



Evidence-based Series #1-17: Section 1

The Role of HER2/*neu* in Systemic and Radiation Therapy for Women with Breast Cancer: A Clinical Practice Guideline

B. Dhesy-Thind, K.I. Pritchard, H. Messersmith, F. O'Malley, L. Elavathil, M. Trudeau, and the Breast Cancer Disease Site Group

A Quality Initiative of the
Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)
Developed by the Breast Cancer Disease Site Group

Report Date: November 10, 2006

Questions

In the absence of trastuzumab systemic therapy:

Endocrine therapy

1. Does the efficacy of tamoxifen (compared with no tamoxifen) depend on HER2/*neu* status?
2. Do the relative efficacies of different tamoxifen durations depend on HER2/*neu* status?
3. Do the relative efficacies of aromatase inhibitors (compared with tamoxifen) depend on HER2/*neu* status?
4. Does the efficacy of ovarian ablation (compared with no ovarian ablation) depend on HER2/*neu* status?

Chemotherapy

5. Does the efficacy of anthracycline-based regimens (compared with non-anthracycline-based regimens) depend on HER2/*neu* status?
6. Do the relative efficacies of different anthracycline-based regimens depend on HER2/*neu* status?
7. Does the efficacy of taxane-containing regimens (compared with non-taxane-containing regimens) depend on HER2/*neu* status?
8. Do the relative efficacies of different taxane-containing regimens depend on HER2/*neu* status?

Chemoendocrine Therapy

9. Does the effect of tamoxifen and chemotherapy (compared with tamoxifen alone) depend on HER2/*neu* status?

Radiation Therapy

10. Does the efficacy of radiation therapy (compared with no radiation therapy) depend on HER2/*neu* status?

Target Population

Women with breast cancer.

Recommendations and Key Evidence

Endocrine Therapy - Tamoxifen

Although the current evidence does not support a definitive recommendation regarding tamoxifen therapy and HER2/*neu* status, the weight of the evidence, especially the Gruppo Universitario Napoletano (GUN) trial (1,2), suggests that the efficacy of tamoxifen may be greater in HER2/*neu*-negative patients than in HER2/*neu*-positive patients. However, the evidence does not support a recommendation against tamoxifen therapy in HER2/*neu*-positive patients. While it is possible that tamoxifen is more effective in HER2/*neu*-negative patients, there is still sufficient evidence that it is effective in HER2/*neu*-positive patients as well.

Endocrine Therapy - Aromatase Inhibitors

The current evidence does not support a definitive recommendation regarding aromatase inhibitor therapy and HER2/*neu* status.

Endocrine Therapy - Ovarian Ablation

The current evidence does not support a definitive recommendation regarding ovarian ablation and HER2/*neu* status.

Chemotherapy - Anthracyclines

Patients with HER2/*neu*-positive breast cancer should be considered for chemotherapy containing an anthracycline instead of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or melphalan and 5-fluorouracil (PF) chemotherapy.

- Ten studies (3-18) of CMF or PF versus an anthracycline-containing chemotherapy were identified that also performed a substudy analysis by HER2/*neu* status. Two of these studies (4,10,14,15) reported a significant interaction between HER2/*neu* status and treatment. A meta-analysis of these studies by HER2/*neu* status found a significant benefit in terms of both overall survival (OS) (hazard ratio [HR] 0.74, 95% confidence interval [CI] 0.63 to 0.87) and disease-free survival (DFS) (HR 0.73, 95% CI 0.63 to 0.85) for the use of anthracycline-based chemotherapy compared to CMF or PF in patients with HER2/*neu*-positive breast cancer, but found no evidence of a benefit in HER2/*neu*-negative patients (HR 1.04 for overall survival, 1.00 for disease-free survival). The interaction between treatment and HER2/*neu* status was found to be significant in the meta-analysis (difference in log OS HRs -0.32 [95% CI -0.51 to -0.12], difference in log DFS HRs -0.29 [95% CI -0.47 to -0.10]).

Qualifying Statements

- Patients with HER2/*neu*-positive breast cancer may derive more benefit from a more intense anthracycline regimen, in terms of dose (i.e., 100 mg/m² epirubicin versus 50 or 60 mg/m² epirubicin) or schedule (i.e., 60 mg/m² epirubicin every 14 days compared to every 21 days), over a less intense one. Four of the identified studies (5,11,19-23) comparing more intense anthracycline-based regimens to less intense ones were identified that also performed a substudy analysis of HER2/*neu* status. Three of these studies (19,21-23) found a significant overall survival benefit for more intense anthracycline regimens versus less intense. A

meta-analysis of these studies by HER2/*neu* status found a significant benefit in terms of DFS (HR 0.53, 95% CI 0.37 to 0.77) for patients with HER2/*neu*-positive breast cancer receiving more intense anthracycline-based chemotherapy. This meta-analysis found no benefit in HER2/*neu*-negative patients (HR 1.09). However, this analysis was found to be sensitive as to which of three different possible sets of hazard ratios were selected in one study (5,11). In that study, the analysis of time-to-progression was conducted using three different methods of HER2/*neu* testing, and the significance of the meta-analysis of the differences in log hazard ratio between the HER2/*neu* subgroups was significant or not significant depending on the choice of testing. Therefore, a firm recommendation was not possible, as absence of interaction could not be definitively rejected.

- The Breast Cancer DSG has produced two separate guidelines on trastuzumab systemic therapy, PG #1-15 (metastatic) and EBS #1-24 (adjuvant), described under “Related Guidelines” below. These guidelines provide important information regarding the use of trastuzumab and anthracyclines sequentially or in combination with regards to concerns about cardiac toxicity. Physicians are encouraged to review the recommendation and qualifying statements in light of the information provided in those guidelines if combination or sequential trastuzumab/anthracycline therapy is being considered. Physicians are discouraged from using trastuzumab concurrently with anthracyclines.

Chemotherapy - Taxanes

The current evidence does not support a definitive recommendation regarding taxane chemotherapy and HER2/*neu* status.

Chemoendocrine Therapy

The current evidence does not support a definitive recommendation regarding chemoendocrine therapy and HER2/*neu* status.

Radiation Therapy

The current evidence does not support a definitive recommendation regarding radiation therapy and HER2/*neu* status.

Related Guidelines

- PG #1-15: *The Role of Trastuzumab (Herceptin®) in the Treatment of Women with HER2/*neu*-overexpressing Metastatic Breast Cancer.*
- EBS #1-24: *The Role of Trastuzumab in Adjuvant and Neoadjuvant Therapy in Women with HER2/*neu*-overexpressing Breast Cancer.*

EVIDENCE-BASED SERIES #1-17

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Evidence-based Series #1-17: Section 2

The Role of HER2/*neu* in Systemic and Radiation Therapy for Women with Breast Cancer: A Systematic Review

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In the absence of trastuzumab systemic therapy:

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1. Does the efficacy of tamoxifen (compared with no tamoxifen) depend on HER2/*neu* status?
2. Do the relative efficacies of different tamoxifen durations depend on HER2/*neu* status?
3. Do the efficacies of aromatase inhibitors (compared with tamoxifen) depend on HER2/*neu* status?
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Chemotherapy

5. Does the efficacy of anthracycline-based regimens (compared with non-anthracycline-based regimens) depend on HER2/*neu* status?
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Chemoendocrine Therapy

9. Does the effect of tamoxifen and chemotherapy (compared with tamoxifen alone) depend on HER2/*neu* status?

Radiation Therapy

10. Does the efficacy of radiation therapy (compared with no radiation therapy) depend on HER2/*neu* status?

INTRODUCTION

During the year 2006 in Canada, an estimated 22,200 women will have been diagnosed with breast cancer, and 5,300 women will have died from the disease (1) Many of these women were probably treated with adjuvant systemic therapies to reduce the risk of recurrence, therapies often associated with significant short- and long-term toxicities. The use of a predictive tumour marker to select treatment is, therefore, attractive as it might spare some patients from receiving ineffective or excessively toxic therapy. The marker HER2/*neu* may play such a role in predicting the response to specific treatments for women with breast cancer.

The HER2/*neu* gene encodes for a 185-kd transmembrane glycoprotein, a member of a family of growth-factor receptors with intrinsic tyrosine kinase activity. The amplification of the HER2/*neu* gene or the overexpression of its protein is observed in 20% to 30% of human breast cancers and is associated with a poor prognosis in patients with primary breast cancer. Amplification and/or overexpression of HER2/*neu* in breast cancer is associated with a number of adverse prognostic factors, including advanced stage, axillary lymph node involvement, absence of estrogen and progesterone receptors, increased S-phase fraction, and high nuclear grade (2,3). Many studies suggest that HER2/*neu* amplification and/or overexpression may be associated with relative sensitivity or resistance to endocrine therapy or chemotherapy. This systematic review was developed to review the evidence for the use of HER2/*neu* amplification and/or overexpression as a predictive marker to guide the selection of systemic and radiation therapy for patients with breast cancer.

The purpose of this evidence-based series is to summarize, review, and provide recommendations regarding the effect of HER2/*neu* status on the efficacy of different systemic therapies in the absence of trastuzumab. The Breast Cancer DSG has produced two separate guidelines on trastuzumab systemic therapy, Practice Guideline #1-15 (metastatic) *The Role of Trastuzumab (Herceptin®) in the Treatment of Women with HER2/neu-overexpressing Metastatic Breast Cancer* and Evidence-Based Series #1-24 (adjuvant) *The Role of Trastuzumab in Adjuvant and Neoadjuvant Therapy in Women with HER2/neu-overexpressing Breast Cancer*. Physicians who are treating HER2/*neu*-positive breast cancer patients should also review those two documents, in addition to this document.

METHODS

This systematic review was developed by Cancer Care Ontario's Program in Evidence-Based Care (PEBC). Evidence was selected and reviewed by four members of the PEBC Breast Cancer Disease Site Group (DSG) and two methodologists.

This systematic review is a convenient and up-to-date source of the best available evidence on the role of HER2/*neu* expression in systemic and radiation therapy for women with breast cancer. The body of evidence in this review is primarily comprised of mature randomized controlled trial data. That evidence forms the basis of a clinical practice guideline developed by the Breast Cancer DSG and published as Section 1 of this Evidence Based Series. The systematic review and companion practice guideline are intended to promote evidence-based practice in Ontario, Canada. The PEBC is editorially independent of Cancer Care Ontario and the Ontario Ministry of Health and Long-Term Care.

Literature Search Strategy

MEDLINE was searched to December 2005 using a disease-specific medical subject heading (MeSH) term ("breast neoplasms"), marker-specific MeSH terms ("receptor, erbB-2" OR "genes, erbB-2" OR "oncogene proteins v-erbB"). The Excerpta Medica database (EMBASE) was similarly searched up to September 2005 using a disease-specific Excerpta Medica Tree (EMTREE) term ("breast cancer") and a marker-specific EMTREE term ("oncogene c erb"). The same, and design-specific EMTREE terms ("clinical study" OR "clinical trial").

Articles containing the trastuzumab Emtree term (“trastuzumab”) were excluded. Search terms for the following publication types and study designs were also included in each strategy: practice guidelines, systematic reviews, meta-analyses, reviews, randomized controlled trials, and controlled clinical trials. Due to the large volume of studies on trastuzumab, articles containing this term in the title or abstract were excluded from both strategies.

Issue 1 (2005) of the Cochrane Library and online conference proceedings from the American Society of Clinical Oncology (ASCO) (<http://www.asco.org/ac/1,1003,12-002095,00.asp>; 1999-2005) and the San Antonio Breast Cancer Symposium (SABCS) (<http://www.sabcs.org/SymposiumOnline/index.asp#abstracts>; 2001-2004) were also searched. The Canadian Medical Association Infobase (<http://mdm.ca/cpgsnew/cpgs/index.asp>) and the National Guidelines Clearinghouse (<http://www.guideline.gov/>) were searched for existing evidence-based practice guidelines. Relevant articles and abstracts were selected and reviewed by one reviewer, and the reference lists from these sources were searched for additional trials, as were the reference lists from relevant review articles.

Inclusion Criteria

Articles on clinical trials were selected for inclusion in this systematic review of the evidence, if they met the following criteria:

- The effects of systemic and/or radiation therapy was analyzed according to HER2/*neu* status in a phase III randomized controlled trial.
- Reported outcomes included disease-free survival, progression-free survival, time-to-progression, objective response rate, or overall survival.
- Clinical trial results were reported in full papers or abstracts. Although data presented in meeting abstracts may not be as reliable and complete as that from papers published in peer-reviewed journals, abstracts can be a source of important evidence from randomized trials and add to the evidence available from fully published studies. These data often appear first in meeting abstracts and may not be published for several years.

