

Classical Cyclophosphamide, Methotrexate, and Fluorouracil Chemotherapy Is More Effective in Triple-Negative, Node-Negative Breast Cancer: Results From Two Randomized Trials of Adjuvant Chemoendocrine Therapy for Node-Negative Breast Cancer

Marco Colleoni, Bernard F. Cole, Giuseppe Viale, Meredith M. Regan, Karen N. Price, Eugenio Maiorano, Mauro G. Mastropasqua, Diana Crivellari, Richard D. Gelber, Aron Goldhirsch, Alan S. Coates, and Barry A. Gusterson

ABSTRACT

Purpose

Retrospective studies suggest that primary breast cancers lacking estrogen receptor (ER) and progesterone receptor (PR) and not overexpressing human epidermal growth factor receptor 2 (HER2; triple-negative tumors) are particularly sensitive to DNA-damaging chemotherapy with alkylating agents.

Patients and Methods

Patients enrolled in International Breast Cancer Study Group Trials VIII and IX with node-negative, operable breast cancer and centrally assessed ER, PR, and HER2 were included (n = 2,257). The trials compared three or six courses of adjuvant classical cyclophosphamide, methotrexate, and fluorouracil (CMF) with or without endocrine therapy versus endocrine therapy alone. We explored patterns of recurrence by treatment according to three immunohistochemically defined tumor subtypes: triple negative, HER2 positive and endocrine receptor absent, and endocrine receptor present.

Results

Patients with triple-negative tumors (303 patients; 13%) were significantly more likely to have tumors > 2 cm and grade 3 compared with those in the HER2-positive, endocrine receptor-absent, and endocrine receptor-present subtypes. No clear chemotherapy benefit was observed in endocrine receptor-present disease (hazard ratio [HR], 0.90; 95% CI, 0.74 to 1.11). A statistically significantly greater benefit for chemotherapy versus no chemotherapy was observed in triple-negative breast cancer (HR, 0.46; 95% CI, 0.29 to 0.73; interaction $P = .009$ v endocrine receptor-present disease). The magnitude of the chemotherapy effect was lower in HER2-positive endocrine receptor-absent disease (HR, 0.58; 95% CI, 0.29 to 1.17; interaction $P = .24$ v endocrine receptor-present disease).

Conclusion

The magnitude of benefit of CMF chemotherapy is largest in patients with triple-negative, node-negative breast cancer.

J Clin Oncol 28:2966-2973. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Recommended principles for the choice of therapies in operable breast cancer include the recognition of diverse subtypes of breast cancer and the identification of particular targets based on genetic signature and immunohistochemistry.¹ Recent studies using DNA microarray profiling have led to classification of invasive breast cancer subgroups with common molecular features²⁻⁴ and the recogni-

tion that breast cancer is a heterogeneous entity.^{5,6} Several molecular subgroups have been proposed: human epidermal growth factor receptor 2 (HER2) –overexpressing/estrogen receptor (ER) –negative and progesterone receptor (PR) –negative tumors, basal-like (ER-, PR-, and HER2-negative disease), and luminal-like (ER and/or PR expression).^{3,4}

An immunohistochemical (IHC) profile based on the degree of expression of ER, PR, and HER2 similarly identifies subgroups of breast

From the Department of Medicine, Research Unit in Medical Senology, and Division of Pathology and Laboratory Medicine, European Institute of Oncology, Università degli Studi di Milano, Milan; Department of Pathological Anatomy, University of Bari, Bari; Centro di Riferimento Oncologico, Aviano, Italy; Department of Mathematics and Statistics, College of Engineering and Mathematical Sciences, University of Vermont, Burlington, VT; International Breast Cancer Study Group Statistical Center, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Frontier Science and Technology Research Foundation, Harvard School of Public Health, Boston, MA; Oncology Institute of Southern Switzerland, Bellinzona; International Breast Cancer Study Group, Bern, Switzerland; University of Sydney, Sydney, Australia; and Division of Cancer Sciences and Molecular Pathology, Faculty of Medicine, University of Glasgow, Glasgow, United Kingdom.

Submitted August 31, 2009; accepted February 18, 2010; published online ahead of print at www.jco.org on May 10, 2010.

Written on behalf of the International Breast Cancer Study Group.

Supported in part by the Swiss Group for Clinical Cancer Research; Cancer Research Switzerland/Oncosuisse; Frontier Science and Technology Research Foundation; The Cancer Council Australia; Australian New Zealand Breast Cancer Trials Group; Grant No. CA-75362 from the National Cancer Institute, National Institutes of Health, Bethesda, MD; Swedish Cancer Society; Foundation for Clinical Research of Eastern Switzerland; grants from the National Health and Medical Research Council of Australia; and Breakthrough Breast Cancer (B.A.G.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Marco Colleoni, MD, Research Unit in Medical Senology, Department of Medicine, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy; e-mail: marco.colleoni@ieo.it.

© 2010 by American Society of Clinical Oncology

0732-183X/10/2818-2966/\$20.00

DOI: 10.1200/JCO.2009.25.9549

cancer patients who will respond to different systemic neoadjuvant and adjuvant treatments.^{1,7} In the neoadjuvant setting, less benefit from the introduction of chemotherapy can be expected among patients whose tumors contain high levels of ER compared with those whose tumors are defined as endocrine receptor absent.^{8,9} Studies in the adjuvant setting in the node-positive population, showed that patients with ER-negative disease derived a greater benefit from modern improvements in chemotherapy regimens when compared with those with ER-positive disease.^{7,10} However, limited information is available in the adjuvant setting on the responsiveness to chemotherapy in the node-negative population.

The aim of this retrospective study was to evaluate the magnitude of the effect of adjuvant classical CMF (cyclophosphamide, methotrexate, and fluorouracil) chemotherapy in a large series of patients with node-negative breast cancer treated within the context of two adjuvant trials according to centrally reviewed IHC subtypes.

