.9 yrs rng 33-77 yrs sd <50 yrs 45 (47.9%) ≥50 yrs 49 (52.1%)	B W H A 100% O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	T <3cm 31 (33%) T 3-5cm 39 (41%) T3 >5 cm 24 (26%) N- 41 (38%) N+ 66 (62%)	Scale reported	ER+ PR+ not reported
Gusterson et al. 2003; rec. # 43690 760 node- pts randomized to periop CMF vs no adj. Tx	HER2- mn md HER2+ rng sd menopausal status: pre- 53% post 52.4% 47.5% 47%	Grp1 Grp2 B W not H A reported O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Mn T HER2+ HER2- T size ≤2cm 41% 57% >2cm unk 53% 40% 6% 3% N0 100% 100%	Scale not reported not	HER2+ HER2- ER+ 36% 51% ER- 41% 32% unk 23% 17% PR+ 24% 37.5% PR- 50% 37.5% unk 26% 25%
Gusterson et al. 2003; rec. # 43690 746 node+ pts randomized to perioperative vs prolonged CMF	HER2- mn md HER2+ rng sd menopausal status: pre- 60% post 50% 60% 40%	Grp1 Grp2 B W not H A reported O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Mn T HER2+ HER2- T size ≤2cm not >2cm # positive nodes: reported 1-3+ 51% 57% ≥4 49% 43%	Scale reported not	HER2+ HER2- ER+ 56% 28% ER- 32% 59% unk 12% 13% PR+ 62% 36% PR- 22% 45% unk 16% 19%
Moliterni et. al. 2003; rec. # 10210 RCT; CMF (Grp 1) versus CMF→A (Grp 2)	Grp2 mn Grp1 md not rng reported sd <51 yr 69% 67%	Grp1 Grp2 B W not H A reported O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 Mn T T stage distribution not reported ~65% <2.1 cm diam. N0 N1 100% 100% N2	Scale reported not	Grp1 Grp2 ER+ 59% 52% ER- 34% 39% unk 7% 9% PR+ 53% 53% PR- 38% 34% unk 9% 13%
Colozza et al. 2005; rec. # 3820 RCT; CMF (Grp 1) vs epirubicin (Grp 2); n=133 each tested for HER2 status	Grp2 age (years): <40 12 9 Grp1 40-50 32 40 >50 56 51 menopausal status: pre 53 53 post 47 47	Grp1 Grp2 B W not H A reported O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 tumor diameter (cm): ≤2 45% 46% 2-5 50% 48% >5 unk 1% 0% 5% 6% N0 20% 23% N1-3 59% 52% N4-9 21% 26%	Scale reported not	Grp1 Grp2 ER+ 56% 55% ER- 41% 44% unk 4% 2% PR+ 63% 63% PR- 33% 35% unk 4% 2%

Data Abstraction Table IIIa-B (continued): Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Hormone Receptor Status
Adjuvant Chemotherapy (continued)						
Pritchard et al. 2006; rec. # 1760 RCT; CMF versus CEF; n=163 FISH+ (Grp 1); n=465 FISH- (Grp2)	Grp2 age (%) ≤29 yr Grp1 Grp2 30-39 4 1 40-49 27 22 ≥50 yr 54 60 all pre-menopausal; ineligible if post-menopausal 15 17	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp2 T1 35% 40% T2 52% 49% T3 Grp1 5% 5% # positive nodes: 0 0 0 1-3 57% 63% 4-10 36% 31% ≤10 7% 7%	Scale reported not	Grp1 Grp2 ER+ 56% 62% ER- 35% 27% unk 9% 12% PR+ not reported
Knoop et al. 2005; rec. # 3450 RCT (n=773); CMF (Grp 1; n=421) vs CEF (Grp 2; n=352)	Grp2 age in years (%) <40 16.4 Grp1 40-49 47.6 50-59 22.0 60-69 14.0 menopausal status pre 69.8 68.5 post 30.2 31.5	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp2 T size, cm (%) 0-2 42.4 39.3 Grp1 2.1-5 49.5 52.4 >5 8.1 8.3 # positive nodes (%) 0 35.6 37.8 1-3 33.3 29.5 >3 31.3 32.7	Scale reported not	Grp1 Grp2 (%) ER+ 27.1 25.0 ER- 66.7 68.2 (%) PR+ not PR- reported
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880; Grp 1, n=542, Dressler analysis; Grp 2, n=469, rest of CALGB 8869[12]	Grp2 mn 50.6 yr 50.4 yr Grp1 md rng pre- 40.7 40.3	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 Mn T (cm) 2.96 2.86 Mn # N+ 4.62 4.68	Scale reported not	Grp1 Grp2 (%) (%) ER+ 68.2 64.8 PR+ 59.1 55.7
Del Mastro et al. 2004, 2005; rec # 48020 Grp 1: n=731, HER2 known Grp 2: n=483 HER2 unknown	Grp2 md 54 54 rng 25-70 26-70 Grp1 <50 35.8% 43.1% 50-59 34.7% 35.6% >59 29.5% 21.3%	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 T1 47.1% 52.6% T2 46.2% 42.2% T3-4 5.3% 4.4% T? 1.4% 0.8% N+ 62.3% 67.7% N- 37.6% 32.3%	Scale reported	Grp1 Grp2 ER+ 54% 49% ER- 43% 38% ER? 3% 13% PR+ 42% 36% PR- 50% 44% PR? 8% 20%
Tanner et al. 2006; rec. # 1820 (n=391 tested for HER2 status; 180 from FEC arm + 211 from CTCb arm)	HER2- <50 years of age: HER2+ n=227 30.8% 69.2% ≥50 years of age: n=164 35.4% 64.6%	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	HER2+ HER2- tumor size: <2 cm 126 27% 73% n= 2-5cm 213 36% 64% >5 cm 37 30% 70% unk 15 47% 53% # positive nodes: 5-7 68 34% 66% 8-9 107 27% 73%	not Scale reported not	+ HER2- ER+ and/or PR+: yes (210) 22% 78% no (148) 49% 51% HER2 unk (33) 27% 73%

[12] Also showed patient populations were similar in 3 CAF dose arms (high, moderate, low); data not abstracted here.

				≥10	216	35% 65%	

Data Abstraction Table IIIa-B (continued): Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Hormone Receptor Status
Adjuvant Chemotherapy (continued)						
Hayes et al. 2007; rec. # 47610 Grp1, n=643 Grp2, n=679 each is random mix of patients from 6 RCT arms[13]	Grp1 Grp2 age, years: <40 20% 20% 40-49 40% 38% 50-59 27% 30% ≥60 12% 12% menopausal status: pre 61% 61% post 39% 39%	Grp1 Grp2 B 8% 9% W 84% 84% H 5% 4% A 2% 2% O 1% 1%	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 tumor size (cm): ≤2 33% 35% >2 66% 64% unk <1% <1% # positive nodes: 1-3 48% 46% 4-9 40% 43% ≥10 12% 11%	Scale reported not	Grp1 Grp2 ER+ 57% 62% ER- 43% 38% PR+ not reported
Martin et al. 2005; rec # 47650 Grp 1: DAC, n=745 Grp 2: FAC, n=746	Grp2 mn Grp1 md 49 49 rng 26-70 23-70 sd	Grp1 Grp2 B W not H reported A O	Grp1 Grp2 I IIa IIb not IIIa IIIb reported IV	Grp1 Grp2 T1 40% 43% T2 52% 51% T3 8% 6% N0 0 0 1-3N$^+$ 63% 62% ≥4 N$^+$ 37% 38%	Scale Grp1 Grp2 100% had Karnofsky score ≥80%	Grp1 Grp2 ER+ &/or PR+ 76% 76% menopausal status: pre: 56% 55% post: 44% 45%
Neoadjuvant (Pre-operative) Chemotherapy						
Learn et al. 2005; rec. # 47640 AC, n=50 AC+D, n=47 AC→D, n=47 pooled data on n=142 evaluated for clin. response	mn 48 yrs md 47 yrs rng 27-73 yrs sd	B W H A 71% O not reported 29%	I 23.6% IIa 39.6% IIb 30.6% IIIa 6.3% IIIb 0 IV 0	clinical tumor diameter ≤2 cm 28.2% >2-≤5 cm 47.2% >5 cm 24.6% N0 61.3% N1 38.7% N≥2 0	Scale reported not	Of n=121 with biopsy specimens available for IHC: ER+ 60.3% ER- 39.7% PR+ 57.9% PR- 42.1%
Arriola et al. 2006; rec # 950 prospective single-arm series; n=232	mn 47 yrs md rng sd	B W not H A reported O	I IIa IIb not IIIa IIIb reported IV	T2 30% T3 70% N0 60% N1 40%	Scale reported	ER+ 67% ER- 29% unk 4% PR+ 52% PR- 43% unk 5%
Park et al. 2003; rec # 9960 retrospective single-arm series; n=67	years <50 82% ≥50 18%	B W not H A reported O	I IIa IIb not IIIa IIIb reported	tumor size (cm): 5-10 91% >10 9%	Scale not reported	ER+ 46% ER- 54% PR status not reported

[13] Also reported data comparing groups 1 and 2 with 1799 patients from CALGB 9344 not included in biomarker analysis; data showed similar baseline characteristics and 5-year outcomes.

Data Abstraction Table IIIa-B (continued): Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Hormone Receptor Status
Neoadjuvant (Pre-operative) Chemotherapy (continued)						
Zhang et al. 2003; rec # 9820 retrospective single-arm series; n=97	md 44.5 yr rng 25-74 yr sd ≥50 44% <50 56%	B W not H reported A O	I IIa IIb not IIIa IIIb reported IV	T1 13% T2 53% ≥T3 34% N- 33% N+ 67%	Scale reported	ER+ 65% PR+ 56%
Tulbah et al. 2002; rec # 11560	HER2+ HER2- age (yr) <50 20 27 >50 2 5 menopausal status: pre 20 25 post 2 7	B W not H reported A O	HER2+ HER2- I 0 0 IIa 1 3 IIb 6 9 IIIa 5 10 IIIb 10 10 IV 0 0	HER2+ HER2- T2 3 7 T3 9 16 T4 10 9 N0 8 9 N1 12 18 N2 2 5	Scale not reported	HER2+ HER2- ER+ 12 16 ER- 9 11 unk 1 5 PR+ 11 11 PR- 10 16 unk 1 5
Tinari et al. 2006; rec # 2300 retrospective single-arm series; n=77	mn md 46.1 yrs rng 25.5-73.7 yrs sd	B W not H reported A O	I IIa IIb not IIIa IIIb reported IV	tumor size (cm): 2-5 75% >5 25%	Scale not reported not	ER+ 62% ER- 38% PR+ 45% PR- 55%

Data Abstraction Table IIIa-B (continued): Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Hormone Receptor Status
Chemotherapy for Advanced or Metastatic Disease						
Harris et al 2006; rec. # 390	mn All md 54.9† rng sd pre post	B All W 20.6† H A O	I II All III not IV reported Unk	T1 T2 All T3 T4 N0 N1 N2 N3 median # mets: 1†	Scale All ECOG performance status of 0,1,2=100	 ER+ and/or All PR+=58†
Di Leo et al 2004; rec. # 5970 Grp 1: A, n=91 Grp 2: T, n=85 patients with tumor blocks tested for HER2 status	Grp2 mn Grp1 md 54 yr 51yr rng sd pre post	Grp1 Grp2 B W Not reported H A O	Grp1 Grp2 I II III IV 100% 100% Unk	Grp1 Grp2 ≥3 sites 46% 51% Visceral Involvement 76% 79%	Scale Grp1 Grp2 Karnofsky all w specimens: 60-70: 15% 15% ≥80: 85% 85% HER2+ subgroup: 60-70: 33% 0	Grp1 Grp2 ER+ not reported PR+ not reported reported (data not shown) other factors similar in HER2 status subgroups of each arm
Konecny et al 2004; rec. # 6740 Grp 1: EC, n=137 Grp 2: ET, n=138 data are for subgroups with known HER2 status	Grp2 Mn Grp1 md 55 55 rng 31-74 29-75 sd pre post	Grp1 Grp2 B W not H A reported O	Grp1 Grp2 I II III IV 100% 100% Unk	Grp1 Grp2 Nuclear Grade 1 2.2 2.9 2 41.6 37.0 3 38.7 46.3 Unk 17.5 13.8 # of met sites 1 35.8 31.9 2 21.2 21.0 ≥3 42.3 42.0 Unk 0.7 5.1	Scale Grp1 Grp2 Karnofsky >60 100 100 Prior adj chemo Yes 40.2 32.6 No 59.1 65.9 unk 0.7 1.5 Prior palliative hormone therapy Yes 14.6 13.8 No 84.7 86.2 unk 0.7 0	Grp1 Grp2 ER+ 52.6 60.9 ER- 37.2 32.6 unk 10.2 6.5 PR+ 48.9 49.3 PR- 40.1 42.7 unk 11.0 8.0

*p<.05; † characteristics of subset with biomarker data, n=165, similar to those of patients w/o biomarker measurements, n=299

Study	Assays (Name)		Criteria for Positivity		Test Results (%)		Comments
Adjuvant Chemotherapy							
Yang et al. 2003 rec. # 8840	FISH IHC	not done Neomarker antibody	FISH IHC	not done strong & complete mem-brane staining in >10% of tumor cells	Pos Equiv Neg 3+ 2+ 1+ 0	36% 0 64%	HER2+ = IHC 3+ by DAKO scoring pre-ASCO/CAP
Gusterson et al. 2003; rec. # 43690	FISH IHC	not done ICR12 monoclonal antibody	FISH IHC	not done strong & complete mem-brane staining at dilution shown to give + signal if ≥3 copies of HER2 gene	Pos Equiv Neg Pos Equiv Neg	 16% of 760 node- pts; 19% of 746 node+ pts none 84% of 760 node- pts; 81% of 746 node+ pts	
Moliterni et. al. 2003; rec. # 10210 RCT; CMF (Grp 1) vs CMF→A (Grp 2)	FISH IHC	not done CB11 antibody	FISH IHC	not done strong membrane staining found equivalent to 3+ by HercepTest	Pos Equiv Neg Pos Neg ND	 Grp 1: 18.2%; Grp 2: 16.2% 75.6% 6.2%	73.3% 10.5%
Colozza et al. 2005; rec. # 3820 RCT; CMF (Grp 1) vs epirubicin (Grp 2); n=133 each tested for HER2 status	FISH IHC	not done CB11 antibody and HercepTest	FISH IHC 	not done >50% CB11+ ≤50% CB11+ CB11 negative HercepTest using DAKO scoring system	 HER+ HER- HER- 3+ 2+ 1+ 0	 Grp1: 28% 41% 31% 7% 10% 75%	Grp2: 41% 36% 23% 9% 7% 11% 71%
Pritchard et al. 2006; rec. # 1760 RCT; CMF versus CEF;	FISH IHC PCR	PathVysion kit CB11 and TAB 250 antibodies (results reported separately from each antibody assay) as described by O'Malley et al. 2001; rec. #13790)	FISH IHC	HER2/CEP17 ≥2.00 complete membrane staining, score ≥5 on Allred semi-quantitative scale	Pos Neg CB11+ CB11- TAB250+ TAB250-	163 (26%) 465 (74%) 124 (20% 510 (80%) 116 (18%) 516 (82%)	also reported concordance rates between the different assays used PCR+ 195 (31%) PCR- 429 (69%)
Knoop et al. 2005; rec. # 3450 RCT (n=805 tested)	FISH IHC	pharmDx HercepTest	FISH IHC	HER2/CEP17 ≥2, as in kit manufacturer's manual; only tested if IHC 2^+ or 3^+ followed instructions in manual for HercepTest kit	Pos Equiv Neg 3+ 2+ 1+ 0	IHC2+: 21.0 6.2 72.8 30.6 10.1 32.7 26.7	IHC3+: 89.4 8.9 1.6 (IHC3+ or FISH+) = HER2+: 32.7% HER2-: 67.3%

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Adjuvant Chemotherapy (continued)				
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880	FISH PathVysion kit IHC CB11 (n=346) or A0-11-854 (n=177) antibodies PCR differential PCR assay as described in Thor et al. 1998	FISH HER2/CEP17 ≥2 IHC ≥50% of invasive cells stained by antibody PCR "unequivocal amplification relative to …normal…and amplified standard controls"	RCT arm: high-dose / mod-dose / low-dose / total FISH Pos 30 (5.7%) / 31 (5.9%) / 30 (5.7%) / 91 (17%) FISH Neg 149 (28.4%) / 136 (26.0%) / 148 (28.2%) / 433 (83%) IHC Pos 44 (8.4%) / 43 (8.2%) / 40 (7.7%) / 127 (24%) IHC Neg 134 (25.6%) / 124 (23.7%) / 138 (26.4%) / 396 (76%) PCR Pos 30 (6.1%) / 31 (6.3%) / 30 (6.1%) / 91 (18%) PCR Neg 131 (26.7%) / 125 (25.5%) / 144 (29.3%) / 400 (82%)	
Del Mastro et al. 2004, 2005; rec. # 48020	FISH not done IHC CB11 antibody all slides scored by one pathologist, blinded to treatment arm & outcome	FISH not done IHC 3+ score on Dako scale: 103 of 731 (14%) with specimens available for assay	Pos Neg FEC_{14} (n=370) / FEC_{21} (n=361) 3+ 50 (13.5%) / 53 (14.7%) 2+ 24 (6.5%) / 23 (6.4%) 1+ 19 (5.1%) / 20 (5.5%) 0 277 (74.9%) / 265 (73.4%)	
Tanner et al. 2006; rec. # 1820 data for n=180 from FEC arm tested for HER2 status	FISH not done CISH Zymed probes (digoxigenin-labeled) IHC not done	CISH ≥6 copies in >30% of invasive carcinoma cells or ratio >2, HER2/CEP17 IHC	Pos Equiv 56 (31%) Neg 0 / 124 (69%) 3+ 2+ 1+ 0	
Hayes et al. 2007; rec. # 47610	FISH PathVysion kit IHC CB11 antibody and HercepTest	FISH HER2/CEP17 ≥2.00 IHC CB11: HER2+ if ≥50% of breast cancer cells were stained; Herceptest: as in Dako manual	Pos Equiv Neg 3+ 2+ 1+ 0	proportions of HER2+ and HER2- patients not reported for any assay method
Martin et al. 2005; rec # 47650	FISH not reported IHC CB11 antibody (only for 12 patients)	FISH HER2/CEP17 ≥2.00 IHC not reported	Pos 319 (21.4%; 20.8%, DAC arm; 22.0% FAC arm) Neg 943 (63.3%; 63.8%, DAC arm; 62.7% FAC arm) ??? 229 (15.4%; 15.4%, DAC arm; 15.3% FAC arm)	

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Neoadjuvant (Pre-operative) Chemotherapy				
Learn et al. 2005; rec. # 47640 n=104 classified for HER2 status	FISH not reported IHC TAB250 antibody (Zymed; South San Francisco, CA) FISH performed on all specimens with "borderline" HER2 IHC scores	FISH not reported IHC not reported	HER2 Pos 41 (39.4% of those tested) HER2 Neg 63 (60.6% of those tested)	
Arriola et al. 2006; rec # 950 n=223 tested by IHC/FISH initial algorithm & by CISH	FISH Oncor/Ventana Inform kit CISH Zymed probe and Spot-Light kit IHC CB11 antibody and HercepTest algorithm: CB11 first, then HercepTest for negatives only, then FISH for discordant IHC results; positives by initial algorithm tested by CISH	CISH >5 copies or ratio>2 for HER2/CEN17 IHC/FISH initial algorithm: $CB11^+$ if complete membrane staining in >10% of cells; $HercepTest^+$ if 2^+ or 3+; $FISH^+$ if >4 signals/cell	>5 copies ratio>2 Pos 18% 14% Neg 82% 86% Pos 19% Neg 81%	
Park et al. 2003; rec # 9960	FISH not done CISH Zymed SPOT-Light HER2 probe, digoxigenin-labeled IHC not done	FISH not done CISH HER2 gene copy # >4, or large gene copy cluster in >50% of cancer cell nuclei IHC not done	Pos 46% Neg 54%	
Zhang et al. 2003; rec # 9820	FISH PathVysion kit IHC AB8 Neomarker antibody n=75 analyzed by IHC n=48 analyzed by FISH n=97 all patients in study	FISH gene copy ratio >2.0, HER2/chromosome 17 centromere IHC strong membrane staining ≥10% of tumor cells	Pos 13% Neg 36% untested 51% 3+ 23% 2+ 9% 1+ 10% 0 35% untested 23%	overall, 28% (n=28) $HER2^+$ defined as 3+ by IHC or FISH+; 72% (n=69) HER2-
Tulbah et al. 2002; rec # 11560 n=54 tested	FISH not done IHC HercepTest	FISH not done IHC scored 0-3+, as in Dako kit guide; for analysis of response, only 3+ was considered HER2+	Pos Equiv Neg 3+ 22 (41%) 2+ 12 (22%) 1+ 8 (15%) 0 12 (22%)	
Tinari et al. 2006; rec # 2300 retrospective single-arm series; n=77	FISH not reported IHC HercepTest (Dako)	FISH not reported; used only if HercepTest scored 2+ IHC scored 0-3+, as in Dako kit guide; positive if IHC scored 3+ or if FISH+ and IHC 2+	Pos 20 (26%) Equiv 0 Neg 57 (74%) 3+ 2+ 1+ 0	