In addition, clinical practice guidelines were included if they addressed relevant topics.

Exclusion Criterion

Trials published in a language other than English were excluded due to the lack of translation resources.

Synthesizing the Evidence

Where possible, meta-analyses were conducted to create summary estimates of the treatment effects by HER2/*neu* status for disease-free and overall survival. The methods described by Parmar et al (4) were used to derive the log-hazard ratio and its standard error. When these values were derived from survival curves, two independent analysts conducted the derivation from the curve, and their results were averaged. Analysis was conducted using the Review Manager software, version 4.2.7 (5). Because the analyzed trials deal with different treatment regimens and patient groups, the assumption, necessary for fixed effects modelling, of a common treatment effect to be measured was not supportable. Therefore, a random effects model was used for all summary estimates. With time-to-event outcomes, analysis was conducted using the generic inverse variance method with random effects. In one case (aromatase inhibitors versus tamoxifen in the neoadjuvant setting), overall response rate was combined via meta-analysis, also using a random-effects model.

In order to formally test the interaction of treatment and HER2/*neu* status for time-to-event outcomes where meta-analysis was performed, the difference between the HER2/*neu*-positive and HER2/*neu*-negative log hazard ratios was taken for each study and analyzed using the generic inverse variance method with random effects. The standard error of this difference was calculated using the following formula:

$$SE_{diff} = \sqrt{SE_{HER2+}^2 + SE_{HER2-}^2}$$

where SE_{diff} is the standard error of the difference in log-hazard ratios, SE_{HER2+} is the standard error of the log-hazard ratio in the HER2/*neu*-positive subgroup, and SE_{HER2-} is the standard error of the log-hazard ratio in the HER2/*neu* negative subgroup. If the estimate of the difference was found to be significantly different from zero, this was interpreted as evidence of an interaction between treatment and HER2/*neu* status.

RESULTS

Thirty-one trials (Table 1) (6-53) and one practice guideline (54,55) were eligible for inclusion in this systematic review of the evidence. Of note, several trials reported relevant findings in more than one article. Tables 2 through 10 summarize the trial eligibility criteria and outcomes, and Table 11 summarizes the therapy regimens evaluated by the included trials.

Critical Appraisal of Selected Studies

The selected studies consist primarily of the reanalysis of the results of completed phase III clinical trials that used stored tissue samples for the determination of HER2/*neu* status. In most cases, the tissue samples were either not available or inappropriate for HER2/*neu* testing. While most of the studies evaluated the differences between the patients tested for HER2/*neu* status and those that were not, the analysis could well be biased if the availability of tissue samples is correlated in some way with treatment or outcome.

The selected studies used a range of HER2/*neu* status assessment methods (based on serum protein, protein overexpression, and gene amplification), as described in Table 2. There is also evidence of variability between observers (56,57) and laboratories (58,59) in the implementation and scoring of immunohistochemistry for HER2/*neu* overexpression, even when the stated method is identical. This variation in assessment of status likely reduces the comparability of the studies on a particular question.

The statistical power necessary to detect an interaction between two variables is greater than that necessary to detect the effect of each variable individually. In addition, as none of the included trials were designed with this interaction as a primary or secondary outcome of interest, their sample sizes were not calculated with these interaction tests in mind. Therefore, in trials where no significant interaction was detected, the magnitude of the outcomes by HER2/*neu* status and treatment should be considered when determining whether there is no clinically meaningful significance or whether the trial was underpowered for this purpose.

Table 1. Studies included in this practice guideline report.

Questions	Table	Number of studies	Reference numbers
Endocrine therapy			
Does the efficacy of tamoxifen (compared with no tamoxifen) depend on HER2/ <i>neu</i> status?	2	4	(6-11)
Do the relative efficacies of different tamoxifen durations depend on HER2/ <i>neu</i> status?	3	1	(12,13)
Do the efficacies of aromatase inhibitors (compared with tamoxifen) depend on HER2/ <i>neu</i> status?	4	3	(14-17)
4. Does the efficacy of ovarian ablation (compared with no ovarian ablation) depend on HER2/ <i>neu</i> status?	5	2	(8,10,18,19)
Chemotherapy			
5. Does the efficacy of anthracycline-based regimens (compared with non-anthracycline-based regimens) depend on HER2/ <i>neu</i> status?	6	10 ^A	(20-32,51-53)
6. Do the relative efficacies of different anthracycline-based regimens depend on HER2/ <i>neu</i> status?	7	5	(22,28,33-39)
7. Does the efficacy of taxane-containing regimens (compared with non-taxane-containing regimens) depend on HER2/ <i>neu</i> status?	8	6	(40-47,50)
8. Do the relative efficacies of different taxane-containing regimens depend on HER2/ <i>neu</i> status?	9	1	(48)
Chemoendocrine therapy			
9. Does the effect of tamoxifen and chemotherapy (compared with tamoxifen alone) depend on HER2/ <i>neu</i> status?	10	1	(49)
Radiation Therapy			
10. Does the efficacy of radiation therapy (compared with no radiation therapy) depend on HER2/ <i>neu</i> status?		0	

^A One abstract (60) was identified that could not be included due to the incomprehensible nature of the report.

Table 2. Trials comparing the efficacy of tamoxifen versus no tamoxifen by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	OS	DFS	Median f/u (years)	OS subgroup analysis			DFS subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
DBCCG 77c (6)	1515 ^A /1716	Tam (1yr) Observation	Postmen., high-risk	NR	HR=NR^B	NR	NR	NR	NR	HR=0.89	HR=0.86	p=NS
GUN (7,9)	358 ^D /433	Tam (2yrs) Observation	Stage I-III	HR=0.68	NR	15 ^E	HR=1.09	HR=0.59	p=0.04	SIR=1.22 SIR=0.86	SIR=0.80 SIR=1.21	p=0.03
Swedish (11)	428/564	Tam (2yrs) Observation	Premen. Stage II	NR	HR=0.77^F	14	NR	NR	NR	HR=0.38 ^G	HR=0.69 ^G	p=NS

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level. All RR's and HR's are presented with ratios <1 favouring the first arm. In some cases, marked with an *, the inverse of the reported ratio is presented here for comparability.

^A Only 1057 steroid receptor-positive patients were included in the HER2/neu DFS subgroup analysis.

^B Overall DFS benefit with tamoxifen reported (p=0.02) but data not shown.

^C 10-year DFS.

^D Only 245 patients were available for the DFS analysis.

^E DFS analysis performed after median f/u of ~14 years.

^F Recurrence-free survival.

^G ER+ patients only.

Abbreviations: CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; DBCCG, Danish Breast Cancer Cooperative Group; DFS, disease-free survival; ER, estrogen receptor; f/u, follow-up; Gos, gosorelin; GUN, Gruppo Universitario Napoletano; HR, hazard ratio; Int, interaction; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; NR, not reported; OS, overall survival; PgR, progesterone receptor; Postmen., postmenopausal; Premen., premenopausal; SIR, standardized incidence ratio; Tam, tamoxifen.

Table 3. Trials comparing the relative efficacy of different tamoxifen regimens by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	OS	DFS	Median f/u (years)	OS subgroup analysis			DFS subgroup analysis		
							HER2+	HER2 -	Int effect	HER2+	HER2-	Int effect
SBCCG (12,13)	577/3545	Tam (5yrs) Tam (2yrs)	Postmen.	HR=0.82	HR=0.82	11	NR	NR	NR	RRR=1.1	RRR=0.62	p=0.25

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level.

Abbreviations: DFS, disease-free survival; f/u, follow-up; HR, hazard ratio; Int, interaction; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; NR, not reported; OS, overall survival; Postmen., postmenopausal; RRR, recurrence rate ratio; SBCCG, Swedish Breast Cancer Cooperative Group; Tam, tamoxifen.

Table 4. Trials comparing the efficacy of aromatase inhibitors versus tamoxifen by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	Median f/u	TTP (months)	ORR	TTP (months) subgroup analysis			ORR subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
DUMC (15,17)	278/337	Letrozole Tamoxifen	Neoadjuvant setting. Postmen., ER+ and/or PgR+	NR	NA	60% 41%	NA	NA	NA	69% 17%^A	53% 40%^A	NR
IMPACT (16)	239/330	Anastrozole Tamoxifen An.+Tam.	Neoadjuvant setting, Postmen.,ER+	NR	NA	37% 36% 39%	NA	NA	NA	58% 22% 31%	NR	NR
PSUHMC (14)	562/907	Letrozole Tamoxifen	Recurrent or metastatic, ER+ and/or PgR+	NR	HR=0.73	32% 21%	HR=0.73	HR=0.70	NR	17% 13%	39% 26%	NR

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level.

^A HER2/neu+ vs. HER2/neu- difference significant for tamoxifen arm.

Abbreviations: An., Anastrozole; DUMC, Duke University Medical Center; ER+, estrogen-receptor positive; f/u, follow-up; IMPACT, Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen; Int, interaction; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; NA, not applicable; NR, not reported; ORR, objective response rate; PgR+, Postmen., postmenopausal; progesterone-receptor positive; PSUHMC, Pennsylvania State University Hershey Medical Center; Tam., tamoxifen; TTP, time-to-progression; vs., versus.

Table 5. Trials comparing the efficacy of ovarian ablation versus no ovarian ablation by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	OS	DFS	Median f/u (years)	OS subgroup analysis			DFS subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
ABCSG 5 (8,10)	572/1034	Tam + Gos CMF	Premen., ER or PgR pos.	RR=0.79	RR=0.71	5	NR	NR	NS	NR	NR	NS
Vietnamese (18,19)	282/709	Ooph+Tam Obs+Therapy ^A	Premenopausal, ER+, operable BC	RR=0.67 ^B	RR=0.58^B	3.7	RR=0.26	RR=0.68	P=0.07 ^C	RR=0.37	RR=0.48	p=0.18 ^C

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level.

^A Patients in this arm were randomized to observation, and if relapse occurred, received the same treatment as those in the first arm.

^B From original report (18) including all patients. Adjusted for number of positive lymph nodes, pathologic tumour size, estrogen-receptor positivity, and histologic grade.

^C p-value is adjusted for number of axillary lymph nodes, pathologic tumour size, histologic grade, and HER2/neu positivity.

Abbreviations: ABCSG, Austrian Breast and Colorectal Cancer Study Group; BC, breast cancer; DFS, disease-free survival; ER+, estrogen-receptor positive; f/u, follow-up; Int, interaction; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; NR, not reported; Obs, observation; Ooph, oophorectomy; OS, overall survival; Premen., premenopausal; RR, rate ratio; Tam, tamoxifen.

Table 6. Trials comparing the efficacy of anthracycline-containing regimens versus non-anthracycline containing regimens by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	OS	DFS	Median f/u (years)	OS subgroup analysis			DFS subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
Belgian ^A (22,28)	354/777	HEC CMF	N+	HR=0.93*	HR=0.93* ^B	6.2	NR	NR	NR	HR=0.70* ^B	HR=1.19* ^B	p=0.57
	354/777	EC CMF	N+	HR=1.22*	HR=1.19* ^B	6.2	NR	NR	NR	HR=0.61* ^B	HR=1.52* ^B	p=0.18
Czech ^C (24)	62/106	AC CMF	pT1c-3a, pN0-1	ND	ND	3.8	NR	NR	NS	NR	NR	NS
DBCCG 89d (51)	805/1195	CEF CMF	See Note ^D	HR=0.776	HR=0.781	8.06/10.40 ^E 8.09/10.28 ^E	HR=0.725	HR=0.818	p=0.63	HR=0.747	HR=0.789	p=0.81
GOIRC (52,53)	266/348	E CMF	N- if ER-, or N+ with ≤ 9 positive nodes	HR=1.14	HR=1.11	8	75.8% ^F 67.6% ^F	84.5% ^F 87.4% ^F	p=0.311	65.9% ^F 70.3% ^F	60.1% ^F 68.6% ^F	p=0.6628
GUN-3 (31,32)	123/220	CMF→EV CMF	Stage II N+ or Stage III	HR=0.87	RR=0.93	10	HR=0.85	HR=1.64	p=0.05	NR	NR	NR
Milan (25)	506	CMF→A CMF	1-3 nodes positive	HR=1.22 ^G	HR=1.26 ^G	15	HR=0.61	HR=1.26	p=0.052	HR=0.83	HR=1.22	p=0.251
NCIC CTG MA.5 ^C (29,30)	602/710	CEF CMF	Premen., N+	77%^H 70%^H	63%^H 53%^H	4.9 ^J	HR=0.78* ^K	HR=1.11* ^K	p=0.22 ^K	HR=0.65* ^K	HR=0.93* ^K	p=0.18 ^K
NSABP B-11 (21,27)	638/707	PAF PF	N+, ER- and/or PgR-	65% ^L 59% ^L	51%^L 44%^L	13.5 ^M	RR=0.66^N	RR=0.90 ^N	p=0.15	RR=0.60^N	RR=0.96 ^N	p=0.02
NSABP B-15 (20,26)	1355/2194	AC ^P CMF	N+, "TAM nonresponsive"	83% 82%	62% 63%	12.4 ^M	RR=0.82 ^N	RR=1.07 ^N	p=0.11	RR=0.84 ^N	RR=1.02 ^N	p=0.19
Spanish ^C (23)	141/989	FAC CMF	Stage I-III	NR	NR	7	72% ^Q 42%^{Q,R}	82% ^Q 84%^{Q,R}	NR	NR	NR	NR

Items in bold are statistically significant comparisons at the α=0.05 level. All RR's and HR's are presented with ratios <1 favouring the first arm. In some cases, marked with an *, the inverse of the reported ratio is presented here for comparability.