PATIENTS AND METHODS

Patients were enrolled in two randomized clinical trials conducted by the International Breast Cancer Study Group (IBCSG), which have been reported elsewhere.^{11,12} Briefly, between 1990 and 1999, IBCSG Trial VIII randomly assigned 1,109 assessable pre-/perimenopausal women with lymph node-negative breast cancer to sequential treatment with six 28-day courses of classical CMF chemotherapy followed by 18 monthly subcutaneous implants of goserelin, six 28-day courses of classical CMF alone, 24 monthly implants of goserelin alone, or no adjuvant treatment. The no-treatment arm was discontinued in 1992 after 46 patients had been assigned to that group. Tamoxifen was not prescribed. From 1988 to 1999, IBCSG Trial IX randomly assigned

1,669 eligible and assessable postmenopausal women with lymph node-negative breast cancer to sequential treatment with three 28-day courses of classical CMF chemotherapy followed by tamoxifen for 57 months or tamoxifen (20 mg/d) alone for 5 years. The primary end point was disease-free survival (DFS). Targeted anti-HER2 therapy was not used in these trials. The intention to perform separate analyses according to ER status was specified in the original protocol. In 1998, a protocol amendment for both trials restricted enrollment to patients with ER-positive tumors on the basis of evidence from other trials that ovarian ablation (in the case of Trial VIII) and tamoxifen alone (in the case of Trial IX) were not effective for patients with ER-negative tumors. Institutional review boards reviewed and approved the protocols, and informed consent was required according to the criteria established within the individual countries.

Central Pathology Review

Retrospective tissue collection was carried out in accordance with institutional guidelines and national laws. More than 80% of patients randomly assigned in Trials VIII and IX had archival tumor material available for review in the IBCSG Central Pathology Laboratory. Central pathology review was conducted without knowledge of patient treatment assignment or outcome. Expression of ER, PR, HER2, and Ki-67 labeling index (LI) in the primary tumors was determined by IHC.^{13,14} Ki-67 LI was assessed using mouse monoclonal antibody MIB-1 (1:200 dilution; Dako, Glostrup, Denmark); the percentage of cells that showed definite nuclear immunoreactivity with MIB-1 among 2,000 invasive neoplastic cells in randomly selected high-power fields ($\times 400$) at the periphery of the tumor was recorded.

Hormone receptor-absent status was defined as $< 1\%$ immunoreactive cells, in accordance with recent reports.^{1,7} Whole tumor sections were incubated with the specific primary mouse monoclonal antibodies to ER (clone 1D5; 1:100 dilution) or PR (clone 1A6; 1:800 dilution) (both from Dako). HER2 immunoreactivity was assessed using a HercepTest kit (Dako), as recommended by the manufacturer, and was scored for the intensity of immunostaining, the completeness of cell membrane staining, and the percentage of

Table 1. Patient and Disease Characteristics

Characteristic	Triple Negative (n = 303)		HER2 Positive, Endocrine Receptor Absent (n = 119)		Endocrine Receptor Present (n = 1,835)		P*	Not Included† (n = 521)	
	No.	%	No.	%	No.	%		No.	%
Menopausal status									
Premenopausal (IBCSG Trial VIII)	136	45	39	33	737	40	.07	195	37
Postmenopausal (IBCSG Trial IX)	167	55	80	67	1,098	60		326	63
Tumor size, cm									
≤ 2	127	42	60	50	1,164	63	< .001	336	64
> 2	173	57	58	49	648	35		175	34
Unknown	3	1	1	1	3	1		10	2
Tumor grade									
1	5	2	2	2	363	20	< .001	129	25
2	62	20	35	30	926	51		209	40
3	236	78	78	66	538	29		164	31
Unknown	0	0	4	3	8	0		19	4
Peritumoral vascular invasion									
Yes	233	77	87	73	1,447	79	.24	393	75
No	44	15	24	20	280	15		70	13
Unknown	26	9	8	7	108	6		58	11
Age, years (mean ± SD)	52.7 ± 10.1		55.0 ± 10.0		54.3 ± 10.0		.03	54.1 ± 10.1	
IBCSG Trial VIII	43.6 ± 5.7		44.2 ± 6.4		44.4 ± 5.4		.28	43.9 ± 5.9	
IBCSG Trial IX	60.1 ± 5.9		60.3 ± 6.4		60.9 ± 6.2		.38	60.2 ± 6.5	

Abbreviations: HER2, human epidermal growth factor receptor 2; IBCSG, International Breast Cancer Study Group; SD, standard deviation.

*P values compare the three tumor subtypes and are based on Fisher's exact test (menopausal status, tumor size, and peritumoral vascular invasion), the χ^2 test (tumor grade), and the Kruskal-Wallis test (age).

†Central review of estrogen receptor, progesterone receptor, and HER2 not available.

immunoreactive neoplastic cells by using a four-tier scale from 0 to 3+, as previously described.¹⁴ HER2-negative status was defined as immunostaining levels 0, 1+, and 2+, and HER2 overexpression was defined as intense and complete membrane staining of > 10% of the tumor cells.

Fluorescent in situ hybridization testing was not performed because of inadequate tumor sections. A patient's tumor was considered triple negative if ER and PR were both absent and HER2 status was negative. The tumor was classified as HER2 positive, endocrine receptor absent if HER2 was overexpressed and ER and PR were both absent, and as endocrine receptor present if either ER or PR or both were present. Patients with missing information regarding ER, PR, and HER2 status were excluded from this analysis (n = 521).

Statistical Analysis

The χ^2 test and Fisher's exact test¹⁵ were used to evaluate associations between tumor subtype and menopausal status (trial), tumor size, tumor grade, and vessel invasion. The Kruskal-Wallis test¹⁶ was used to evaluate associations with age categories.

DFS was defined as the time from random assignment to the first failure (including relapse, second primary, or death). Time to relapse was defined as the time from random assignment to any breast cancer relapse (local, regional, or distant, including contralateral breast events). Overall survival (OS) was defined as the time from random assignment to death from any cause. Patients who were event free at the time of last follow-up were censored at that time.

