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ORIGINAL REPORT

C-MYC Alterations and Association With Patient Outcome in Early-Stage HER2-Positive Breast Cancer From the North Central Cancer Treatment Group N9831 Adjuvant Trastuzumab Trial

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ABSTRACT

Purpose

Findings from the human epidermal growth factor receptor 2 (HER2) –positive National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 trial suggested that *MYC/HER2* coamplification (> 5.0 copies/nucleus) was associated with additional benefit from adjuvant trastuzumab in patients with early-stage breast cancer. To further explore this relationship, we investigated associations between *MYC* amplification and disease-free survival (DFS) in a similar adjuvant trastuzumab HER2-positive breast cancer trial—North Central Cancer Treatment Group (NCCTG) N9831.

Patients and Methods

This analysis included 799 patients randomly assigned to receive chemotherapy alone or with concurrent trastuzumab on N9831. Fluorescence in situ hybridization (FISH) was performed by using a dual-probe mixture for *MYC* and centromere 8 (*MYC*:CEP8) on tissue microarrays. *MYC* amplification was prespecified as *MYC*:CEP8 ratio > 2.2 or average *MYC* copies/nucleus > 5.0. Exploratory variables included polysomy 8.

Results

In comparing DFS (median follow-up, 4.0 years) between treatments, patients with *MYC*:CEP8 ratio ≤ 2.2 (n = 618; 77%) and > 2.2 (n = 181; 23%) had hazard ratios (HRs) of 0.46 ($P < .001$) and 0.67 ($P = .33$), respectively (interaction $P = .38$). Patients with *MYC* copies/nucleus ≤ 5.0 (n = 534; 67%) and > 5.0 (n = 265; 33%) had HRs of 0.52 ($P = .002$) and 0.48 ($P = .02$), respectively (interaction $P = .94$). Patients with *MYC*:CEP8 ratio < 1.3 with normal chromosome 8 copy number (n = 141; 18%) and ≥ 1.3 or < 1.3 with polysomy 8 (n = 658; 82%) had HRs of 0.66 ($P = .28$) and 0.44 ($P < .001$), respectively (interaction $P = .23$). Patients with *MYC* copies/nucleus < 2.5 (n = 130; 16%) and ≥ 2.5 (n = 669; 84%) had HRs of 1.07 ($P = .87$) and 0.42 ($P < .001$), respectively (interaction $P = .05$).

Conclusion

We did not confirm the B31 association between *MYC* amplification and additional trastuzumab benefit. Exploratory analyses revealed potential associations between alternative *MYC*/chromosome 8 copy number alterations and differential benefit of adjuvant trastuzumab.

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INTRODUCTION

The combination of chemotherapy and trastuzumab significantly prolongs survival of patients with breast cancer with human epidermal growth factor 2 (HER2) –positive tumors in adjuvant and metastatic settings.[1-4] However, many women who receive trastuzumab develop resistance within 1 year, and 15% to 25% of women diagnosed with HER2-positive, early-stage disease develop tumor relapse within 3 years, despite therapy.[5] Thus, identifying patients who would respond best to trastuzumab is critical to the appropriate management of patients with HER2-positive breast cancer.

Copy number anomalies of *c-myc* (*MYC*) have been reported to be potential predictors of response

to HER2-targeted therapies.[2,6,7] *MYC*, located on chromosome 8, is a proto-oncogene with a central role in proliferation and malignant transformation of human and animal cells.[8] Its protein product (MYC) is a transcription factor that critically participates in most aspects of normal cellular function, including replication, proliferation, metabolism, differentiation, and apoptosis.[8,9] Because aberrations of MYC play a key role in malignant transformation, most types of malignancies in humans have been reported to have amplification and/or overexpression of MYC with varying frequencies.[10] In human breast cancer, *MYC* has been shown to be amplified with reported frequencies between 1% to 94% (average, approximately 16%).[8] *MYC* amplification has typically been associated with poor prognosis of breast cancer.[8]

MYC acts as a downstream target of HER2-driven proliferative signals in breast cancer cells in vitro.[11] In human breast tumors, *MYC*

Table 1. Patient/Disease Characteristics by *MYC*:CEP8 Ratio

Characteristic	*MYC*:CEP8 Ratio ≤ 2.2 (n = 618; 77%) No.	%	*MYC*:CEP8 Ratio > 2.2 (n = 181; 23%) No.	%	*P**
Age, years					
Median	50		49		
Range	23-80		27-71		
Age group, years					.21
< 40	100	16	37	20	
40-49	203	33	56	31	
50-59	191	31	62	34	
≥ 60	124	20	26	14	
Race					.29
White	541	88	153	85	
Other	77	12	28	15	
Menopausal status					.20
Premenopausal or age < 50 years	322	52	104	57	
Postmenopausal or age ≥ 50 years	296	48	77	43	
ER status					.03
Positive	284	46	100	55	
Negative	334	54	81	45	
PgR status					.008
Positive	210	34	81	45	
Negative	408	66	100	55	
Surgery					.97
Breast-conserving	221	36	65	36	
Mastectomy	397	64	116	64	
Nodal status					.88
No. of positive nodes					
1-3	253	41	65	36	
4-9	158	26	50	28	
10+	92	15	28	15	
No positive nodes	30	5	11	6	
Positive sentinel node	41	6	14	8	
Negative sentinel node	44	7	13	7	
Predominant tumor histology					.04
Ductal	578	94	177	98	
Lobular	20	3	2	1	
Other	20	3	1	0.6	
Missing	0		1	0.6	
Histologic tumor grade (Elston/SBR)					.004
Well/intermediate	190	31	36	20	
Poor	428	69	145	80	
Pathologic tumor size, cm					.22
< 2	204	33	51	28	
≥ 2	414	67	130	72	
Received hormonal treatment					.43
Yes	285	46	77	43	
No	330	54	102	57	
Missing	3		2		

Abbreviations: *MYC*:CEP8, *MYC* and centromere 8 ratio; ER, estrogen receptor; PgR, progesterone receptor; SBR, Scarff-Bloom-Richardson.
**P* values from the χ^2 test.

amplification has been associated with *HER2* amplification,[12,13] and *HER2*-amplified breast tumors were shown to have a 2.5-fold or greater increased likelihood of having *MYC* amplification.[12] Patients with breast cancer who had *MYC/HER2* coamplification were observed to have substantially worse outcomes than patients who had single-gene amplification,[7,12] even after standard chemotherapy, in the National Surgical Adjuvant Breast and Bowel Project (NSABP) Cooperative Group B28 trial.[7] It was then hypothesized that *MYC* amplification may confer resistance to trastuzumab therapy in patients with HER2-positive breast cancer. However, a preliminary report from the adjuvant trastuzumab NSABP B31 trial (hereafter B31) showed that patients with *MYC/HER2* coamplification in their primary breast tumors, defined as average copies/nucleus > 5.0 (n = 471; time to first recurrence hazard ratio [HR], 0.24; 13 *v* 51 events; $P < .001$), benefited significantly more ($P = .007$) from trastuzumab than did patients with only *HER2* amplification (n = 1,078; HR, 0.63; 55 *v* 82 events; $P = .007$), although a significant benefit of trastuzumab was observed in both *MYC*-amplified and nonamplified patients.[7]