Study	Assays (Name)		Criteria for Positivity		Test Results (%)			Comments
Neoadjuvant (Pre-operative) Chemotherapy (continued)								
Chemotherapy for Advanced or Metastatic Disease								
Harris et al 2006; rec. # 390	FISH IHC	Vysis PathVysion kit (Vysis Inc, Downers Grove, IL) Monoclonol antibody CB11 (Biogenex, San Ramon, CA); HercepTest (Dako Corp, Carpinteria, CA)	FISH	Ratio of HER2 to CEP17 signal ≥ 2.0.	Pos Neg	26 74		Cohen's kappa = 83.0% (SE 5.3%) for FISH vs CB11; 72.0%(SE6.2%) for Hercep-Test (0-1 vs 2-3) vs FISH; 79.2%(SE6.0%) for Hercep-Test (0-2 vs 3) vs FISH; 70.0%(SE6.3%) for Hercep-Test (0-1 vs 2-3) vs CB11; 84.2%(SE5.4%) for Hercep-Test (1-2 vs 3) vs CB11. By CB11, 9% of African American women are HER2+ vs 20% of Caucasian women (p=0.08).
			IHC	CB11: moderate to strong intensity staining in ≥10% of invasive carcinoma cells.	Pos Equiv Neg	20 80		
				Herceptest score of 3+; i.e., complete membrane staining of >10% tumor cells	0 1 2 3	40 28 11 21		
Di Leo et al 2004; rec. # 5970	IHC FISH UK)	CB-11 (Novocastra, Newcastle, Spectrum Orange HER-2/ Spectrum Green CEP17 (PathVysion, Vysis, Downers Grove, IL)	IHC→FISH: FISH done if IHC stained membranes in ≥ 1% of invasive cells; HER2+ if signal ratio, HER2/CEP17 ≥2			Grp1	Grp2	
					Pos	16%	25%	
					unknown	14%	16%	
					Neg	69%	59%	
Konecny et al 2004; rec. # 6740	FISH IHC	PathVision HER-2 Neu & CEP17 probes (Vysis, Downers Grove, IL) not done	FISH	≥2 HER-2/neu genes per Chromosome 17 Centromere		Grp1	Grp2	
					Pos	35.8	34.8	
					Equiv Neg	64.2	65.2	

Study	Primary Outcomes	Secondary Outcomes	Criteria for Tumor Response or Progression	Independent Response Assessor?	F/U Frequency/Duration
Adjuvant Chemotherapy					
Yang et al. 2003 rec. # 8840	DFS	no others reported by HER2 status	not specified	not mentioned	not reported
Gusterson et al. 2003; rec. # 43690	DFS, OS	no others reported by HER2 status	not specified	not mentioned	6 yrs median F/U duration frequency not reported
Moliterni et. al. 2003; rec. # 10210	RFS, OS	no others reported by HER2 status	relapse: any new manifestation of disease (locoregional, distant or contralateral)	not mentioned	q3wk during Tx (1 yr), then q6mo for 5 yr, then q12 mo; median F/U duration, 178 mo
Colozza et al. 2005; rec. # 3820	RFS, OS	no others reported by HER2 status	relapse: any new manifestation of disease (locoregional, distant or contralateral)	not mentioned	8 years median F/U duration (range, 6.4-9.5 years); frequency not reported
Pritchard et al. 2006; rec. # 1760	RFS, OS	no others reported by HER2 status	recurrence: local, chest wall, regional or distant disease; but contralateral = new primary	not mentioned	q3mo to end of year 2, then q6mo to end of year 5, then yearly; median F/U 10 yrs; minimum F/U 9 years
Knoop et al. 2005; rec. # 3450	RFS, OS	no others reported by HER2 status	recurrence: local, regional, distant, 2nd malignancy or death	not mentioned	median estimated potential F/U: 8 yr for RFS, 10 yr for OS; F/U frequency not reported
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880	DFS, OS	no other outcomes reported by HER2 status	for DFS, event = documented relapse or death; progression not defined	not mentioned	F/U frequency not reported; median F/U duration, 9 years
Del Mastro et al. 2004, 2005; rec. # 48020	EFS, OS	no others reported	for EFS, event = local or distant relapse, 2nd breast primary, or death	not mentioned for events, but HER2 scoring blinded	frequency not reported; median F/U duration, 6.7 years
Tanner et al. 2006; rec. # 1820	RFS, OS	no others reported by HER2 status	RFS: breast cancer specific survival from randomization to first recurrence	not mentioned (but CISH analyses blinded to outcome)	not reported (but may be in original report, ref. 16)
Hayes et al. 2007; rec# 47610	DFS	OS; no others by HER2 status	event = first local or distant recurrence or death	not mentioned	F/U frequency not reported; F/U duration “approximately 10 years”
Martin et al. 2005; rec # 47650	DFS	OS, adverse events, QOL; none reported by HER2 status	not specified	not mentioned	day 21 of each cycle, then every 6 mos for 5 years, then annually; median F/U 55 months
Neoadjuvant (Pre-operative) Chemotherapy					
Learn et al. 2005; rec. # 47640	pathologic and clinical responses	no other outcomes reported	pCR: no residual invasive cells in resected tumor or nodes; cCR: 100% tumor regression; cPR: 50-99% tumor regression; ORR: cCR + cPR no response (cNR): <50% regression and <25% growth progressive disease (PD): ≥25% tumor growth	not mentioned for either response or HER2 assays	clinical responses evaluated after 12 weeks of chemotherapy but before surgery; pathologic response determined on resected tumor and nodes

Study	Primary Outcomes	Secondary Outcomes	Criteria for Tumor Response or Progression	Independent Response Assessor?	F/U Frequency/Duration
Neoadjuvant (Pre-operative) Chemotherapy (continued)					
Arriola et al. 2006; rec # 950	pathologic complete response (pCR)	clinical response; other outcomes not reported	pCR: no invasive tumor cells; clinical response: >50% ↓, product of maximal diameters; progression: 25% ↑, product of maximal diameters;	not relevant?	physical exam q3wk during neoadj Tx; repeat mammography ≥15 days post 4th cycle of doxorubicin to assess clinical response; pathologic response assessed after surgery
Park et al. 2003; rec # 9960	clinical & pathologic responses	other outcomes not reported	pCR: no invasive cancer; cCR: not defined PR: >50% ↓ tumor volume; SD: <50% ↓ tumor volume	not relevant?	physical exam q1wk during neoadj Tx; repeat MRI before surgery after completion of 4 chemoTx cycles
Zhang et al. 2003; rec # 9820	pathologic, clinical, & imaging responses (imaging responses by mammography, ultrasonography, or both)	DFS; other outcomes not reported	pCR: no invasive tumor in resected breast tissue or nodes MRD: <1 cm diam in breast & no positive nodes ERD: >1 cm diam in breast or any positive node cCR: no evidence of tumor in breast or lymph nodes; cPR: >50% ↓ in tumor size cSD: <50% ↓ to <25% ↑ in tumor size; cPD: >25% ↑ in tumor size	not relevant? path. good responses = pCR + MRD; clin. good responses = cCR + cPR	physical exam at baseline & at start of each neoadjuvant chemoTx cycle to assess clinical responses; imaging at baseline & between end of final chemoTx cycle & surgery to assess imaging responses; pathologic responses assessed by microscopic evaluation of resected breast tissue and nodes; n=61 (63%) had mastectomy, 36 (37%) had breast conserving surgery; median F/U for DFS: 33 months
Tulbah et al. 2002; rec # 11560	pathologic and clinical responses	DFS, OS; other outcomes not reported	pCR, pPR, pSD, pPD as described by Feldman et al (ref. 27); cCR, cPR, cSD, cPD as previously described (ref. 19); events for DFS: local, regional, or distant recurrence; contralateral breast cancer; any other primary cancer; or death	not mentioned for response or progression; HER2 assessor blinded to outcome	F/U frequency not described; median F/U duration, 25 (±7) months
Tinari et al. 2006; rec # 2300	pathologic responses	reported RFS, but not by HER2 status	pCR: no invasive tumor in resected breast tissue or nodes MRD: no gross tumor, microscopic invasive tumor in ≤2 high-powered fields PR: >50% ↓ in all lesions SD: <25% Δ in size, each lesion PD: >25% ↑ in size, any lesion tumor response (TR): pCR or MRD overall response (OR): pCR, MRD or PR	not mentioned for response or progression; HER2 assessor blinded to outcome	physical exam, bilateral mammography, blood chemistry, CEA, CA 15-3, chest X-ray every 3 months for 2 years, every 6 months thereafter; bone scan every 6 months; pathologivc response to treatment assessed after tumor excision and axillary lymph node dissection (median of 10 nodes excised per patient)

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-D (continued): Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Criteria for Tumor Response or Progression	Independent Response Assessor?	F/U Frequency/Duration
Chemotherapy for Adavanced or Metastatic Disease					
Harris et al 2006; rec. # 390	Radiographic response	TTF, OS	Disappearance of all lesions (CR) or ≥ 50% reduction in sum of the products of bidimensional measurements of all lesions, with improvement or no change in any nonmeasurable lesions (PR)	Not reported	"Std staging studies" after every 3 cycles; median follow-up=8.3 years
Di Leo et al 2004; rec. # 5970	TTP	OS; clinical/imaging response	WHO criteria; ORR=%CR+%PR	Not reported	Before, during, and every 3 mos after treatment, to progression or death. Median FU=23 months.
Konecny et al 2004; rec. # 6740	did not specify primary outcome; reported clinical/ imaging response rates, PFS, OS (each from randomization)		CR=disappearance of all radiographically or visually apparent tumor; PR=>50% decrease (2D measurements) of all measurable lesions; ORR=%CR+%PR; Progression= >25% increase in any measurable lesion or appearance of new lesion	Not routinely	FU every 12 weeks; F/U duration not reported

Study	Time to Event Outcomes												
Adjuvant Chemotherapy													
Yang et al. 2003 rec. # 8840 CMF; single-arm series	Outcome OS TTP PFS RFS DFS	Grp HER2+ HER2- HER2+ HER2- HER2+ HER2- HER2+ HER2-	N 34 60	Med (mos) 6-7 years not reached	1 yr	2.5 yr ~60% ~90%	3 yr	4 yr	5 yr 53% 86%	Test log rank	p <0.01	HR (95%CI)	Comments also reported stratified log rank that adjusted for nodal status (p=0.002)
Gusterson et al. 2003; rec. # 43690 760 node- pts randomized to periop CMF (Tx) or no adj Tx (Cx)	Outcome OS (HER2+) OS (HER2-) TTP PFS RFS DFS(HER2+) DFS (HER2-)	Grp Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx	N 64 54 436 206 64 54 436 206	Med (mos) not reached not reached not reached not reached not reached not reached not reached not reached	2 yr ~84% ~86% ~90% ~85%	3 yr ~68% ~75% ~85% ~77%	4 yr ~65% ~73% ~80% ~72%	5 yr ~62% ~70% ~77% ~70%	6 yr 76±5 79±6 85±2 87±2 61±6 68±7 71±2 68±3	Test Cox prop hazards Cox prop hazards Cox prop hazards Cox prop hazards	p NS NS NS NS	HR (95%CI) 1.15 (0.54-2.46) 1.04 (0.68-1.61) 1.22 (0.66-2.25) 0.82 (0.61-1.09)	Comments unadjusted univariate analyses; adjusted results also NS unadjusted univariate analyses; adjusted results also NS
Gusterson et al. 2003; rec. # 43690 746 node+ pts randomized to periop (Cx) or prolonged (Tx) CMF	Outcome OS (HER2+) OS (HER2-) TTP PFS RFS DFS(HER2+) DFS (HER2-)	Grp Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx Tx Cx	N 85 55 406 200 85 55 406 200	Med (mos) not reported not reported not reached not reached ~36 ~24 >72 ~40	2 yr ~60% ~50% ~80% ~63%	3 yr ~50% ~42% ~70% ~55%	4 yr ~43% ~35% ~63% ~45%	5 yr ~40% ~30% ~57% ~40%	6 yr 46±6 40±7 71±2 61±4 38±5 29±6 52±3 36±4	Test Cox prop hazards Cox prop hazards Cox prop hazards Cox prop hazards	p NS 0.01 NS <0.0001	HR (95%CI) 1.15 (0.62-1.54) 0.69 (0.52-0.92) 0.77 (0.51-1.16) 0.57 (0.46-0.72)	Comments unadjusted univariate analyses; adjusted analyses gave similar results unadjusted univariate analyses; adjusted analyses gave similar results

Study	Time to Event Outcomes												
Adjuvant Chemotherapy (continued)													
Moliterni et. al. 2003; rec. # 10210 RCT; CMF (Cx) versus CMF→A (Tx)	Outcome	Grp	N	Med (mos)	2 yr	4 yr	6 yr	8 yr	10 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	45	>192	~92%	~83%	~73%	~68%	64%	Cox		0.61 (0.32-1.16)	
		Cx	50	~170	~90%	~80%	~63%	~57%	54%	model			
	OS (HER2-)	Tx	203	>192	~97%	~90%	~86%	~83%	76%	Cox		1.26 (0.89-1.79)	
		Cx	208	>192	~97%	~94%	~90%	~83%	77%	model			
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS(HER2+)	Tx	45	>192	~85%	~75%	~62%	~58%	55%	Cox		0.83 (0.46-1.49)	
		Cx	50	~102	~85%	~65%	~62%	~52%	46%	model			
	RFS (HER2-)	Tx	203	~162	~90%	~80%	~65%	~60%	56%	Cox		1.22 (0.91-1.64)	
		Cx	208	>192	~90%	~80%	~74%	~65%	59%	model			
	DFS	Tx											
		Cx											
Colozza et al. 2005; rec. # 3820 RCT; CMF (Cx) vs epirubicin (Tx); n=133 each group tested for HER2 status	Outcome	Grp	N	Med (mos)		4 yr	6 yr	% at 8 yr±SD		Test	p	HR (95%CI)	Comments: data at 8 yrs from Table 4; n per HER2+ group disagrees with Tables 1 & 2 CMF HER2+ versus CMF HER2- p=0.024 all other comparisons not significant, including epirubicin HER2+ versus epirubicin HER2- p=0.24
	OS (HER2+)	Tx	54	not reached		~89%	~80%	75.8±5.8		log			
		Cx	37	not reached		~77%	~70%	67.6±7.7		rank			
	OS (HER2-)	Tx	79	not reached		~90%	~87%	84.5±4.1		log			
		Cx	96	not reached		~93%	~90%	87.4±3.4		rank			
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS(HER2+)	Tx	54					60.1±6.9		log			
		Cx	37					68.6±7.2		rank			
	RFS (HER2-)	Tx	79					65.9±5.4		log			
		Cx	96					70.3±4.7		rank			
	DFS	Tx											
		Cx											
Pritchard et al. 2006; rec. # 1760 RCT; CMF (Cx) versus CEF (Tx); HER2 status by FISH results	Outcome	Grp	N	Med (yrs)	2 yr	4 yr	6 yr	8 yr	10 yr	Test	p	HR (95%CI)	Comments
	OS ($HER2^+$)	Tx	75	not reached	~93%	~70%	~62%	~58%	~57%	log	0.06	0.65 (0.42-1.02)	
		Cx	88	~5.3	~92%	~62%	~47%	~46%	~45%	rank			
	OS ($HER2^-$)	Tx	237	not reached	~93%	~83%	~75%	~67%	~63%	log	NS	1.06 (0.83-1.44)	
		Cx	228	not reached	~93%	~80%	~75%	~67%	~62%	rank			
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS ($HER2^+$)	Tx	75	not reached	~77%	~67%	~58%	~57%	~56%	log	0.003	0.52 (0.34-0.80)	
		Cx	88	~2.5	~63%	~43%	~42%	~34%	~31%	rank			
	RFS ($HER2^-$)	Tx	237	~10	~81%	~67%	~60%	~54%	~50%	log	NS	0.91 (0.71-1.18)	
		Cx	228	~10	~81%	~64%	~58%	~54%	~50%	rank			

Study	**Time to Event Outcomes**												
Adjuvant Chemotherapy (continued)													
Knoop et al.	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
2005; rec. # 3450	OS (HER2+)	Tx	120							Cox	0.09	0.73 (0.50-1.05)	HR's and 95% CI's all
		Cx	143							proportional hazards			adjusted by multivariate
RCT (n=805);	OS (HER2-)	Tx	249							Cox	0.23	0.82 (0.59-1.13)	analysis (see Table 3F)
CMF (Cx) versus		Cx	293							proportional hazards			
CEF (Tx)	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS ($HER2^+$)	Tx	120							Cox	0.10	0.75 (0.53-1.06)	
		Cx	143							proportional hazards			
	RFS ($HER2^-$)	Tx	249							Cox	0.10	0.79 (0.60-1.05)	
		Cx	293							proportional hazards			
	DFS	Tx											
		Cx											
	DSS	Tx											
		Cx											
Dressler et al.	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
2005, rec. #	OS $HER2^+$	high	44	>108		~97%		~97%	93% (86-100)				
4280; Thor et al.	by IHC	mod	43	~87		~93%		~66%	58% (47-75)				
1998, rec. #		low	40	~96		~90%		~66%	63% (49-80)				
40880	OS $HER2^-$	high	134	~100		~93%		~80%	74% (67-81)				
	by IHC	mod	124	>108		~96%		~86%	78% (80-92)				
separate survival		low	138	~100		~93%		~80%	74% (67-81)				
curves show	PFS	Tx											
similar results for		Cx											
HER2 status by	DFS $HER2^+$	high	44	>108		~97%		~90%	87% (74-96)				
IHC, FISH, and	by IHC	mod	43	~36		~60%		~47%	47% (34-64)				
PCR; only		low	40	~66		~65%		~58%	53% (39-71)				
abstracted data	DFS $HER2^-$	high	134	>108		~83%		~70%	64% (56-73)				
for HER2 by IHC	by IHC	mod	124	>108		~83%		~70%	65% (57-74)				
		low	138	~90		~78%		~63%	59% (51-68)				
	DSS	Tx											
		Cx											