^A These studies were conducted on the same population, one using IHC to determine HER2/neu status, the other using FISH. Only the FISH results are reported here.

^B Event-free survival

^C HER2/neu analysis in abstract form.

^D (Premen., N-, grade 2 or 3 tumours ≤ 5 cm) or (premen., recept- or unknown, with >5 cm or N+) or (postmen., recept-, >5 cm or N+)

^J In original report (30). Median follow-up not reported in abstract of HER2/neu analysis.

^K Both IHC and FISH were used to determine HER2/neu status. Only the FISH results are reported here. In report, HR's were expressed as CMF/CEF hazards, the inverse is presented here for consistency with other studies.

^L Five-year survival or disease-free survival

^M "Average time on study"

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^E Value before the slash is the median follow-up with respect to RFS. The value after the slash is the median follow-up with respect to OS.

^F 8 year survival or relapse-free survival.

^G Hazard ratio adjusted for HER2/*neu* status, age, tumour stage, estrogen receptor status, and p53, and stratified by progesterone receptor status.

^H Five-year overall survival or relapse-free survival. Includes all subjects from original report (30).

^N Adjusted for age, clinical tumour size, pathologic lymph node status and ER expression.

^P Study had three arms: AC x4, CMF x 6, or AC→CMF. Only the AC and CMF arms are compared by HER2/*neu* status.

^Q Five-year overall survival.

^R HER2/*neu*⁺ vs. HER2/*neu*⁻ difference significant for CMF arm.

Abbreviations: →, followed by; A, doxorubicin; C, cyclophosphamide; CTG, Cancer Trials Group; DBCCG, Danish Breast Cancer Cooperative Group; DFS, disease-free survival; E, epirubicin; ER-, estrogen receptor negative; f/u, follow-up; F, 5-fluorouracil; FISH, fluorescence in-situ hybridization; GOIRC, Gruppo Oncologico Italiano di Ricerca Clinica; GUN, Gruppo Universitario Napoletano; H, higher dose; HR, hazard ratio; IHC, immunohistochemistry; Int, interaction; n_{HER} , number of patients with HER2/*neu* status known included in analysis; n_{total} , total number of patients in study; NCIC, National Cancer Institute of Canada; ND, no difference; NR, not reported; NS, not significant; NSABP, National Surgical Adjuvant Breast and Bowel Project; OS, overall survival; P, melphalan (L-PAM); PgR-, progesterone receptor negative; Premen., premenopausal; RFS, recurrence-free survival; RR, relative risk; TAM, tamoxifen; V, vincristine; vs., versus.

Table 7. Trials comparing the relative efficacy of different anthracycline containing regimens by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	Median f/u (years)	OS	DFS	OS subgroup analysis			DFS subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
More versus less intense anthracycline therapy												
CALGB 8541/8869 (35)	992/1549	HCAF CAF LCAF	Stage II	9.3	Significantly improved with HCAF or CAF		87%^A 66%^A 63%^A	77%^A 82%^A 78%^A	p<0.001	71%^A 52%^A 50%^A	65%^A 66%^A 60%^A	p=0.001
FASG 05 (36,37)	332/565	FEC100 FEC50	Node-positive	10.1	10-year RRR=29%	10-year RRR=24%	NR	NR	NR	55.4% 37.1%	38.9% 35.8%	NR
GONO-MIG1(33) ^B	731/1214	FEC14 FEC21	High risk node-negative or node-positive	6.7	HR=0.65^C	NR	HR=0.59	HR=0.79	p=0.38	HR=0.54 ^D	HR=0.91 ^D	p=0.12
Belgian (22,28) ^E	354/777	HEC EC	Node-positive	6.2	HR=0.76*	HR=0.78* ^D	NR	NR	NR	HR=1.08* ^D	HR=0.75* ^D	p=0.53
Anthracycline versus anthracycline plus high dose												
Dutch (34,38,39)	801/885	FEC+HD ^F FEC	Operated breast cancer w/ >4 positive nodes	7.25	HR=0.85	HR=0.84 ^G	NR	HR=0.72	NR	HR=1.26 ^G	HR=0.68^G	P=0.006^G

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level. All RR's and HR's are presented with ratios <1 favouring the first arm. In some cases, marked with an *, the inverse of the reported ratio is presented here for comparability.

^A Five-year survival or disease-free survival.

^B Abstract and presentation only.

^C Adjusted for node status, tumour grade, progesterone receptor status, and HER2/neu status.

^D Event-free survival.

^E The study was conducted using two different methods of HER2/neu status determination; IHC and FISH. Only the FISH results are reported here.

^F Regimen was five cycles of FEC vs. four cycles of FEC followed by one cycle of high-dose cyclophosphamide, thiotepa and carboplatin. Hormone-receptor positive patients received tamoxifen in both arms.

^G Relapse-free survival.

Abbreviations: A, doxorubicin; C, cyclophosphamide; CALGB, Cancer and Leukemia Group B; DFS, disease-free survival; E, epirubicin; f/u, follow-up; F, 5-fluorouracil; FASG, French Adjuvant Study Group; FISH, fluorescence in-situ hybridization; GONO-MIG, Gruppo Oncologico Nord Ovest Mammella Intergruppo; H, higher-dose; HD, high dose regimen of cyclophosphamide, thiotepa, and carboplatin; HR, hazard ratio; IHC, immunohistochemistry; Int, interaction; L, low-dose; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; ND, no difference; NR, not reported; OS, overall survival; RRR, relative risk reduction; vs versus.

Table 8. Trials comparing the efficacy of taxane-containing regimens compared with non-taxane containing regimens by HER2/neu status.

Trial	n _{HER} /n _{total}	Arms	Population	Median f/u (years)	OS	ORR	PFS/OS subgroup analysis			ORR subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
EORTC 10923 (41,43)	114/331	A T	Progressive metastatic cancer with no prior chemotherapy	NR	18.3 m 15.6 m	40% 24%	NR	NR	NR	39% ^A 24% ^A	41% ^A 24% ^A	NR
TAX303 (46)	176/326	A D	Metastatic breast cancer, previously treated with adj. or metastatic CMF	1.9	14 m 15 m	33.3% 47.8%	0.88/1.47 ^B	0.77/0.64 ^B	p=0.62/p=0.10	27% 67%	35% 40%	p=0.03
AGO (42,45)	275/560	EC ET	Progressive metastatic cancer with no prior chemotherapy	NR	NR	41% 46%	7.1/16.4 ^C 10.5/21.4	10.4/33.1 ^C 9.6/27.5	p=0.11/p=0.14	46% 76% ^D	33% 50% ^D	p=0.26
SBG (40,44)	131/283	MF D	Progressive metastatic cancer with anthracycline failure	NR	ND	24% 53%	ND ^E	ND ^E	NR	33% 53%	18% 53%	NR
BCIRG 001 (50)	1250/1491	FAC DAC	Adjuvant, at least one node positive	4.6	HR 1.47 ^F	NR	HR 1.67 ^F	HR 1.32 ^F	p=0.41 ^F	NR	NR	NR
University of Texas (47)	104/144	AC AC→D	Neoadjuvant, (T1C-T3, N0, M0) or (T1-3, N1, M0)	NR	NR	55% 82%	NA	NA	NA	75% 78%	51% 81%	NR

Items in bold are statistically significant comparisons at the α=0.05 level.

^A Calculated from HER2/neu data by IHC score, using 0-1+ as HER2/neu negative and 2-3+ as HER2/neu positive.

^B HER2/neu+ vs. HER2/neu- time-to-progression and OS hazard ratio, A vs. Docetaxel.

^C HER2/neu+ vs. HER2/neu- median PFS and OS in months. Difference significant for EC arm.

^D HER2/neu+ vs. HER2/neu- ORR difference significant for ET arm.

^E No statistically significant difference for both OS and time-to-progression.

^F Only disease-free survival results reported by HER2/neu status. Inverse of reported hazard ratios shown in the table in order to maintain consistent listing of non-taxane containing arm first.

Abbreviations: A, doxorubicin; AGO, Arbeitsgemeinschaft für Gynäkologische Onkologie; C, cyclophosphamide; D, docetaxel; E, epirubicin; EORTC, European Organization for Research and Treatment of Cancer; F, 5-fluorouracil; f/u, follow-up; HR, hazard ratio, first listed arm versus second listed arm; Int, interaction; m, months; M, methotrexate; n_{HER}, number of patients with HER2/neu status known included in analysis; n_{total}, total number of patients in study; ND, no difference; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SBG, Scandinavian Breast Group; T, paclitaxel; vs., versus.

Table 9. Trials comparing the relative efficacy of different taxane containing regimens.

Trial	n _{HER} /n _{total}	Arms	Population	Median f/u	OS	OS subgroup analysis		
						HER2+	HER2 -	Int effect
CALBG 9342 (48) ^A	175/474	T175 T210 T250	Stage IV	NR	NR	“There was no relationship between HER2 status and OS”		NR

^A Abstract only.

Abbreviations: CALGB, Cancer and Leukemia Group B; f/u, follow-up; Int, interaction; n_{HER}, number of patients with HER2/*neu* status known included in analysis; n_{total}, total number of patients in study; NR, not reported; OS, overall survival; T, paclitaxel.

Table 10. Trials comparing the efficacy of tamoxifen and chemotherapy, compared with tamoxifen alone, by HER2/*neu* status.

Trial	n _{HER} /n _{total}	Arms	Population	Med f/u	OS	DFS	OS subgroup analysis			4 yrs DFS subgroup analysis		
							HER2+	HER2-	Int effect	HER2+	HER2-	Int effect
SWOG S8814 (49)	595/1477	Tam CAF+Tam	ER-positive, node-positive	NR	HR=1.29	HR=1.43	NR	NR	NR	^A 41%/56% ^A 74%/75%	^A 81%/82% ^A 84%/84%	^A p=0.09/p=0.21

Items in bold are statistically significant comparisons at the $\alpha=0.05$ level.

^A HER2/*neu* overexpression was measured by immunohistochemistry using two different monoclonal antibodies: MAb1 and CB11. The MAb1 results are presented before the slash, the CB11 results after.

Abbreviations: CAF, cyclophosphamide, doxorubicin, and 5-fluorouracil; f/u, follow-up; Int, interaction; n_{HER}, number of patients with HER2/*neu* status known included in analysis; n_{total}, total number of patients in study; NR, not reported; OS, overall survival; SWOG, Southwest Oncology Group; Tam, tamoxifen.

Table 11. Summary of therapy regimens evaluated by included trials.