To further explore these early findings, we investigated disease-free survival (DFS) according to *MYC* to centromere 8 ratio (*MYC*:CEP8), *MYC* gene copy number, and chromosome 8 copy number in patients with early-stage breast cancer randomly assigned to receive chemotherapy alone or chemotherapy with concurrent trastuzumab in the North Central Cancer Treatment Group (NCCTG) N9831 intergroup adjuvant trastuzumab phase III trial (hereafter N9831).

PATIENTS AND METHODS

Patients

The N9831 trial had three arms: arm A, doxorubicin and cyclophosphamide followed by weekly paclitaxel; arm B, same as arm A but followed by 1 year of sequential trastuzumab; arm C: same as arm A but with 1 year concurrent trastuzumab started the same day as weekly paclitaxel. An updated joint analysis of N9831 and B31 revealed that patients treated with concurrent trastuzumab had a lower number of events (breast cancer recurrence, second primary cancer, or death before recurrence) compared with the control group (222 *v* 397; DFS HR, 0.48; $P < .001$). Trastuzumab therapy was associated with a 35% reduction in the risk of death ($P < .001$).[3]

All patients tested for HER2 protein overexpression or gene amplification at a central laboratory were included in these analyses. Outcome data of patients in arm B had not been released by the study's independent data monitoring committee at time of analysis and are not included in this report. In accordance with assurances filed with and approved by the Department of Health and Human Services, local institutional review boards approved N9831, and all patients signed informed consent. The Mayo Institutional Review Board and the Correlative Science Committee of the North American Breast Cancer Group (NABCG) approved this translational study.

Tissue Microarrays

Tissue microarrays (TMAs) were constructed as part of the translational study component of N9831 by using an ATA-27 automated TMA construction system (Beecher Instruments, Silver Spring, MD). Hematoxylin and eosin–stained and HER2-stained slides from all blocks were first reviewed by a pathologist to demarcate representative areas of invasive tumor. From each formalin-fixed, paraffin-embedded tissue block, one tissue biopsy (0.6-mm diameter and 2.8-mm depth) was placed approximately 1.3 mm from the next one on each of three recipient TMAs in a random fashion according to National Cancer Institute (NCI) –recommended guidelines. Each TMA contained biopsies from non-neoplastic human liver, placenta, and tonsil control tissues (Data Supplement). We also examined the concordance between TMA and whole-section *MYC* fluorescence in situ hybridization (FISH) analyses of 84 independent breast tumors and observed a concordance of 90% and 96% for average *MYC* copies/nucleus and *MYC*:CEP8 ratio, respectively.[14]

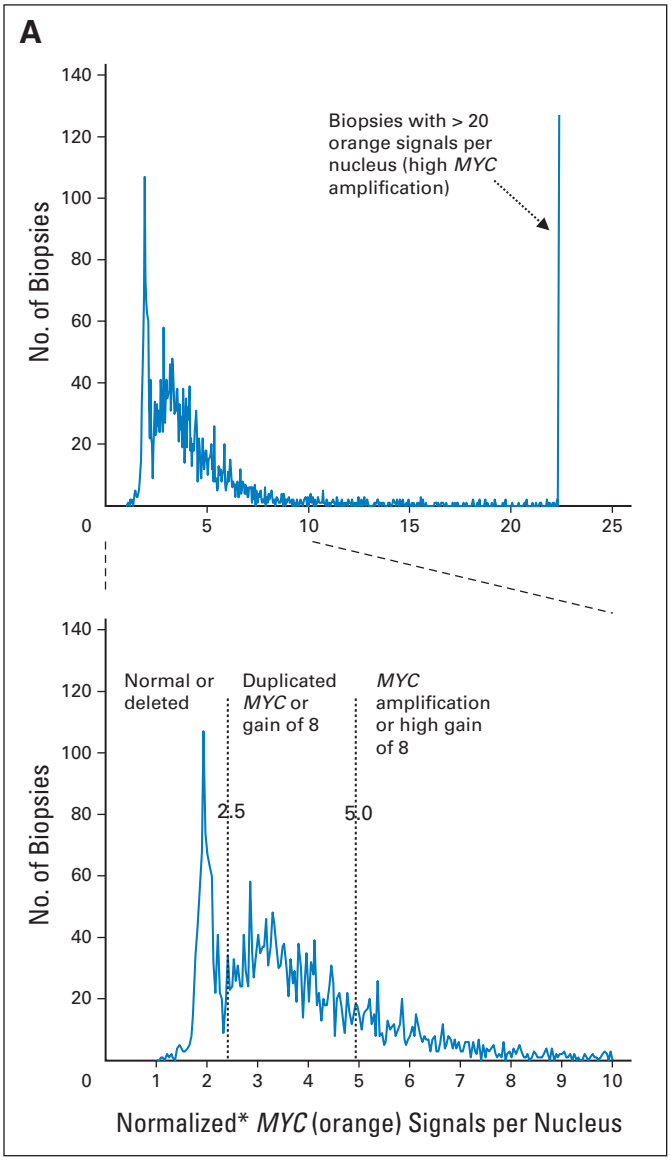

Fig 1. Distribution of the average *MYC* signals per nucleus and *MYC* and centromere 8 (*MYC*:CEP8) ratios for all evaluable patients on tissue microarrays (TMAs). (A) Distribution of the average *MYC* signals per nucleus for all evaluable biopsies on TMAs. (*)The average *MYC* signals per nucleus were normalized to the average *MYC* signal per nucleus found in non-neoplastic biopsies (Data Supplement) to account for nuclear truncation as a result of tissue sectioning. (B) Distribution of *MYC*:CEP8 ratios for 1,404 evaluable patients represented on TMAs. Amplified *MYC*: > 6 *MYC* signals in > 40% of invasive nuclei, *MYC*:CEP8 ratio > 2.2; select cases with small clones of *MYC* amplification (> 10 *MYC* signals in > 5% and < 40% of invasive nuclei) or with low *MYC* amplification with high aneusomy were considered amplified according to the pathologist's interpretation but had an *MYC*:CEP8 ratio < 2.2. Duplicated *MYC*: *MYC*:CEP8 ratio > 1.30 and without *MYC* amplification. Polysomy 8: ≥ 3 CEP8 signals in > 30% of invasive nuclei (ratio > 0.80 and < 1.30). −8, −*MYC*: loss of chromosome 8 and deleted *MYC*, 1 *MYC* and CEP8 signal in > 60% of invasive nuclei and an *MYC*:CEP8 ratio < 0.80. NACA: normal for all chromosome 8 anomalies; ≥ 3 CEP8 signals in < 30% nuclei and 1 CEP8 signal in < 60% nuclei (*MYC*:CEP8 ratio > 0.80 and < 1.30). Amplified *MYC*, n = 207 (14%); duplicated *MYC*, n = 551 (39.2%); polysomy 8, n = 443 (31.5%); -8, -*MYC*, n = 32 (2.3%); NACA, n = 171 (12.2%).