Study	**Time to Event Outcomes**												
Adjuvant Chemotherapy (continued)													
Del Mastro et al. 2004, 2005; rec # 48020 Tx = FEC_{14} Cx = FEC_{21}	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS (HER2+)	Tx	50	>72	~100%	~100%	~96%	~92%	89.9%	prop	0.22	0.59 (0.26-1.37)	for all FEC_{14}, $HER2^+$ vs $HER2^-$: EFS, HR=1.21 (0.65-2.24) p=0.54; OS, HR=1.85 (0.88-3.89), p=0.103
		Cx	53	>72	~98%	~89%	~85%	~81%	75.1%	hazards			
	OS (HER2-)	Tx	320	>84	~100%	~99%	~96%	~95%	91.9%	prop	0.34	0.79 (0.49-1.28)	
		Cx	308	>84	~100%	~99%	~96%	~94%	90.7%	hazards			
	PFS	Tx											
		Cx											
	EFS ($HER2^+$)	Tx	50	>72	~100%	~98%	~85%	79%	77.7%	prop	0.092	0.54 (0.27-1.11)	for all FEC_{21}, $HER2^+$ vs $HER2^-$: EFS, HR=2.07 (1.27-3.38), p=0.003; OS, HR2.47 (1.34--4.57), p=0.004
		Cx	53	>72	~91%	~82%	~68%	~67%	62.5%	hazards			
	EFS ($HER2^-$)	Tx	320	>84	~100%	~93%	~90%	~85%	81.5%	prop	0.57	0.91 (0.65-1.27)	
		Cx	308	>84	~98%	~93%	~87%	~83%	80.9%	hazards			
	DSS	Tx											
		Cx											
Tanner et al. 2006; rec. # 1820 only reported statistical comparisons of FEC versus CTCb, not HER2+ versus HER2- in same treatment arm	Outcome	Grp	N	Med (mos)	2 yr	3 yr	4 yr	5 yr	6 yr	Test	p	HR (95%CI)	Comments
	OS	$HER2^+$	56	~54	~79%	~64%	~58%	~46%	~41%	not			
		$HER2^-$	124	>84	~94	~83%	~74%	~68%	~64%	reported			
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS	$HER2^+$	56	~48	~68%	~62%	~50%	~46%	~46%	not			
		$HER2^-$	124	>84	~84%	~74%	~67%	~66%	~65%	reported			
	DFS	Tx											
		Cx											
Hayes et al. 2007; rec. # 47610 AC→P (Tx) vs. AC alone (Cx) HER2 status based on CB11 IHC test results; data from K-M curves of Fig. 1; Groups 1 & 2 randomly and separately selected from n=3121 in CALGB 9344 RCT	Outcome	Grp	N	Med (mos)	3 yr	6 yr	9 yr	Test	p	HR (95%CI)	Comments:		
	OS $HER2^+$ Group 1	Tx		not reached	~87%	~75%	~70%	Cox multi-variate regression	0.17	0.61	HR's & p's: interaction of HER2+ status with effect of adding paclitaxel (from Table 2); for Groups 1 & 2 pooled, n=1322: HR for OS = 0.57, p = 0.01		
		Cx		~108	~75%	~62%	~50%						
	OS $HER2^-$ Group 1	Tx		not reached	~87%	~77%	~70%						
		Cx		not reached	~87%	~74%	~66%						
	OS $HER2^+$ Group 2	Tx		not reached	~92%	~78%	~78%	Cox multi-variate regression	0.03	0.52			
		Cx		~72	~70%	~50%	~49%						
	OS $HER2^-$ Group 2	Tx		not reached	~92%	~80%	~68%						
		Cx		not reached	~85%	~76%	~63%						
	DFS $HER2^+$ Group 1	Tx		not reached	~80%	~69%	~62%	Cox multi-variate regression	0.15	0.63	Groups 1 & 2, pooled HR for DFS = 0.59, p = 0.01		
		Cx		~66	~60%	~48%	~47%						
	DFS $HER2^-$ Group 1	Tx		not reached	~80%	~70%	~65%						
		Cx		~126	~80%	~67%	~55%						
	DFS $HER2^+$ Group 2	Tx		not reached	~87%	~72%	~67%	Cox multi-variate regression	0.03	0.52			
		Cx		~48	~53%	~48%	~45%						
	DFS $HER2^-$ Group 2	Tx		not reached	~80%	~75%	~69%						
		Cx		~126	~78%	~65%	~60%						

Study	Time to Event Outcomes												
Adjuvant Chemotherapy (continued)													
Martin et al. 2005; rec # 47650 Tx = DAC Cx = FAC	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	DFS $HER2^+$	Tx	155							Cox prop hzrds models		0.60 (0.41-0.88)	K-M DFS curves not shown separately by HER2 status; p values not reported
		Cx	164										
	DFS $HER2^-$	Tx	475									0.76 (0.59-1.00)	
		Cx	468										
	DFS HER2 unknown	Tx	115									0.72 (0.45-1.17)	
		Cx	114										
Neoadjuvant (Pre-operative) Chemotherapy													
Learn et al. 2005; rec. # 47640	did not report time-to-event outcome												
Arriola et al. 2006; rec # 950	did not report time-to-event outcomes												
Park et al. 2003; rec # 9960	did not report time-to-event outcomes												
Zhang et al. 2003; rec # 9820 FAC neoadjuvant chemoTx, n=97; n=78 also given post-op chemoTx (44, paclitaxel; 26, FAC; 8, only doxorubicin) 8 lost to F/U after locoregional Tx	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	Tx											
		Cx											
	TTP	Tx											
		Cx											
	PFS	Tx											
		Cx											
	RFS	Tx											
		Cx											
	DFS HER2+		28	48 (for all patients)	~90%	~83%	~60%	~45%		not specified	NS	not reported	
	HER2-		69		~90%	~80%	~70%	~60%					
Tulbah et al. 2002; rec # 11560	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS $HER2^+$		22	not reached	~95%	~79%	~66%	~66%		log rank	0.31		if HER2+ = IHC 2+ or 3+ OS favored HER2-: 90% vs 79%, p=0.051
	$HER2^-$		32	not reached	~97%	~97%	~72%	~72%					
	TTP	Tx											
		Cx											
	DFS HER2+		21	34.5±7.8 (all 52 pts)	~88%	~75%	~75%	0		log rank	0.43		if HER2+ = IHC 2+ or 3+ difference in DFS still not statistically significant (p=0.09)
					~92%	~83%	~52%	~52%					
	DSS HER2	Tx	31										
		Cx											
Tinari et al. 2006; rec # 2300	did not report time-to-event outcome by HER2 status												

Study	**Time to Event Outcomes**													
Chemotherapy for Advanced or Metastatic Disease														
390, Harris et al 2006	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	10yr	Test	p	HR (95%CI)	Comments
	OS	CB11+	30	11.3							Log rank	0.14		
		CB11-	126	13.1										
		FISH+	37	10.9							Log rank	0.26		
		FISH-	109	13.1										
		HercepTest 2+/3+	46	11.5							Log rank	0.84		
		HercepTest 0/1+	105	13.2										
5970, Di Leo et al 2004 Grp 1: A Grp 2: T	Outcome	Grp	N	Med (mos)	6 mos	1 yr	1.5 yr	2 yr	2.5 yr	Test	p	HR (95%CI)	Comments	
	OS HER2+	1	15	10.8	~.85	~.3	No line	No line	No line	Cox regression	.33	1.47(0.68-3.15)	In full TAX 303 trial, no statistically significant differences between Tx arms with respect to OS or TTP	
		2	21	14.4	~.95	~.6	~.46	No line	No line					
	OS HER2-	1	63	16.9	~.8	~.72	~.5	~.3	No line	Cox regression	.07	0.64(0.40-1.03)		
		2	50	12.6	~.8	~.6	~.32	~.28	0					
					3 mos	6 mos	9 mos	12 mos	15 mos	18 mos				
	TTP HER2+	1	15	4.7	~.75	~.4	~.25	~.15	~.15	0	Cox regression	.73	0.88(0.43-1.82)	
		2	21	7.0	~.75	~.6	~.15	~.1	~0	No line				
	TTP HER2-	1	63	5.9	~.74	~.5	~.35	~.25	~.15	<.1	Cox regression	.22	0.77(0.52-1.16)	
		2	50	5.0	~.74	~.45	~.2	~.1	<.1	No line				
	PFS	Tx												
		Cx												

Study	Time to Event Outcomes											
Chemotherapy for Advanced or Metastatic Disease (continued)												
6740, Konecny et al 2004	Outcome	Grp	N	Med (mos) (95%CI)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI) Comments
	OS	1										
	HER2+		49	16.4(12.1-20.1)	~.65	~.3	~.25	~.25		log	0.010	
Grp 1: EC	HER2-		88	33.1(20.9-50.6)	~.78	~.57	~.45	~.4		rank		
Grp 2: ET		2										
	HER2+		48	21.4(15.3-27.3)	~.74	~.45	~.25	~.1		log	0.463	
	HER2-		90	27.5(17.1-35.2)	~.7	~.55	~.35	~.2		rank		
	HER2+	1	49	16.4(12.1-20.1)	~.6	~.3	~.25	~.25		log	0.319	
		2	48	21.4(15.3-27.3)	~.7	~.4	~.25	~.1		rank		
	HER2-	1	88	33.1(20.9-50.6)	~.78	~.58	~.43	~.4		log	0.292	
		2	90	27.5(17.1-35.2)	~.7	~.55	~.35	~.15		rank		
	TTP	Tx										
		Cx										
	PFS	1										
	HER2+		49	7.1(4.1-9.3)	~.2	~.08	~.08			log	0.010	
	HER2-		88	10.4(6.9-14.9)	~.54	~.22	~.12			rank		
		2										
	HER2+		48	10.5(8.1-11.9)	~.35	~.1	~.05			log	0.584	
	HER2-		90	9.6(7.5-11.3)	~.35	~.15	~.08			rank		
	HER2+	1	49	7.1(4.1-9.3)	~.2	~.08	~.08			log	0.116	
		2	48	10.5(8.1-11.9)	~.35	~.1	~.05			rank		
	HER2-	1	88	10.4(6.9-14.9)	~.47	~.25	~.1			log	0.350	
		2	90	9.6(7.5-11.3)	~.52	~.13	~.08			rank		

Study	Design/ Outcome/ Model	Candidate Method to Identify Candidates	Univariate Results, Variable (p value)	Predictors/ Methods to Select Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Adjuvant Chemotherapy								
Yang et al. 2003 rec. # 8840	**Predictors/**			not reported				
Gusterson et al. 2003; rec. # 43690	RCT OS & DFS Cox proportional hazards	not specified **Selected**	varied by outcome & groups compared	menopausal status; tumor size; ER, PR & nodal status; grade; assigned Tx; selection method not described	not mentioned	varied by outcome & groups compared	not described	not reported
Moliterni et. al. 2003; rec. # 10210	RCT RFS, OS Cox semi-parametric	not specified	not reported	Tx; HER2 status; interaction term; T size; ER, PR, & p53 status; selection method not described	Schoenfeld plots tested prop hzrd; interaction term included	RFS: HR=0.68, p, NS; OS: HR=0.48, p=0.052 (both for Tx x HER2 interaction)	bootstrap procedure ***[need David's help for Results]***	***not certain; need David's input***
Colozza et al. 2005; rec. # 3820	RCT OS Cox model	not specified	not reported	T size; ER, PR, & HER2 status; ≤0.10 in univar. analysis	proportional hazards untested; interaction term included	RFS: HR=1.02 CI: 0.40-2.58, p, NS; OS: HR=1.61 CI: 0.64-4.01, p, NS; both for Tx x HER2 interaction	not described ***David: Do we also need #'s for Tx & HER2 status (top 2 rows, table 5?)***	not mentioned
Pritchard et al. 2006; rec. # 1760 interaction, Tx x HER2 status by FISH	RCT RFS, OS Cox proportional hazards	not specified	RFS: 1.79 (1.08-2.96; p=0.02) OS: 1.66 (0.97-2.85; p=0.07)	age, # + nodes ER status, surgery type, T size; selection method not described	unclear if tested prop hazards; interaction term included	RFS: HR=1.96, CI: 1.15-3.36, p=0.01 OS: HR=2.04, CI: 1.14-3.65, p=0.02	not described	not mentioned
Knoop et al. 2005; rec. # 3450 RCT (n=805); CMF (Cx) versus CEF (Tx)	RCT RFS, OS Cox proportional hazards	age, tumor size, nodal, menopausal & ER status, grade; method of identifying candidates not described	not reported	RFS & OS: T size, nodal & menopausal status; stratified for: grade, ER & TOP2A status; used backward	assumption assessed graphically & w time-depend. component; interaction term included	RFS: W_1=0.6 p, NS (0.81) OS: W_1=0.23 p, NS (0.63)	not described ***[need David to check these entries]***	not mentioned

Study	Design/ Outcome/ Model	Candidate Predictors/ Method to Identify Candidates	Univariate Results, Variable (p value) Selected	Predictors/ Methods to Select Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Adjuvant Chemotherapy (continued)								
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880	3-arm RCT; DFS, OS (only DFS reported); proportional hazards	SQRT_#N^+, T size, meno-pause status, CAF dose, HER2 status, CAF dose x HER2 status interaction term	not reported	SQRT_#N^+, T size, meno-pause status, CAF dose, HER2 status, CAF dose x HER2 status interaction term	proportional hazards test not mentioned; interaction term included in each proportional hazards model	HR for interac-tion terms: HER2 by IHC: 0.418 (0.188-0.930), p=0.0003 HER2 by FISH: 0.919 (0.814-1.038), p=0.033 HER2 by PCR 0.585 (0.253-1.352), p=0.043	not described	not mentioned
Del Mastro et al. 2004, 2005; rec. # 48020	RCT EFS, OS proportional hazards	Tx arm, age, ER, PR, HER2 & menopause status, tumor grade, proliferative activity	EFS, OS see Table 3E for results	step-down procedure based on likelihood ratio test; retined if p<0.15	proportional hazards test not mentioned; interaction term included in each proportional hazards model	EFS HR, vs FEC_{21} $HER2^-$: $HER2^+$, 2.04; FEC_{14} $HER2^-$, 0.85; $HER2^+$, 0.91; $p_{interact}$ = 0.12 OS HR vs FEC_{21} $HER2^-$,: $HER2^+$, 2.41; FEC_{14} $HER2^-$, 0.72; $HER2^+$, 1.13; $_{interact}$ = 0.38	not described	not mentioned
Tanner et al. 2006; rec. # 1820	not reported or done (only reported multivariate Cox regression analysis of outcomes by TOP2A amplification status in HER2+ subgroup)							
Hayes et al. 2007; rec. # 47610	RCT DFS, OS Cox propor-tional hazards	not specified	not reported	use of P; A dose; SQRT_#N^+, T size; menopause, ER, & HER2 status; $HER2^+$ x P use interaction; selection method not described	prop. hazards test not mentioned; interaction term included not described	DFS: HR=0.59 p=0.01 OS: HR=0.57 p=0.01 (both HR's for Groups 1 & 2 pooled)	not described	not mentioned

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-F (continued): Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method to Identify Candidates	Univariate Results, Variable (p value) Selected	Predictors/ Methods to Select Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Adjuvant Chemotherapy (continued)								
Martin et al. 2005; rec # 47650	RCT DFS Cox pro-portional hazards	separate uni-variate anal. on # N^+, ER/PR status, HER2 status, meno-paus status; each adjusted for age, Tsize	see table 3E; p values not reported	not mentioned or described	prop. hazards test not mentioned; included interaction test	ratio of hazard ratios = 0.85; p=0.41	not described	not mentioned
Neoadjuvant Chemotherapy								
Learn et al. 2005; rec. # 47640	did not report time-to-event outcomes							
Arriola et al. 2006; rec # 950	did not report time-to-event outcomes							
Park et al. 2003; rec # 9960	did not report time-to-event outcomes							
Zhang et al. 2003; rec # 9820	did not report regression modeling for time-to-event outcomes							
Tulbah et al. 2002; rec # 11560	did not report regression modeling for time-to-event outcomes							
Tinari et al. 2006; rec # 2300	did not include HER2 as predictor in regression modeling for time-to-event outcome							

Study	Design/ Outcome/ Model	Candidate Predictors/ Method to Identify Candidates	Univariate Results, Variable (p value)	Predictors/ Methods to Select Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Chemotherapy for Advanced and Metastatic Disease								
390, Harris et al 2006	Cox **Selected**	No info	TTF: HR(95%CI); p value HER2+ by CB11: 1.44(0.97-2.15), 0.68 HER2+ by FISH: 1.22(0.85-1.76), 0.29 HercepTest 2-3: 1.02(0.73-1.43), 0.90 HercepTest 3: 1.34(0.91-1.98), 0.14 OS: HR(95%CI); p value HER2+ by CB11: 1.34(0.91-1.99), 0.41 HER2+ by FISH: 1.24(0.86-1.79), 0.25 HercepTest 2-3: 1.04(0.74-1.46), 0.83 HercepTest 3: 1.11(0.76-1.64), 0.59	Age, performance, ER and PR status, # met sites, disease free interval, prior adj chemo, HER2,	No	HercepTest- (0-1 vs 2-3): TTF HR=0.97 (95%CI:0.68-1.40), p=0.88; OS HR=0.93 (95%CI:0.65-1.35), p=0.71	NR	NR
5970, Di Leo et al 2004	Cox	candidates not specified; selected as known prognos-factors & by results of uni-variate analysis	reported in Table 3E;l interaction HER2*Tx for TTP NS (p=.62); HER2*Tx for OS NS (p=.10)					

Study	Design/ Outcome/ Model	Candidate Predictors/ Method to Identify Candidates	Univariate Results, Variable (p value) Selected	Predictors/ Methods to Select Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Chemotherapy for Advanced and Metastatic Disease (continued)								
6740, Konecny et al 2004 Grp 1: EC Grp 2: ET	Cox	Not reported	RR failure Grp2/Grp1 For HER2+= 0.71(0.47-1.09, p=0.118) RR death Grp2/Grp1 For HER2+= 0.78(0.47-1.28, p=.320) RR failure Grp2/Grp1 For HER2-= 1.17(0.84-1.62, p=352) RR death Grp2/Grp1 For HER2-= 1.24(0.83-1.87, p=.294) PFS: T*HER2 p=0.090; OS: T*HER2 p=0.152	Stepwise regression Age, histology, mets site, # mets, prior Tx, nuclear grade, ER/PR status Grp2/Grp1	Both assessed Grp2/Grp1	RR of failure Grp2/Grp1 for HER2+= 0.65 (0.42-1.02, p=0.062) RR death For HER2+= 0.60(0.36-1.02, p=0.059) RR failure For HER2-= 1.10(0.77-1.57, p=0.590) RR death For HER2+= 1.10(0.70-1.72, p=0.680) PFS: adjusted T*HER2: p=0.109 OS: adjusted T*HER2: p=0.136	Not reported	Yes

Grp2/Grp1

Study	Tumor Response	Quality of Life
Adjuvant Chemotherapy		
Yang et al. 2003; rec. # 8840	not reported	not reported
Gusterson et al. 2003; rec. # 43690	not reported	not reported
Moliterni et. al. 2003; rec. # 10210	not reported	not reported
Colozza et al. 2005; rec. # 3820	not reported	not reported
Pritchard et al. 2006; rec. # 1760	not reported	not reported
Knoop et al. 2005; rec. # 3450	not reported	not reported
Dressler et al. 2005, rec. # 4280	not reported	not reported
Del Mastro et al. 2004, 2005; rec. # 48020	not reported	not reported
Tanner et al. 2006; rec. # 1820	not reported	not reported
Hayes et al. 2007; rec. # 47610	not reported	not reported
Martin et al. 2005; rec # 47650	not reported	not reported
Neoadjuvant Chemotherapy		
Learn et al. 2005; rec. # 47640; n=104 classified for HER2 status	Grp N pCR ORR (cCR+cPR) Test p Comments: for ORR data,by multivariate analysis, AC, $HER2^+$ vs $HER2^-$, p=0.06; AC+D, $HER2^+$ vs $HER2^-$, p=0.99 $HER2^+$, AC 32 22% 75% logistic regression NS $HER2^+$, AC+D 9 22% 78% $HER2^-$, AC 37 24% 51% <0.05 HER^-, AC+D 26 24% 81%	not reported
Arriola et al. 2006; rec # 950	Grp N pCR PR SD PD NE Test p Comments all 229 27 0.03 $HER2^+$ Whitney “association of with pCR”	not reported
Park et al. 2003; rec # 9960	Grp N pCR PR OR (CR+PR) NR (PD+NE) Test p Comments $HER2^+$ 31 5 (16%) 22 (71%) 27 (87%) 4 (13%) Fisher’s exact 0.013 $HER2^-$ 36 0 17 (47%) 17 (47%) 19 (53%)	not reported
Zhang et al. 2003; rec # 9820	Grp N cCR+cPR cNR p RR 95%CI pCR+MRD ERD p RR 95%CI tests $HER2^+$ 28 93% 7% 0.14 1.2 1.1-1.4 Mann-Whitney 18% 82% 0.53 1.4 0.54-3.67 Fisher’s exact & $HER2^-$ 69 78% 22% 13% 87% asymptotic	not reported