Trial	Reference in this report	Assessment Method	Criteria for Positive	Therapy regimen
ABCSG 5 (8,10)	Tables 2 and 5	Unstated, likely IHC based on criteria for positive	“Strongly positive (+++)”	HER2/neu Tam + Gos: Tam (20 mg/day orally for 5 years) plus Gos (3.6 mg q 28 days for 3 years). CMF: C (600 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) days 1 and 8, q 28 days for 6 cycles.
AGO (42,45)	Table 8	FISH using Vysis Pathvision HER2/ <i>neu</i> and centromeric chromosome 17 probes	≥2 HER2/ <i>neu</i> signals per centromeric chromosome 17 signals per nucleus	EC: E (60 mg/m ²) and C (600 mg/m ²) q 3 weeks for maximum 10 cycles. ET: E (60 mg/m ²) and T (175 mg/m ²) q 3 weeks for maximum 10 cycles.
BCIRG 001 (50)	Table 8	FISH, specific probes not reported	>2 HER2/ <i>neu</i> signals per centromeric chromosome 17 signals per nucleus	DAC: D (75 mg/m ²), A (50 mg/m ²), and C (500 mg/m ²) q 3 weeks for 6 cycles FAC: F (500 mg/m ²), A (50 mg/m ²), and C (500 mg/m ²) q 3 weeks for 6 cycles
Belgian (22,28) ^A	Tables 6 and 7	FISH using Vysis Pathvision HER2/ <i>neu</i> and centromeric chromosome 17 probes	>2 HER2/ <i>neu</i> signals per centromeric chromosome 17 signals per nucleus	HEC: E (100 mg/m ²) and C (830 mg/m ²) q 3 weeks for 8 cycles. EC: E (60 mg/m ²) and C (500 mg/m ²) q 3 weeks for 8 cycles. CMF: C (100 mg/m ² days 1 through 14), M (40 mg/m ² on days 1 and 8), and F (600 mg/m ² on days 1 and 8) q 4 weeks for 6 cycles.
CALBG 9342 (48)	Table 9	IHC using CB11 antibody and FISH using Vysis Pathvision	Not reported	T175: T (175 mg/m ²) q 3 weeks. T210: T (210 mg/m ²) q 3 weeks. T250: T (250 mg/m ²) q 3 weeks.
CALGB 8541/8869 (35)	Table 7	IHC using CB11 antibody on some specimens and an unstated antibody on others ^B	Estimated ≥50% of cells stained	HCAF: C (600 mg/m ²), A (60 mg/m ²), on day 1, and F (600 mg/m ²) on days 1 and 8, q 28 days for 4 cycles. CAF: C (400 mg/m ²), A (40 mg/m ²), on day 1, and F (400 mg/m ²) on days 1 and 8, q 28 days for 6 cycles. LCAF: C (300 mg/m ²), A (30 mg/m ²), on day 1, and F (300 mg/m ²) on days 1 and 8, q 28 days for 4 cycles.
Czech (24)	Table 6	IHC using CB11 antibody	Not reported	AC: A (60 mg/m ²) and C (600 mg/m ²) q 21 days for 4 cycles. CMF: C (500 mg/m ²), M (40 mg/m ²) and F (600 mg/m ²) days 1 and 8 q 28 days for 4 cycles.

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Trial	Reference in this report	Assessment Method	Criteria for Positive	Therapy regimen
DBCCG 77c (6)	Table 2	IHC using Dako A485 antibody	"Distinct membranous immunostaining"	Tam (1 yr): Tam (10 mg 3 times/day) for 1 year Observation
DBCCG 89d	Table 6	IHC using Dako antibody, with FISH using Dako probe on 2+ IHC results	3+ on IHC, or 2+ and two-fold amplification on FISH	CEF: C (600 mg/m ²), E (60 mg/m ²), and F (600 mg/m ²) on day 1 q 21 days CMF: C (600 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) on day 1 q 21 days Patients were also randomized to pamidronate (150 mg twice daily for 4 years) or observation in 2x2 factorial design
DUMC (15,17)	Table 4	IHC using 3B5 antibody	2+ or 3+ on 0-3+ scale ^C	Letrozole: Letrozole (2.5 mg/day) for 4 months Tamoxifen: Tam (20 mg/day) for 4 months
Dutch (34,38,39)	Table 7	IHC using 3B5 antibody	3+ on 0-3+ scale ^C	FEC+ HD: As FEC arm, except only 4 cycles, followed by one cycle of C (6 g/m ²), thiotepa (480 mg/m ²), and carboplatin (1600 mg/m ²), FEC: F (500 mg/m ²), E (90 mg/m ²), and C (500 mg/m ²) q 3 weeks for 5 cycles.
EORTC 10923 (41,43)	Table 8	IHC using CB11 antibody	2+ or 3+ on 0-3+ scale ^C	A: A (75 mg/m ²) q 3 weeks for maximum 7 cycles. T: T (200 mg/m ²) q 3 weeks for maximum 7 cycles.
FASG 05 (36,37)	Table 7	IHC using unstated antibody	2+ or 3+ on 0-3+ scale ^C	FEC100: F (500 mg/m ²), E (100 mg/m ²), and C (500 mg/m ²) q 3 weeks for 6 cycles. FEC50: F (500 mg/m ²), E (50 mg/m ²), and C (500 mg/m ²) q 3 weeks for 6 cycles.
GOIRC (52,53)	Table 6	IHC using CB11 antibody	>50% stained tumour cells	E: E (30 mg/m ²) q week for 16 weeks. CMF: C (600 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) days 1 and 8 q 28 days for 6 cycles.

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Trial	Reference in this report	Assessment Method	Criteria for Positive	Therapy regimen
GONO-MIG1 (33)	Table 7	IHC using CB11 antibody	3+ on 0-3+ scale ^C	FEC14: As FEC21, except q 14 days. FEC21: F (600 mg/m ²), E (60 mg/m ²), and C (600 mg/m ²) q 21 days.
GUN (7,9)	Table 2	IHC using MAb-1 antibody	Estimated >10% of cells with membrane staining	Tam (2 yrs): Tam (30 mg/day) for 2 years Observation
GUN-3 (31,32)	Table 6	IHC using MAb-1 antibody	Not reported	CMF→EV: C (100 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) for 3 cycles followed by E (75 mg/m ²) and V (1.4 mg/m ² on days 1 and 8) for 3 cycles. CMF: C (100 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) for 6 cycles.
IMPACT (16)	Table 4	IHC using Dako antibody, with FISH using Vysis probe on 2+ IHC results	3+ on IHC, or 2+ and two-fold amplification on FISH	Anastrozole: Anastrozole (1 mg daily) for 12 weeks before surgery. Tamoxifen: Tam (20 mg daily) for 12 weeks before surgery. An.+Tam.: As above combined.
Milan (25)	Table 6	IHC using CB11 antibody	“strong membrane labelling”	CMF→A: As CMF, except for 8 cycles, followed by A (75 mg/m ²) q 3 weeks for 4 cycles. CMF: C (600 mg/m ²), M (40 mg/m ²), and F (600 mg/m ²) q 3 weeks for 12 cycles.
NCIC CTG MA.5 (29,30) ^C	Table 6	FISH, specific probes not reported	Not reported	CEF: C (75 mg/m ² orally days 1 through 14), E (60 mg/m ² days 1 and 8), and F (500 mg/m ² days 1 and 8) q month for 6 months. CMF: C (100 mg/m ² orally days 1 through 14), M (40 mg/m ² days 1 and 8), and F (600 mg/m ² days 1 and 8) q month for 6 months.
NSABP B-11 (21,27)	Table 6	IHC using both mAb-1 and pAb-1 antibodies	“any tumour cell showed definite membrane staining resulting in a so-called fishnet appearance”	PAF: As PF, with A (30 mg/m ² on days 1 and 21). PF: P (4 mg/m ² days 1 through 5) and F (300 mg/m ² days 1 through 5) q 6 weeks for 17 cycles.
NSABP B-15 (20,26)	Table 6	IHC using both TAB250/mAb-1 and pAb-1 antibodies	“any tumour cell showed definite membrane staining resulting in a so-called fishnet appearance”	AC: A (60 mg/m ²) and C (600 mg/m ²) q 21 days for 4 cycles. CMF: C (100 mg/m ² orally days 1 through 14), M (40 mg/m ² days 1 and 8), and F (600 mg/m ² days 1 and 8) q month for 6 months.

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Trial	Reference in this report	Assessment Method	Criteria for Positive	Therapy regimen
PSUHMC (14)	Table 4	Immunoassay for pre-treatment serum HER2/ <i>neu</i> ECD (Immuno 1, Bayer Corporation)	>15 ng/ml of serum HER2/ <i>neu</i> ECD ^D	Letrozole: Letrozole (2.5 mg/day). Tamoxifen: Tamoxifen (20 mg/day).
SBCCG (12,13)	Table 3	IHC using Dako antibody	2+ or 3+ on 0-3+ scale ^C	Tam (5 yrs): Tam (20 or 40 mg/day, depending on center) for 5 years. Tam (2 yrs): Tam (20 or 40 mg/day, depending on center) for 2 years.
SBG (40,44)	Table 8	IHC using Dako antibody	2+ or 3+ on 0-3+ scale ^C	D: D (100 mg/m ²) q 3 weeks until progression or toxicity. MF: M (200 mg/m ²) and F (600 mg/m ²) q 3 weeks until progression or toxicity.
Spanish (23)	Table 6	IHC using CB11 antibody	"tumours expressing HER2 in 50% or more of .cells"	FAC: F (500 mg/m ²), A (50 mg/m ²), and C (500 mg/m ²) q 3 weeks for 6 cycles. CMF: C (600 mg/m ²), M (60 mg/m ²), and F (600 mg/m ²) q 3 weeks for 6 cycles.
Swedish (11)	Table 2	IHC using CB11 antibody	3+ on 0-3+ scale ^C	Tam (2 yrs): Tam (dose not stated) for 2 years. Observation
SWOG S8814 (49)	Table 10	IHC using MAb1 and CB11	Not reported	Tam: Tam (20 mg/d) for 5 years. CAF+Tam: C (100 mg/m ² orally days 1 through 14), A (30 mg/m ² days 1 and 8), and F (500 mg/m ² days 1 and 8) q 29 days for 6 cycles, plus Tam as above.
TAX303 (46)	Table 8	FISH using Vysis probes on specimens with >1% staining on IHC using CB11	Ratio of HER2 signals to centromeric 17 signals >2	A: A (75 mg/m ² q 3 wks) D: Docetaxel (100 mg/m ² q 3 weeks)
University of Texas (47)	Table 8	IHC using TAB250, with confirmation of borderline specimens by FISH	Not stated	AC: A (60 mg/m ²) and C (600 mg/m ²) q 21 days for 4 cycles. AC→D: As above, plus docetaxel (100 mg/m ²) q 27 days for 4 cycles. ^E

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Trial	Reference in this report	Assessment Method	Criteria for Positive	Therapy regimen
Vietnamese (18,19)	Table 5	IHC using both TAB250 and Dako antibodies	Scoring used by Chang et al, (61)	Ooph+Tam: Oophorectomy plus Tam (20 mg/day) for 5 years. Obs+Therapy: Observation, followed by above if recurrence.

^A The study was conducted using both IHC and FISH to determine HER2/*neu* status. Only the FISH results are reported here. Details on schedule and dosages of treatment arms taken from initial study report (62).

^B Two different sets of patients, separated in time, are included in this study. CB11 was used in the second set because the antibody used with the first set was no longer available.

^C With some variation between studies, this scale was as follows: 0, completely negative; 1+ faint perceptible staining; 2+, moderate staining of entire membrane in >10% of tumour cells, 3+, strong circumferential staining of entire membrane. The variation between studies in the exact definition of each score is considered minimal compared to the variation between antibodies used and completely different scoring systems.

^D Cut off derived from the mean plus two standard deviations of serum HER2/*neu* ECD in 245 healthy women.

^E Given as neoadjuvant therapy. Roughly half of patients in AC arm received docetaxel as the AC→Docetaxel arm, but received the docetaxel in the adjuvant, not neoadjuvant, setting, after the response measures reported in this document were collected.

Abbreviations: A, doxorubicin; ABCSG, Austrian Breast and Colorectal Cancer Study Group; AGO, Arbeitsgemeinschaft für Gynäkologische Onkologie; An., anastrozole; C, cyclophosphamide; CALGB, Cancer and Leukemia Group B; D, docetaxel; DBCCG, Danish Breast Cancer Cooperative Group; DUMC, Duke University Medical Center; E, epirubicin; ECD, extracellular domain; EORTC, European Organization for Research and Treatment of Cancer; F, 5-fluorouracil; FASG, French Adjuvant Study Group; FISH, fluorescence in situ hybridization; GOIRC, Gruppo Oncologico Italiano di Ricerca Clinica; GONO-MIG, Gruppo Oncologico Nord Ovest Mammella Intergruppo; Gos, gosorelin; GUN, Gruppo Universitario Napoletano; IHC, immunohistochemistry; NCIC CTG, National Cancer Institute of Canada Cancer Trials Group; NSABP, National Surgical Adjuvant Breast and Bowel Project; Ooph, oophorectomy; PSUHMC, Pennsylvania State University Hershey Medical Center; : q, every; SBCCG, Swedish Breast Cancer Cooperative Group; SBG, Scandinavian Breast Group; T, paclitaxel; Tam, tamoxifen.

Outcomes

Endocrine Therapy

Question #1: Does the efficacy of tamoxifen (compared with no tamoxifen) depend on HER2/neu status?

Reports of HER2/neu subgroup analyses for three trials (6,7,9,11) of tamoxifen versus observation were identified (see Table 2). Two of these trials (6,11) reported no significant interaction between HER2/neu status and the treatment arm (tamoxifen versus [vs.] no tamoxifen) for any outcome.