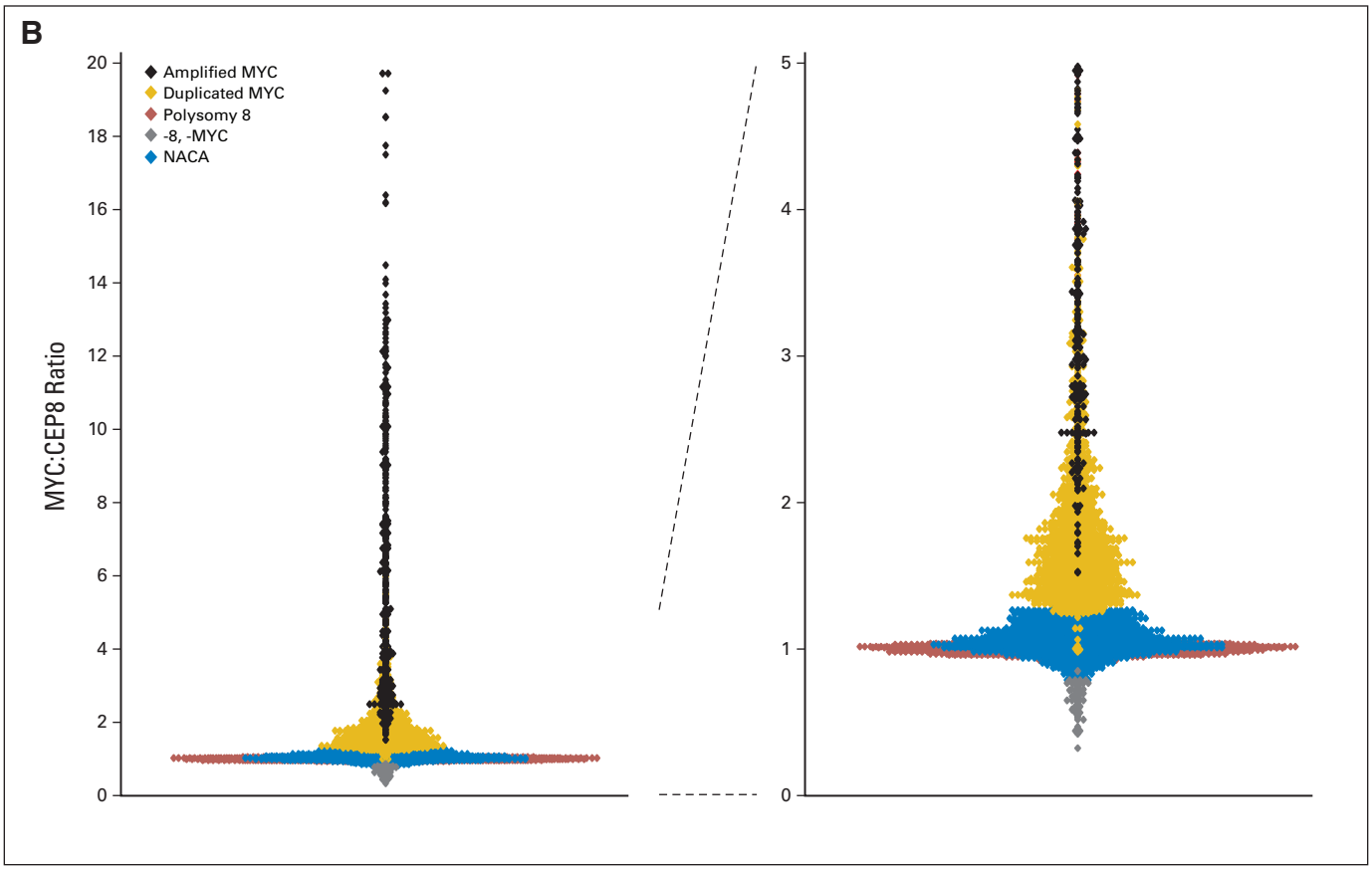

Fig 1. (continued)

MYC *Testing Methods*

FISH analysis was performed on deparaffinized 5-μm TMA sections by using the *c-MYC* locus–specific probe labeled with Spectrum Orange (*MYC*) and the chromosome 8 centromere probe (D8Z2) labeled with Spectrum Green (CEP8; Abbott Molecular, Des Plaines, IL). Standard laboratory protocols and quality control measures were followed. The TMA biopsies were scanned by two certified cytogenetic technologists to detect any subpopulations of amplified cells by using individual filters. Thirty representative nuclei from the invasive tumor were scored for both orange (*MYC*) and green (CEP8) signals by each technologist (total N = 60), and the signals were counted by using a dual filter. Overall evaluation was performed by a board-certified pathologist (R.P.K., R.B.J., W.R.S., or K.K.). When the orange *MYC* signals were clearly amplified (large clouds of amplification), we assigned ≥ 20 orange signals and counted the green CEP8 signals. For such cases, the ratio was defined as 20 divided by the average number of green signals per cell.