Study	Tumor Response									Quality of Life
Neoadjuvant Chemotherapy (continued)										
Tulbah et al. 2002; rec # 11560	Grp HER2^{+} 3+ HER2^{-}	N 21 31	pCR 6 (29%) 7 (23%)	PR	SD	PD	NE	Test	p NS	Comments also NS if IHC 2+ and considered HER2+	not reported
Tinari et al. 2006; rec # 2300	Grp all HER2^{+} HER2^{-}	N 77 20 57	TR* 23.4%	OR* 72.7%	SD 3.9%	PD 0	NE	Test	p	Comments see Table 3H: Response Regression Modeling	not reported
Chemotherapy for Advanced or Metastatic Disease										
390, Harris et al 2006	HER2 by CB11 Pos Neg HER2 FISH Pos Neg CepTest Pos (2-3, 0-1) Neg CepTest Pos (3) Neg (0-2)	N 30 126 37 109 46 105 30 121	CR+PR (%) 23 24 22 25 35 18 23 23	p 0.70 0.96							not reported
5970, Di Leo et al 2004 Grp 1: A Grp 2: T	Grp 1 HER2+ HER2- HER2 unk All HER2+ HER2- HER2 unk All	N 15 63 13 91 21 50 14 85	%(CR+PR) 27 0.026 35 31 33 0.98 67 40 36 46	A versus T OR (95%CI) p HER2+ 5.50(1.28-23.69) 0.04 HER2-1.24(0.58-2.68) 0.70 HER2 unk 1.25(0.25-6.24) 1.00 All 1.72(0.94-3.18) 0.09 (In full TAX 303 trial, response rates were 48% with docetaxel (n=161), 33% with doxorubicin (n=165), p=0.008)						not reported

* OR= overall response (cPR + minimal residual disease + PR); TR = tumor response (cPR + minimal residual disease);

Study	Tumor Response									Quality of Life
Chemotherapy for Advanced or Metastatic Disease (continued)										
6740, Konecny et al 2004 Grp1: EC Grp 2: ET	Grp	N	CR+PR(95%CI)	SD	PD	NE	Test	p	Comments	not reported
	HER2+	97	60(51-70)				chi sq	0.004		
	HER2-	178	41(34-49)							
	Grp 1									
	HER2+	49	45(32-60)				chi sq	0.130		
	HER2-	88	33(22-43)							
	Grp 2									
	HER2+	48	76(63-88)				chi sq	0.005		
	HER2-	90	50(39-61)							
	HER2+									
	Grp1	49	45(32-60)				chi sq	0.004		
	Grp2	48	76(63-88)							
	HER2-									
	Grp1	88	33(22-43)				chi sq	0.002		
	Grp2	90	50(39-61)							

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-H: Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Adjuvant Chemotherapy								
Yang et al. 2003 rec. # 8840	**Predictors/**		**Predictors/**		not reported or done			
Gusterson et al. 2003; rec# 43690					not reported or done			
Moliterni et. al. 2003; rec# 10210					not reported or done			
Colozza et al. 2005; rec. # 3820		**Selected**			not reported or done			
Pritchard et al. 2006; rec. # 1760					not reported or done			
Knoop et al. 2005; rec. # 3450					not reported or done			
Dressler et al. 2005, rec. # 4280					not reported or done			
Del Mastro et al. 2004, 2005; rec. # 48020					not reported or done			
Tanner et al. 2006; rec. # 1820					not reported or done			
Hayes et al. 2007; rec# 47610					not reported or done			
Martin et al. 2005 rec. # 47650					not reported or done			
Neoadjuvant Chemotherapy								
Learn et al. 2005; rec. # 47640	RCT ORR logistic regression	T size; nodal status; ER, PR, HER2, & p53 status; method for identifying not described	$HER2^-$ ORR: 81%, AC+D 51%, AC, p<0.05; $HER2^+$ ORR: 78% AC+D, 75%, AC, p=0.79	not specified or described	not mentioned	ORR, $HER2^-$ adjusted odds ratio, AC+D/AC: 3.5 (95% CI: 1.2-13.0); p<0.05	not described	not mentioned
Arriola et al. 2006; rec # 950	prospective series; pCR logistic regression	method not specified; ER, PR, Ki67 & HER2 status, tumor grade, histology	HER2 status & pCR: p=0.03	forward stepwise logistic regression	not applicable?	not reported	not described	not reported

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Neoadjuvant Chemotherapy (continued)								
Park et al. 2003; rec # 9960	retrospective series; response; linear regression	method not specified; HER2 & topoII amplification	HER2: p=0.013 topoII: p=0.011	not specified	not applicable?	95% responders in HER2/topoII (n=18), p=0.038	not described	not reported
Zhang et al. 2003; rec # 9820	not reported or done							
Tulbah et al. 2002; rec# 11560	not reported or done							
Tinari et al. 2006; rec # 2300	retrospective series; tumor response (cPR + MRD); univariate logistic regression	method not specified; HER2 & topoII amplification; age, T size, ER & PR status, grade, Ki67 status	HER2: OR = 5.28 (95% CI: 1.57-19.6); p=0.008	not reported co-amplified	not mentioned	not reported	not described	not reported
Chemotherapy for Advanced or Metastatic Disease								
390, Harris et al 2006	not reported or done							
5970, Di Leo et al 2004	RCT; ORR; logistic regression	candidates: KPS, # mets, visceral involvement, HER2 status; selected as known prognostic factors	For HER2+ pts given docetaxel, OR=3.12 (1.11-8.80), p=.03; i.e., HER2*Tx is stat. signif.	Tx, HER2, Karnofsky score, # met sites; visceral involvement; Tx*HER2; Tx*visceral; stat. signif. in univariate analysis	Interactions included	Outcome=response NR Tx*HER2: OR Gp2/Gp1=3.64 (1.39-9.54); p=.01 (only SS factor). Remains SS after adjust for visceral and Tx*visceral		NR

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Chemotherapy for Advanced or Metastatic Disease (continued)								
6740, Konecny et al 2004 Grp 1: EC Grp 2: ET	Logistic regression	(95%CI:	OR(HER2+ vs HER2-) =2.19 1.31-3.65) p=0.003 OR (Grp2 vs Grp 1 for HER2+) =3.65(95%CI: 1.51-8.86); p=0.004) OR (Grp2 vs Grp1 for HER2-) =2.07 (95%CI: 1.10-3.90, p=0.002) Interaction Tx*HER2: P=0.308	Selected Predictors/ included	Interactions	Adjusted interaction Tx*HER2: p=0.256 OR (Grp2 Vs Grp 1 For HER2+) =3.64 (95% CI: 1.48-8.92; P=0.005) OR (Grp2 Vs Grp 1 For HER2-) =1.92 (95% CI: 1.01-3.64; P=0.046)	not mentioned	yes, but not shown

Question 3: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-I: Adverse Events

Toxicity Type	**Study**	**Severity or Grade**	**Results**
Chemotherapy			
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 3: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-I (continued): Adverse Events

Toxicity Type	**Study**	**Severity or Grade**	**Results**
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-J: Study Quality Ratings

Study	Prospective design	Prespecified hypotheses about relation of marker to oucome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Adjuvant Chemotherapy									
Yang et al. 2003; rec. # 8840	N	N	N	Y	?	Y	Y	?	NA
Gusterson et al. 2003; rec. # 43690	Y	N	Y	Y	?	Y	N	med: 6 yrs	? ? N Y N N
Moliterni et. al. 2003; rec. # 10210	Y	N	Y	Y	?	Y	Y	med: 14.8 yrs	? ? Y N ? Y
Colozza et al. 2005; rec. # 3820	Y	N	Y	Y	Y	Y	N	med: 8 yrs	? ? N N ? N
Pritchard et al. 2006; rec. # 1760	Y	N	Y	Y	?	Y	Y	med: 10 yrs	? ? ? ? ? N
Knoop et al. 2005; rec. # 3450	Y	N	Y	Y	?	Y	N	med: 10 yrs	? ? Y ? ? N
Dressler et al. 2005, rec. # 4280; Thor et al. 1998, rec. # 40880	Y	Y	Y	Y	Y	Y	N	med: 9 yrs	Y ? ? N Y N
Del Mastro et al. 2004, 2005; rec. # 48020	Y	N	Y	Y	Y	Y	N	med: 6.7 yrs	? ? ? ? ? ?

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen

Data Abstraction Table IIIa-J (continued): Study Quality Ratings

Study	Prospective design	Prespecified hypotheses about relation of marker to oucome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Adjuvant Chemotherapy (continued)									
Tanner et al. 2006; rec. # 1820	Y	N	Y	Y	Y	Y	N	?	NA
Hayes et al. 2007; rec. # 47610	Y	Y	Y	Y	Y	Y	Y	med: ~10 yrs	? ? ? ? ? ?
Martin et al. 2005; rec # 47650	Y	Y	Y	N	?	Y	Y	med: 4.6 yrs	? ? ? ? ? ?
Neoadjuvant (Pre-operative) Chemotherapy									
Learn et al. 2005; rec. # 47640	Y	N	N	N	?	Y	N	pCR at resection	? ? NA ? ? ?
Arriola et al. 2006; rec # 950	Y	Y	Y	Y	?	Y	Y	pCR at resection	? ? NA ? ? ?
Park et al. 2003; rec # 9960	N	N	N	Y	?	Y	Y	pCR at resection	? ? ? ? ? ?
Zhang et al. 2003; rec # 9820	N	N	N	N	?	Y	Y	pCR at resection	NA
Tulbah et al. 2002; rec # 11560	N	N	N	Y	Y	Y	Y	pCR at resection	NA
Tinari et al. 2006; rec # 2300	N	N	N	Y	Y	Y	Y	pCR at resection	? ? NA Y ? ?

Question 3a: Tissue HER2 Results to Guide Selection of Chemotherapy Regimen
Data Abstraction Table IIIa-J (continued): Study Quality Ratings

Study	Prospective design	Prespecified hypotheses about relation of marker to oucome	Large, well-defined, represent-ative study population	Marker assay methods well-described	Blinded assessment of marker in relation to outcome	Homogeneous treatment(s), either randomized or rule-based selection	Low rate of missing data (≤ 15%)	Sufficiently long follow-up	Well-described, well-conducted multivariate analysis of outcome: 1) clear candidate variable selection, 2) clear, appropriate model-building guidelines, 3) assumptions tested, 4) standard prognostic variables included, 5) continuous variables well handled, 6) validation 1) 2) 3) 4) 5) 6)
Chemotherapy for Advanced or Metastatic Disease									
390, Harris et al 2006	Y	N	Y	Y	Y	Y	N	med: 8.3 yrs	N N N Y ? N
5970, Di Leo et al 2004	Y	N	Y	Y	Y	Y	N	med: 23 months	N N N ? ? N
6740, Konecny et al 2004	Y	N	Y	Y	?	Y	N	?	N N Y Y ? N

Question 3b: Tissue HER2 Results to Guide Selection of Hormonal Therapy

Data Abstraction Table IIIb-A: Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Neoadjuvant Hormonal Therapy						
None						
Adjuvant Hormonal Therapy						
Ryden et al 2005, multicenter, Sweden, 1986-1991	RCT (secondary analysis)	Stage II invasive cancer, premenopausal or <50 years old. Includes HR+ and HR-	564	428	136 (64, no specimens; 72, not assessable by IHC). Another 55 not assessable by FISH. Baseline prognostic factors similar in groups with or without specimens.	TAM for 2 yrs vs no TAM; also mastectomy or breast-conserving surgery + radiotherapy <2% pts received additional adjuvant chemotherapy (polychemotherapy, n=8; goserelin, n=1). Evenly distributed across arms.
Knoop et al. 2001, 27 sites, all in Denmark?, 8/77-11/82	RCT (secondary analysis of Danish Breast Cancer Cooperative Group's 77c protocol)	Adjuvant, postmenopausal, "high risk" (positive axillary lymph nodes, tumor > 5 cm, skin or deep facia involvement) (Note: eligibility did not depend on hormone receptor status)	1716	1515	201 (167, no specimens; 33, unevaluable ; 1,,unaccounted for. Baseline prognostic factors & outcomes similar in groups with or without specimens.)	TAM thrice daily for 1 year vs observation. All patients treated with mastectomy, lower ALND, and radiotherapy. 8% of the TAM pts were HER2-positive, vs. 14% of the observation arm (p=0.001) (per email from Dr. Knoop)
Metastatic Hormonal Therapy						
Arpino et al. 2004, multicenter, US?, 1982-1987	Prospective uncontrolled, SWOG protocol 8228 and ancillary study 9314	First-line Tx for metastatic disease, ER+; prior adjuvant TAM or chemo completed > 3 mos before relapse	349	136	213 (134, no specimens; 7, inevaluable specimens; 4, lost to F/U, 68, assays unsuccessful)	TAM twice daily until disease progression (failure), 10 mg (n=56) or 10 mg/m^2 (n=149) :

Data Abstraction Table IIIb-B: Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Neoadjuvant Hormonal Therapy						
None						
Adjuvant Hormonal Therapy						
Ryden et al 2005	TAM Cx Med 45 45 Rge 25-57 26-57	Not reported	Tumor size TAM Cx Med 25 22 (p=0.03) Rge 5-75 2-50	Lymph node status(%) TAM Cx Neg 30 26 p 0.4 1+-3+ 50 49 ≥4+ 20 24 Unk <1 <1 Nottingham histologic grade (%) TAM Cx 1 10 12 p 0.6 2 39 44 3 45 42 Not Done 6 2	Not reported	TAM Cx ER-/PR- 30 26 ER-/PR+ 8 10 ER+/PR- 4 ER+/PR+ 54 57 P=0.6 Not done 4 2
Knoop et al. 2001	All % HER2+ in each age grp <50 <1 14 50-59 22 26 60-69 45 18 70-79 30 15 80-89 4 3 p=.001 med for all=66 range for all=45-88	Not reported	Tumor size(%) < 20 mm 19 21-50 mm 56 >50 mm 25 NR <1 Med=40 mm Rge=0-250 mm	Degree of anaplasia (%) I 26 II 47 III 15 Other 11 NR 1	Not reported	All % HER2+ in each group ER- 34 3 ER+ 66 11 p=0.001 PR- 57 27 PR+ 43 7 p=0.001

Data Abstraction Table IIIb-B (continued): Patient Characteristics

Study	Age	Race (%)	Disease Stage	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Metastatic Hormonal Therapy						
Arpino et al. 2004	Her2+ Her2- Age < 65 66% 57% ≥65 34% 43% pre 16% 12% post 84% 88%	Grp1 Grp2 B not W H reported A O	Grp1 Grp2 I II III not IV reported Unk	Grp1 Grp2 Mn T T1 T2 T3 T4 not N0 reported N1 N2 N3 Unk	Scale Grp1 Grp2 not reported	Her2+ Her2- ER+ 100% 100% PR+ 78% 96% PR- 19% 4%

*p<.05

Data Abstraction Table IIIb-C: HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Neoadjuvant Hormonal Therapy				
None				
Adjuvant Hormonal Therapy				
Ryden et al 2005	FISH Ventana Medical Systems, Tucson, AZ IHC Pathway CB-11, 760-2694 (Ventana Medical Systems, Inc)	FISH 6+ signals/tumor cell IHC HercepTest 3+	Pos 13 Neg 87 3+ 15 2+ 9 1+ 17 0 59	Correlation between IHC 3+ and Fish amplification, r=0.82, p<0.001; κ=0.84. 8% of ER+ tumors were HER2 3+ or HER2 amplified. Among HER2 2+ tumors, 1 was amplified.
Knoop et al. 2001	FISH not done IHC Polyclonal, code #A485, Dako	FISH IHC Description unclear But appears cases high positive if ≥50% cancer Cells w/ membrane staining, roughly comparable to HercepTest 3+. Membrane staining <50% tumor cells called low positive HER2.	Pos 18 Equiv Neg 82 3+ 2+ 1+ 0	Of 18% positive, 40% were low + and 60%, hi +.
Metastatic Hormonal Therapy				
Arpino et al. 2004	FISH PathVysion HER-2/neu DNA Probe (Visis Inc, Downer's Grove, IHC Monoclonal antibody TAB 250 (Triton, Alameda, CA)	FISH HER-2/CEP17 ratio _2 IHC complete membrane staining in ≥10% tumor cells	Pos 24 Neg 76 Pos 21 Neg 79	2 "readers" for FISH; 1 "reader" for IHC Results using IHC reported elsewhere[14] concordance: 85%

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IL)

[14] Results of TAM*HER2 measured using IHC and definitions of clinical responses were published in Elledge RM, Green S, Ciocca D et al. Clin Cancer Res 1998;4:7-12. (Note: longer duration of response required by Arpino et al. 2004)

Question 3b: Tissue HER2 Results to Guide Selection of Hormonal Therapy
Data Abstraction Table IIIb-D: Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Criteria for Tumor Response or Progression	Independent Response Assessor?	F/U Frequency/Duration
Neoadjuvant Hormonal Therapy					
None					
Adjuvant Hormonal Therapy					
Ryden et al 2005	Recurrence free survival	None	Not reported	Not mentioned	Median for pts with no breast cancer event=14 yrs (range: 0-17 yrs). F/U annually for 5 yrs then every 18 mos.
Knoop et al. 2001	Disease free survival	None	not reported	not mentioned	Not reported; but 10 year DFS included in tables.
Metastatic Hormonal Therapy					
Arpino et al. 2004	Response	Time to failure and overall survival	response = cCR or cPR or cSD cCR: no evidence of disease for ≥6 mos.[2] cPR: ≥50% ↓ in cross-sectional area of all measurable lesions[2] cSD: <cPR but not cPD[2] cPD: ≥25% ↑ in cross-sectional area of all measurable lesions or appearance of new lesions[2]	not mentioned	"Nearly all" tumor blocks >10 years old and some >20 years old. F/U frequency and duration not reported

Study	Time to Event Outcomes													
Neoadjuvant Hormonal Therapy														
None														
Adjuvant Hormonal Therapy														
Ryden et al 2005	Outcome	Grp	N	Med (mos)	5 yr	10yr	15 yr	Test	p	HR (95%CI)	Comments			
	RFS HER2+ (IHC 3+)													
ER+ pts		Tx	8		~0.7	~0.7	~0.7	LR	0.2	0.38 (0.08-1.79)	No stat diff in RFS between HER2+			
		Cx	13		~0.4	~0.4	~0.4				and HER2- pts (measured by IHC or			
	RFS HER2- (IHC 0-2+)										FISH) among untreated pts.			
		Tx	115		~0.75	~0.7	~0.65	LR	0.07	0.69(0.46-1.03)	VEGFR2 status was predictive of			
		Cx	124		~0.7	~0.6	~0.55				TAM efficacy.			
	RFS HER2+ (FISH)													
		Tx			Data not reported			LR	0.14	0.21 (0.03-1.67)				
		Cx			Data not reported									
	RFS HER2- (FISH)													
		Tx			Data not reported			LR	0.14	0.73(0.47-1.12)				
		Cx			Data not repoted									
Knoop et al. 2001 (univariate)	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	10yr	Test	p	HR (95%CI)	Comments
	<u>DFS:</u>													
	HER2 -	Tx								34(2)	LR	.0001		Bonferroni p=.0006
Tam=Tx; Obs=Cx	Or low +	Cx								26(2)				
	(n=1005)													
	Her 2 hi +	Tx							63(11)	37(12)	LR	.5		Bonferroni p=.5
	(n=52)	Cx								35(8)				
								Cox	HER2+ (n=54): RR Tam vs Cx=0.89 (95%CI:0.63-1.27) HER2- (n=998): RR Tam vs Cx=0.86 (95%CI:0.78-0.93)					
						57(2)								
								MV Cox	HER2 and HER2*TAM: Not significant (p Values not reported)					
					43(2)									
	NOTE: Analysis limited to steroid receptor positive pts. Standard error is in parentheses. LR=log-rank test of differences in DFS probabilities for pats with the variables in question when treated with tam or not.													
					41(9)									

Question 3b: Tissue HER2 Results to Guide Selection of Hormonal Therapy
Data Abstraction Table IIIb-E (continued): Time to Event Outcomes

Study	**Time to Event Outcomes**													
	Metastatic Hormonal Therapy													
Arpino et al. 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	10yr	Test	p	HR (95%CI)	Comments
	TTF	HER2-	104	7	~.35	~.18	~.08	~.08	0	0	LR	.007		HER2+ pts had lower
		HER2+	32	5	~.20	~.03	No line	No line	No line					median ER levels, even
	OS	HER2-	104	31	~.85	~.60	~.50	~.25	~.20	~.05	LR	.07		when all pts ER+
		HER2+	32	25	~.90	~.52	~.30	~.20	~.08	~.05				
	HER2+ as predictor of TTF											0.54	1.15	adjusted
	HER2+ as predictor of survival										MV Cox	0.97	0.99	adjusted
											MV Cox			