The Gruppo Universitario Napoletano (GUN) trial (7,9), which compared the efficacy of two years of tamoxifen versus observation in patients with stage I-III breast cancer, found a significant benefit to overall survival for tamoxifen (HR 0.68, 95% C.I. 0.51-0.91). The trial also found significant interaction between the treatment arm (tamoxifen vs. observation) and HER2/neu status for both overall survival ($p=0.04$) and disease-free survival ($p=0.03$), with a greater benefit with tamoxifen reported in HER2/neu-negative patients.

The Austrian Breast and Colorectal Cancer Study Group (ABCSSG) trial does not strictly address Question #1 but does involve a study arm containing tamoxifen and one that does not (Table 5).

Meta-Analysis

Only one study (6) out of the four identified provided sufficient information from which to derive log-hazard ratios and their standard errors for disease-free survival. One study (11) provided sufficient information on disease-free survival for estrogen receptor-positive patients but not for the entire patient population. Only one study (7,9) provided sufficient information for overall survival. Therefore, no meta-analysis was conducted of these trials.

Question #2: Do the relative efficacies of different tamoxifen durations depend on HER2/neu status?

The Swedish Breast Cancer Cooperative Group (SBCCG) trial (12,13) was the only trial identified that evaluated the impact of HER2/neu expression on the response to different tamoxifen durations (Table 3). The trial reported no significant interaction, for any outcome, between HER2/neu status and the efficacy of five versus two years of tamoxifen therapy.

Question #3: Do the efficacies of aromatase inhibitors (compared with tamoxifen) depend on HER2/neu status?

Two trials, one led by the Duke University Medical Centre (DUMC) (15,17) and one by the Penn State University Hershey Medical Centre (PSUHMC) (14), evaluated the efficacy of letrozole compared with tamoxifen according to HER2/neu status. The IMPACT trial (16) compared anastrozole, tamoxifen, and the combination of the two, in the neoadjuvant setting. These trials are summarized in Table 4. None of these trials reported any significant interaction between HER2/neu status and treatment arm (aromatase inhibitors vs. tamoxifen) for any outcome.

Meta-Analysis

Two of the included trials (15-17) were conducted in the neoadjuvant setting and were considered suitable for pooling through meta-analysis. The objective response rate (ORR) was reported in both of these trials for both HER2/neu-positive and HER2/neu-negative patients. The pooled odds ratio for objective response among HER2/neu-positive patients was 7.86 (95% CI 2.38 to 25.92), with the value over one indicating a greater response among those treated with aromatase inhibitors over those treated with tamoxifen. Among HER2/neu-negative patients, the pooled odds ratio for objective response was 1.19 (95% CI 0.58 to 2.45).

Question #4: Does the efficacy of ovarian ablation (compared with no ovarian ablation) depend on HER2/neu status?

Two trials evaluated some form of ovarian ablation by HER2/*neu* status. Neither of these trials directly addresses the question of ovarian ablation alone versus observation, but they do address the question of ovarian ablation in combination versus no ovarian ablation as initial therapy. One trial, reported by Love et al, (18,19) and conducted in hospitals in Vietnam and China, analysed the efficacy of oophorectomy and tamoxifen versus observation with oophorectomy and tamoxifen in the event of relapse. The other is the ABCSG-5 trial (8,10) mentioned above, which evaluated tamoxifen and goserelin versus chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). These trials are summarized in Table 5. Neither trial reported significant interaction between HER2/*neu* status and treatment arm (ovarian ablation vs. no ovarian ablation) for any outcome. As the two studies identified were considered clinically heterogeneous, no meta-analysis was attempted.

Chemotherapy

Question #5: Does the efficacy of anthracycline-based regimens (compared with non-anthracycline-based regimens) depend on HER2/neu status?

Summary of Studies

Ten trials, all conducted in the adjuvant setting, analysed the efficacy of anthracycline-based regimens versus non-anthracycline-based regimens according to HER2/*neu* status (20-32,51-53) (Table 6). Eight of these trials (20,22-26,28-30,51-53) reported no significant interaction between HER2/*neu* status and treatment arm (anthracycline-containing regimen versus non-anthracycline-containing regimen) for any outcome.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-11 trial compared the efficacy of melphalan, doxorubicin, and 5-fluorouracil (PAF) with melphalan and 5-fluorouracil (PF) in node-positive, estrogen- and/or progesterone-negative patients (21,27). This trial found a significant benefit to five-year disease-free survival (51% vs. 44%, $p=0.007$) with the inclusion of doxorubicin in the regimen. When analyzed by HER2/*neu* status, the trial found a significant benefit in HER2/*neu*-positive patients with the inclusion of doxorubicin in the regimen, in terms of overall survival (relative risk [RR] 0.66, $p=0.01$) and disease-free survival (RR 0.60, $p=0.001$). No significant benefit was found in HER2/*neu*-negative patients. A significant interaction was found between HER2/*neu* status and treatment arm (PAF vs. PF) for disease-free survival ($p=0.02$).

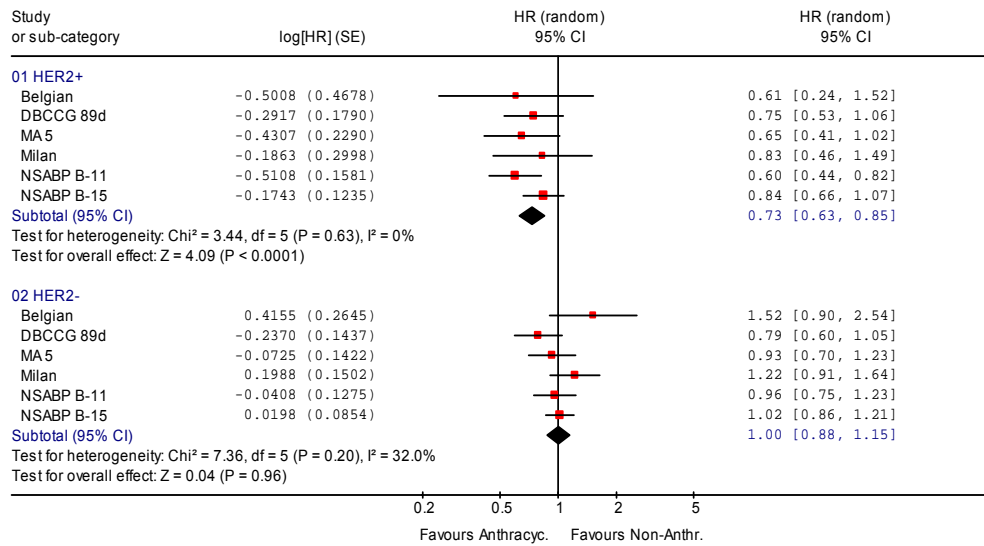
In the GUN-3 trial reported by De Laurentis et al and De Placido et al (31,32), CMF followed by epirubicin plus vincristine was found to be not significantly different from CMF alone in terms of overall survival or disease-free survival. In the subgroup analysis of HER2/*neu* status, there was a significant ($p=0.05$) interaction between HER2/*neu* status and treatment arm (CMF plus epirubicin and vincristine vs. CMF alone) for overall survival, with HER2/*neu*-positive patients benefiting more from the anthracycline-containing regimen than HER2/*neu*-negative patients do.

Meta-Analysis

To estimate the overall effect of HER2/*neu* status on the efficacy of anthracycline-based regimens, a meta-analysis was conducted on both disease-free and overall survival. Six of the studies (20-22,25-30,51) provided sufficient information to derive a log-hazard ratio and its standard error for disease-free survival for both HER2/*neu*-positive and HER2/*neu*-negative individuals. Five of the studies (20,21,25-27,29,30,51) plus two additional studies (31,32,52,53) provided sufficient information to derive a log-hazard ratio and its standard error for overall survival for both HER2/*neu*-positive and HER2/*neu*-negative individuals. The results of these analyses are shown in Figures 1 and 2.

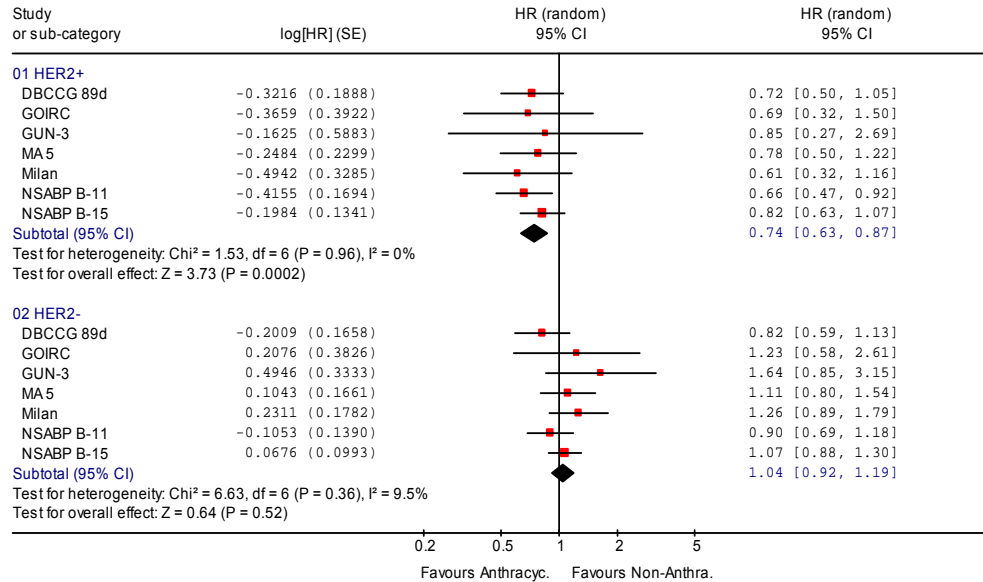
The inclusion of the trial by Di Leo et al (listed as “Belgian” in the figures) trial (22,28) in the meta-analysis proved difficult. Not only did the researchers evaluate two separate dosage levels of epirubicin and cyclophosphamide (as shown in Table 11) but they also used the following three separate methods of evaluating the HER2/*neu* status of the included patients: fluorescence in-situ hybridization (FISH), immunohistochemistry (IHC) using CB11 and 4D5 antibodies, and IHC using a cocktail of TAB250 antibody and pAb1 polyclonal serum. Therefore, six different sets of hazard ratios from this study could have been selected for inclusion. The results in Figures 1 and 2 reflect the inclusion of the lower-dose epirubicin and cyclophosphamide versus CMF comparison, with HER2/*neu* status established by FISH.

Figure 1. Meta-analysis of disease-free survival hazard ratios (HR) in trials comparing anthracycline vs. non-anthracycline-based regimens by HER2/*neu* status.



References: Belgian (22,28), DBCCG 89d (51), Milan (25), MA.5 (29,30), NSABP B-11 (21,27), NSABP B-15 (20,26).
 Abbreviations: CI, confidence interval; DBCCG, Danish Breast Cancer Cooperative Group; HR, hazard ratio; NSABP, National Surgical Adjuvant Breast and Bowel Project; SE, standard error; vs., versus.

Figure 2. Meta-analysis of overall survival hazard ratios (HR) in trials comparing anthracycline vs. non-anthracycline-based regimens by HER2/neu status.



References: DBCCG 89d (51), GOIRC (52,53), GUN-3 (31,32), Milan (25), MA.5 (29,30), NSABP B-11 (21,27), NSABP B-15 (20,26).

Abbreviations: CI, confidence interval; DBCCG, Danish Breast Cancer Cooperative Group; GOIRC, Gruppo Oncologico Italiano di Ricerca Clinica; GUN, Gruppo Universitario Napoletano; HR, hazard ratio; NSABP, National Surgical Adjuvant Breast and Bowel Project; SE, standard error; vs., versus.

In both of the meta-analyses, there was very little statistical heterogeneity, with I² being no more than 6.1% and the p-value of the Chi² test of heterogeneity no less than 0.37. A treatment benefit for anthracycline-based regimens appears to be present in HER2/*neu*-positive patients in terms of both overall survival (HR 0.74, 95% CI 0.63 to 0.87) and disease-free survival (HR 0.73, 95% CI 0.63 to 0.85). This benefit does not appear to be present in HER2/*neu*-negative patients (overall survival HR 1.04, disease-free survival HR 1.00).

The difference in log-hazard ratios between HER2/*neu* subgroups was also analyzed, as described in the Methods section, and was found to be significant for both disease-free and overall survival. For disease-free survival, the difference was estimated as -0.29 (95% CI -0.47 to -0.10), and for overall survival, the difference was estimated as -0.32 (95% CI -0.51 to -0.12). There was no statistical heterogeneity in these estimates (I² 0%, Chi² test of homogeneity p>0.58).