Three different cut point methods were used to classify *MYC* and CEP8 copy number anomalies: (1) a prespecified method that defined *MYC* amplification as *MYC*:CEP8 ratio > 2.2, similar to the *HER2*:CEP17 ratio established by the 2007 American Society of Clinical Oncologists/College of American Pathologists guidelines[15] and recommended by the Correlative Science Committee of the NABCG; (2) a prespecified method that defined *MYC* amplification as an average of > 5.0 *MYC* copies/nucleus, which was used in the B31 cohort[7]; and (3) exploratory cut points. Exploratory cut points included (1) *MYC*:CEP8 ratio ≥ 1.3 or *MYC*:CEP8 ratio < 1.3 with polysomy 8 (p8; at least three CEP17 signals in $> 30\%$ of nuclei; Data Supplement); (2) ≥ 2.5 average *MYC* copies/nucleus; (3) high *MYC* gain (> 5.0 average *MYC* gene copies/nucleus); (4) low *MYC* gain (2.5 to 5 average *MYC* copies/nucleus); and (5) normal or loss of *MYC* (< 2.5 average *MYC* copies/nucleus). Although not prespecified, the chosen exploratory cut points closely match *MYC* and centromere 8 cut points established for prostate cancer alterations by using the same probe set.[16,17]

Statistical Methods

DFS was the primary end point of N9831 and was defined as local, regional, or distant recurrence, contralateral breast cancer, another primary cancer (except squamous or basal cell carcinoma of the skin, carcinoma in situ of the cervix, or lobular carcinoma in situ of the breast), or death from any cause. The duration of DFS was defined as the time from registration to the first DFS event. DFS was estimated by the Kaplan-Meier method. Comparisons between arms A and C within subgroups were performed by using Cox proportional hazards models stratified by nodal status (one to three *v* four to nine *v* ≥ 10 positive nodes *v* positive sentinel node only *v* negative sentinel node with no axillary nodal dissection *v* axillary nodal dissection with no positive nodes) and hormone receptor status (estrogen receptor–positive and/or progesterone receptor–positive *v* negative for both receptors). The ability of *MYC* to predict differential trastuzumab benefit between *MYC* subgroups was tested by using Cox proportional hazards models (also stratified by nodal status and hormone receptor status), which included a treatment arm by *MYC* subgroup interaction term. The maximum FISH *MYC*:CEP8 ratio or *MYC* gene copy number of the triplicate TMA biopsies was used for all analyses associated with patient outcome.

RESULTS

Study Patients

The trial enrolled 2,289 patients into arms A (1,232 patients) and C (1,057 patients) of which 1,490 patients (arm A, 816; arm C, 674) were excluded from this analysis for the following reasons: not HER2-positive by central pathology review (arm A, 109; arm C, 84); canceled before initiating therapy (arm A, 15; arm C, seven); did not meet eligibility criteria (arm A, 21; arm C, 17); withdrew consent (arm A, 39;

Table 2. No. of NCCTG N9831 Patients According to Average *MYC* Copies/Nucleus and *MYC*:CEP8 Ratio Categories

Patient Tumor Status	Average *MYC* Copies/Nucleus	*MYC*:CEP8 Ratio						
		< 1.3, Disomy 8	< 1.3, p8*	1.3-2.2	> 2.2		Total	
					No.	%	No.	%
All patients (N = 799)	< 1.5	4	0	1	0		5	
	1.5 to < 2.5	107	4	14	0		125	
	2.5 to 5.0	30	196	153	25		404	
	> 5.0	0	29	80	156		265	33
	Total	141	229	248	181	23	799	
HER2 FISH-amplified patients only (n = 716)	< 1.5	4	0	1	0		5	
	1.5 to < 2.5	95	3	12	0		110	
	2.5 to 5.0	27	171	137	21		356	
	> 5.0	0	27	72	146		245	34
	Total	126	201	222	167	23	716	

Abbreviations: NCCTG, North Central Cancer Treatment Group; *MYC*:CEP8, *MYC* and centromere 8 ratio; HER2, human epidermal growth factor receptor 2; FISH, fluorescent in situ hybridization.
*p8, polysomy of chromosome 8: ≥ 3 CEP17 signals in > 30% of nuclei.

arm C, nine); no consent for future translational analysis (arm A, 62; arm C, 52); no or inadequate tissue block for inclusion on TMAs (arm A, 537; arm C, 478); and included on TMAs but was a technical failure (arm A, 33; arm C, 27). Of the 2,289 patients, 799 (arm A, 416; arm C, 383) were evaluable for *MYC* gene and CEP8 copy number alterations. The median follow-up time was 4.0 years (arm A, 4.2 years with 104 DFS events; arm C, 3.7 years with 47 DFS events; includes all follow-up available through August 24, 2009). The clinicopathologic characteristics and outcomes of the 799 patients enrolled on arms A and C reported herein were similar to the 1,490 patients on arms A and C excluded from analysis (Data Supplement). The clinicopathologic characteristics of the 799 patients whose tumors had *MYC*:CEP8 ratios ≤ 2.2 and > 2.2 are shown in Table 1. Patients whose tumors had *MYC*:CEP8 ratio > 2.2 appear to have a higher rate of hormone receptor positivity, ductal histology, and poor histologic tumor grade than patients whose tumors had *MYC*:CEP8 ratio ≤ 2.2. The clinicopathologic characteristics of the 799 patients whose tumors had average *MYC* copies/nucleus ≤ 2.2 and > 2.2 are shown in the Data Supplement.

Distribution of Average MYC *Signals Per Nucleus and* MYC*:CEP8 Ratio*

Figure 1 shows the distribution of average *MYC* gene signals per nucleus for all evaluable N9831 biopsies (Fig 1A) and of *MYC*:CEP8 ratios for all evaluable patients represented on the TMAs (Fig 1B). According to the pathologist's (R.B.J.'s) interpretation, several different *MYC* and chromosome 8 copy number anomalies were observed, including *MYC* amplification (15%), *MYC* duplication (38%), p8 (32%), and *MYC* and chromosome 8 loss (2.3%; Fig 1B). Of the 799 tumors, 181 tumors (23%) had *MYC*:CEP8 ratio > 2.2, and 265 (33%) and 404 tumors (51%) had > 5.0 and 2.5 to 5 average *MYC* copies/nucleus, respectively (Table 2). Of the patients whose tumors had average 2.5 to 5.0 *MYC* copies/nucleus (n = 477), 73% (349 of 477) had either *MYC*:CEP8 ratio < 1.3 with p8 (n = 156) or *MYC*:CEP8 ratio 1.3 to 2.2 (n = 196; Table 2). Figure 2 illustrates representative FISH signal patterns of select *MYC* anomalies including *MYC* amplification with p8 (Fig 2A), low-level *MYC* amplification with disomy 8 (Fig 2B), and duplicated *MYC* with p8 (Fig 2C).