Question 3b: Tissue HER2 Results to Guide Selection of Hormonal Therapy
Data Abstraction Table IIIb-F: Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value) Selected	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Neoadjuvant Hormonal Therapy								
None								
Adjuvant Hormonal Therapy								
Ryden et al 2005	Cox	Not reported	ER+/HER2-(IHC) P=0.07 ER+/HER2+(IHC) P=0.2 ER+/PR+/HER2-(IHC), P=0.03 ER+/PR+/HER2+(IHC), p=0.03	Not certain; prob Age, tumor size, Node status, NHG, HER2 status, TAM, TAM*HER2. Analyzed ER+ and ER+/PR+ tumors Separately.	Not reported	ER+: TAM*HER2(IHC) p=0.4 ER+/PR+ TAM*HER2(IHC) p=0.3	Not reported	Not reported
Knoop et al. 2001 (among steroid receptor positive pts)	Cox		Tam vs Cx for HR+ Within HER2+ and - subgroups RR HER2+=0.89 (95% CI: 0.63-1.27) RR HER2-=0.86 (95% CI: 0.78-0.93)	Tumor size, proportion node pos, histologic grade, p53 status, HER2 status, EGFR status, tamoxifen, and interactions betw. TAM and p53, EGFR, And HER2	Interactions included	HER2 and HER2*TAM not significant	Not reported	Not reported
Metastatic Hormonal Therapy								
Arpino et al. 2004	Cox* single-arm retrospec-tive; TTF & OS *partially non-parametric	signif predictors of TTF in SWOG 8228, HER 1, HER 2		menopausal status, disease-free interval, ER and PR levels; HER1, HER2		TTF HR 1.15 P .54 OS HR .99 P .97	Not reported	Not reported

Question 3: Tissue HER2 Results to Guide Treatment Regimen Selection

Data Abstraction Table IIIb-G: Tumor Response and Quality of Life

Study	Tumor Response	Quality of Life
Neoadjuvant Hormonal Therapy		
None		
Adjuvant Hormonal Therapy		
Ryden et al 2005	Not reported	Not reported
Knoop et al. 2001	not reported	not reported
Metastatic Hormonal Therapy		
Arpino et al. 2004	Grp N cCR+cPR+cSD PD NE Test p Comments **HER2- 56% 44% χ^2 NS** **HER2+ 32 47% 53%** **104**	not reported

Question 3: Tissue HER2 Results to Guide Treatment Regimen Selection

Data Abstraction Table IIIb-H: Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Neoadjuvant Hormonal Therapy								
None	**Predictors/**		**Predictors/**					
Adjuvant Hormonal Therapy								
Ryden et al 2005	not done or reported							
Knoop et al. 2001	not done or reported							
Metastatic Hormonal Therapy								
Arpino et al. 2004	not done or reported	**Selected**						

DSS=disease-specific survival; OS=overall survival; prop haz=proportional hazard

Data Abstraction Table IIIb-I: Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Hormonal Therapy			
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Data Abstraction Table IIIb-I (continued): Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 3: Tissue HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IIIb-J: Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged

Question 3: Tissue HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IIIb-K: Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Indepen-dently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknow-ledged
Hormonal Therapy								
Chemotherapy								

COMMENTS:

13310, Knoop et al. 2001	"Strong precautions have to be taken when the predictive value of HER2 is analyzed in ER-positive patients because of the inverse correlation between HER2 and ER (p. 3381)…Unfortunately, this large study does not have enough statistical power to finally confirm of disconfirm whether a small group of steroid receptor positive patients is resistant to or have a detrimental response to tamoxifen treatment (p. 3383)."

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-A: Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Gasparini et al. 2007, Italy, multicenter; 12/00 – 09/04	PII RCT	untreated MBC, t-IHC 2+/3+ (1^{st}-line metastatic disease)	124 enrolled (61 grp 1, 63 grp 2); allocation concealment: A	123 for efficacy and toxicity, 118 for ORR	1 for efficacy and toxicity, 6 for ORR	Grp 1: paclitaxel; Grp 2: paclitaxel + trastuzumab
Im et al. 2005, Korea, multicenter	PII, single-arm	MBC no previous CHT for metastatic disease (1^{st}-line)	40	39 for toxicity, 38 for response	1 for toxicity, 2 for response (refused further tx)	Epirubicin + docetaxel
Fornier et al. 2005, USA, 1 center	RET analysis of PII	MBC, HER2 overexpressing and non-overexpressing	55 of 95 in trial who had 1^{o} tumor tested for tHER2	55		Paclitaxel + trastuzumab
Muller et al. 2004, Germany, multicenter	RCT	1^{st}-line tx for MBC	103 of 597 in trial	101	2	Grp1: epirubicin + paclitaxel (ET, n=47, 62% sHER2+);Grp2: epirubicin + cyclophosphamide (EC, 54, 65% n=sHER2+)
Luftner et al. 2004, Germany, 1 center	PII	stage IV BC, 1 or 2 prev CHT (1 anthra-cycline-based)	35	35		Dose-intensified paclitaxel (1^{st}-line 6%, 2^{nd}-line 60%, 3^{rd}-line 34%)
Sandri et al. 2004, Italy, 1 center	Clinical trial	stage IV BC, $\geq$ 1 prev CHT for met dis (2^{nd}-line+)	64	39	25	Cyclophosphamide + methotrexate
Colomer et al. 2004, Spain, 7 centers	PII	progressive advanced BC, no 1^{o} tx for mets (1^{st}-line)	43	43 for toxicity 42 for efficacy	1	Paclitaxel + gemcitabine
Burstein et al. 2003, US, 17 centers	PII	stage IV BC, IHC HER2 3+ or FISH+, no prev CHT for met dis (1^{st}-line)	55	54 (43 had sHER2 values at baseline and after 1 tx cycle)	1 (did not receive protocol-based tx)	Trastuzumab + vinorelbine

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-A (continued): Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Lipton et al. 2003, mulitnational, multicenter	RCT	postmenopausal locally advanced, (stage IIIB) loco-regionally recurrent BC, MBC, ER+/PR? and/or PR+/ER? (1st-line)	562 of 907 allocation concealment: B	562		Grp1: letrozole (n=283) Grp2: tamoxifen (n=279)
Esteva et al. 2002, US, 1 center	PRO CS	MBC overexpressing tHER2, w/ or w/o previous tx for met dis, but no prior trastuzumab	30	30		Trastuzumab + docataxel
Colomer et al. 2000, Spain, 1 center	PRO CS	MBC, no previous CHT for met dis (1st-line)	77	55	3	Doxorubicin+ paclitaxel
Colomer et al. 2006, Spain, 6 centers	PII	advanced BC (1st-line)	52	47		IV vinorelbine+ IV gemcitabine
Yamauchi et al. 1997, US, ? centers	RCT	MBC (1st-line)	94 of 369	94		3 doses of droloxifene

Data Abstraction Table IV-B: Patient Characteristics

Study	Age/Menopausal Status	Race (%)	Disease Stage	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Gasparini et al. 2007	Grp2 mn md 54.27 56.02 Grp1 rng 30-71 32-72 sd pre 31.7 33.2 post 68.3 66.8	Grp1 Grp2 B W H A O	Grp1 Grp2 I IIa IIb IIIa IIIb IV	Grp1 Grp2 # met sites 1 33.4 39.7 2 40.0 33.3 3 13.3 14.3 4 8.3 7.9 >4 5.0 4.8	Scale Grp1 Grp2 ECOG 0 81.7 80.9 1 13.4 12.8 2 4.9 6.3	Grp1 Grp2 E-/P- 31.7 49.2 E+/P+ 36.7 36.5 E+/P- 16.7 4.8 E-/P+- 10.0 4.8 E+/P? 1.7 ?? 3.3 4.8
Im et al. 2005	Grp1 mn md 49 rng 35-70 sd pre 41.0 post 58.9	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# involved organs 1 43.6 2 30.8 ≥3 25.6	Scale Grp1 ECOG 0 20.5 1 53.8 2 25.6	
Fornier et al. 2005	mn md 51 Grp1 rng 33-67 sd pre post	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# met sites Grp1 md 2 rng 1-4	KPS Grp1 md 90 rng 70-100	
Muller et al. 2004	Grp1+2 mn md 56 rng 33-73 sd pre post	Grp1+2 B W H A O	Grp1+2 I IIa IIb IIIa IIIb IV 100	Grp1+2 visc dis 80	Scale Grp1+2	Grp1+2 E+ 61
Luftner et al. 2004	mn 48 md Grp1 rng 31-63 sd pre 9 post 91	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# involved organs Grp1 1 26 2 31 >2 43	Scale Grp1	E+/P+ 34% E-&P- 17 2 49 Grp1

Data Abstraction Table IV-B (continued): Patient Characteristics

Study	Age/Menopausal Status	Race (%)	Disease Stage	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
7280, Sandri et al. 2004	mn 56 md Grp1 rng 36-81 sd 11 pre post	B W Grp1 H A O	I Ia Grp1 IIb IIIa IIIb IV 100	# involved organs Grp1 1 25.6 2 38.6 ≥3 35.8	Scale Grp1 Grp1	 Grp1
7960, Colomer et al. 2004	mn md 53 Grp1 rng 29-72 sd pre post	B W Grp1 H A O	I Ia Grp1 IIb IIIa IIIb IV 100	# met sites Grp1 md 3 rng 1-6	Scale Grp1 md 100 rng 70-100	 E+ 49 E- 28 Grp1 ? 23
9100, Burstein et al. 2003	mn md 54.5 Grp1 rng 29-82 sd pre post	B W Grp1 H A O	I Ia Grp1 IIb IIIa IIIb IV 100	# met sites Grp1 md 3 rng 1-6	Scale Grp1 ECOG 0 70 1 28 2 2	 E+P+ 37 E+/P- 11 Grp1 E-/P+ 7 E-/P-- 44
9520, Lipton et al. 2003	Grp2 mn md 65 63 Grp1 rng 42-94 31-90 sd pre post	Grp1 Grp2 B W 95 97 H A O	Grp1 Grp2 I 12 13 Iia 22 18 Iib 13 15 IIIa 7 6 IIIb 8 12 IV 30 28 ? 7 8	Grp1 Grp2 # met sites 1 53 55 2 37 34 3 10 11	Scale Grp1 Grp2 KPS md 90 90 rng 50- 50- 100 100	Grp1 Grp2 E+&P+ 38 40 E+/P+ 28 27 E?/P? 34 33

Data Abstraction Table IV-B (continued): Patient Characteristics

Study	Age/Menopausal Status	Race (%)	Disease Stage	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Esteva et al. 2002	mn md Grp1 45 rng Grp1 33-78 sd pre post	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# met sites 1 Grp1 16 2 40 3 16 ≥4 26	Scale Grp1 KPS(%) 90 63 80 20 70 16	Grp1
Colomer et al. 2000	mn md rng Grp1 sd pre 34.5 post 65.5	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# met sites 1 Grp1 55.2 ≥2 44.8	Scale Grp1	E+ 66.7 E- 33.3 Grp1
Colomer et al. 2006	mn md Grp1 64 rng Grp1 34-81 sd pre post	B W Grp1 H A O	I IIa Grp1 IIb IIIa IIIb IV 100	# met sites md Grp1 2 rng 1-4	Scale Grp1 ECOG 0 41.2 1 47.1 2 11.8	E+ 67.3 E- 32.7 Grp1
Yamauchi et al. 1997	Grp1+2+3 < 64 46.8 ≥ 64 53.2 pre post	Grp1+2+3 B W H A O	Grp1+2+3 I IIa IIb IIIa IIIb IV 100	Grp1+2+3 # met sites 1 44.7 2 37.2 ≥3 18.1	Scale Grp1+2+3	Grp1+2+3 E++ 26.7 E+ 28.7 P++ 20.2 P+ 13.8

Data Abstraction Table IV-C: HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Gasparini et al. 2007	Serum: Bayer ADVIA Centaur, at baseline FISH IHC: HercepTest	Serum > 15 ng/ml FISH IHC 3+	Pos Equiv Neg Pos Equiv Neg Pos 68.3 Equiv 31.7 Neg	No info on % sHER2+/-
Im et al. 2005	Serum: Oncogene ELISA FISH IHC: Biogenix CB11 Ab	Serum ≥ 15 2+ FISH IHC ≥ 10% tumor cells with intense membrane staining	Pos 14.8 Equiv Neg 85.2 Pos Equiv Neg Pos Equiv Neg	sHER2 tested in 27 of 40
Fornier et al. 2005	Serum: Bayer Immuno 1 FISH PathVysion (in 44 of 55) IHC: HercepTest/CB11	Serum > 15 FISH ≥ 2 IHC 2+/3+, HercepTest 2+/3+, CB11	Pos 69 Equiv Neg 31 Pos 41 Equiv Neg 59 Pos 47 Equiv Neg 53 Pos 36 Equiv Neg 64	
Muller et al. 2004	Serum: Oncogene Science ELISA FISH IHC: CB11	Serum ≥ 15 FISH IHC 3+	Pos 37 Equiv Neg 63 Pos Equiv Neg Pos 31 Equiv Neg 69	tHER2 tested in 29 of 103

Data Abstraction Table IV-C (continued): HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Luftner et al. 2004	Serum: Oncogene Science	Serum ≥ 15	Pos 63 Equiv Neg 37	
	FISH	FISH	Pos Equiv Neg	
	IHC:	IHC	Pos Equiv Neg	
Sandri et al. 2004	Serum: Bayer Immuno 1	Serum ≥ 15	Pos ? Equiv Neg ?	
	FISH	FISH	Pos Equiv Neg	
	IHC: Dako HercepTest	IHC > 10% cancer cells with intense to moderate staining	Pos 35 Equiv Neg 65	tHER2 tested in 20 of 39
Colomer et al. 2004	Serum: Oncogene ELISA	Serum ≥ 30 ng/ml	Pos 29.3 Equiv Neg 70.7	
	FISH	FISH	Pos Equiv Neg	
	IHC:	IHC	Pos Equiv Neg	
Burstein et al. 2003	Serum: Oncogene Science	Serum	Pos ? Equiv Neg ?	
	FISH ?	FISH	Pos 18 Equiv Neg	
	IHC: ?	IHC 3+	Pos 80 Equiv 2 Neg	

Data Abstraction Table IV-C (continued): HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Lipton et al. 2003	Serum: Immuno 1 assay	Serum ≥ 15	Pos 29.2 Equiv Neg 70.8	
	FISH	FISH	Pos Equiv Neg	
	IHC:	IHC	Pos Equiv Neg	
Esteva et al. 2002	Serum: Immuno 1	Serum ≥ 15	Pos 70 Equiv Neg 30	
	FISH PathVysion	FISH +	Pos 85.7 Equiv Neg 14.3	FISH done in 28 of 30
	IHC: Neomarkers mAb e2-4001	IHC 3+	Pos 79.2 Equiv Neg 20.8	IHC done in 24 of 30
Colomer et al. 2000	Serum: Calbiochem ELISA	Serum 450 fmol/ml	Pos 43.6 Equiv Neg 56.4	sHER2 done in 55 of 58
	FISH	FISH 0,2+	Pos Equiv Neg	
	IHC: CB11	IHC	Pos 27.5 Equiv Neg 72.5	IHC done in 40 of 58
Colomer et al. 2006	Serum: Oncogene Science ELISA	Serum > 30	Pos 29.8 Equiv Neg 70.2	
	FISH	FISH	Pos Equiv Neg	
	IHC:	IHC	Pos Equiv Neg	

Data Abstraction Table IV-C (continued): HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Yamauchi et al. 1997	Serum: Oncogene Science ELISA	Serum ≥ 5000 U/ml	Pos 34 Equiv Neg 66	
	FISH	FISH	Pos Equiv Neg	
	IHC:	IHC	Pos Equiv Neg	

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-D: Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
Gasparini et al. 2007	ORR	Safety profile, TTP, duration of response	WHO	NR	Med: 16.6 mo
Im et al. 2005	Response	Response duration (RD), TTP, overall survival, toxicity	WHO	NR	Med 22.5 mo
Fornier et al. 2005	Correlation between sHER2 and tHER2	Response	CR=disappearance; PR=>50%↓, ≥ 4 wks; MR=25-49%↓; SD=<25%↓; PD=≥25%↑	NR	≥ 4 wk
Muller et al. 2004	OS	ORR, PFS	UICC	NR	Med 8.9 mo (0.5-36)
Luftner et al. 2004	ORR	PFS, duration of response	Internationally accepted criteria (Miller et al. 1981)	NR	≥ 4 wk
Sandri et al. 2004	Clinical benefit (PR+SD)	TTP, OS	WHO	NR	2 mo
Colomer et al. 2004	Toxicity, response	Response, TTP	WHO	NR	26 mo
Burstein et al. 2003	ORR	Toxicity, TTF	RECIST	NR	8 wk
Lipton et al. 2003	TTP	ORR, CB	IUAC	NR	3 mo
Esteva et al. 2002	Response	Toxicity	ECOG	NR	≥ 8 wk
Colomer et al. 2000	Response	Response duration	WHO	Yes	med 23 mo
Colomer et al. 2006	ORR	Toxicity, PFS	WHO	NR	?
Yamauchi et al. 1997	Response	TTP, OS	?	NR	?

Study	**Time to Event Outcomes**												
Gasparini et al. 2007													
Im et al. 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	
	TTP	sHER2+	4	2.8							<0.001		
		sHER2-	19	8.3									
	RD	sHER2+	3	1.5	0						<0.001		
		sHER2-	13	6.7	~43	~33							
	OS	sHER2+	4	12.4	~50	~26					0.076		
		sHER2-	23	not reached	~72	~56							
Fornier et al. 2005													
Muller et al. 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	sHER2+/EC	19	~8.4	~50	~15	~0			LR	0.092		
		sHER2-/EC	35	~22	~77	~40	~15						
		sHER2+/ET	18	~16	~60	~10	~0			LR	NS		
		sHER2-/ET	29	~14	~65	~10	~0						
	PFS	sHER2-/EC	35	~7	~30	~0	~0			LR	NS		
		sHER2-/ET	29	~9	~21	~0	~0						
		sHER2+/EC	19	~12	~21	~0	~0			LR	0.0341		
		sHER2+/ET	18	~9	~28	~0	~0						
Luftner et al. 2004	Outcome	Grp	N	Mn (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	
	Resp Dur	sHER2+	9	6.0	~0						0.042		
		sHER2-	5	2	~60								
	PFS	sHER2+	22	3	~3						0.098		
		sHER2-	13	4	~10								
Sandri et al. 2004	Outcome	Grp	N	Mn (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	sHER2+	?	2	~0	~0	~0			LR	0.007		
		sHER2-	?	11	~34	~12	~7						
	OS	sHER2+	?	11	~47	~0	~0			LR	<0.001		
		sHER2-	?	16	~84	~49	~42						
Colomer et al. 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	Resp Dur	sHER2+	5	7.9	~40	~0					0.04		
		sHER2-	24	14.4	~55	~37							

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-E (continued): Time to Event Outcomes

Study	**Time to Event Outcomes**												
Burstein et al. 2003	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
9520, Lipton et al. 2003	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	sHER2+	164										
		letrazole	87	6.1	~28	~7	~6	~4		Cox	0.0596	0.73 (0.53,1.01)	
		tamoxiven	77	3.3	~17	~5	~3						
		sHER2-	398										
		letrazole	196	12.2	~53	~29	~20	~14		Cox	0.0019	0.70 (0.56,0.88)	
		tamoxiven	202	8.5	~38	~20	~10	~8					
	TTF	sHER2+	164										
		letrazole	87	6.0						Cox	0.0418		
		tamoxiven	77	3.2									
		sHER2-	398										
		letrazole	196	11.6						Cox	0.0066		
		tamoxiven	202	6.2									
11880, Esteva et al. 2002	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
14870, Colomer et al. 2000	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	Resp Dur	sHER2+	15	7.5	~26						0.035		
		sHER2-	24	11	~50	~35				MV Cox	0.06		
22050, Colomer et al. 2006	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
26670, Yamauchi et al. 1997	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	sHER2+	32	~3	~13	~13				Cox	0.0003	0.36 (0.21, 0.63) adjusted	
		sHER2-	62	~8	~43	~28							
	OS	sHER2+	32	~28	~74	~54				Cox	0.003	0.35 (0.17, 0.70) adjusted	
		sHER2-	62		~92	~63							