In order to determine whether the choice of arms and testing methods included from the Di Leo et al study made any difference in the disease-free survival results, a sensitivity analysis was conducted. The choice of dose level and testing method included in the analysis made little difference in the overall estimate of effect; the estimated hazard ratios and their confidence intervals differed by no more than 0.04, and the difference of log-hazard ratios was statistically significant in all cases. Therefore, the choice of arm from this trial made no difference to the results of the analysis.

As not all the identified studies could be included in the meta-analysis, there was concern that publication bias might be an issue, that is, that the unincluded studies, if included, would alter the conclusions. To address this concern, a funnel plot of the differences in log-hazard ratios of the included studies was reviewed, and the “trim and fill” methods described by Duval and Tweedle (63) were used to evaluate any asymmetry present. In the case of disease-free survival, no asymmetry in the funnel plot was identified. In the case of overall survival, evidence of possible publication bias was present, with two studies (GUN3 and Milan) possibly contributing to the asymmetry as identified by the iterative method of Duval and Tweedle. Two

imputed studies were added to the analysis, and, taking these imputed studies into account, the difference between the log-hazard ratios was still significant (-0.27 [95% CI -0.46 to -0.08]). It is, therefore, less likely that further publications of currently unpublished data from any of the studies identified in this systematic review or other studies will affect this result.

Question #6: Do the relative efficacies of different anthracycline-based regimens depend on HER2/neu status?

Five trials, all in the adjuvant setting, analysed the effect of different anthracycline-based regimens according to HER2/neu status (22,28,33-39) (Table 7). Note that, while most of these trials compared more versus less intense anthracycline regimens, one trial (34,38,39) compared anthracycline-based therapy to anthracycline plus high-dose chemotherapy. That trial is included in this section for simplicity of organization but should be considered separately. Two of these trials (22,28,33) reported no significant interaction between HER2/neu status and treatment arm (more intense versus less intense anthracycline-based regimen) for any outcome, and one trial (36,37) did not report a significance test for interaction.

The Cancer and Leukemia Group B (CALGB) 8541/8869 trials (35) compared the efficacy of the following three different regimens: a high-dose cyclophosphamide (C) (600 mg/m²), doxorubicin (A) (60 mg/m²), and 5-fluorouracil (F) (600 mg/m²) regimen for four cycles (HCAF); a moderate-dose (C 400 mg/m², A 60 mg/m², F 400 mg/m²) regimen for six cycles (CAF); and a low-dose (C 300 mg/m², A 30 mg/m², F 300 mg/m²) regimen for four cycles (LCAF). It is important to note that the dose used in the HCAF arm in this trial is now generally considered the standard dose, while the CAF and LCAF arm doses are no longer common. This trial, which compared these regimens in patients with stage II cancer, found that there were significantly improved overall survival and disease-free survival with HCAF and CAF compared to LCAF. A subgroup analysis of this trial (35) reported by Thor et al found a significant interaction between HER2/neu status and treatment arm (HCAF vs. CAF vs. LCAF) for both five-year overall survival (p<0.001) and five-year disease-free survival (p=0.001). HER2/neu-positive patients benefited from HCAF similarly to HER2/neu negative patients but had worse outcomes on the CAF and LCAF regimens (see Table 7).

More recently, Dressler et al (64) reported on a further study of a subset of patients included in the above CALGB 8541/8869 analysis. In this study, HER2/neu status was also established, using FISH and polymerase chain reaction (PCR), in 523 patients who had been included in the original study and whose status had been established by IHC. The outcomes were compared, based on the method of HER2/neu-status identification. This study demonstrated that the significant interaction between HER2/neu status and treatment arm for disease-free survival was present regardless of the method used, FISH (p=0.033), IHC (p=0.0003), or PCR (p=0.043). Also of note, this study found that those patients whose tumours were considered HER2/neu-positive by both FISH and IHC experienced the largest magnitude benefit, in terms of disease-free survival, from the HCAF arm (see Table 12). As this study included only a subset of the patients from the study reported by Thor et al, the analysis by Thor et al was considered to have greater statistical power and is abstracted in Table 7 and used in the meta-analysis found below.

Table 12. Hazard ratios for disease-free survival, HCAF vs. CAF/LCAF arms, by HER2/*neu*-status identification method, CALGB 8541/8869 trial.

	FISH+	FISH-
IHC+	3.42 (1.43 to 8.19)	1.70 (0.63 to 4.60)
IHC-	0.96 (0.23 to 3.97)	1.02 (0.74 to 1.40)

Reference: (64)

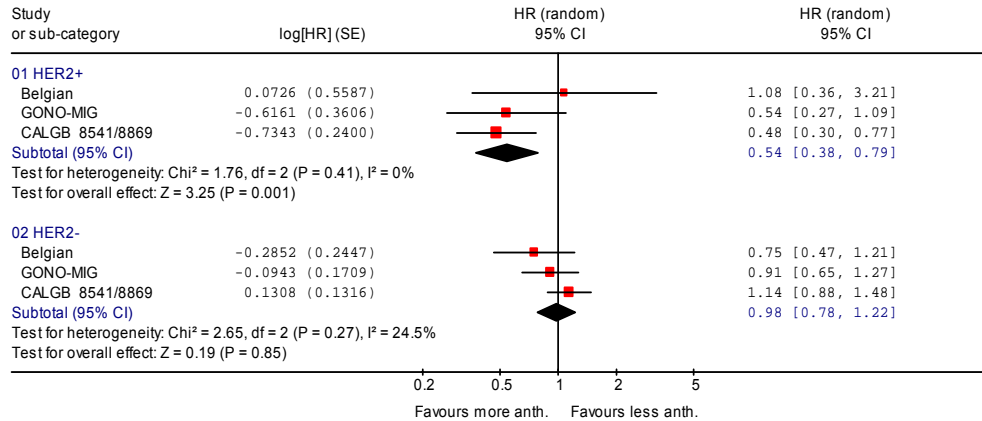
Abbreviations: CAF, cyclophosphamide, doxorubicin, and 5- fluorouracil; CALGB, Cancer and Leukemia Group B; FISH, fluorescence in situ hybridization; H, higher dose; IHC, immunohistochemistry; L, lower dose; vs., versus.

In the trial reported by Rodenhuis et al, in association with the Netherlands Working Party on Autologous Transplantation of Solid Tumours, (34,38,39) the efficacy of high-dose chemoendocrine therapy compared with normal dose was analyzed according to HER2/*neu* status. As noted above, this trial is very different in purpose from the other trials reported in this section and is included in this section for the purpose of simplicity. Originally reported in 2003, the updated trial results were published in 2005. The trial examined patients with operated breast cancer who had more than four positive nodes. All patients in the trial received tamoxifen when appropriate. This trial compared a regimen of five cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) versus four cycles of the same with one final high-dose cycle of cyclophosphamide, thiotepa, and carboplatin. The trial found no significant difference in either overall or relapse-free survival. However, the trial found a significant benefit to relapse-free survival (HR 0.68, p=0.002) and overall survival (HR 0.72, p=0.02) in the high-dose arm among HER2/*neu*-negative patients. No significant benefits were found among HER2/*neu*-positive patients. The interaction between treatment arm and HER2/*neu* status was significant for relapse-free survival (p=0.006).

Meta-analysis

Four of the identified studies compared more intense anthracycline-based regimens to less intense ones (22,28,33,35-37). To estimate the overall effect of HER2/*neu* status on the efficacy of the more intense versus less intense anthracycline-based regimens, a meta-analysis was conducted on median disease-free survival. Three of the studies provided sufficient information to derive a log-hazard ratio and its standard error for disease-free survival for both HER2/*neu*-positive and HER2/*neu*-negative individuals. In the case of the CALGB 8541/8869 trial (35), the high-dose versus medium-dose arms comparison was included in the analysis. In the case of the Belgian trial (22,28), the results based on FISH analysis of HER2/*neu* status were used. The results of this analysis are shown in Figure 3.

Figure 3. Meta-analysis of disease-free survival hazard ratios (HR) in trials comparing different anthracycline-based regimens by HER2/neu status.



References: Belgian (22,28), CALGB (35), GONO-MIG (33)

Abbreviations: CI, confidence interval; GONO-MIG, Gruppo Oncologico Nord Ovest Mammella Intergruppo; CALGB, Cancer and Leukemia Group B; HR, hazard ratio; SE, standard error.

In this meta-analysis, there was no statistical heterogeneity in the HER2/*neu*-positive estimate (Chi² test for homogeneity p=0.41, I² 0%), and little in the HER2/*neu*-negative estimate (Chi² test for homogeneity p=0.27, I² 24.5%). A treatment benefit for more intense versus less intense anthracycline-based regimens appears to be present in HER2/*neu*-positive patients in terms of time-to-progression (HR 0.54, 95% CI 0.38 to 0.79). This benefit does not appear present in HER2/*neu*-negative patients (time-to-progression HR 0.98).

The difference in log disease-free survival hazard ratios between HER2/*neu* subgroups was also analyzed, as described in the Methods section. This difference was not found to be significant -0.51 (95% CI -1.12 to 0.09). There was some statistical heterogeneity in these estimates (I² 43.8%, Chi² test of homogeneity p=0.17).

As there was little difference in disease-free survival between the moderate- and low-dose arms in the CALGB trial, a sensitivity analysis was not conducted regarding the choice of arm from that trial. However, a sensitivity analysis was conducted regarding the choice of HER2/*neu*-testing method used to generate the estimates for the Belgian trial. In this case, the choice of method did make a considerable difference in the resulting estimates. When the results based on IHC using CB-11 and 4D5 antibody were included instead of FISH, a significant treatment interaction was detected (difference in log-hazard ratios -0.72, 95% CI -1.15 to -0.30). The results based on IHC using the cocktail method were of less magnitude than the FISH results (difference in log-hazard ratios -0.44, 95% CI -1.11 to 0.23).

Question #7: Does the efficacy of taxane-containing regimens (compared with non-taxane-containing regimens) depend on HER2/*neu* status?

Five trials (40-47) analysed the effect of taxane-containing regimens versus non-taxane regimens according to HER2/*neu* status (Table 8). Four of these trials (40-46) were in the metastatic setting, and one (47) was in the neoadjuvant setting. Three of these trials (40-45) reported no significance in the test of interaction between HER2/*neu* status and treatment arm (taxane vs. non-taxane-based therapy) for any outcome.

Di Leo et al reported a retrospective analysis of the TAX303 trial (46). This trial compared single-agent docetaxel to single-agent doxorubicin in the treatment of patients with metastatic breast cancer who had been treated previously with adjuvant or first-line metastatic CMF. The TAX303 trial found a significant difference between the regimens in terms of ORR (docetaxel 47.8% vs. doxorubicin 33.3%, p=0.008) but not in terms of median time-to-progression or overall survival. The analysis found a significant interaction between HER2/*neu*

status and treatment arm (docetaxel vs. doxorubicin) for ORR ($p=0.03$); patients with HER2/*neu*-positive breast cancers who received docetaxel experienced a significantly higher ORR than those on doxorubicin, while there was no difference among patients with HER2/*neu*-negative breast cancer.

Meta-Analysis

No meta-analysis was performed, as either the taxane-containing and non-taxane arms were not comparable or, where they were comparable (single-agent taxane vs. single-agent anthracycline), only one study (46) provided sufficient information to derive log-hazard ratios and their standard errors for disease-free or overall survival.

Question #8: Do the relative efficacies of different taxane-containing regimens depend on HER2/*neu* status?

Only one trial, the CALBG 9342 trial, reported in abstract form, analysed the efficacy of different taxane-containing regimens according to HER2/*neu* status. This trial compared the efficacy of three different paclitaxel dosages (175 mg/m², 210 mg/m², and 250 mg/m²) and found no relationship between HER2/*neu* status and overall survival (Table 9).

Chemoendocrine

Question #9: Does the effect of tamoxifen and chemotherapy (compared with tamoxifen alone) depend on HER2/*neu* status?

Only one trial, the Southwest Oncology Group S8814 trial, reported in abstract form, analysed the efficacy of tamoxifen and chemotherapy compared with tamoxifen alone according to HER2/*neu* status (Table 10). This trial found no significant interaction between treatment and HER2/*neu* status.

Question #10: Does the efficacy of radiation therapy (compared with no radiation therapy) depend on HER2/*neu* status?

There were no studies identified that evaluated the efficacy of radiation therapy compared to no radiation therapy by HER2/*neu* status that met the inclusion and exclusion criteria.