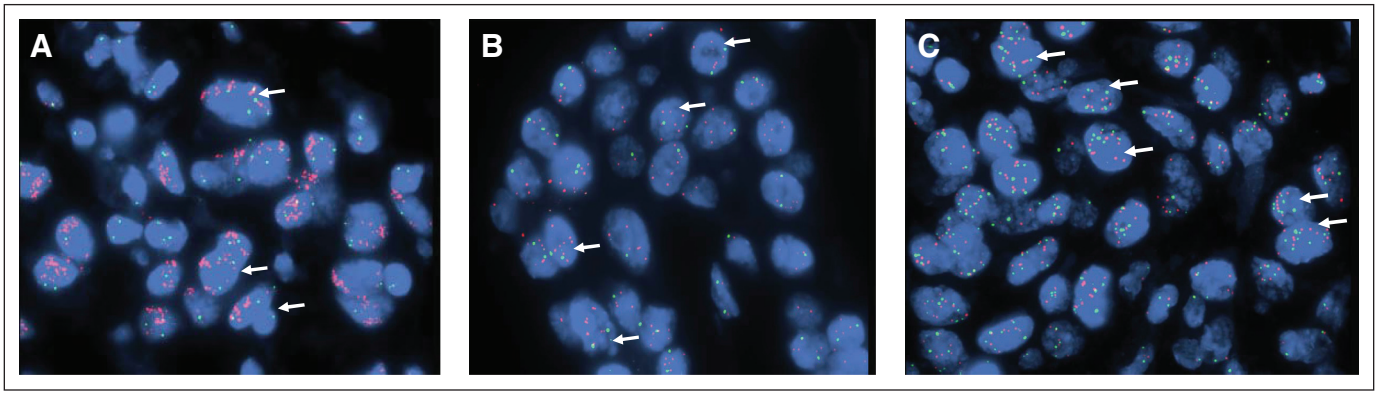

Fig 2. Representative fluorescence in situ hybridization (FISH) signal patterns of select *MYC* and chromosome 8 copy number anomalies. White arrows indicate nuclei representative of the respective FISH signal patterns for the different copy number anomalies. (A) *MYC* amplification with polysomy 8. The average *MYC* signals per nucleus was 21, the *MYC*:CEP8 ratio was 8.0, and the percentage of cells with ≥ 3 CEP8 signal was 60. (B) Low amplification of *MYC* with disomy 8. The average *MYC* signals per nucleus was 5.9, the *MYC*:CEP8 ratio was 3.5, and the percentage of cells with ≥ 3 CEP8 signals was 4.4. (C) Duplicated *MYC* with polysomy 8. The average *MYC* signals per nucleus was 5.2, the *MYC*:CEP8 ratio was 1.6, and the percentage of cells with ≥ 3 CEP8 signals was 91.

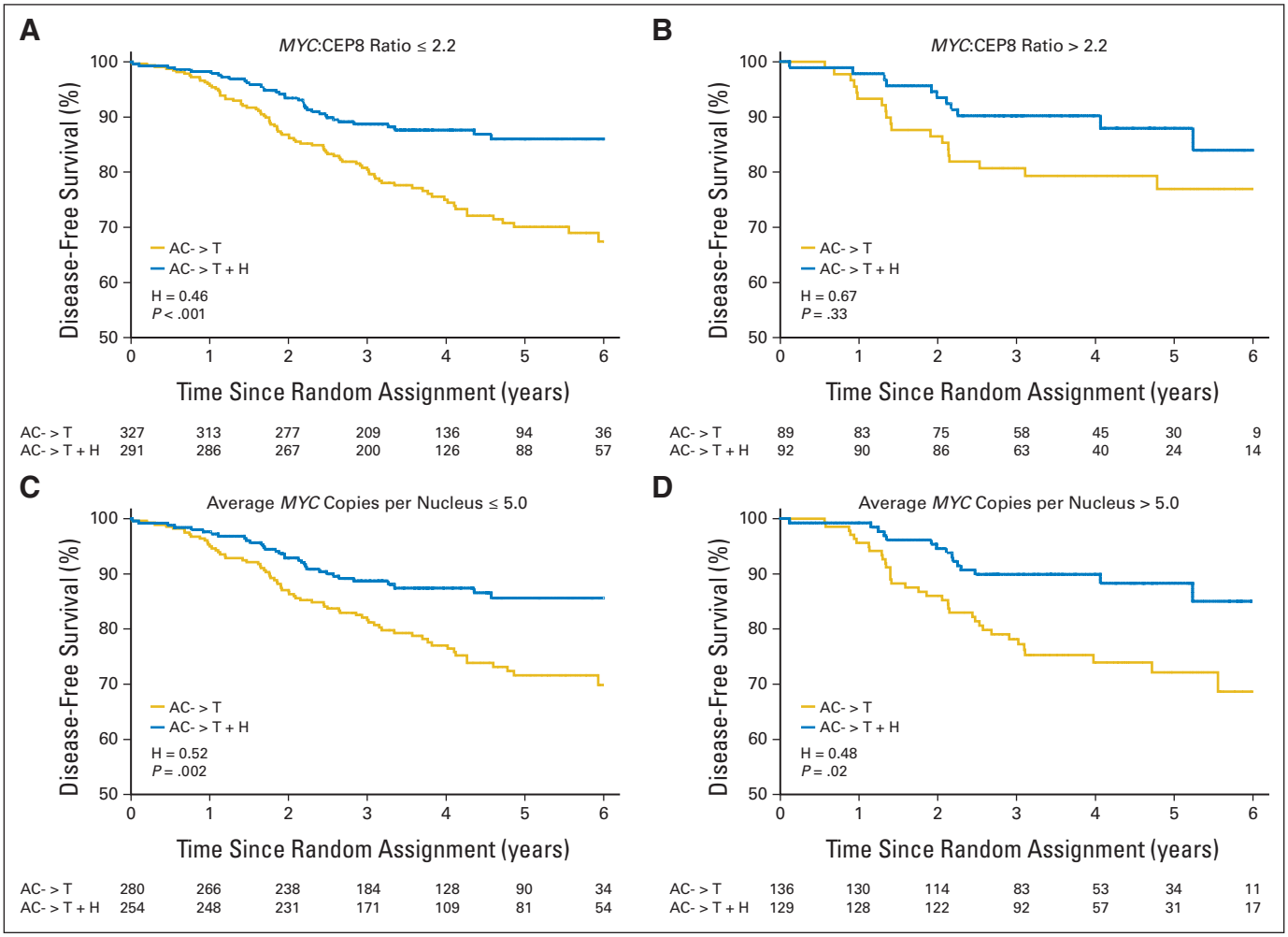

Fig 3. Disease-free survival by *MYC* amplification: (A) by *MYC*:CEP8 ratio ≤ 2.2, (B) by *MYC*:CEP8 ratio > 2.2, (C) by average *MYC* copies per nucleus ≤ 5.0, and (D) by average *MYC* copies per nucleus > 5.0. HR, hazard ratio; A, doxorubicin; C, cyclophosamide; T, paclitaxel; H, trastuzumab.