Time to relapse was evaluated using cumulative incidence analysis and competing-risks regression analysis.¹⁷ For these analyses, second non-breast primaries and deaths without relapse were treated as competing events. Tumor subtype status was evaluated as a treatment-effect modifier after adjustment for other prognostic factors. The treatment comparison was chemotherapy versus no chemotherapy.

Separate models were run according to menopausal status (defined by trial population). Model-based hazard ratios (HRs) and 95% CIs for the chemotherapy treatment effect were estimated according to tumor subtype. Wald tests were used to determine the statistical significance of the interaction effect of treatment group and tumor subtype. DFS and OS were estimated using the product-limit method.¹⁸ The log-rank test¹⁹ was used to compare treatment groups in terms of DFS and OS.

RESULTS

This analysis was conducted on the 2,257 patients for whom IHC-defined tumor subtype was available (82.6% of the patients included in Trials VIII and IX). Baseline characteristic of patients with centrally assessed ER, PR, and HER2 and those of patients not included in the analysis due to unavailability of central review were comparable (Table 1), thus minimizing potential selection bias. A total of 303 patients (13%) had triple-negative breast cancer, 119 (5%) had HER2-positive, endocrine receptor-absent disease, and 1,835 (81%) had endocrine receptor-present disease. A total of 1,255 women (56%) were randomly assigned to receive chemotherapy, and the remaining 1,002 (44%) were assigned to no chemotherapy. The median follow-up was 11 years (maximum: 18.6 years).

Tumors larger than 2 cm and grade 3 tumors were more commonly observed among patients with triple-negative disease, followed by those with HER2-positive, endocrine receptor-absent disease, and those with endocrine receptor-present disease, respectively (57% v 49% v 35%; $P < .001$; tumor > 2 cm and 78% v 68% v 29%; $P < .001$; grade 3).

Table 2 shows the numbers of relapse events available for each subtype along with 10-year cumulative incidence rates. The cumulative incidence rates in patients with endocrine receptor-present disease were similar in those patients who received chemotherapy when compared with those allocated no chemotherapy (19% v 20%). By

Table 2. Cumulative Incidence Estimates of Relapse According to Tumor Subtype, Menopausal Status, and Treatment Group

Treatment and Subtype	No. of Patients	No. of Relapse Events	10-Year Cumulative Incidence of Relapse \pm SE
All Patients			
Triple negative			
No CMF	133	49	36 \pm 4
CMF	170	38	21 \pm 3
HER2 positive, endocrine receptor absent			
No CMF	54	22	41 \pm 7
CMF	65	17	27 \pm 6
Endocrine receptor present			
No CMF	815	171	20 \pm 1
CMF	1,020	203	19 \pm 1
Premenopausal patients (IBCSG Trial VIII)			
Triple negative			
No CMF	49	21	39 \pm 7
CMF	87	25	27 \pm 5
HER2 positive, endocrine receptor absent			
No CMF	12	6	50 \pm 15
CMF	27	11	43 \pm 10
Endocrine receptor present			
No CMF	263	69	27 \pm 3
CMF	474	100	21 \pm 2
Postmenopausal patients (IBCSG Trial IX)			
Triple negative			
No CMF	84	28	34 \pm 5
CMF	83	13	15 \pm 4
HER2 positive, endocrine receptor absent			
No CMF	42	16	38 \pm 8
CMF	38	6	16 \pm 6
Endocrine receptor present			
No CMF	552	102	17 \pm 2
CMF	546	103	18 \pm 2

Abbreviations: HER2, human epidermal growth factor receptor 2; IBCSG, International Breast Cancer Study Group; CMF, cyclophosphamide, methotrexate, and fluorouracil.

contrast, a large difference in terms of incidence of events for chemotherapy versus no chemotherapy was observed in patients with triple-negative (21% v 36%) or HER2-positive, endocrine receptor-absent subtypes (27% v 41%). Table 3 shows the results of multivariable competing-risk regression analyses predicting time to relapse. No clear effect was observed in the endocrine receptor-present subtype (HR, 0.90; 95% CI, 0.74 to 1.11). The magnitude of the chemotherapy effect was low both in patients with HER2-positive, endocrine receptor-present disease (220 patients [10%]; HR, 0.73; 95% CI, 0.42 to 1.25) and in those with HER2-negative, endocrine receptor-present disease (1,576 patients [71%]; HR, 0.93; 95% CI, 0.75 to 1.16). In contrast, a significant reduction in the HR for chemotherapy versus no chemotherapy was observed for the triple-negative subtype (HR, 0.46; 95% CI, 0.29 to 0.73; interaction $P = .009$ relative to endocrine receptor-present subtype). A smaller effect was observed for the HER2-positive, endocrine receptor-absent subtype (HR, 0.58; 95% CI, 0.29 to 1.17; interaction $P = .24$ relative to endocrine receptor-present subtype).

Table 3. Group-Specific Treatment HRs for Relapse Based on Competing-Risks Regression

Tumor Subtype	HR (CMF v No CMF)	95% CI	<i>P</i> *	Overall Interaction <i>P</i> †
All patients				
Triple negative	0.46	0.29 to 0.73	.009	.022
HER2 positive, endocrine receptor absent	0.58	0.29 to 1.17	.24	
Endocrine receptor present	0.90	0.74 to 1.11	—	
Premenopausal patients (IBCSG Trial VIII)				
Triple negative	0.51	0.27 to 0.98	.22	.47
HER2 positive, endocrine receptor absent	0.68	0.22 to 2.14	.79	
Endocrine receptor present	0.80	0.59 to 1.08	—	
Postmenopausal patients (IBCSG Trial IX)				
Triple negative	0.38	0.19 to 0.76	.01	.0091
HER2 positive, endocrine receptor absent	0.37	0.13 to 1.03	.06	
Endocrine receptor present	1.01	0.77 to 1.33	—	

NOTE. The treatment comparison was CMF (cyclophosphamide, methotrexate, and fluorouracil) chemotherapy v no CMF chemotherapy. Hazard ratio (HR) estimates are adjusted for peritumoral vascular invasion, tumor size, and tumor grade. For the “all patients” comparison, the HRs are also adjusted for menopausal status.