LR

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-F: Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Gasparini et al. 2007	PII RCT PTP Cox PH	unclear **Selected**	NR	treatment arm, subgroup defined by each clinical or biomarker variable	PH assumption NR; preselected 1st-order interactions investigated	interactions: tHER2 (0.0938) sHER2+ (0.0538) non-visc (0.7845) E+/P+ (0.7845) #lesions (0.7355) ECOGPS (0.4643) EGFR (0.9607) CA 15.3 (0.1209) tHER2Δ (0.2684) EGFRΔ (0.1984) CA15.3Δ (0.0666)	NR	NR
Im et al. 2005								
Fornier et al. 2005								
Muller et al. 2004								
Luftner et al. 2004								
Sandri et al. 2004								
Colomer et al. 2004								
Burstein et al. 2003								
Lipton et al. 2003	RCT TTP, TTF Cox PH	treatment effects within sHER2 sub-Groups	NA	sHER2 subgroups	PHA?	subgroup analyses (see Table 4E)	NR	NR
Esteva et al. 2002								

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-F (continued): Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Colomer et al. 2000	PRO PS Resp dur, Cox PH	NR	menop (0.74) relapse (0.83) adjCHT (0.85) #mets (0.53) CA15-3 (0.03) sHER2 (0.03)	selection methods NR	PHA?	menop (0.81) relapse (0.95) adjCHT (0.64) #mets (0.37) CA15-3 (0.21) sHER2 (0.04)	NR	NR
Colomer et al. 2006		**Selected**						
Yamauchi et al. 1997	RCT TTP, OS Cox PH	age dom dis site sHER2 time to recurr E receptor P receptor # met site	NR	stepwise	PHA?/ no	TTP: sHER2 (0.0003) P++ (0.02) sHER2 (0.003) P++ (0.002) DF > 5yr (0.01)	NR	NR

OS:

Data Abstraction Table IV-G: Tumor Response and Quality of Life

Study	**Tumor Response (%)**										**Quality of Life**					
Gasparini et al. 2007	Grp	N	CR	PR	SD	PD		Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Im et al. 2005	Grp	N	CR	PR	SD	PD		Test	p	Comments						
	sHER2+	4	0	75	25			ChiSq	0.45							
	sHER2-	23	13.0	43.4	26.1	17.4										
Fornier et al. 2005	Grp	N	Response		No response			Test	p	Comments						
	sHER2									Response=						
	+	38	50		50			FE	1.0	CR+PR						
	-	17	47		43											
	Δ<15	25	68		32			FE	0.005							
	Δ≥15	13	15		85											
	Δ≥55%	25	68		32			FE	0.015	OR 4.25						
	Δ<55%	30	33		67					95% CI: 1.37-13.19						
Muller et al. 2004	Grp	N	CR+PR		SD	PD		Test	p	Comments						
	sHER2+/ET	18	50.0		33.3	16.7		ChiSq	NS							
	sHER2-/ET	26	46.2		38.5	15.4										
	sHER2+/EC	17	29.4		35.3	35.3		ChiSq	0.059							
	sHER2-/EC	31	41.9		35.5	22..6										
Luftner et al. 2004	Grp	N	CR+PR		SD	PD		Test	p	Comments						
	sHER2+	22	40.9		36.4	22.7		MH	0.40	mn dur 25.7 wks						
	sHER2-	19	38.5		30.8	30.8				mn dur 65.2 wks (p=0.042)						
Sandri et al. 2004	Grp	N	CR	PR	SD	PD	NE	Test	p	Comments						
Colomer et al. 2004	Grp	N	Response		No response			Test	p	Comments						
	sHER2+	15	42		58			FE	0.02							
	sHER2-		83		17											
Burstein et al. 2003	Grp	N	No progression			Progression		Comments								
	sHER2+	26						AU ROC=0.8947								
	sHER2-	?						BL or Δ in sHER2 do not not predict response, but no ↓ in sHER2 predicted progression								
		?														

Data Abstraction Table IV-G (continued): Tumor Response and Quality of Life

Study	Tumor Response (%)								Quality of Life
Lipton et al. 2003	Grp	N	CR+PR		SD+PD	Test	p	Comments	
	sHER2+	164							
	letrazole		17			log regr	0.4507		
	tamoxifen		13		87				
	sHER2-								
	letrazole		39	83		log regr	0.0078		
	tamoxifen	398	26		74				
	Grp	N	CR+PR	61	SD PD	Test	p	Comments	
	sHER2+	164							
	letrazole		33			log regr	0.3057		
	tamoxifen		26		74				
	sHER2-								
	letrazole		57	67		log regr	0.0162		
	tamoxifen	398	45		55				
Esteva et al. 2002 *Relevant to both Q4 and Q3	Grp	N	CR+PR			Test	p	Comments	
	sHER2+	21	76	43		FE	0.04		
	sHER2-		33		67				
	IHC 3+	19	63	SD+PD		FE	0.99		
	IHC 0-2+	5	60	24	40				
	FISH+	24	67			FE	0.60		
	FISH-	4	50	37	50				
Colomer et al. 2000 *Relevant to both Q4 and Q3	Grp	N	CR	PR	No response	Test	p	Comments	
	sHER2+	24	0	62		Chi sq	0.021		
	sHER2-	31	26	52					
	IHC+	11	9	55	37	Chi sq	0.219		
	IHC-	28	18	64	23				
Colomer et al. 2006	Grp	N	CR+PR	36	No response	Test	p	Comments	
	sHER2+	14	50	18	50		0.9		
	sHER2-		48.5		51.5				
Yamauchi et al. 1997	Grp	N	Response		No response	Test	p	Comments	
	sHER2+	32	9			FE	0.00001		
	sHER2-		56		44				

62 91 ?

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-H: Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Gasparini et al. 2007	PII RCT ORR logistic	unclear **Selected**	NR **Predictors/**	treatment arm, subgroup de-fined by each clinical or bio-marker variable	preselected 1st-order interactions investigated	interactions: tHER2 (0.0035) sHER2+ (0.6044 non-visc (0.8639) E+/P+ (0.3079) #lesions (0.0669) ECOGPS (0.1688) EGFR (0.5996) CA 15.3 (0.3415) tHER2Δ (0.983) EGFRΔ (0.8283) CA153Δ (0.6524)	NR	NR
Im et al. 2005								
Fornier et al. 2005								
Muller et al. 2004								
Luftner et al. 2004								
Sandri et al. 2004								
Colomer et al. 2004								
Burstein et al. 2003								
Lipton et al. 2003	RCT ORR, CB logistic	treatment effects within sHER2 sub-groups	NA	sHER2 sub-groups	yes	subgroup analyses (see Table 4G)	NR	NR
Esteva et al. 2002								

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-H (continued): Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Colomer et al. 2000	PRO PS ORR, logistic	NR	menop (0.39) relapse (0.79) adjCHT (0.91) #mets (0.32) CA15-3 (0.36) sHER2 (0.01)	selection methods NR	NA	menop (0.15) relapse (0.57) adjCHT (0.88) #mets (0.35) CA15-3 (0.83) sHER2 (0.03)		
Colomer et al. 2006		**Selected**						
Yamauchi et al. 1997	RCT Response logistic	age dom dis site sHER2 time to recurr E receptor P receptor # met site	NR	stepwise	no	sHER2 (0.0001) no hx CHT (0.01) soft site (0.01) E++ (0.04)	NR	NR

Data Abstraction Table IV-I: Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-J: Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged
Gasparini et al. 2007	+	+	+	+	+	Good	Yes, industry funded
Muller et al. 2004	?	?	+	+	+	?	-
Lipton et al. 2003	+	-	+	+	+	Fair	+
Yamauchi et al. 1997	?	?	+	+	+	?	+

Question 4: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table IV-K: Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Independently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknowledged
Im et al. 2005	+	+	+	+ (NA/-)	+	+	+	+
Fornier et al. 2005	+	+	+	+ (-)	+	+	+	+
Luftner et al. 2004	+	+	+	+ (-)	+	- (no AEs)	+	+
Sandri et al. 2004	+	+	+	+ (-)	+	- (no AEs)	+	+
Colomer et al. 2004	+	+	+	+ (-)	+	+	+	-
Burstein et al. 2003	+	+	+	+ (-)	+	+	+	+
Esteva et al. 2002	+	+	+	+ (-)	+	+	+	+
Colomer et al. 2000	+	-	+	+ (+)	+	- (no AEs)	+	+
Colomer et al. 2006	+	+	+	+ (-)	+	+	+	+

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Krug et al. 2005	Phase 2 RCT	Untreated advanced (Stage 3b or 4) nonsmall cell without prior chemotherapy. Prior RT must have been completed 3 weeks prior to study entry	64	64 (Grp1:31, Grp2:34)		Docetaxel+trastuzumab (Grp1) or palitaxel+trastuzamab (Grp2)
Cappuzzo et al. 2005	Prospective Clinical Trial	NSCLC with locally advanced or metastatic disease which progressed after chemotherapy/ medical contraindications to chemotx	102	101	1	Gefitinib
Hirsch et al. 2005	Prospective Clinical Trial	Stage IIIb or IV BAC or adeno with BAC features 1st –line CHT (101) or 2nd+-line (36)	145	56	89	Gefitinib until progression or prohibitive toxicity
Pelosi et al. 2005	RET CS	Stage I NSCLC with no (neo)adjuvant tx and stage I-III NET, treated	345 (NSCLC 207 (NET)	Same (retrospective study)	0	No neoadjuvant tx for NSCLC. All pts had radical surgery+mediastinal LN dissection
Saad et al. 2004	RET CS	Unselected patients with stage I conventional and bronchioloalveolar adenocarcinoma	100	100	0	complete surgical resection, no chemotherapy or radiotherapy
Langer et al. 2004	PII	Recurrent, stage IV, or stage IIIB NSCLC, HercepTest 1+/2+/3+	56	53	3	Trastuzumab, paclitaxel and carboplatin
Cappuzzo et al. 2003	PRO CS	Stage IIIB/IV NSCLC, pretreated with 1st-line platinum-based CHT and RT	63	63		2nd+-line gefitinib
Koukourakis et al. 1999	RET CS	Surgically treated NSCLC	216	189 with survival data	27	Treated with surgery alone

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-A-Lung Cancer (continued): Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
Koukourakis et al. 2000	RET CS	Operable NSCLC T1,2-N0,1	112	112	0	Surgery alone without RT or Chemotx
Graziano et al. 1998	PII	Stage IIIA lung cancer with ipsilateral mediastinal node involvement	66	46 had pre-study IHC staining for Her-2. 1 additional case was not accounted for	20	Two cycles of cisplatin-etoposide then surgical resection ten two additional cycles of cisplatin-etoposide then radiation to the thorax
Pfeiffer et al. 1996	RET CS	NSCLC treated surgically without adjuvant radiotx. Two pt had adjuvant cytotoxic tx	186	Same (RET CS)	0	Radical surgery (152) Macroscopic tissue was left in 34 pts

Data Abstraction Table V-B-Lung Cancer: Patient Characteristics

Study	Age	% Female	Race (%)	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Krug et al. 2005	Grp1 Grp2 mn md rng sd	Grp1 Grp2 48 76 (p=0.03) Her-2+ Her-2- 65% 62%	Grp1 Grp2 B W H A O	Grp1 Grp2 Ia Ib IIa IIb IIIa IIIb 10% 18% IV 90% 82% LD ED Her2+ Her2- Iiib 10 16 IV 90 84	Scale Grp1 Grp2 KPS 90% 35 26 80% 52 62 70% 13 12 Her2+ Her2- 70% 30 2 (p=0.02)	Grp1 Grp2 Bone Mets 37 21 Wt Loss>5% 10 18 Her2+ Her2- Bone Mets 30 27 ((p=0.77) Wt Loss>5% 25 9 (p=0.12)
Cappuzzo et al. 2005	mn md rng sd Grp1	Grp1	B W H A O Grp1	Ia Ib IIa IIb IIIa IIIb IV LD ED Grp1	Scale Grp1	Smoking Grp1 Grp2 .
Hirsch et al. 2005	All pt mn md 68 rng 34-88 sd	All pt 51%	All pt B W H A O	All PT Ia Ib IIa IIb IIIa IIIb 11% IV 89% LD ED	Scale All pt SWOG 0 89% 1/2 11%	Smoking

Data Abstraction Table V-B-Lung Cancer (continued): Patient Characteristics

Study	Age	% Female	Race (%)	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Pelosi et al. 2005	NSCLC NET male mn 63.4 58.7 md 64 63 rng 35,82 16,80 sd 8.1 14.4 female mn 61.6 52.6 md 62 54 rng 41,80 15,75 sd 8.8 15.4	NCLC NET 11.0% 37.2%	Grp1 B W H A O	NSCLC Ia 43 Ib 57 IIa IIb IIIa IIIb IV LD ED NET pIa 47 pIb 18 pIIa 9.7 pIIb 8.2 pIIIa 15.5 pIIIb <1	Scale Grp1	Smoking Grp1 Grp2 Information was available but not recorded
Saad et al. 2004	Grp1 Grp2 mn 64 67 md rng sd 11 7	Grp1 Grp2 38 46	Grp1 Grp2 B W H A O	Grp1 Grp2 Ia 46 68 Ib 54 32 IIa IIb IIIa IIIb IV LD ED	Scale Grp1 Grp2	Smoking Grp1 Grp2
Langer et al. 2004	Grp1 mn md 59 rng 31-77 sd	Grp1 49.1	Grp1 B 3.8 W 92.4 H A O 3.8	Grp1 Ia Ib IIa IIb IIIa IIIb 9.4 IV 81.2 Rec 9.4	Scale Grp1 ECOG 0 52.8 1 47.2	Smoking Grp1 Grp2

Data Abstraction Table V-B-Lung Cancer (continued): Patient Characteristics

Study	Age	% Female	Race (%)	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
Cappuzzo et al. 2003	mn 58.5 md Grp1 rng 31-79 sd	Grp1 36.5	B W Grp1 H A O	Ia Ib Grp1 IIa IIb IIIa IIIb 17.5 IV 82.5 LD ED	Scale Grp1 ECOG 0 25 1 64 2 11	Smoking Grp1 Grp2
Koukourakis et al. 1999	mn md Grp1 rng sd <60 25.5% >60 74.5%	Grp1 16.7	B W Grp1 H A O	T1 29.2 T2 Grp1 70.8 N0 56.5 N1 43.5 Grade 1,2 47.2 3 52.8	Scale Grp1	Smoking Grp1 Grp2
Koukourakis et al. 2000	mn md 63 Grp1 rng 45-76 sd	Grp1 20.5	B W Grp1 H A O	T1 37 T2 Grp1 63 N0 62.5 N1 37.5	Scale Grp1	Smoking Grp1 Grp2
Graziano et al. 1998	mn md Grp1 rng sd	Grp1	B W Grp1 H A O	Grp1 Ia Ib IIa IIb IIIa 100 IIIb IV LD ED	Scale Grp1	Smoking Grp1 Grp2
Pfeiffer et al. 1996	mn md 61 Grp1 rng 42-79 sd	Grp1 29.6% (55/186)	B W Grp1 H A O	Ia 46.2 Ib Grp1 25.8 IIIa 22.0 IIIb 1.6 IV 4.3 LD ED	Scale Grp1	Smoking Grp1 Grp2

Data Abstraction Table V-C-Lung Cancer: HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Krug et al. 2005	Serum: FISH standard fashion at Albany Medical Center IHC: HerceptTest (Dako)	Serum FISH IHC 2-3+ 0-1+	Pos Equiv Neg Pos Equiv Neg Pos 20/64 (31%) Equiv Neg 44/64 (69%)	
Cappuzzo et al. 2005	Serum: FISH PathVysion Her-2 DNA probe kit (Vysis) IHC: HerceptTest (Dako)	Serum FISH ≥ 4 copies ≥40% of cells ≤ 4copies > 40% of cells IHC score 200-400	Pos Equiv Neg Pos 23/101 (22.8%) Equiv Neg 78/101 (77.2%) Pos 5/72 (6.9%) Equiv Neg 67/72 (93.1%)	
Hirsch et al. 2005	Serum: FISH PathVysion Her-2 DNA probe kit (Vysis) IHC:	Serum FISH ≥ 4 copies ≥ 40% cells _ 4 copies >40% of cells IHC	Pos Equiv Neg Pos 17/56 (30%) Equiv Neg 39/56 (70%) Pos Equiv Neg	
Pelosi et al. 2005	Serum: FISH: PathVysion Her-2 DNA Probe kit (Vysis) IHC: rabbit polyclonal antiserum to HER2 (Dako, Envision plus-HRP) membrane only	Serum FISH IHC (only if FISH+) 288/345 (83.5%) Only 5 cases had gene amplification and were IHC 2+/3+. These were the tumors used for the survival analysis	Pos NSCLC NET Equiv Neg Pos Neg 2+3+ 29/345 (8.4%) 11/207 (5.3%) 1+ 28/345 (8.1%) 0 196/207 (94.7%)	

0

Data Abstraction Table V-C-Lung Cancer (continued): HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Saad et al. 2004	Serum:	Serum	Pos Equiv Neg	
	FISH	FISH	Pos Equiv Neg	
	IHC: rabbit polyclonal, Dako	IHC complete/almost complete staining of entire cell periphery in > 10%	Pos Equiv Neg	AC: 38, BAC: 18
Langer et al. 2004	Serum:	Serum	Pos Equiv Neg	
	FISH	FISH	Pos Equiv Neg	
	IHC: Dako HercepTest	IHC 1+	Pos Equiv Neg	100
Cappuzzo et al. 2003	Serum:	Serum	Pos Equiv Neg	
	FISH	FISH	Pos Equiv Neg	
	IHC: Dako Herceptest	IHC 2+	Pos Equiv Neg	16.3 18.6 65.1
Koukourakis et al. 1999	Serum:	Serum 3+	Pos Equiv Neg	
	FISH	FISH	Pos Equiv Neg	
	IHC: monoclonal antibody NCL-CB11(Novocastra Laboratories)	IHC strong diffuse staining negative/weak staiining	Pos Equiv Neg	166 (76.9%) 50 (23.1%)

Data Abstraction Table V-C-Lung Cancer (continued): HER2 Measurement Methods

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Koukourakis et al. 2000	Serum: FISH IHC: monoclonal antibody NCL-CB11(Novocastra Laboratories	Serum FISH IHC strong (>70% cells) weak (25-70% cells) less than 25% of cells	Pos Equiv Neg Pos Equiv Neg Pos 55 (49%) Equiv 31 (28%) Neg 26 (23%)	membrane staining was not analyzed
Graziano et al. 1998	Serum: FISH IHC: polyclonal rabbit HER-2 antiserum (DBW-2).	Serum FISH IHC membrane or membrane and cytoplasmiic reactivity throughout the tissue	Pos Equiv Neg Pos Equiv Neg Pos 22% (10/46) Neg 78% (36/46)	
Pfeiffer et al. 1996	Serum: FISH IHC: A485 (Dako, Denmark)	Serum section FISH IHC >=80% <80% 0 cells	Pos Equiv Neg Pos Equiv Neg high 26% (49) low 58% (108) Neg 16% (29)	

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-D-Lung Cancer: Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
Krug et al. 2005	Response rate	OS	Standard bidimensional criteria for measurable disease or improvement for patients with evaluable disease	NR	56 mo
Cappuzzo et al. 2005	Objective respones	TTP	Response evaluation criteria in Solid Tumors Group Criteria	Yes	2 mo
Hirsch et al. 2005	Response and overall survival		RECIST	NR	> 24 mo
Pelosi et al. 2005	Prevalence and prognosis of FISH her-2 abnormalities in lung CA (both NET and NSCLC	OS, DFS	NR	NR	NET: 53.3 +/-53.6 months NSCLC: 72.6 +/-49.3 months
Saad et al. 2004	OSOverall surval				AC: 52 ± 20 mo; BAC: 40 ± 17 mo
Langer et al. 2004	Toxicity	Response, OS, PFS	?	NR	med 34 mo
Cappuzzo et al. 2003	Response	TTP, OS	RECIST	NR	> 19 mo
Koukourakis et al. 1999	OS		NR	NR	med 42 mo (18-84)
Koukourakis et al. 2000	OS		NR	NR	med 46 mo (2-96)
Graziano et al. 1998	Response, DFS, OS		NR	NR	
Pfeiffer et al. 1996	OS				66 mos (40-119)