Associations Between Copy Number Anomalies and DFS

DFS by *MYC* amplification is defined as *MYC*:CEP8 ratio > 2.2. In comparing DFS between treatment arms, patients whose tumors had *MYC*:CEP8 ratio ≤ 2.2 (Fig 3A) and > 2.2 (Fig 3B) had HRs of 0.46 (n = 618; 36 *v* 85 events; $P < .001$) and 0.67 (n = 181; 11 *v* 19 events; $P = .33$), respectively. These HRs were not significantly different (interaction $P = .38$). We also investigated whether *MYC*:CEP8 ratio was associated with different outcomes for patients who were given standard chemotherapy alone. Within arm A, a nonsignificant HR of 0.73 (95% CI, 0.44 to 1.20) was observed for patients with a *MYC*:CEP8 ratio > 2.2 compared with those who had a ratio of ≤ 2.2 (Data Supplement).

DFS by *MYC* amplification is defined as average *MYC* copies/nucleus > 5.0. In comparing DFS between treatment arms, patients whose tumors had average *MYC* copies/nucleus ≤ 5.0 (Fig 3C) and > 5.0 (Fig 3D) had HRs of 0.52 (n = 534; 32 *v* 69 events; $P = .002$) and 0.48 (n = 265; 15 *v* 35 events; $P = .02$), respectively. These HRs were not significantly different (interaction $P = .94$). Within arm A, an HR of 1.0 (95% CI, 0.66 to 1.51) was observed for those patients with > 5.0 compared with those with ≤ 5.0 average *MYC* copies/nucleus (Data Supplement).

DFS by Alternate Cut Points

Considering alternate cut points, patients whose tumors had *MYC*:CEP8 ratio < 1.3 with normal 8 (Fig 4A) and ≥ 1.3 or < 1.3 with p8 (Fig 4B) had HRs of 0.66 (n = 141; 12 *v* 18 events; $P = .28$) and 0.44 (n = 658; 35 *v* 86 events; $P < .001$), respectively. These HRs were not significantly different (interaction $P = .23$). The HRs for patients whose tumors had *MYC*:CEP8 ratio < 1.3 with p8 and 1.3 to 2.2 were 0.38 (95% CI, 0.19 to 0.76; n = 229) and 0.40 (95% CI, 0.21 to 0.77; n = 248), respectively (Fig 4C).

Patients whose tumors had average *MYC* copies/nucleus < 2.5 (Fig 4D) and ≥ 2.5 (Fig 4E) had HRs of 1.07 (n = 130; 13 *v* 16 events; $P = .87$) and 0.42 (n = 669; 34 *v* 88 events; $P < .001$), respectively. These HRs are significantly different (interaction $P = .05$). The HRs for patients whose tumors had average *MYC* copies/nucleus 2.5 to 5.0 and > 5.0 were 0.38 (95% CI, 0.22 to 0.64; n = 404) and 0.48 (95% CI, 0.25 to 0.89; n = 265), respectively (Fig 4F).

DISCUSSION

In human breast cancer, *MYC* is a commonly amplified oncogene with diverse functions and biologic effects, and it has been associated

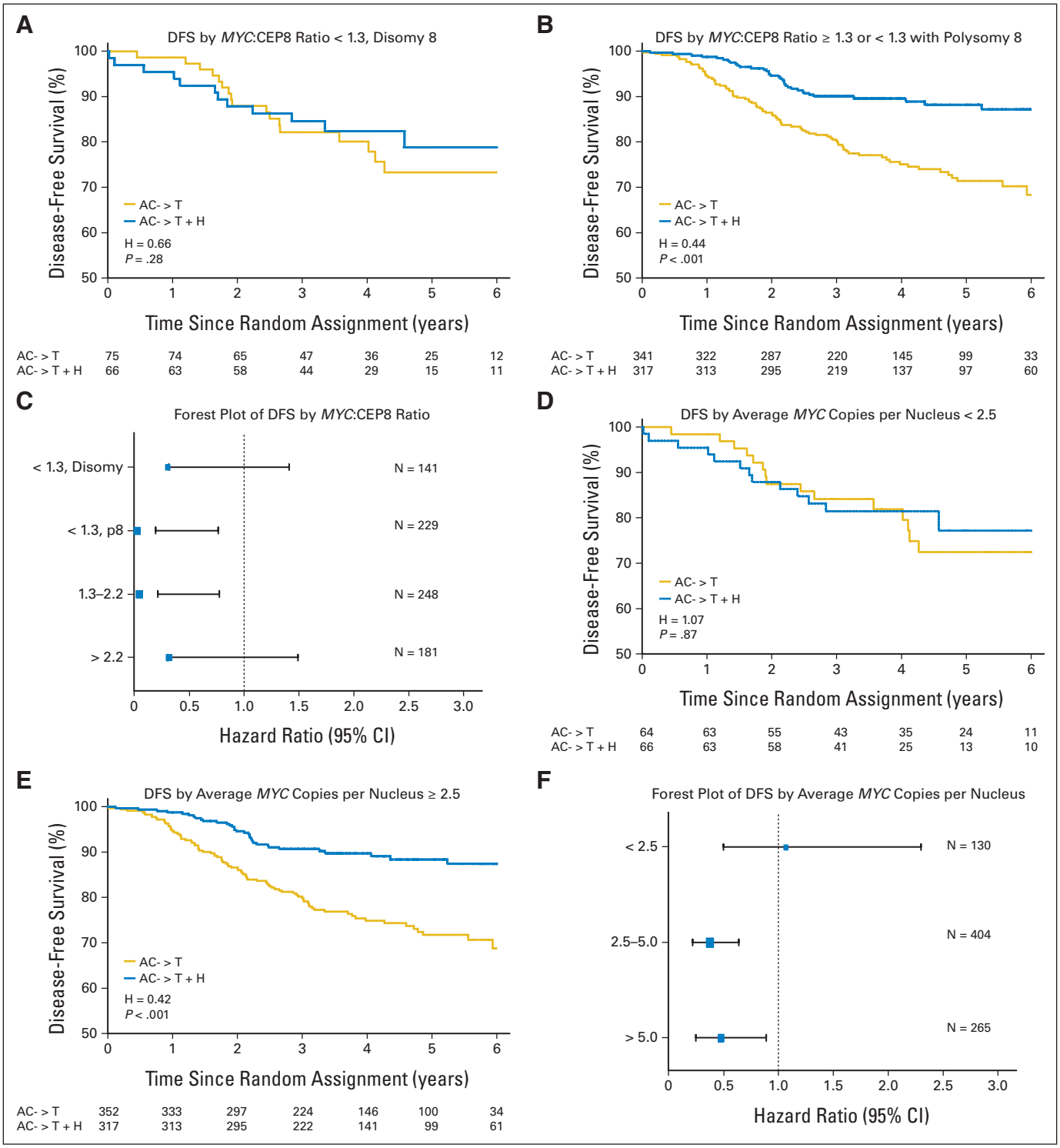

Fig 4. Disease-free survival (DFS) by alternate cut points (A) by *MYC*:CEP8 ratio < 1.3 with disomy 8 and (B) by *MYC*:CEP8 ratio ≥ 1.3 or < 1.3 with polysomy 8. (C) Forest plot of DFS by *MYC*:CEP8 ratio. (D) DFS by average *MYC* copies per nucleus < 2.5 and (E) ≥ 2.5. (F) Forest plot of DFS by average *MYC* copies per nucleus. HR, hazard ratio; A, doxorubicin; C, cyclophosamide; T, paclitaxel; H, trastuzumab.