Abbreviations: HER2, human epidermal growth factor receptor 2; IBCSG, International Breast Cancer Study Group.

*All *P* values are interaction *P* values that assess heterogeneity of HRs relative to the HR in the endocrine receptor–present group.

†Overall interaction *P* value assesses heterogeneity across all three groups.

We conducted an exploratory analysis evaluating the magnitude of the chemotherapy effect in endocrine receptor–present disease and either HER2 positive and/or Ki-67 high ($\geq 14\%$; 517 patients), and in endocrine receptor–present disease and not HER2 positive or Ki-67 high (1,028 patients), in accordance with recently reported data.⁷ The magnitude of the chemotherapy effect was not significant in both groups (HR, 0.96; 95% CI, 0.74 to 1.23 and HR, 0.83; 95% CI, 0.53 to 1.30, respectively).

We also looked at relative risk reduction with chemotherapy in the HER2-negative group ($n = 2,046$) versus the HER2-positive group ($n = 361$). The respective HRs for chemotherapy versus no chemotherapy (competing-risks regression analysis after adjustment for peritumoral vascular invasion, tumor size, tumor grade, and menopausal status) were 0.77 (95% CI, 0.64 to 0.93) and 0.70 (95% CI, 0.47 to 1.06). No significant interaction was detected between treatment and HER2 status ($P = .68$).

Figures 1 and 2 illustrate the estimated subtype-specific cumulative incidence of relapse over time according to treatment group for all patients (Fig 1) and for premenopausal patients (Figs 2A through 2C) and postmenopausal patients (Figs 2D through 2F). DFS and OS for each subtype according to treatment are shown in Figure 3. Chemotherapy significantly improved 10-year DFS in the triple-negative subtype (73% v 57%; $P = .007$), whereas no effect was observed in the endocrine receptor–present subtype (74% v 71%; $P = .25$).

Our analysis defined ER and PR according to absent or present, using at least 1% of stained cells as the cutoff for hormone receptor present. We also reanalyzed using the more arbitrary but often

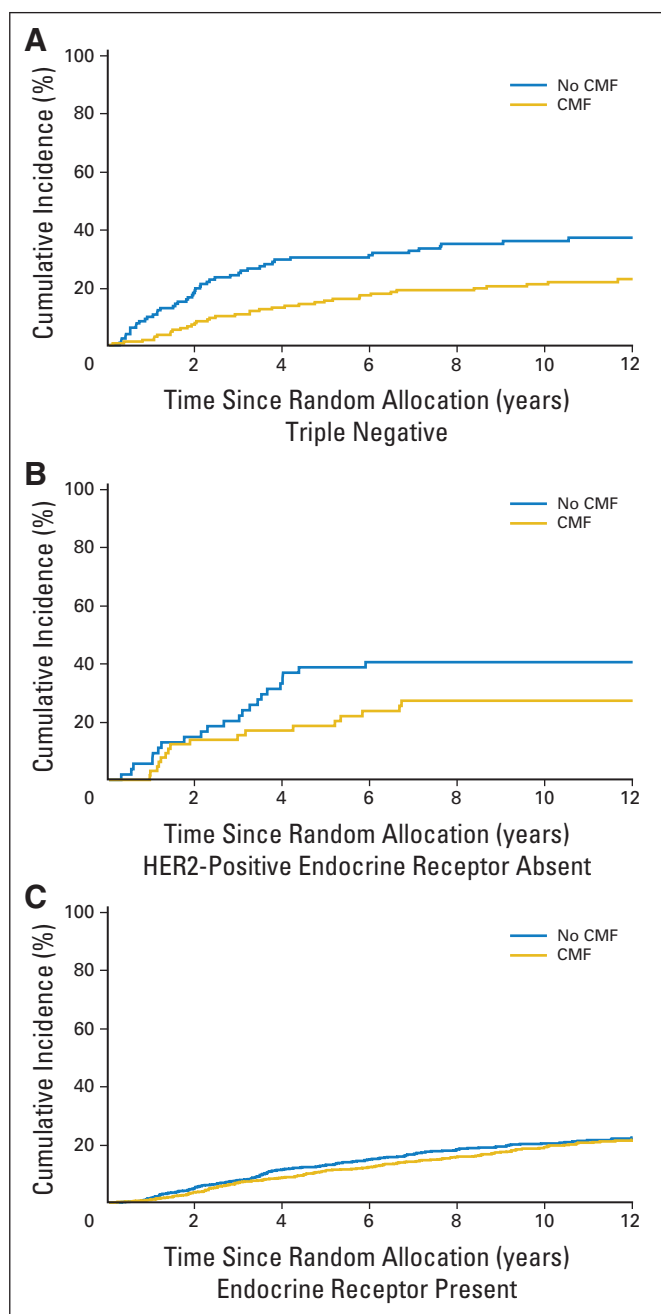


Fig 1. Estimated subtype-specific cumulative incidence of relapse over time according to treatment group (CMF [cyclophosphamide, methotrexate, and fluorouracil] chemotherapy v no CMF chemotherapy) for patients with (A) triple-negative, (B) human epidermal growth factor receptor 2 (HER2)–positive, endocrine receptor–absent, or (C) endocrine receptor–present tumors.

used 10% cutoff to define hormone receptor positive ($\geq 10\%$) and negative ($< 10\%$). The results were similar and are included in the Appendix, in Appendix Tables A1–A4 (online only), and Appendix Figures A1–A3 (online only).