Study													
Krug et al. 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC+	20	14.3	~65	~30	~20	~20			NS		IHC 2+/3+
		IHC-	45	14.9	~59	~33	~16	~10					
Cappuzzo et al. 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	23	20.8	60.9	~38					0.056		discrep-
		FISH-	78	8.4	37.2	~15							ancies
	TTP	FISH+	23	9.05	34.8						0.02		discrep-
		FISH-	78	2.7	9.0	~5							ancies
Hirsch et al. 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	17	16	~64	~26					0.80		Cox HER2
		FISH-	39	13	~61	~35							not entered
Pelosi et al. 2005	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	FISH+	5	> 14 yrs	100	~80	~60	~60	~60	LR	NS		
		FISH-	340	> 14 yrs	94	~88`	~80	~72	~70				
	DFS	FISH+	5	> 14 yrs	~80	~60	~60	~60	~60	LR	NS		
		FISH-	340	~13 yrs	~86	~77`	~74	~70	~65				
Saad et al. 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	AC-IHC+	19	~24	~81	~50	~18	~0		?	<0.001		Cox: HER2
		AC-IHC-	31	~43	~96	~75	~54	~41					independent
		BAC-IHC+	9	~30	~91	~63	~27	~0	~0	?	<0.001		Cox: HER2
		BAC-IHC-	41	~39	~100	~100	~50	~30	~19				independent
Langer et al. 2004	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC3+	8	10.9	37.5	25					0.77		
		IHC2+	23	8.6	26.1	13.5							
		IHC1+	22	14.3	59.1	11.4							
	PFS	IHC3+	8	2.7	-						0.34		
		IHC2+	23	3.8	~9								
		IHC1+	22	3.9	~6								
Cappuzzo et al. 2003	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	TTP	IHC 2+/3+	15	3.5							NS		
		IHC 0 /1+	28	3.7									
	OS	IHC 2+/3+	15	5.7							NS		
		IHC 0 /1+	28	6.8									
Koukourakis et al. 1999	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC+									0.51		Cox HER2
		IHC-											not entered
Koukourakis et al. 2000	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC+									NS		
		IHC-											

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-E (continued): Time to Event Outcomes

Study													
Graziano et al. 1998	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC+	10	10.5							0.617		
		IHC-	37	17.5									
Pfeiffer et al. 1996	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	IHC-none	29	~34	~75	~66	~42	~32	~20	LR	NS	0.89 (0.56-1.41)	
		IHC-low	108	~24	~58	~50	~38	~33	~25				
		IHC-high	49	~24	~75	~50	~40	~30	~25				

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Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-F-Lung Cancer: Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Krug et al. 2005								
Cappuzzo et al. 2005	**Predictors/**							
Hirsch et al. 2005								
Pelosi et al. 2005								
Saad et al. 2004	RET CS OS CPHM	**Selected**	signif:	age, sex, recurrence, angiolymphatic invasion, HER2, p53,		independent predictors: recurrence, angiolymphatic invasion, p53, HER2, VEGF		
Langer et al. 2004								
Cappuzzo et al. 2003		HER2 p53 VEGF						
Koukourakis et al. 1999								
Koukourakis et al. 2000								
Graziano et al. 1998								
Pfeiffer et al. 1996								
		VEGF						

Data Abstraction Table V-G-Lung Cancer: Tumor Response and Quality of Life

Study	**Tumor Response (%)**									**Quality of Life**					
Krug et al. 2005	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Cappuzzo et al. 2005	Grp FISH+ FISH-	N 23 78	CR+PR 34.8 6.4		SD	PD	Test	p 0.001 0.08	Comments Cox MV adjusted for EGFR mutation HR 0.22 (95% CI: 0.04, 1.21)	Scale	Domain	F/U	Grp	n	mn±sd
	Grp FISH+ FISH-	N 23 78	CR+PR+SD 56.5 33.3				Test	p 0.04	Comments						
Hirsch et al. 2005	Grp FISH+ FISH-	N 11 28	CR+PR 36% 46%	ChiSq	SD	PD	Test	p >.05	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Pelosi et al. 2005	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Saad et al. 2004	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Langer et al. 2004	Grp IHC3+ IHC2+ IHC1+	N 8 23 21	Response 25 17.4 33.3	ChiSq			Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Cappuzzo et al. 2003	Grp HER2 2+/3+ HER2 0/1+	N 15 28	CR	PR 13.3 14.3	SD 26.7 50.0	PD	Test	p 0.126	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Koukourakis et al. 1999	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Koukourakis et al. 2000	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Graziano et al. 1998	Grp HER2+ HER2-	N 10	Response 30 33			ChiSq	Test FE	p 0.999	Comments	Scale	Domain	F/U	Grp	n	mn±sd
Pfeiffer et al. 1996	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-H-Lung Cancer: Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
Krug et al. 2005								
Cappuzzo et al. 2005	**Predictors/**		**Predictors/**					
Hirsch et al. 2005								
Pelosi et al. 2005								
Saad et al. 2004								
Langer et al. 2004		**Selected**						
Cappuzzo et al. 2003								
Koukourakis et al. 1999								
Koukourakis et al. 2000								
Graziano et al. 1998								
Pfeiffer et al. 1996								

Data Abstraction Table V--Lung Cancer: Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n % 3%
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-J-Lung Cancer: Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged
Krug et al. 2005	yes	No	yes	yes	yes	fair	yes

Question 5: HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-K-Lung Cancer: Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Indepen-dently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknow-ledged
Cappuzzo et al. 2005	+	-	+	+	+	+	+	+
Hirsch et al. 2005	+	-	+	+	+	+	+	+
Pelosi et al. 2005	+	+/-	+	+ (?)	+	+	+	+
Saad et al. 2004	+	+	+	+	+	+	+	-
Langer et al. 2004	+	+	+	+ (?)	+	+	+	+
Cappuzzo et al. 2003	+	+	+	+(?)	-	+	+	-
Koukourakis et al. 1999	+	-	+	+	+	-	+	+
Koukourakis et al. 2000	+	-	+	+ (?)	+	-	?	+
Graziano et al. 1998	+	-	+	+ (?)	+	+/-	+	+
Pfeiffer et al. 1996	+	-	+	+	+	+/-	+	-

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-L, Ovarian Cancer. Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)		Treatment Regimen (Agents)
Camilleri-Broet et al., 2004	RCT	Histologically-proven advanced epithelial ovarian carcinoma	164	117	47	no histology slides	cisplatin + epirubicin + cyclophosphamide (2 different doses of cyclophosphamide combined)
Bookman et al., 2003	PII, single-arm	recurrent or persistent epithelial ovarian or primary peritoneal carcinoma, tIHC 2+/3+	45 of 95 who had tIHC 2+/3+	41	2 2	inadequate pathology or wrong primary tumor did not receive tx	trastuzumab
Bowman et al., 2002	PII, single-arm	previously treated relapsed ovarian carcinoma	60	50	9 1	no CT evidence of disease unsatisfactory baseline scan	letrozole
Campos et al., 2001	retrospective	previously treated relapsed and refractory ovarian carcinoma	72	48	24	blocks not available	liposomal doxorubicin
Hengstler et al., 1999	retrospective	primary epithelial ovarian carcinoma	77	77	0		carboplatin or cisplatin + cyclophosphamide
Di Leo et al., 1995	PII, single-arm	previously treated relapsed and refractory stage III-IV epithelial ovarian carcinoma	78	42	6 30	deemed ineligible before study entry tumor samples unavailable	mitoxantrone + ifosfamide

Data Abstraction Table V-M, Ovarian Cancer. Patient Characteristics

Study	Age	Race (%)	Disease Stage	Performance Status	Histologic Grade	Histologic Type
Camilleri-Broet et al., 2004	Grp1 mn md 59 rng 23-70 sd	B W Grp1 H A O	Ia Ib Grp1 IIa IIb IIIa 4 IIIb 25 IIIc 65 IV 22 (1 missing)	Scale Grp1 WHO 0 27 1 60 2 8 missing 5	Tumor Grade Grp1 1 (well diff) 0 2 (moderately diff w/out nuclear atypia) 13 3 (moderately diff w/nuclear atypia) 40 4 (poorly diff or undiff w/nuclear atypia) 29 missing	Tumor Type Grp1 serous or mixed 63 epithelial clear cell 3 endometrioid undiff carcinoma 26 8
Bookman et al., 2003	Grp1 mn md 59 rng 44-82 sd	B 4.9 W Grp1 92.7 H 2.4 A O	Ia Ib Grp1 Iia Iib IIIa IIIb IV	Scale Grp1 GOG 0 61 1 39	Tumor Grade Grp1 1 (well diff) 2.4 2 (moderately diff) 22.0 3 (poorly diff) 75.6 18	Tumor Type Grp1 serous 65.9 clear cell 17.1 endometrioid 7.3 other 9.8 (2 mixed epithelial, 1 TCC, 1 undiff)
Bowman et al., 2002	Grp1 mn md 65 rng 43-83 sd	B W Grp1 H A O	Ia Ib Grp1 IIa IIb IIIa IIIb IV	Scale Grp1 WHO 100 0-2	Tumor Grade Grp1 well diff 5 moderately diff 22 poorly diff 65 not documented 8	Tumor Type Grp1 serous 72 endometrioid other (not defined) 10 18
Campos et al., 2001	Grp1 mn md 57 rng 31-77 sd	B W Grp1 H A O	Initial presentation Grp1 I/II 6 III 72 IV 22	Scale Grp1 NR 0 25 1 28 2 18 3 3 Unk 26	Tumor Grade Grp1 poorly diff 58	Tumor Type Grp1 serous/papillary 81 endometrioid clear cell 7 mixed 7 other (not defined) 3 8

Data Abstraction Table V-M, Ovarian Cancer. Patient Characteristics (continued)

Hengstler et al., 1999	Grp1 mn md rng sd	B W H A O	Grp1 Ia Ib IIa IIb IIIa IIIb IV	FIGO I + II 20 III+IV 50	Scale Grp1 WHO GI-GIII	Tumor Type Grp1 serous nonserous
Di Leo et al., 1995	Grp1 mn md 57 rng 39-74 sd	B W H A O	Grp1 Ia Ib IIa IIb IIIa IIIb IV	Scale Grp1 ECOG 0 40 1 49 2 11	Tumor Grade Grp1 well diff 15 moderately diff 28 poorly diff 38 not documented 19	Tumor Type Grp1 serous/papillary 83 mucinous endometrioid clear cell 3 8 6

Data Abstraction Table V-N, Ovarian Cancer. HER2 Measurement Methods

Study	Assays (Name)		Criteria for Positivity		Test Results (%)		Comments
Camilleri-Broet et al., 2004	Serum		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC	Novocastra Labs CB11	IHC	plasma membranes of > 10% cells moderately or strongly labeled	Pos Equiv Neg	16 ? ?	95 patients, variability observed within some tumors (not defined)
Bookman et al., 2003	Serum	Genentech ELISA	Serum	> 2.60 ng/ml	Pos Equiv	33	24 of 41 pts had sHER2 data
	FISH				Neg	67	
			FISH		Pos Equiv Neg		
	IHC	LabCorp 4D5 ± CB11					
			IHC	2+/3+	Pos Equiv Neg	34 66	
Bowman et al., 2002	Serum		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC	Neomarkers CB11	IHC	continuous variable with no cutoff	Pos Equiv Neg		
Campos et al., 2001	Serum		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC	IMPATH with Dako HercepTest	IHC	$\geq$ 1+	Pos Equiv Neg	9 91	

Data Abstraction Table V-N, Ovarian Cancer. HER2 Measurement Methods (continued)

Study	Assays (Name)	Criteria for Positivity	Test Results (%)	Comments
Hengstler et al., 1999	Serum	Serum	Pos	c-erbB-2 (HER2) expression analyzed by reverse
			Neg	semiquantitative PCR
	FISH	FISH	Pos	
			Equiv	
		Equiv	Neg	
	IHC	IHC continuous	Pos	
			Equiv	
			Neg	
Di Leo et al., 1995	Serum	Serum	Pos	
			Equiv	
			Neg	
	FISH	FISH	Pos	
			Equiv	
			Neg	
	IHC Triton Diagnostics pAb1	IHC ≥ 10% staining	Pos 14	
			Equiv	
			Neg 86	

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-O, Ovarian Cancer. Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
9040, Camilleri-Broet et al., 2004		Response, OS, PFS	≥ 50% reduction in product of tumor measurements and no appearance of new lesions, evaluated clinically or by laparotomy		med 68 mo
10820, Bookman et al., 2003	ORR (CR + PR)	PFS, toxicity	CR = disappearance for ≥ 4 wks PR = ≥ 50%↓ ID = ≥ 50%↑ PD = increasing disease or death due to disease prior to tumor assessment		
11720, Bowman et al., 2002		ORR, TTP, OS, failure-free at 12 wks	UICC Rustin's (CA125)		med 24 mo (rng 11-35 mo)
13450, Campos et al., 2001		ORR, CA-125, OS, TTPtoxicity	Rustin's (CA125) For pts with measurable disease: CR = disappearance of measurable disease and CA125 < 35 U/ml for ≥ 30 days after tx PR = > 50%↓ of measurable disease for ≥ 30 days and CA125 ↓ > 50% SD = response < PR without PD for at least 3 wk PD = > 25%↑ of measurable disease and CA125 ↑ > 25% or new disease sites on clinical exam For pts w/out measurable disease: CR = normalization of CA125 and complete resolution of pleural ascites/fluid (if present) PR = > 50%↓ of CA125 with reduction of ascites/pleural fluid PD = same as pts with measurable disease		med 22 mo
15150, Hengstler et al., 1999	OS				
17300, Di Leo et al., 1995		Toxicity, ORR, TTF, OS	WHO criteria for response (not shown in paper)		

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-p, Ovarian Cancer. Time to Event Outcomes

Study	**Time to Event Outcomes**												
Camilleri-Broet et al., 2004 cisplatin + epirubicin + cyclophospha-mide	Outcome PFS OS 	Grp HER2+ pts HER2- pts HER2+ pts HER2- pts	N 15 102 15 102	Med (mos) 12 15 25 35	1 yr ~58 ~70 ~86 ~92	2 yr ~8 ~30 ~46 ~60	3 yr 0 ~20 ~20 ~46	4 yr ~9 0 ~36	5 yr ~6 ~28	Test Cox Cox Cox Cox	p 0.02 0.02 0.02 0.04	RR (95%CI) 2.13 (1.13-4.01) 2.08 (1.11-3.91) 2.07 (1.03-4.17) 2.07 (1.03-4.15)	Comments UV MV UV MV
Bookman et al., 2003 trastuzumab	Outcome PFS OS	Grp	N 41 41	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments no relation between tHER2 expression level and PFS/OS
Bowman et al., 2002 letrozole	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Campos et al., 2001 liposomal doxorubicin	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
Hengstler et al., 1999 carboplatin or cisplatin + cyclophospha-mide	Outcome OS	Grp low HER2 interm HER2 high HER2	N 37 19 21	Med (mos) 100.8 63.6 16.8	1 yr ~80 ~93 ~63	2 yr ~75 ~81 ~25	3 yr ~72 ~74 ~15	4 yr ~60 ~55 ~5	5 yr ~52 ~50 ~5	Test LR	p 0.0001	HR (95%CI)	Comments complete grp
Di Leo et al., 1995 mitoxantrone + ifosfamide	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-Q, Ovarian Cancer. Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Predictors/ Method for Identifying Candidates	Univariate Results, Variable (p value)	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
9040, Camilleri-Broet et al., 2004	RET/ PFS, OS/ Cox	?	?	based on UV PFS: PS, ascites, residual lesion size, HER2 OS: PS, ascites, residual lesion size, HER2	?	PFS:ascites (<0.01), HER2 OS: ascites (0.04), HER2	N	?
10820, Bookman et al., 2003		**Selected**						
11720, Bowman et al., 2002					(0.02)			
13450, Campos et al., 2001					(0.04)			
15150, Hengstler et al., 1999								
17300, Di Leo et al., 1995	RET/ TTF, OS/ Cox recurs part	imaging, tumor grade, residual tumor volume, age, disease sites, tumor responsiveness, p53, HER2	TTF: disease detectable on imaging (0.02) OS: (0.01)	TTF: disease detectable on imaging OS: same	?	TTF: disease detectable on imaging OS: same		

Data Abstraction Table V-R, Ovarian Cancer. Tumor Response and Quality of Life

Study	**Tumor Response (%)**										**Quality of Life**					
9040, Camilleri-Broet et al., 2004	Grp	N	CR	PR	SD	PD		Test	p	Comments no relation between tHER2 expression level and response;	Scale	Domain	F/U	Grp	n	mn±sd
10820, Bookman et al., 2003	Grp	N	CR	PR	SD	PD		Test	p 0.023	Comments IHC3+ more likely to experience cycle 1 toxicity	Scale	Domain	F/U	Grp	n	mn±sd
11720, Bowman et al., 2002	Grp	N	CR+PR		SD	PD	?	Test 0.026	p	Comments high HER2 (not defined) associated with CA125 progression	Scale	Domain	F/U	Grp	n	mn±sd
13450, Campos et al., 2001	Grp IHC+ IHC- IHC?	N 4 30 24	CR 50 30 17	PR	SD 11 29	PD 57 50	 2 4	Test FE	p 0.579	Comments ≥50%↓ in CA125	Scale	Domain	F/U	Grp	n	mn±sd
15150, Hengstler et al., 1999	Grp	N	CR+PR		SD+PD			Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
17300, Di Leo et al., 1995	Grp IHC+ IHC-	N 8 14	CR 25 14	PR	SD 75 86	PD		Test FE	p 0.602	Comments	Scale	Domain	F/U	Grp	n	mn±sd

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-S, Ovarian Cancer. : Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
9040, Camilleri-Broet et al., 2004	**Predictors/**		**Predictors/**					
10820, Bookman et al., 2003								
11720, Bowman et al., 2002								
13450, Campos et al., 2001		**Selected**						
15150, Hengstler et al., 1999								
17300, Di Leo et al., 1995								

Data Abstraction Table V-T, Ovarian Cancer. Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-U, Ovarian Cancer. Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-V, Ovarian Cancer. Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Independently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknow-ledged
9040, Camilleri-Broet et al., 2004	+/-	+	+	+ (-)	+	+/-	+	+
10820, Bookman et al., 2003	+	+	+	+ (-)	- ?	+	+	+
11720, Bowman et al., 2002	+	-	+	+ (-)	-	+	+	+
13450, Campos et al., 2001	+	-	+	+ (-)	-?	+	+/-	+
15150, Hengstler et al., 1999	+	-	+	+ (-)	+	-	+	-
17300, Di Leo et al., 1995	+	+	+	+ (-)	+?	+/-	+	+

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-W, Prostate Cancer. Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
1320, Nishio et al., 2006	retrospective	bone metastatic prostate cancer	50	49	1, indeterminate tHER2 staining	maximal androgen blockade as follows: n = 47 antiandrogens + LH-RH agonists n = 3 antiandrogens + bilateral orchiectomy
16450, Arai et al., 1997	retrospective	histologically diagnosed, untreated prostate cancer	71	33 of 40 with stage D2 disease	38, not followed for response	all pts with stage D2 disease received antiandrogen tx, including bilateral orchiectomy, leuprorelin acetate, or DES
18080, Fox et al., 1994	Retrospective	untreated prostate cancer	45	45	0	expectant

Data Abstraction Table V-X, Prostate Cancer. Patient Characteristics

Study	Age	Race (%)	Disease Stage	Performance Status	PSA	Gleason Score
1320, Nishio et al., 2006	HER2+ (n = 21) mn 74.4 md 72 rng 63-85 sd 6.5 HER2- (n = 28) mn 73.0 md 72 rng 61-91 sd 7.9	B W H A O	HER2+ HER2- T1c 4.8 3.6 T2a 4.8 14.3 T2b 23.8 25.0 T3a 0 0 T3b 9.5 10.7 T4 52.4 46.4 Tx 4.8 0 N0 52.4 67.9 N1 42.9 32.1 N2 4.8 0	Scale Grp1	Grp1 HER2+ mn 798.5 md 426 rng 34-3780 sd 1076.2 HER2- mn 1242.3 md 270.4 rng 37-10060 sd 2323.6	HER2+ HER2- 7 4.8 10.7 8 42.9 28.6 9 38.1 60.7 10 14.3 0
16450, Arai et al., 1997	Grp1 mn md rng sd	B W H A O	Whitmore-Jewett system B 8.5 C 26.8 D1 8.5 D2 56.3	Scale Grp1	mn md rng sd	
18080, Fox et al., 1994	Grp1 mn 65 md rng 54-75 sd	B W H A O	A1 100	Scale Grp1	Grp1 mn md rng sd	Grp1 all <4