with both poor and good prognoses.[8,9] Preliminary evidence from the B31 trial suggested that *MYC* amplification may predict additional benefit of adjuvant trastuzumab in patients with early-stage HER2-positive breast cancer.[7] Inhibition of HER2 signaling has been hypothesized to turn on the proapoptotic function of dysregulated MYC,[18,19] resulting in a higher rate of apoptosis in tumors with copy number gain. Constant overexpression of MYC without concomitant increase in survival or proliferation-promoting growth factors (eg, transforming growth factor α [TGF-α] and G1 cyclins) may cause a tumor to be more sensitive to apoptotic stimuli or to

chemotherapy, which may be reflected in a better prognosis.[8,20] Conversely, because MYC induces cell proliferation and HER2-positive breast tumors tend to have a high proliferation index,[21-23] those tumors with alterations for both genes may be more susceptible to the growth inhibitory synergistic effects observed with the combination of chemotherapy and trastuzumab.[24-27]

To further explore the B31 relationship between *MYC* amplification and sensitivity to trastuzumab, we evaluated the associations between *MYC*/chromosome 8 copy number anomalies and benefit of adjuvant trastuzumab in N9831. *MYC* amplification (*MYC*:CEP8 ratio $>$ 2.2) was observed in 23% of patients. *MYC* amplification (average copies/nucleus $>$ 5.0) was observed in 33% of patients, similar to the 30% reported for B31 using the same cut point.[7] We also observed other copy number anomalies with relatively high frequencies. *MYC* duplication and p8 were observed in 39% and 32% of patients, respectively. Earlier studies did not report on the frequencies or impact of other possible alterations of *MYC* in breast cancer. We found significant associations between *MYC* amplification and hormone receptor positivity, ductal histology, and poor histologic tumor grade but not between *MYC* amplification and nodal status or tumor size.

Literature findings[8] are inconsistent regarding associations between *MYC* amplification and clinicopathologic characteristics. However, a meta-analysis[28] demonstrated that only the correlation of *MYC* amplification (typically defined as *MYC*:CEP8 ratio $\geq$ 2.0) with progesterone receptor negativity was statistically significant. The cohort of patients in N9831 were all HER2 positive, and hormone receptor testing was not centrally performed, which may account for the discrepant hormone receptor findings in our study. In addition, high *MYC* gene expression levels have been correlated with large breast tumors but also with better survival.[29] This may be because proliferative cells are more sensitive to chemotherapy. Colon cancers with low levels of *MYC* amplification have been reported to respond better to adjuvant chemotherapy than those without gene amplification.[30] However, we did not observe a significant correlation between *MYC* amplification and tumor size nor between *MYC* amplification and DFS advantage in N9831 patients treated with only chemotherapy.

In contrast to the preliminary B31 results that showed patients with *MYC*/*HER2* coamplification (average copies/nucleus $>$ 5.0) benefited significantly more ($P = .007$) from trastuzumab than patients with only *HER2* amplification, we did not observe a significant association between *MYC* amplification, defined as *MYC*:CEP8 ratio $>$ 2.2 or average copies/nucleus $>$ 5.0 and greater benefit in terms of prolonged DFS from trastuzumab in N9831. Important differences between the two studies include end points (time to first recurrence and overall survival in B31 *v* DFS in N9831) and number of patients examined (1,549 in B31 and 799 in N9831). In many circumstances, statistical anomalies could cause significant interaction findings, which may not hold up in independent validation. Both studies also found that patients with either *MYC*-amplified or nonamplified tumors significantly benefited from trastuzumab, limiting the clinical predictive impact of *MYC* amplification (as conventionally defined) on additional benefit of trastuzumab.

Although we could not corroborate the B31 findings strictly on the basis of *MYC* amplification defined as $>$ 5.0 average copies/nucleus, we observed differential benefit of trastuzumab in groups of patients with alternative *MYC* and chromosome 8 copy number alterations. We observed that patients whose tumors had *MYC*:CEP8 ratio $\geq$ 1.3 or *MYC*:CEP8 ratio $<$ 1.3 with p8 or whose tumors had $\geq$ 2.5 average *MYC* copies/nucleus appeared to derive more benefit from trastuzumab than those whose tumors had no *MYC* copy number alterations or loss of *MYC* ($<$ 2.5 average *MYC* copies/nucleus). This suggests that *MYC* duplication (or low-level relative gain of *MYC*) and gain of chromosome 8 are perhaps both responsible for predicting additional benefit of trastuzumab.

These N9831 data imply that alternate *MYC*/chromosome 8 copy number alterations may be associated with differential benefit of trastuzumab and are consistent with an alternate hypothesis that *MYC* may be a surrogate for other genes located on chromosome 8. In addition to the proto-oncogene *MYC*, other genes present in the 8q24 amplicon may contribute directly to the outcome of patients harboring the large-scale genomic rearrangement involving 8q24 amplification.[31-33] For example, genes mapping to 8q24 (*RAD21, KIAA0196, TAF2, FAM49B,* and *C8ORF53*) were significantly enriched in a 17-gene model predicting prostate cancer systemic progression.[32] In addition, 8q24 was one of four highly aberrant chromosomal regions identified by gene expression microarray studies that selected *CYC1*, *SIAHBP*, and *SCRIB* as potential oncogenes.[31] *SIAHBP* (FIR) has been shown to be involved in a complex that regulates *MYC* gene expression.[34] Ongoing MYC protein analyses and whole-genome expression profiling of N9831 tumors will provide important information regarding the relationship between MYC and other pertinent genes and the benefit of adjuvant trastuzumab.

Overall, our data suggest that alternative *MYC* and chromosome 8 copy number anomalies may identify subgroups of HER2-positive tumors that are responsive or nonresponsive to trastuzumab. Further investigation into the role of MYC, its regulators, its downstream effectors, and other genes located on chromosome 8 is required to fully elucidate the prognostic and/or predictive utility of MYC in HER2-positive breast cancer. Understanding the full extent of the oncogenic effects of 8q24 amplification is critical to the development of more effective, targeted therapies for patients with breast cancer that exhibit this genetic aberration.