DISCUSSION

Recognition of factors predictive of response has become crucial for understanding treatment effects in the adjuvant treatment of breast

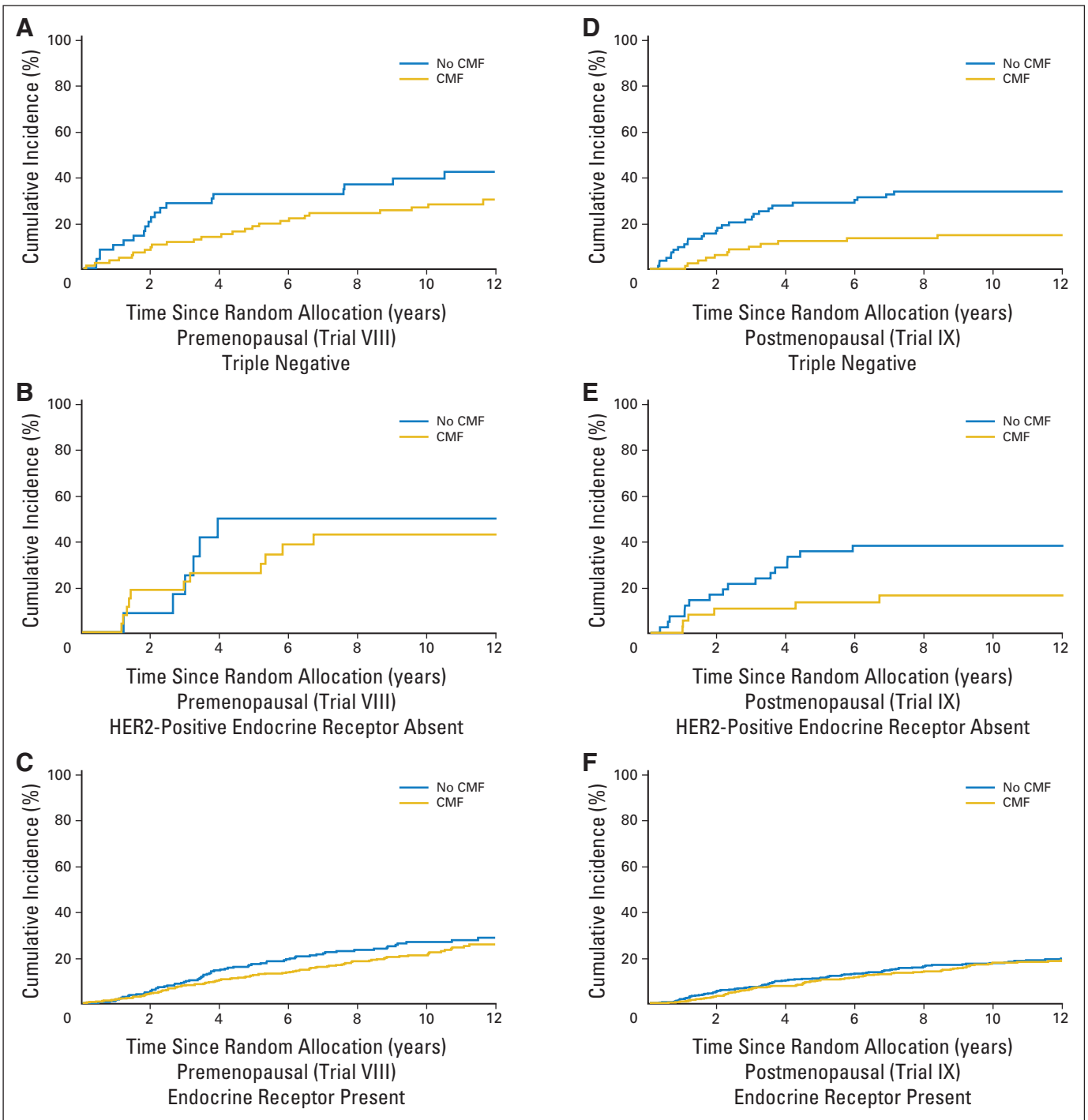


Fig 2. Estimated subtype-specific cumulative incidence of relapse over time according to treatment group (CMF [cyclophosphamide, methotrexate, and fluorouracil] chemotherapy v no CMF chemotherapy) for premenopausal patients in International Breast Cancer Study Group (IBCSG) Trial VIII with (A) triple-negative tumors, (B) human epidermal growth factor receptor 2 (HER2) –positive, endocrine receptor–absent tumors, or (C) endocrine receptor–present tumors and for postmenopausal patients in IBCSG Trial IX with (D) triple-negative tumors, (E) HER2-positive, endocrine receptor–absent tumors, or (F) endocrine receptor–present tumors.

cancer. Recent developments include increasing attention to predictive factors and to systemic therapies prescribed in homogeneous groups of patients according to the higher chance of response.¹

Intrinsically different subtypes of breast cancer have been identified on the basis of genetic profile and IHC determination of selected targets.^{3,7,20} Given the paramount importance of using targeted ther-

apies wherever possible for adjuvant treatment, timely, accurate, and reliable histopathologic assessment that identifies and quantifies the target is essential.

Emerging experimental and clinical studies have shown that steroid hormone receptor expression and epidermal growth factor receptor (EGFR; mainly HER2) pathways, alone or in combination, play