Other Clinical Practice Guidelines

ASCO published a clinical practice guideline on the use of tumour markers in breast and colorectal cancer in 1997 and updated these recommendations in 2000 (55). These updated guidelines, developed by an update committee, were based on a literature review of English language literature from 1994 to 1999, but the exact methods used in this search were not described. The recommendations were developed by the committee through a consensus process. The recommendations from the 2000 guideline applicable to HER2/*neu* can be summarized as follows:

- All primary breast cancers should be evaluated for HER2/*neu* overexpression at the time of diagnosis or recurrence. Measures of HER2/*neu* amplification may “be of value.”
- Clinical laboratories should provide detailed reports of HER2/*neu* testing, including details of quality controls, scoring, reproducibility, sensitivity, specificity, and clinical validity.
- High levels of HER2/*neu* expression may be used to identify patients who may particularly benefit from anthracycline-based chemotherapy, but they should not be used to exclude people from this therapy.
- HER2/*neu* status should not be used in determining whether or not to offer endocrine therapy.

- HER2/*neu* status should not be used in determining whether or not to offer taxane-based chemotherapy.
- Routine use of HER2/*neu* overexpression to identify early breast cancer patients with a higher risk of relapse is not recommended. (*It should be noted that this recommendation was made prior to the recent evidence of the efficacy of adjuvant trastuzumab.*)
- Measurement of the extracellular domain of HER2/*neu* is not recommended.

The update committee declined to make any recommendation for or against adjuvant CMF chemotherapy for those with HER2/*neu* overexpressing cancers.

DISCUSSION

Endocrine Therapy - Tamoxifen

Three trials (6-11) were identified that evaluated the efficacy of tamoxifen by HER2/*neu* status; two compared tamoxifen to observation, one compared tamoxifen plus goserelin to CMF, and one compared five years versus two years of tamoxifen. In addition, two trials (14,15,17) of aromatase inhibitors and tamoxifen reported results of interest to the question of tamoxifen interaction with HER2/*neu* status. The weight of the identified evidence, especially the GUN trial, suggests that the efficacy of tamoxifen is greater in HER2/*neu*-negative patients than in positive patients. However, while there is evidence to suggest tamoxifen is more effective in HER2/*neu*-negative patients, there is insufficient evidence to suggest tamoxifen is ineffective in HER2/*neu*-positive patients. Therefore, no definitive recommendations can be made at this time.

Endocrine Therapy - Aromatase Inhibitors

Two trials (14,15,17) were identified that evaluated the efficacy of letrozole compared with tamoxifen according to HER2/*neu* status and one (16) that evaluated anastrozole. Trials that evaluated exemestane were not identified. None of these trials reported significance testing of the interaction between HER2/*neu* status and treatment, although the PSUHMC trial suggests that, in the metastatic setting, letrozole benefit to ORR may be more pronounced in patients with HER2/*neu*-negative breast cancer.

Full results regarding HER2/*neu*-status subgroup analysis have not yet been published from the following large trials of aromatase inhibitors: ATAC¹ (65), IES² (66), and BIG³ 1-98 (67). An abstract from the 2003 San Antonio Breast Cancer Symposium (SABCS) by Dowsett et al (68) reported on a subgroup analysis of the ATAC trial comparing time-to-recurrence by estrogen-receptor (ER) and progesterone-receptor (PR) status. The abstract reported a marginally significant difference ($p=0.05$) between the ER+/PR+ (HR 0.82, 95% CI 0.65-1.03) and ER+/PR- (HR 0.48, 95% CI 0.33 to 0.71) subgroups. As HER2/*neu* overexpression and amplification are highly correlated with ER+/PR- status (68), these results may reflect an interaction between HER2/*neu* status and anastrozole therapy. However, an abstract from the 2005 SABCS by Viale et al (69), detailing the results by ER and PR status from the BIG 1-98 trial, reported no obvious difference between ER+/PR+ (HR 0.84, 95% CI 0.69 to 1.03) and the ER+/PR- (HR 0.83, 95% CI 0.62 to 1.10) subgroups comparing letrozole versus tamoxifen, and so this relationship may not hold across all aromatase inhibitors. If and when HER2/*neu* subgroup analyses are published from these trials, it may then be possible to make a definitive statement regarding the interaction of aromatase inhibitor therapy and HER2/*neu* status.

¹ Aromatase, tamoxifen, alone or in combination

² Intergroup Exemestane Study

³ Breast International Group

Endocrine Therapy - Ovarian Ablation

Two trials evaluated some form of ovarian ablation by HER2/*neu* status. Neither of these trials reported significant interaction between HER2/*neu* status and efficacy.

Chemotherapy - Anthracyclines

Ten trials (20-32,51-53), all conducted in the adjuvant setting, analysed the efficacy of anthracycline-based regimens versus non-anthracycline-based regimens according to HER2/*neu* status. While only two of these trials (21,27,31,32) reported statistically significant interaction between HER2/*neu* status and treatment arm (anthracycline-containing regimen versus non-anthracycline-containing regimen) for an efficacy outcome, all of these trials were consistent in showing a trend for HER2/*neu*-positive patients experiencing greater benefit from anthracycline-based therapy than HER2/*neu*-negative patients. Additionally, the evidence suggests that HER2/*neu*-negative patients gain no benefit from anthracycline-based chemotherapy compared to CMF. This is borne out by the meta-analysis of the trials, where a significant benefit from anthracycline-based therapy in terms of disease-free and overall survival was found in HER2/*neu*-positive patients only.

Four trials (22,28,33,35-37) evaluated more intensive (either higher dose or dose-dense) anthracycline-based regimens versus less intensive ones. The trials included in the meta-analysis varied in terms of the anthracycline used (doxorubicin or epirubicin), and the type of intensification (increased dosage versus shorter time frame at same dosage). The evidence from these trials and a meta-analysis of three of them support the conclusion that more intense anthracycline regimens may provide more benefit in HER2/*neu*-positive patients. These results are not conclusive; a significant interaction could not be established definitively in the meta-analysis, as the choice of testing method used in the Belgian trial (22,28) made a considerable difference in the results. It should be noted that the testing method most similar to that used in the other two included trials, IHC by CB-11 antibody, yielded the least statistical heterogeneity of the three results that could have been included, and also was the only result that implied a statistically significant interaction. However, as there were fewer than 30 patients per arm in the HER2/*neu*-positive subgroup, this association with testing may well be simply an artefact of low numbers.

One trial (34,38,39) examined the efficacy of FEC versus FEC combined with high-dose chemotherapy. This trial found a significant interaction in terms of disease-free survival, with HER2/*neu*-negative patients being the only patients who received any significant benefit from the addition of high-dose chemotherapy. It is important to note that the FEC regimen received in both arms differed only by a single cycle (four cycles in the high-dose chemotherapy arm versus five cycles in the other arm), and so this interaction is likely solely due to the high-dose chemotherapy.

Chemotherapy - Taxanes

Four trials (40-46) conducted in the metastatic setting and one trial (47) in the neoadjuvant setting evaluated the efficacy of taxane-containing regimens versus non-taxane regimens according to HER2/*neu* status. One other trial (48) evaluated the efficacy of three different dose levels of paclitaxel. Overall, these trials provide no evidence that taxane-based therapy, compared to non-taxane based therapy, is either more or less efficacious based on HER2/*neu* status.

Chemoendocrine Therapy

One trial (49) evaluated the efficacy of tamoxifen and chemotherapy compared with tamoxifen alone according to HER2/*neu* status. This trial found no significant interaction between therapy and HER2/*neu* status, but its results were consistent with the tamoxifen trials identified above.

Radiation Therapy

There were no studies identified that evaluated the efficacy of radiation therapy compared to no radiation therapy by HER2/*neu* status that met the inclusion and exclusion criteria.

ONGOING TRIALS

A search was made of the National Cancer Institute clinical trials database on the Internet (http://www.cancer.gov/search/clinical_trials/) for ongoing clinical trials that mention HER2/*neu* status in their titles. None were identified as appropriate to the questions posed in this review. However, few of the trials included in this review were designed primarily to investigate the role of HER2/*neu* status; the HER2/*neu* status investigation was a substudy or additional analysis of an existing trial. Therefore, it is likely that there are ongoing and completed trials that will analyze the effect of HER2/*neu* status that cannot be identified prior to the publication of the findings of the trial.

CONCLUSIONS

There is a growing body of evidence, especially pertaining to anthracycline-based regimens, that the effectiveness of systemic therapy is related to the HER2/*neu* status of the breast cancer being treated. With the recent results regarding the effectiveness of adjuvant trastuzumab (70,71), it is expected that the number of patients who are tested for HER2/*neu* overexpression or amplification will increase greatly. More clinicians will have access to this information and will be considering it when deciding which systemic therapy options should be offered to their patients. Because of the relatively low statistical power of interaction tests, a clinically significant interaction between HER2/*neu* status and treatment is possibly not being detected in the existing substudy analyses of randomized clinical trials. Therefore, questions regarding the interaction between HER2/*neu* status and some forms of systemic therapy, such as aromatase inhibitors, taxanes, and ovarian ablation, may not be completely answered unless trials are specifically designed, and powered, to answer them.

CONFLICT OF INTEREST

The lead authors of this document were asked to report any conflicts of interest. BD, LE, FO, KP, HM, declared that they had no potential conflicts; MT reported receiving per-patient funding for clinical trials.

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Evidence-based Series #1-17: Section 3

The Role of HER2/neu in Systemic and Radiation Therapy for Women with Breast Cancer: Guideline Development and External Review— Methods and Results

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A Quality Initiative of the
Program in Evidence-Based Care (PEBC), Cancer Care Ontario (CCO)
Developed by the Breast Cancer Disease Site Group

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THE PROGRAM IN EVIDENCE-BASED CARE

The Program in Evidence-Based Care (PEBC) is an initiative of the Ontario provincial cancer system, Cancer Care Ontario (CCO) (1). The PEBC mandate is to improve the lives of Ontarians affected by cancer, through the development, dissemination, implementation, and evaluation of evidence-based products designed to facilitate clinical, planning, and policy decisions about cancer care.

The PEBC supports a network of disease-specific panels, called Disease Site Groups (DSGs) and Guideline Development Groups (GDGs), mandated to develop the PEBC products. These panels are comprised of clinicians, methodologists, and community representatives from across the province.

The PEBC is well known for producing evidence-based practice guideline reports, using the methods of the Practice Guidelines Development Cycle (1,2). The PEBC reports consist of a comprehensive systematic review of the clinical evidence on a specific cancer care topic, an interpretation of and consensus agreement on that evidence by our DSGs and GDGs, the resulting clinical recommendations, and an external review by Ontario clinicians in the province for whom the topic is relevant. The PEBC has a formal standardized process to ensure the currency of each clinical practice guideline report, through the routine periodic review and evaluation of the scientific literature and, where appropriate, the integration of that literature with the original clinical practice guideline information.

The Evidence-based Series

Each Evidence-based Series is comprised of three sections.

- *Section 1: Clinical Practice Guideline.* This section contains the clinical recommendations derived from a systematic review of the clinical and scientific literature and its interpretation by the DSG or GDG involved and a formalized external review by Ontario practitioners.

- *Section 2: Systematic Review.* This section presents the comprehensive systematic review of the clinical and scientific research on the topic and the conclusions reached by the DSG or GDG.
- *Section 3: Guideline Development and External Review: Methods and Results.* This section summarizes the guideline development process and the results of the formal external review by Ontario practitioners of the draft version of the clinical practice guideline and systematic review.

DEVELOPMENT OF THIS EVIDENCE-BASED SERIES

Development and Internal Review

This evidence-based series was developed by the Breast Cancer Disease Site Group (DSG) of Cancer Care Ontario's PEBC. The series is a convenient and up-to-date source of the best available evidence on the role of HER2/*neu* in systemic and radiation therapy for women with breast cancer, developed through systematic review, evidence synthesis, and input from practitioners in Ontario. A list of the members of the Breast Cancer DSG can be obtained at <http://www.cancercare.on.ca/>.

Report Approval Panel

Prior to the submission of this evidence-based series report for external review, the report was reviewed and approved by the PEBC Report Approval Panel, which consists of two members, including an oncologist, with expertise in clinical and methodology issues. Key issues raised by the Panel included:

- The language used to describe interactions was inconsistent and somewhat confusing.
- There was some concern regarding the meta-analytic method (pooled difference in log-hazard ratios) used to test the interaction of HER2/*neu* status and treatment arm. Supporting information regarding the validity of this method was requested.
- The discussion and qualifying statement regarding tamoxifen therapy in HER2/*neu*-positive patients was of concern. It was suggested that the authors elaborate on this issue and provide more context and guidance.
- There was concern that the funnel-plot and "trim and fill" analysis of trials of anthracycline versus non-anthracycline-based therapy regimens was excessive and not required in order to inform the clinical recommendations.
- The fact that the document did not in any way address trastuzumab systematic therapy was of concern. This was especially the case with regard to a qualifying statement in the recommendations suggesting that more intense anthracycline regimens may be more beneficial to HER2/*neu*-positive patients. Concern was expressed that, elsewhere, the Breast Cancer DSG was suggesting that these patients should receive trastuzumab and that the DSG had raised concerns regarding the cardiac toxicity of trastuzumab and anthracycline combination therapy.