Grp1 Grp1

Data Abstraction Table V-Y, Prostate Cancer. HER2 Measurement Methods

Study	Assays (Name)		Criteria for Positivity		Test Results (%)		Comments
1320, Nishio et al., 2006	Serum		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC	K5205 (Dako HercepTest)	IHC	weak to moderate or strong complete membrane staining in > 10% of tumor cells (> 1+)	Pos Equiv Neg	42 2 56	
16450, Arai et al., 1997	Serum	SV2-61γ and 6G10 (Eiken Chemical Co. Tokyo, Japan)	Serum	> 3.7 ng/mL	Pos Equiv Neg	30 70	71 treated pts had serum assay data plus 161 additional patients
	FISH		FISH		Pos Equiv Neg		
	IHC		IHC		Pos Equiv Neg		
18080, Fox et al., 1994	Serum		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC	CB11(Novacostra Lab)	IHC	membrane or cytoplasmic staining in > 10% of tumor cells	Pos Equiv Neg	36 64	

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-Z, Prostate Cancer. Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
1320, Nishio et al., 2006		cause-specific survival (CSS) RFS			med48.7 mo rng 6.9-79.4 mo
16450, Arai et al., 1997					
18080, Fox et al., 1994		OS			rng 3-216 mo (3-12 mo intervals)

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-AA, Prostate Cancer. Time to Event Outcomes

Study													
	PFS												
1320, Nishio et al., 2006	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	CSS	HER2+	21	~ 32	100	~ 60	~ 41	~ 39	21.2	log-rank	0.0084		
		HER2-	28	NR	~ 92	~ 80	~ 70	~ 60	63.2				
	RFS	HER2+	21	~ 9	~ 42	~ 32	23.8	~ 15	0	log-rank	0.0485		
		HER2-	28	~ 19	~ 73	~ 45	35.7	~ 28	~ 23				
16450, Arai et al., 1997	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	PFS	HER2+	11	~ 9	~ 30	~ 10	~ 10			log-rank	<0.05		
		HER2-	22	~ 15	~ 60	~ 38	~ 38	~ 38					
18080, Fox et al., 1994	Outcome	Grp	N	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test	p	HR (95%CI)	Comments
	OS	HER2+	16	~ 35	~ 87	~ 80	~ 47	~ 38	~ 38	W-G	0.0316		
		HER2-	29	~ 162	~ 93	~ 88	~ 89	~ 84	~ 84				

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-BB, Prostate Cancer. Time to Event Outcome Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Predictors/ Methods for Selecting Predictors for Multivariate Model	Proportional Hazards Assumption Assessed?/ Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
1320, Nishio et al., 2006	**Predictors/**							
16450, Arai et al., 1997								
18080, Fox et al., 1994								

Selected

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection

Data Abstraction Table V-CC, Prostate Cancer. Tumor Response and Quality of Life

Study	**Tumor Response (%)**									**Quality of Life**					
1320, Nishio et al., 2006	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
16450, Arai et al., 1997	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
18080, Fox et al., 1994	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-DD, Prostate Cancer. Response Regression Modeling

Study	**Design/ Outcome/ Model**	**Candidate Method for Identifying Candidates**	**Univariate Results, Variable (p value)**	**Methods for Selecting Predictors for Multivariate Model**	**Interactions Considered?**	**Multivariate Model Results, Variable (p value)**	**Discrimination/ Validation Methods/ Results**	**Calibration/ Goodness of Fit**
1320, Nishio et al., 2006	retrospective **Predictors/**		**Predictors/**					
16450, Arai et al., 1997	case series							
18080, Fox et al., 1994	retrospective							
		Selected						

Data Abstraction Table V-EE, Prostate Cancer. Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-FF, Prostate Cancer. Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-GG, Prostate Cancer. Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Indepen-dently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknow-ledged
1320, Nishio et al., 2006	+	+	+/-	+ (-)	+ ?	+/-	+	-
16450, Arai et al., 1997	-	-	+/-	+ (-)	+	-	+	+
18080, Fox et al., 1994	+	-	+/-	+ (-)	+	+	+	+

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-HH, Head and Neck Cancer. Design, Enrollment and Treatment

Study	Design	Therapeutic Setting	n, Enrolled (Randomized)	n, Evaluated	n, Withdrawn (Lost to F/U)	Treatment Regimen (Agents)
24520, Nagler et al., 2003 1 center in Israel	RET CS	Malignant salivary tumors	36	36		Surgery, no adjuvant therapy
25490, Khan et al., 2002 1981-1992, 1 US center	RET CS	SCC of oral cavity (57%) or oropharynx (43%)	77	56	14 incomplete f/u; 10 samles unvailable	Primary surgical excision and EBRT with curative intent

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-II, Head and Neck Cancer. Patient Characteristics

Study	Age	% Female	Race (%)	Disease Stage	Performance Status	Comorbidities or Other Prognostic Factors
24520, Nagler et al., 2003	mn 56 md rng 15-79 sd 4 >60 45%	Grp1 45	Jews 97 Arabs 3 Grp1	Ia Ib IIa IIb IIIa IIIb IV Grp1	Scale Grp1	Tumor Grade Grp1
25490, Khan et al., 2002	mn md rng sd 41-56 25% 56-59 25% 59-66 25% 66-79 25%	Grp1 18	B 16 W 84 H A O Grp1	T1-2 34 T3-4 66 N0 18 N1 43 N2-3 39 II 9 III 28 IV 63 Grp1	Scale Grp1	Tumor Grade Grp1

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection

Data Abstraction Table V-JJ, Head and Neck Cancer. HER2 Measurement Methods

Study	Assays (Name)		Criteria for Positivity		Test Results (%)		Comments
24520, Nagler et al., 2003	Serum:		Serum		Pos Equiv Neg		
	FISH		FISH		Pos Equiv Neg		
	IHC:	Dako polyclonal	IHC	> 10% staining, moderate to strong	Pos Equiv Neg	28 72	
25490, Khan et al., 2002	Serum:		Serum		Pos Equiv Neg		
	FISH	PathVysion	FISH (1/2)	HER2:CEP17 > 2	Pos Equiv Neg	4/16 (25%)	quantifiable signal in 16 of 19
	IHC:	CB 11, > 10% staining, moderate (2+), strong (3+), intense (4+)	IHC	≥ 2+	Pos Equiv Neg	12/67 (18%) 4/67 (6%) 53/67 (79%)	discrepant text says 67 or 69 total tested

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-KK, Head and Neck Cancer. Outcome Assessment

Study	Primary Outcomes	Secondary Outcomes	Response Criteria	Independent Response Assessor	F/U Frequency/Duration
24520, Nagler et al., 2003	OS				
25490, Khan et al., 2002	Local recurrence, regional recurrence, distant recurrence, DFS, OS			NR	

?

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-LL, Head and Neck Cancer. Time to Event Outcomes

Study	**Time to Event Outcomes**												
24520, Nagler et al., 2003	Outcome OS	Grp IHC+ IHC-	N 10 26	Med (mos)	1 yr	2 yr	3 yr	4 yr	5 yr	Test LR	p 0.0004	HR (95%CI)	Comments
25490, Khan et al., 2002	Outcome DFS OS OS	Grp IHC+ vs - IHC+ vs - FISH disom/- FISH polysom FISH+	N ? ? 47 5 4	Med (mos) 5.8 3.1 2.2	1 yr	2 yr	3 yr	4 yr	5 yr	Test UV Cox UV Cox LR	p 0.15	RR (95%CI) 0.83 (0.29, 2.4) 1.4 (0.62, 3.3)	Comments LR test for combined di-somic, non-overexpressed + polysomic

30
72

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-MM, Head and Neck Cancer. Time to Event Outcome Regression Modeling

Study	**Design/ Outcome/ Model**	**Candidate Method for Identifying Candidates**	**Univariate Results, Variable (p value)**	**Predictors/ Methods for Selecting Predictors for Multivariate Model**	**Proportional Hazards Assumption Assessed?/ Interactions Considered?**	**Multivariate Model Results, Variable (p value)**	**Discrimination/ Validation Methods/ Results**	**Calibration/ Goodness of Fit**
24520, Nagler et al., 2003	**Predictors/**							
25490, Khan et al., 2002								

43
40
0

Selected

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection

Data Abstraction Table V-NN, Head and Neck Cancer. Tumor Response and Quality of Life

Study	Tumor Response (%)									Quality of Life					
24520, Nagler et al., 2003	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd
25490, Khan et al., 2002	Grp	N	CR	PR	SD	PD	Test	p	Comments	Scale	Domain	F/U	Grp	n	mn±sd

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-OO, Head and Neck Cancer. Response Regression Modeling

Study	Design/ Outcome/ Model	Candidate Method for Identifying Candidates	Univariate Results, Variable (p value)	Methods for Selecting Predictors for Multivariate Model	Interactions Considered?	Multivariate Model Results, Variable (p value)	Discrimination/ Validation Methods/ Results	Calibration/ Goodness of Fit
24520, Nagler et al., 2003	**Predictors/**		**Predictors/**					
25490, Khan et al., 2002								

Selected

Data Abstraction Table V-PP, Head and Neck Cancer. Adverse Events

Toxicity Type	Study	Severity or Grade	Results
Treatment-related mortality			F/U (mo) Grp1 n % Grp2 n %
Nausea			F/U (mo) Grp1 n % Grp2 n %
Vomiting			F/U (mo) Grp1 n % Grp2 n %
Anorexia			F/U (mo) Grp1 n % Grp2 n %
Lethargy			F/U (mo) Grp1 n % Grp2 n %
Neurosensory			F/U (mo) Grp1 n % Grp2 n %
Hearing loss			F/U (mo) Grp1 n % Grp2 n %
Cardiac ischemia			F/U (mo) Grp1 n % Grp2 n %
Diminished LVEF			F/U (mo) Grp1 n % Grp2 n %
Arrhythmias			F/U (mo) Grp1 n % Grp2 n %
Bronchopulmonary			F/U (mo) Grp1 n % Grp2 n %
Dermatologic			F/U (mo) Grp1 n % Grp2 n %
Kidney			F/U (mo) Grp1 n % Grp2 n %
Anemia			F/U (mo) Grp1 n % Grp2 n %
Thrombocytopenia			F/U (mo) Grp1 n % Grp2 n %
Leukopenia or neutropenia			F/U (mo) Grp1 n % Grp2 n %
Infection			F/U (mo) Grp1 n % Grp2 n %
Other			F/U (mo) Grp1 n % Grp2 n %

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-QQ, Head and Neck Cancer. Randomized Trial Study Quality Ratings

Study	Initial Assembly of Comparable Groups	Low Loss to Followup, Maintenance of Comparable Groups	Measurements Reliable, Valid, Equal	Interventions Comparable/ Clearly Defined	Appropriate Analysis of Results	Overall Rating	Funding/ Sponsorship Source Acknowledged

Question 5: Serum HER2 Results to Guide Treatment Regimen Selection
Data Abstraction Table V-RR, Head and Neck Cancer. Case Series/Single Arm Trial Study Quality Ratings

Study	Clearly Defined Question	Well-Described Study Population	Well-Described Intervention	Use of Validated Outcome Measures (Indepen-dently Assessed)	Appropriate Statistical Analysis	Well-Described Results	Discussion/ Conclusions Supported by Data	Funding/ Sponsorship Source Acknow-ledged
24520, Nagler et al., 2003								
25490, Khan et al., 2002								

Appendix D. Technical Expert Panel (TEP) and Reviewers

Technical Expert Panel (TEP)

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Sidney Kimmel Cancer Center
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How to Complete and Submit Your Review

Thank you for taking the time to review the draft of the evidence report, "HER2 Testing to Mange Patients with Breast Cancer or Other Solid Tumors."

We are counting on you to:

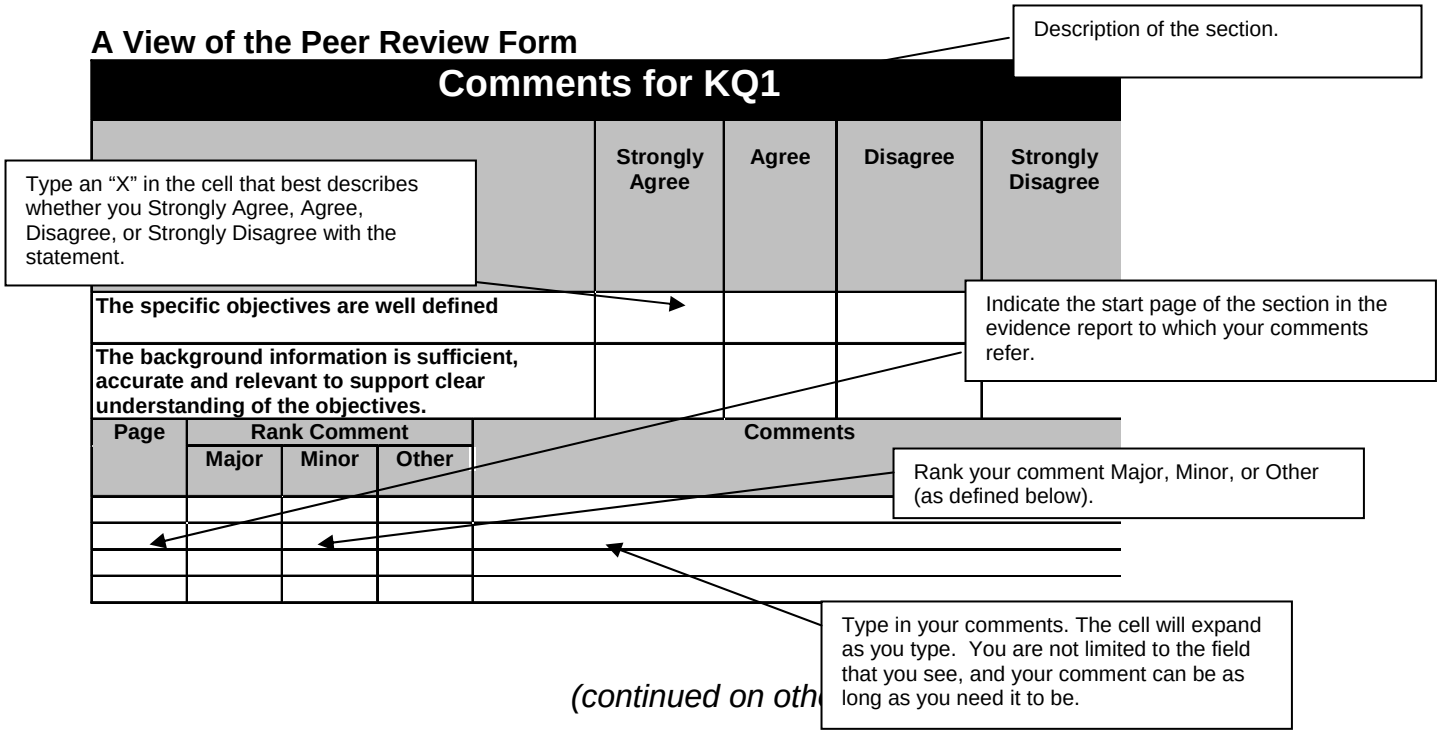
- Review the enclosed draft;
- Make your comments in the enclosed electronic peer-review response form (on CD-ROM);
- Return your comments electronically AND return the confidential draft as soon as possible but no later than Friday, April 11, 2008. (**Please contact us if your schedule does not permit meeting this deadline.**)

FOR ASSISTANCE WITH ANY PART OF THIS PROCESS, PLEASE contact Elizabeth De La Garza 312.297.5623, or elizabeth.delagarza@bcbsa.com.

Please, before you start:

1. **Review these instructions.**
2. **Open the Word® table document** on the enclosed CD-ROM and save a version on your system so that you may enter and save comments (the document on the CD is "read only"). This is the form where you will eventually enter your comments and viewing it may help you organize your comments while you review the report.
3. **Call us immediately if electronic use of the form is a problem—**we can work with many problems you might encounter.
4. Thank you and good luck formulating your review!

A View of the Peer Review Form



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Enter your comments into the form

1. **Open the file "Peer Review Form – HER2" on the enclosed CD-ROM**. This file is a Microsoft® Word® document that contains 11 separate tables, one for each subsection of the draft and one for general or miscellaneous comments. PLEASE SAVE A COPY OF THE FILE ON YOUR SYSTEM TO WORK IN (the file on CD-ROM is "read only")
2. Enter an "X" in the cell that best describes whether you Strongly Agree, Agree, Disagree, or Strongly Disagree with each statement about the section.
3. For your comments, if any, enter the *start*-page number that your comment(s) will address. (Column A)
4. Rank each comment by entering an X under Major, Minor or Other. (Definitions of Major, Minor, and Other are at the bottom of each table)
5. Make your comments. The Comment cell will expand as you write. Your comment may be as long as you like. To add another comment: cite another start-page-number, rank the comment, and write. Make as many comments per section as you choose. If you run out of rows for a particular table(s), you can add additional rows to complete your commentary.
6. **Save your work often!** When you stop working, save and close the document. (In the File menu, left-click Save, and again in the File menu, left-click Close.)

Develop your comments

The most helpful comments will be specific, will explain how an issue affects the report, and will recommend improvements to the report. Please rank each of your comments as **Major, Minor, or Other**:

- **Major comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
- **Minor comment:** A criticism that identifies an important but not critical aspect of presentation, interpretation or analysis.
- **Other comment:** Positive feedback, or any comments not of a substantive nature. Please do NOT comment on grammatical or formatting errors—these will be caught in final edits.

Send your review back to BCBSA

If at all possible, please e-mail your completed Word® file to elizabeth.delagarza@bcbsa.com. Or, you may save your comments to diskette or CD-ROM and return it in the mailer provided. You have agreed to return the confidential draft at the same time. Use the return packaging and the return address slip provided. The cost for shipping these items will be paid by BCBSA.

FOR HELP CONTACT Elizabeth De La Garza, 312.297.5623 or elizabeth.delagarza@bcbsa.com.

THANK YOU FOR YOUR PARTICIPATION.

Comments for Structured Abstract/Executive Summary

	Strongly Agree	Agree	Disagree	Strongly Disagree
The structured abstract and executive summary clearly and adequately summarize the key points and findings of this report.				

Page	Rank Comment			Comments
	Major*	Minor*	Other *	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
* **Minor Comment:** A criticism that identifies an important but not critical aspect of presentation, interpretation or the analysis of the report.
* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

	Strongly Agree	Agree	Disagree	Strongly Disagree
The specific objectives are well defined.				
The background information is sufficient, accurate and relevant to support clear understanding of the objectives.				

Page	Rank Comment			Comments
	Major*	Minor*	Other *	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

	Strongly Agree	Agree	Disagree	Strongly Disagree
The methods and procedures used to develop the analysis have been clearly developed.				
The methods are appropriate.				

Page	Rank Comment			Comments
	Major*	Minor*	Other *	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 1				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 2				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 3a				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 3b				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
* **Minor Comment:** A criticism that identifies an important but not critical aspect of presentation, interpretation or the analysis of the report.
* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 4				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

Key Question 5				
	Strongly Agree	**Agree**	**Disagree**	**Strongly Disagree**
The background provides accurate and sufficient information for this portion of the evidence review.				
The key questions address the appropriate concerns.				
The overview accurately represents the evidence.				
The text provides satisfactory closure to the presented evidence.				

Page	**Rank Comment**			**Comments**
	Major*	**Minor***	**Other ***	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
* **Minor Comment:** A criticism that identifies an important but not critical aspect of presentation, interpretation or the analysis of the report.
* **Other:** Positive feedback, or any comments not of a substantive nature. Please do not comment on grammatical or formatting errors—these will be caught in final edits.

	Strongly Agree	Agree	Disagree	Strongly Disagree
The interpretation of results is accurate and appropriate.				
The description of the limitations of the evidence is balanced and appropriate.				

Page	Rank Comment			Comments
	Major*	Minor*	Other *	

* **Major Comment:** A criticism on the substance of the report that identifies a critical error in reasoning or presentation or makes a very important suggestion that will result in a change in the interpretation or analysis of the report.
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