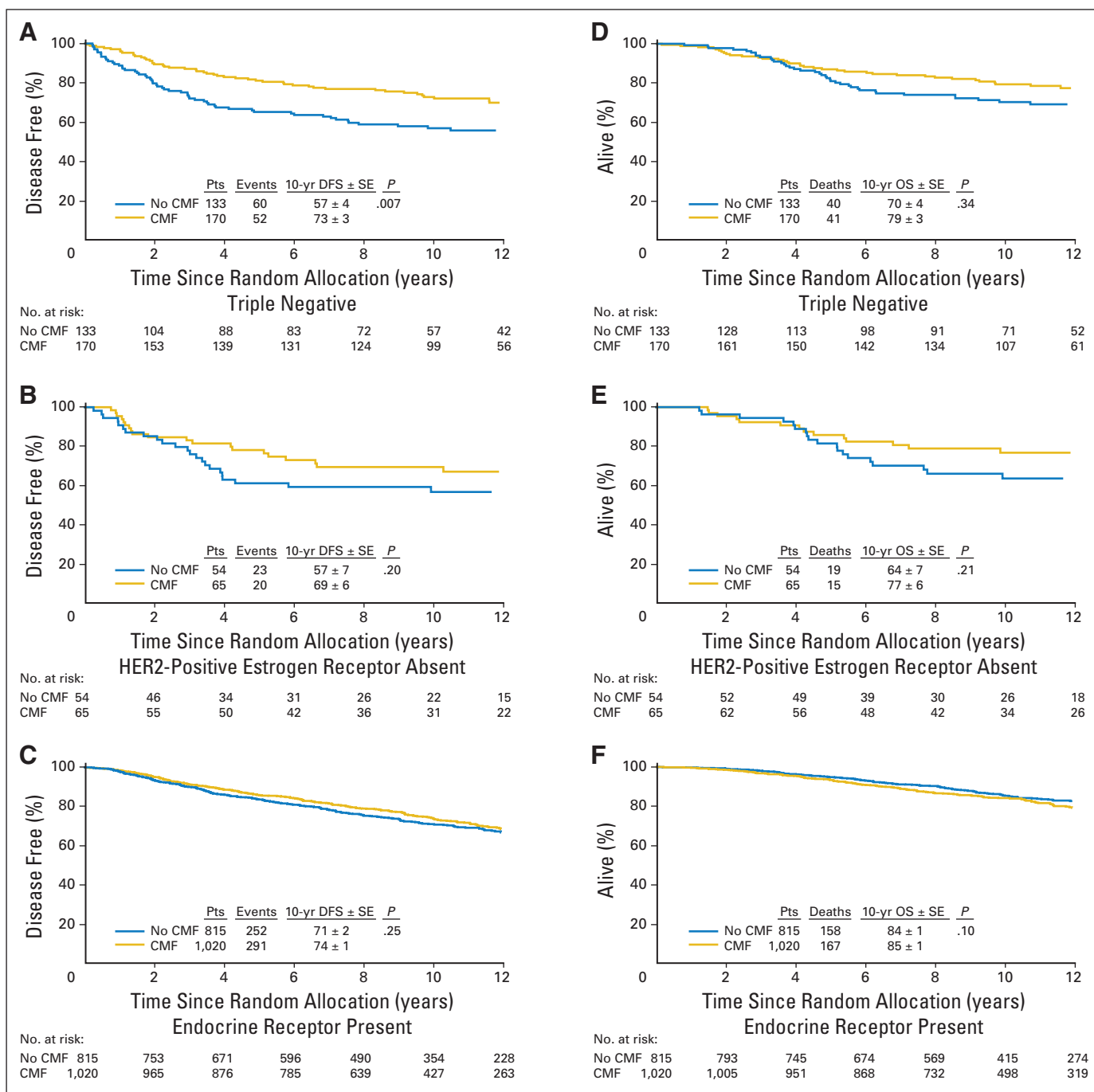


Fig 3. Disease-free survival (DFS) and overall survival (OS) comparing patients assigned to CMF (cyclophosphamide, methotrexate, and fluorouracil) chemotherapy with patients assigned to no CMF chemotherapy with triple-negative (A,D), human epidermal growth factor receptor 2 (HER2) –positive, endocrine receptor–absent (B,E), and endocrine receptor–present (C,F) subtypes. Pts, patients; yr, year.

key roles in the response to therapy in early breast cancer.²¹⁻²³ We therefore present our results to explore the question of chemotherapy responsiveness in early breast cancer, focused on tumor subtypes defined according to accepted cutoffs for ER, PR, and HER2 that have known clinical relevance and are used in regular clinical practice for treatment decision making.

This study provides useful insights into the treatment of breast cancer because it is based on a large population of patients with

node-negative breast cancer treated within the context of randomized clinical trials with standardized treatments and follow-up. The longer (> 10 years) median follow-up is particularly important in a population of node-negative patients. Moreover, a central pathology review using modern procedures gave a consistent and accurate assessment of the biologic features of primary breast cancers.

For several years, the correlation between the effects of adjuvant chemotherapy and degree of steroid hormone expression was studied,

but the interpretation of these results remains controversial.^{24,25} The association between endocrine-responsiveness and outcome may be confounded in retrospective analyses by the large variation in chemotherapy regimens, patient features, methods, and cutoffs used for the determination of hormone receptors.

Recently published data from retrospective analyses of trial data from Cancer and Leukemia Group B²⁶ found that the additional benefit of adding a taxane to adjuvant chemotherapy was not seen in patients with HER2-negative, ER-positive tumors, while the US Breast Cancer Intergroup review of 6,644 node-positive breast cancer patients who received adjuvant chemotherapy supports the value of extensive modern adjuvant chemotherapy in patients with tumors classified as ER negative.¹⁰ More recently, other authors reported that patients with ER-negative tumors (both triple negative and HER2 positive) showed a better response to taxane-containing chemotherapy than to chemotherapy that does not contain taxane,⁷ again supporting a possible increased chemosensitivity of those tumors that did not express steroid hormone receptors.

The results of this study are in line with these findings, supporting a larger effect for a particular chemotherapy regimen (classical CMF) in a specific tumor subtype: triple negative. However, this study differs from others already reported. Only a minority of published studies have a median follow-up exceeding 5 years, while we report results after a median of 11 years of follow-up. Such a prolonged follow-up is particularly important for the assessment of delayed events seen among patients with endocrine-responsive disease.²⁷ Moreover, the present study focuses on a node-negative population, which has a lower metastatic potential. Finally, our analysis evaluates breast cancer subtype as a treatment-effect modifier for a comparison of chemotherapy versus no chemotherapy, an approach which is not commonly reported in the literature.