In response to the Panel's concerns, the authors took the following actions:

- The language used to describe interactions was harmonized and made consistent throughout the document.
- The discussion and qualifying statement regarding tamoxifen and HER2/*neu* status was altered to more clearly describe the current state of the evidence.
- Additional text was added to Section 1 and Section 2 regarding trastuzumab therapy. The purpose of this text was, first, to make it clear that the purpose of this guideline was to review the literature regarding systemic therapy in the absence of trastuzumab therapy and, second, to direct the reader to the two other PEBC guidelines (PG #1-15 and EBS #1-24) that addressed trastuzumab therapy, making it clear that the

recommendations in this evidence-based series should be interpreted in light of the recommendations and evidence in those documents.

- The discussion of the results of the funnel plot and “trim and fill” analysis was made more concise, and additional justification was added to the text.
- A response was provided for the Panel clarifying the rationale behind the meta-analytic methods used and justifying the use of a funnel-plot analysis.

External Review by Ontario Clinicians

Following the review and discussion of Sections 1 and 2 of this evidence-based series and the review and approval of the report by the PEBC Report Approval Panel, the Breast Cancer DSG circulated the clinical practice guideline and systematic review to clinicians in Ontario for review and feedback. Box 1 summarizes the draft clinical recommendations and supporting evidence developed by the Panel.

<p>BOX 1: DRAFT RECOMMENDATIONS (approved for external review July 4, 2006)</p> <p>Target Population Women with breast cancer.</p> <p>Recommendations and Key Evidence Endocrine Therapy - Tamoxifen Although the current evidence does not support a definitive recommendation regarding tamoxifen therapy and HER2/<i>neu</i> status, the weight of the evidence, especially the Gruppo Universitario Napoletano (GUN) trial (3,4), suggests that the efficacy of tamoxifen may be greater in HER2/<i>neu</i>-negative patients than in HER2/<i>neu</i>-positive patients. However, the evidence does not support a recommendation against tamoxifen therapy in HER2/<i>neu</i>-positive patients. While it is possible that tamoxifen is more effective in HER2/<i>neu</i>-negative patients, there is still sufficient evidence that it is effective in HER2/<i>neu</i>-positive patients as well.</p> <p>Endocrine Therapy - Aromatase Inhibitors The current evidence does not support a definitive recommendation regarding aromatase inhibitor therapy and HER2/<i>neu</i> status.</p> <p>Endocrine Therapy - Ovarian Ablation The current evidence does not support a definitive recommendation regarding ovarian ablation and HER2/<i>neu</i> status.</p> <p>Chemotherapy – Anthracyclines Patients with HER2/<i>neu</i>-positive breast cancer should be considered for chemotherapy containing an anthracycline instead of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or melphalan and 5-fluorouracil (PF) chemotherapy.</p> <ul style="list-style-type: none"> • Ten studies (5-20) of CMF or PF versus an anthracycline-containing chemotherapy were identified that also performed a substudy analysis by HER2/<i>neu</i> status. Two of these studies (6,12,16,17) reported a significant interaction between HER2/<i>neu</i> status and treatment. A meta-analysis of these studies by HER2/<i>neu</i> status found a significant benefit in terms of both overall survival (OS) (hazard ratio [HR] 0.74, 95% confidence interval [CI] 0.63 to 0.87)

and disease-free survival (DFS) (HR 0.73, 95% CI 0.63 to 0.85) for the use of anthracycline-based chemotherapy compared to CMF or PF in patients with HER2/*neu*-positive breast cancer, but found no evidence of a benefit in HER2/*neu*-negative patients (HR 1.04 for overall survival, 1.00 for disease-free survival). The interaction between treatment and HER2/*neu* status was found to be significant in the meta-analysis (difference in log OS HRs -0.32 [95% CI -0.51 to -0.12], difference in log DFS HRs -0.29 [95% CI -0.47 to -0.10]).

Qualifying Statements

- Patients with HER2/*neu*-positive breast cancer may derive more benefit from a more intense anthracycline regimen, in terms of dose or schedule, over a less intense one. Four of the identified studies (7,13,21-25) comparing more intense anthracycline-based regimens to less intense ones were identified that also performed a substudy analysis of HER2/*neu* status. Three of these studies (21,23-25) found a significant overall survival benefit for more intense anthracycline regimens versus less intense. A meta-analysis of these studies by HER2/*neu* status found a significant benefit in terms of DFS (HR 0.53, 95% CI 0.37 to 0.77) for patients with HER2/*neu*-positive breast cancer receiving more intense anthracycline-based chemotherapy. This meta-analysis found no benefit in HER2/*neu*-negative patients (HR 1.09). However, this analysis was found to be sensitive as to which of three different possible sets of hazard ratios were selected in one study (7,13). In that study, the analysis of time-to-progression was conducted using three different methods of HER2/*neu* testing, and the significance of the meta-analysis of the differences in log hazard ratio between the HER2/*neu* subgroups was significant or not significant depending on the choice of testing. Therefore, a firm recommendation was not possible, as absence of interaction could not be definitively rejected.
- The Breast Cancer DSG has produced two separate guidelines on trastuzumab systemic therapy, PG #1-15 (metastatic) and EBS #1-24 (adjuvant), described under “Related Guidelines” below. These guidelines provide important information regarding the use of trastuzumab and anthracyclines sequentially or in combination with regards to concerns about cardiac toxicity. Physicians are encouraged to review the recommendation and qualifying statements in light of the information provided in those guidelines if combination or sequential trastuzumab/anthracycline therapy is being considered.

Chemotherapy - Taxanes

The current evidence does not support a definitive recommendation regarding taxane chemotherapy and HER2/*neu* status.

Chemoendocrine Therapy

The current evidence does not support a definitive recommendation regarding chemoendocrine therapy and HER2/*neu* status.

Radiation Therapy

The current evidence does not support a definitive recommendation regarding radiation therapy and HER2/*neu* status.

Methods

Feedback was obtained through a mailed survey of 105 practitioners in Ontario, including 68 medical oncologists and 37 surgeons or radiation oncologists. The survey consisted of items evaluating the methods, results, and interpretive summary used to inform the draft recommendations and whether the draft recommendations should be approved as a practice guideline. Written comments were invited. The survey was mailed out on July 10, 2006. Follow-up reminders were sent at two weeks (post card) and four weeks (complete package mailed again). The authors reviewed the results of the survey.

Results

Fifty responses were received out of the 105 surveys sent (47.6% response rate). Responses include returned completed surveys as well as phone, fax, and email responses. Of the practitioners who responded, 40 indicated that the report was relevant to their clinical practice, and they completed the survey. Key results of the practitioner feedback survey are summarized in Table 1.

Table 1. Responses to eight items on the practitioner feedback survey.

Item	Number (%) ^A		
	Strongly agree or agree	Neither agree nor disagree	Strongly disagree or disagree
The rationale for developing a guideline, as stated in the "Introduction" section of the report, is clear.	34 (85%)	4 (10%)	1 (2.5%)
There is a need for a guideline on this topic.	32 (80%)	4 (10%)	2 (5%)
The literature search is relevant and complete.	35 (87.5%)	3 (7.5%)	0 (0%)
The results of the trials described in the report are interpreted according to my understanding of the data.	35 (87.5%)	1 (2.5%)	1 (2.5%)
The draft recommendations in the report are clear.	34 (85%)	2 (5%)	2 (5%)
I agree with the draft recommendations as stated.	30 (75%)	6 (15%)	2 (5%)
This report should be approved as a practice guideline.	26 (65%)	9 (22.5%)	3 (7.5%)
If this report were to become a practice guideline, how likely would you be to make use of it in your own practice?	Very likely or likely	Unsure	Not at all likely or unlikely
	28 (70%)	6 (15%)	4 (10%)

^A Percentages out of 40 total respondents that indicated the report was relevant to their clinical practice or who left that question blank (two respondents). Percentages may not sum to 100% due to missing data.

Summary of Written Comments

Eleven respondents (27.5%) provided written comments. Several comments reported inconsistencies or errors in the text of the document, which were corrected. In addition to those editorial comments, the main points of comments regarding the content of the evidence-based series are listed below, with the response from the authors:

1. Three respondents stated that a practice guideline on the issue of relationship of HER2/*neu* status to systemic therapy was premature; the evidence is not yet sufficient to warrant the creation of this Evidence-based series. RESPONSE: While the evidence is still insufficient to warrant recommendations on most of the questions contemplated in this evidence-based series, there is sufficient evidence to provide at least some clinical guidance on two. Moreover, there are two important products produced during the practice guideline development cycle, the clinical practice guideline and the systematic review of the available evidence. The systematic review is not in any way made less valuable by the fact that the identified evidence is not sufficient to produce the sort of clinical guidance one might hope to be able to provide. It is only possible to say with

certainty that the evidence is insufficient after the systematic review process is complete. No changes.

2. One respondent stated that the evidence in the document was sufficient to recommend aromatase inhibitors over tamoxifen in HER2/*neu*-positive patients. An additional respondent stated that lower quality evidence was available that also supported a recommendation of aromatase inhibitor over tamoxifen in this population, and should be included in the review as the randomized trial evidence was insufficient. RESPONSE: The authors did not feel that the quality of non-randomized data was sufficient to include the data in this systematic review. This systematic review is limited to phase III randomized controlled trials. The current data do not support a definitive recommendation of aromatase inhibitors over tamoxifen in HER2/*neu*-positive patients. However, two studies evaluating neoadjuvant use of aromatase inhibitors (the DUMC and IMPACT) did demonstrate higher response rates in HER2/*neu*-positive patients receiving an aromatase inhibitor as compared to tamoxifen. As such, aromatase inhibitors may be the preferred treatment in these patients in the neoadjuvant setting. No changes.
3. One respondent stated that the updated data from the NCIC CTG MA.5 trial (26) should be included. RESPONSE: This publication falls outside the time period of the literature search and, therefore, was not eligible for inclusion. It has been reviewed by the authors and was not expected to alter the conclusions of this evidence-based series. However, these updated results will be included in the updated version of this document submitted for peer-reviewed publication and will also be reflected in an update to this document that will be released with that submission.
4. Two respondents stated that the guideline provided no real guidance for practice, especially with regard to the treatment of HER2/*neu* negative patients. RESPONSE: The clinical guidance provided in this document cannot exceed what the evidence identified in the systematic review supports. At this time, there is simply insufficient evidence to provide to clinicians definitive guidance on a number of relevant clinical questions. No changes.
5. One respondent stated that additional explanation should be provided in the qualifying statement for the recommendation about anthracycline-based chemotherapy, regarding more versus less intense anthracycline regimens, as to what is meant by “more intense” and “less intense”. RESPONSE: Additional explanation was added to the qualifying statement.
6. Two respondents indicated a need for providing more context regarding the use of trastuzumab in relation to the recommendations provided in the document. RESPONSE: This review consists of studies conducted in the absence of trastuzumab therapy, as stated in the Introduction. However, two additional clinical practice guidelines are available that discuss the role of trastuzumab in the treatment of HER2/*neu*-positive breast cancer, PG #1-15 *The Role of Trastuzumab (Herceptin®) in the Treatment of Women with HER2/neu-overexpressing Metastatic Breast Cancer* and EBS #1-24 *The Role of Trastuzumab in Adjuvant and Neoadjuvant Therapy in Women with HER2/neu-overexpressing Breast Cancer*. As further evidence becomes available, both this document and the two related documents will be updated. No changes.
7. One respondent expressed concern regarding the meta-analytical methods used. The respondent stated that the conclusions were based on analysis of the subgroup hazard ratios, and not on a meta-analysis of the interaction effects in the individual trials. RESPONSE: The authors are not aware of any currently available meta-analytical techniques, other than individual patient data meta-analysis, that would allow for the interaction effects in the trials to be directly pooled. The authors have attempted to approach this problem, however, through the analysis of the differences in log hazard

ratios, which at least combine the subgroup hazard ratios into a single analysis. The conclusions in this evidence-based series are not based on the direct analysis of the subgroup hazard ratios but on the analysis of the differences. No changes.

Conclusion

This report reflects the integration of feedback obtained through the external review process with final approval given by the Breast Cancer DSG and the Report Approval Panel of the PEBC. Updates of the report will be conducted as new evidence informing the question of interest emerges.

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