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REFERENCES

1. Perez EA, Palmieri FM, Brock SM: Trastuzumab. Cancer Treat Res 151:181-196, 2009
2. Perez EA, Reinholz MM, Dueck AC, et al: C-MYC amplification and correlation with patient outcome in early stage HER2+ breast cancer from the NCCTG adjuvant intergroup trial N9831. Cancer Res 69:76s, 2009 (suppl; abstr 56)
3. Perez EA, Romond EH, Suman VJ, et al: Updated results of the combined analysis of NCCTG N9831 and NSABP B-31 adjuvant chemotherapy with/without trastuzumab in patients with HER2-positive breast cancer. J Clin Oncol 25:6s, 2007 (suppl; abstr 512)
4. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353: 1673-1684, 2005
5. Bedard PL, de Azambuja E, Cardoso F: Beyond trastuzumab: Overcoming resistance to targeted HER-2 therapy in breast cancer. Curr Cancer Drug Targets 9:148-162, 2009
6. Chen Y, Olopade OI: MYC in breast tumor progression. Expert Rev Anticancer Ther 8:1689-1698, 2008
7. Kim C, Bryant J, Horne Z, et al: Trastuzumab sensitivity of breast cancer with co-amplification of HER2 and cMYC suggests pro-apoptotic function of dysregulated cMYC in vivo. Breast Cancer Res Treat 94:S6, 2005 (abstr 46)
8. Liao DJ, Dickson RB: C-Myc in breast cancer. Endocr Relat Cancer 7:143-164, 2000
9. Lutz W, Leon J, Eilers M: Contributions of Myc to tumorigenesis. Biochim Biophys Acta 1602: 61-71, 2002
10. Nesbit CE, Tersak JM, Prochownik EV: MYC oncogenes and human neoplastic disease. Oncogene 18:3004-3016, 1999
11. Neve RM, Sutterlüty H, Pullen N, et al: Effects of oncogenic ErbB2 on G1 cell cycle regulators in breast tumour cells. Oncogene 19:1647-1656, 2000
12. Al-Kuraya K, Schraml P, Torhorst J, et al: Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 64: 8534-8540, 2004
13. Park K, Kwak K, Kim J, et al: c-myc amplification is associated with HER2 amplification and closely linked with cell proliferation in tissue microarray of nonselected breast cancers. Hum Pathol 36:634-639, 2005
14. Reinholz MM, Dueck AC, Lingle WL, et al: The concordance between NCCTG's and NSABP's C-myc FISH assays. J Clin Oncol 26:757s, 2008 (suppl; abstr 22110)
15. Wolff AC, Hammond ME, Schwartz JN, et al: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25:118-145, 2007
16. Qian J, Hirasawa K, Bostwick DG, et al: Loss of p53 and c-myc overrepresentation in stage T(2-3)N(1-3)M(0) prostate cancer are potential markers for cancer progression. Mod Pathol 15:35-44, 2002
17. Sato K, Qian J, Slezak JM, et al: Clinical significance of alterations of chromosome 8 in high-grade, advanced, nonmetastatic prostate carcinoma. J Natl Cancer Inst 91:1574-1580, 1999
18. Adhikary S, Eilers M: Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 6:635-645, 2005
19. Evan GI, Wyllie AH, Gilbert CS, et al: Induction of apoptosis in fibroblasts by c-myc protein. Cell 69:119-128, 1992
20. Liao DJ, Natarajan G, Deming SL, et al: Cell cycle basis for the onset and progression of c-Myc-induced, TGFalpha-enhanced mouse mammary gland carcinogenesis. Oncogene 19:1307-1317, 2000
21. Rudolph P, Olsson H, Bonatz G, et al: Correlation between p53, c-erbB-2, and topoisomerase II alpha expression, DNA ploidy, hormonal receptor status and proliferation in 356 node-negative breast carcinomas: Prognostic implications. J Pathol 187: 207-216, 1999
22. Trihia H, Murray S, Price K, et al: Ki-67 expression in breast carcinoma: Its association with grading systems, clinical parameters, and other prognostic factors—A surrogate marker? Cancer 97:1321-1331, 2003
23. Yerushalmi R, Woods R, Ravdin PM, et al: Ki67 in breast cancer: Prognostic and predictive potential. Lancet Oncol 11:174-183, 2010
24. Arteaga CL: Can trastuzumab be effective against tumors with low HER2/Neu (ErbB2) receptors? J Clin Oncol 24:3722-3725, 2006
25. Henson ES, Hu X, Gibson SB: Herceptin sensitizes ErbB2-overexpressing cells to apoptosis by reducing antiapoptotic Mcl-1 expression. Clin Cancer Res 12:845-853, 2006
26. Menendez JA, Mehmi I, Lupu R: Trastuzumab in combination with heregulin-activated Her-2 (erbB-2) triggers a receptor-enhanced chemosensitivity effect in the absence of Her-2 overexpression. J Clin Oncol 24: 3735-3746, 2006
27. Pero SC, Shukla GS, Cookson MM, et al: Combination treatment with Grb7 peptide and doxorubicin or trastuzumab (Herceptin) results in cooperative cell growth inhibition in breast cancer cells. Br J Cancer 96:1520-1525, 2007
28. Deming SL, Nass SJ, Dickson RB, et al: C-myc amplification in breast cancer: A meta-analysis of its occurrence and prognostic relevance. Br J Cancer 83:1688-1695, 2000
29. Bièche I, Laurendeau I, Tozlu S, et al: Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. Cancer Res 59:2759-2765, 1999
30. Augenlicht LH, Wadler S, Corner G, et al: Low-level c-myc amplification in human colonic carcinoma cell lines and tumors: A frequent, p53-independent mutation associated with improved outcome in a randomized multi-institutional trial. Cancer Res 57:1769-1775, 1997
31. Buness A, Kuner R, Ruschhaupt M, et al: Identification of aberrant chromosomal regions from gene expression microarray studies applied to human breast cancer. Bioinformatics 23:2273-2280, 2007
32. Nakagawa T, Kollmeyer TM, Morlan BW, et al: A tissue biomarker panel predicting systemic progression after PSA recurrence post-definitive prostate cancer therapy. PLoS One 3:e2318, 2008
33. Yao J, Weremowicz S, Feng B, et al: Combined cDNA array comparative genomic hybridization and serial analysis of gene expression analysis of breast tumor progression. Cancer Res 66:4065-4078, 2006
34. Liu J, Kouzine F, Nie Z, et al: The FUSE/FBP/FIR/TFIIH system is a molecular machine programming a pulse of c-myc expression. Embo J 25:2119-2130, 2006