A potential limitation of this study might be related to the regimen most commonly used in the past (ie, classical CMF) and its duration (three courses in the postmenopausal population). However, there is evidence that CMF chemotherapy is not inferior to anthracycline-containing therapy in patients with HER2-negative breast cancer.²⁸ In particular, in a study that compared two regimens with the same schedule and duration—cyclophosphamide, epirubicin, and fluorouracil (CEF) and classical CMF—patients whose tumors do not amplify or overexpress HER2 received virtually no benefit from CEF compared with CMF.^{29,30} More recently, a retrospective analysis from a randomized trial supports the hypothesis that anthracycline-containing adjuvant chemotherapy regimens may be inferior to adjuvant classical CMF in women with a core basal phenotype (negative for hormone receptors and HER2 and positive for CK5/6 or EGFR).³⁰ In particular, in the anthracycline-containing chemotherapy (CEF) arm, patients with core basal tumors had worse survival outcome (HR, 1.8; log-rank $P = .02$) relative to the other biologic subtypes. Conversely, in the classical CMF arm, there was no significant difference (HR, 0.9; $P = .7$). The majority of the above-mentioned trials evaluated the magnitude of the effect of anthracycline-containing chemotherapy in a node-positive population. In this study, we showed that classical CMF chemotherapy significantly improved treatment outcome in a specific patient population characterized by a lower potential for metastatic disease such as a node-negative population.

Although no direct comparison with other regimens is available within this study, the impressive magnitude of the observed effect

supports the hypothesis that the classical CMF regimen, originally formulated on the basis of the highly effective MOPP (mechlorethamine, vincristine, procarbazine, prednisone) regimen, may still represent a reasonable choice of treatment in selected patients. Moreover, long-term results and other information available to date show that the classical CMF regimen is safe³¹ and has little long-term toxicity.³² Whether newer forms of chemotherapy may be beneficial in this group of patients is one of the research priorities in this field.

There are several potential explanations for the different responses to chemotherapy according to tumor subtype. The triple-negative subtype is characterized by high expression of the proliferation cluster of genes.³ A higher proliferative index as measured by Ki-67 LI expression has been observed in this subtype compared with the endocrine-responsive subtype.³³ Potential targets for chemotherapeutic agents are present in triple-negative tumors, the majority of which overexpress EGFR and endothelial growth factors,³⁴ though the response to agents targeting EGFR in breast cancer has been disappointing.³⁵ However, *in vitro* chemosensitivity studies have found that human cells lacking BRCA1, and to some extent other triple-negative cells, may be sensitive to drugs that cause double-strand breaks in DNA³⁶ such as alkylating agents. Tumors with a high proliferation rate may also be particularly sensitive to the antimetabolites used in the CMF regimen.

The question of duration of adjuvant chemotherapy for breast cancer has been directly addressed in several trials.³⁷⁻³⁹ Most of these were small and therefore unsuitable for detecting differences of even modest magnitude. Furthermore, results in the node-negative population are lacking.

It should be emphasized that the tumor subtypes identified in this analysis include heterogeneous groups of tumors, and that the identification of further tumor subtypes amenable to targeted treatments represents a research priority.⁴⁰ In this study, we explored the role of HER2 overexpression and Ki-67 expression in the definition of subgroups within the luminal population, as recently reported,⁷ but we were not able to identify subgroups with different responsiveness to chemotherapy. Possible reasons for these results include the low number of events registered in studies focusing on a node-negative population.

In conclusion, this study confirms that the efficacy of adjuvant systemic therapy for early breast cancer depends on variable features, including those of the tumor, the patient, and the treatment itself. We demonstrated that the magnitude of the effect of chemotherapy in early breast cancer significantly correlates with tumor subtypes identified by IHC. The results of this study provide substantial additional evidence to support the concept that further progress in the adjuvant treatment of breast cancer will require studies dedicated to specific niches of patients selected through the identification of treatment targets.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Marco Colleoni, Giuseppe Viale, Aron Goldhirsch, Barry A. Gusterson

Administrative support: Karen N. Price, Aron Goldhirsch

Provision of study materials or patients: Marco Colleoni, Giuseppe Viale, Eugenio Maiorano, Mauro G. Mastropasqua, Diana Crivellari, Aron Goldhirsch, Alan S. Coates, Barry A. Gusterson

Collection and assembly of data: Meredith M. Regan, Diana Crivellari, Barry A. Gusterson

Data analysis and interpretation: Marco Colleoni, Bernard F. Cole, Meredith M. Regan, Richard D. Gelber, Barry A. Gusterson

Manuscript writing: Marco Colleoni, Bernard F. Cole, Giuseppe Viale, Meredith M. Regan, Karen N. Price, Diana Crivellari, Alan S. Coates, Barry A. Gusterson

Final approval of manuscript: Marco Colleoni, Bernard F. Cole, Giuseppe Viale, Meredith M. Regan, Karen N. Price, Eugenio Maiorano, Mauro G. Mastropasqua, Diana Crivellari, Richard D. Gelber, Aron Goldhirsch, Alan S. Coates, Barry A. Gusterson

REFERENCES

- Goldhirsch A, Ingle JN, Gelber RD, et al: Thresholds for therapies: Highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer. *Ann Oncol* 20:1319-1329, 2009
- Perou CM, Sorlie T, Eisen MB, et al: Molecular portraits of human breast tumours. *Nature* 406:747-752, 2000
- Sorlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 98:10869-10874, 2001
- Sorlie T, Tibshirani R, Parker J, et al: Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 100:8418-8423, 2003
- Yehiely F, Moyano JV, Evans JR, et al: Deconstructing the molecular portrait of basal-like breast cancer. *Trends Mol Med* 12:537-544, 2006
- Rakha EA, El-Sayed ME, Green AR, et al: Prognostic markers in triple-negative breast cancer. *Cancer* 109:25-32, 2007
- Hugh J, Hanson J, Cheang MC, et al: Breast cancer subtypes and response to docetaxel in node-positive breast cancer: Use of an immunohistochemical definition in the BCIRG 001 trial. *J Clin Oncol* 27:1168-1176, 2009
- Colleoni M, Viale G, Zahrieh D, et al: Expression of ER, PgR, HER1, HER2, and response: A study of preoperative chemotherapy. *Ann Oncol* 19:465-472, 2008
- Kaufmann M, Hortobagyi GN, Goldhirsch A, et al: Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: An update. *J Clin Oncol* 24:1940-1949, 2006
- Berry DA, Cirincione C, Henderson IC, et al: Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. *JAMA* 295:1658-1667, 2006
- International Breast Cancer Study Group (IBCSG): Endocrine responsiveness and tailoring adjuvant therapy for postmenopausal lymph node-negative breast cancer: A randomized trial. *J Natl Cancer Inst* 94:1054-1065, 2002
- International Breast Cancer Study Group, Castiglione-Gertsch M, O'Neill A, et al: Adjuvant chemotherapy followed by goserelin versus either modality alone for premenopausal lymph node-negative breast cancer: A randomized trial. *J Natl Cancer Inst* 95:1833-1846, 2003
- Viale G, Regan MM, Maiorano E, et al: Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: Predictive value of centrally reviewed expression of estrogen and progesterone receptors—International Breast Cancer Study Group. *J Clin Oncol* 26:1404-1410, 2008
- Viale G, Regan MM, Mastropasqua MG, et al: Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. *J Natl Cancer Inst* 100:207-212, 2008
- Fisher RA: The logic of inductive inference. *J R Stat Soc* 98:39-84, 1935
- Kruskal WH, Wallis WA: Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 47:583-621, 1952
- Fine JP, Gray RJ: A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 94:496-509, 1999
- Kaplan EL, Meier P: Nonparametric estimation from incomplete observation. *J Am Stat Assoc* 53:457-481, 1958
- Peto R, Pike MC, Armitage P, et al: Design and analysis of randomized clinical trials requiring prolonged observation of each patient: II. Analysis and examples. *Br J Cancer* 35:1-39, 1977
- Regan MM, Viale G, Mastropasqua MG, et al: Re-evaluating adjuvant breast cancer trials: Assessing hormone receptor status by immunohistochemical versus extraction assays. *J Natl Cancer Inst* 98:1571-1581, 2006
- Barrett-Lee PJ: Growth factor signalling in clinical breast cancer and its impact on response to conventional therapies: A review of chemotherapy. *Endocr Relat Cancer* 12:S125-S133, 2005 (suppl 1)
- Arpino G, Weiss H, Lee AV, et al: Estrogen receptor-positive, progesterone receptor-negative breast cancer: Association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst* 97:1254-1261, 2005
- Guarneri V, Broglio K, Kau SW, et al: Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. *J Clin Oncol* 24:1037-1044, 2006
- Roché H, Fumoleau P, Spielmann M, et al: Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: The FNCLCC PACS 01 Trial. *J Clin Oncol* 24:5664-5671, 2006
- Martin M, Pienkowski T, Mackey J, et al: Adjuvant docetaxel for node-positive breast cancer. *N Engl J Med* 352:2302-2313, 2005
- Hayes DF, Thor AD, Dressler LG, et al: HER2 and response to paclitaxel in node-positive breast cancer. *N Engl J Med* 357:1496-1506, 2007
- Saphner T, Tormey DC, Gray R: Annual hazard rates of recurrence for breast cancer after primary therapy. *J Clin Oncol* 14:2738-2746, 1996
- Gennari A, Sormani MP, Pronzato P, et al: HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: A pooled analysis of randomized trials. *J Natl Cancer Inst* 100:14-20, 2008
- Pritchard KI, Shepherd LE, O'Malley FP, et al: HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med* 354:2103-2111, 2006
- Cheang MC, Chia SK, Tu D, et al: Anthracyclines in basal breast cancer: The NCIC-CTG trial MA5 comparing adjuvant CMF to CEF. *J Clin Oncol* 27:11s, 2009 (suppl; abstr 519)
- Goldhirsch A, Coates AS, Colleoni M, et al: Adjuvant chemoendocrine therapy in postmenopausal breast cancer: Cyclophosphamide, methotrexate, and fluorouracil dose and schedule may make a difference—International Breast Cancer Study Group. *J Clin Oncol* 16:1358-1362, 1998
- Colleoni M, Price KN, Castiglione-Gertsch M, et al: Mortality during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluorouracil—International Breast Cancer Study Group. *Lancet* 354:130-131, 1999
- Urruticoechea A, Smith IE, Dowsett M: Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol* 23:7212-7220, 2005
- Viale G, Rotmensz N, Maisonneuve P, et al: Invasive ductal carcinoma of the breast with the “triple-negative” phenotype: Prognostic implications of EGFR immunoreactivity. *Breast Cancer Res Treat* 116:317-328, 2009
- Gusterson BA, Hunter KD: Should we be surprised at the paucity of response to EGFR inhibitors? *Lancet Oncol* 10:522-527, 2009
- James CR, Quinn JE, Mullan PB, et al: BRCA1, a potential predictive biomarker in the treatment of breast cancer. *Oncologist* 12:142-150, 2007
- International Breast Cancer Study Group: Duration and reintroduction of adjuvant chemotherapy for node-positive premenopausal breast cancer patients. *J Clin Oncol* 14:1885-1894, 1996
- Sauerbrei W, Bastert G, Bojar H, et al: Randomized 2 x 2 trial evaluating hormonal treatment and the duration of chemotherapy in node-positive breast cancer patients: An update based on 10 years' follow-up—German Breast Cancer Study Group. *J Clin Oncol* 18:94-101, 2000
- Colleoni M, Gelber S, Simoncini E, et al: Effects of a treatment gap during adjuvant chemotherapy in node-positive breast cancer: Results of International Breast Cancer Study Group (IBCSG) Trials 13-93 and 14-93. *Ann Oncol* 18:1177-1184, 2007
- Gusterson B: Do ‘basal-like’ breast cancers really exist? *Nat Rev Cancer* 9:128-134, 2009