Associations between *ERBB2* amplification and progression-free survival and overall survival in advanced stage, suboptimally-resected epithelial ovarian cancers: A Gynecologic Oncology Group study☆

John Farley[a,1], Sartoru Fuchiuji[b,1], Kathleen M. Darcy[c], Chunqiao Tian[c], William J. Hoskins[d], William P. McGuire[e], Parviz Hanjani[f], David Warshal[g], Benjamin E. Greer[h], Jerome Belinson[i], Michael J. Birrer[j,*,2]

[a]Departments of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

[b]Center for Cancer Research, National Cancer Institute, 37 Convent Drive, Bldg 37, Room 1130, Bethesda, MD 20892, USA

[c]GOG Statistical and Data Center, Elm and Carlton Street, Buffalo NY 14263-0001, USA

[d]Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

[e]Harry and Jeanette Weinberg Cancer Institute, Franklin Square Hospital Center, Baltimore, MD 21237, USA

[f]Abington Memorial Hospital, Abington, PA 19001, USA

[g]Department of Obstetrics and Oncology, Cooper Hospital/University Medical Center, Camden, NJ 08103, USA

[h]Department of Gynecologic Oncology, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA 98109, USA

[i]Department of Gynecology, A-81, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA

[j]Center for Cancer Research, NCI, 37 Convent Drive, Bethesda, MD 20892, USA

Abstract

Objective(s).—The Gynecologic Oncology Group (GOG) examined the association between *ERBB2* amplification and clinical covariates, tumor response, disease status post-chemotherapy, progression-free survival (PFS), and overall survival (OS) in epithelial ovarian cancer (EOC).

Methods.—Women with suboptimally-resected, advanced stage EOC who participated in GOG-111, a multi-center randomized phase III trial of cyclophosphamide + cisplatin versus paclitaxel + cisplatin, and provided a tumor block through the companion protocol GOG-9404 were eligible. *ERBB2* amplification was examined using fluorescence *in situ* hybridization (FISH) with probes for *ERBB2* and the centromere of chromosome 17 (CEP17).

Results.—*ERBB2* amplification, defined as >2 copies of *ERBB2*/CEP17, was a rare event in EOC with 7% (9/133) of women exhibiting between 2.2 and 33.7 copies of *ERBB2*/CEP17, and was not associated with patient age, race, GOG performance status, stage, cell type, grade, measurable disease status, volume of ascites, tumor response or disease status post-chemotherapy. Women with >2 verses 2 copies of *ERBB2*/CEP17 did not have a reduced risk of disease progression (hazard ratio [HR] = 0.56; 95% confidence interval [CI] = 0.27–1.16; $p = 0.120$) or death (HR = 0.57; 95% CI = 0.26–1.23; $p = 0.152$), and *ERBB2* amplification was not an independent prognostic factor for PFS or OS. *ERBB2* amplification, defined as >4 copies of *ERBB2*/nuclei, was observed in 9% (12/133) of women with levels ranging from 4.2 to 49.2 copies of *ERBB2*/nuclei, and was associated with older age and volume of ascites, but not with the other clinical covariates or outcome.

Conclusion(s).—*ERBB2* amplification is a rare event and has no predictive or prognostic value in suboptimally-resected, advanced stage EOC treated with platinum-based combination chemotherapy.

Keywords

ERBB2; Gene amplification; Ovary; Carcinogenesis; FISH

Introduction

Ovarian cancer is the second most common gynecologic malignancy, and the leading cause of cancer related death among the gynecologic malignancies [1,2]. It is estimated that 21,650 new cases of ovarian cancer will be diagnosed in the United States in 2008 and that 15,520 women will die from the disease. Overexpression of the *ERBB2* proto-oncogene occurs in 11–30% of epithelial ovarian cancers (EOC) [3-10]. Expression of *ERBB2* has traditionally been evaluated by immunohistochemistry with inconsistent prognostic results for epithelial ovarian cancer [5-9]. Some studies of EOC associate increased *ERBB2* expression intensity with decreased median and overall survival [5,7]. Other studies have shown no relationship between *ERBB2* expression and survival among epithelial ovarian cancer [8].

Because of these disparate results, fluorescence *in situ* hybridization (FISH) analysis of *ERBB2* amplification has been applied to a series of EOCs in an attempt to alleviate the inherent difficulty in quantifying immunohistochemical staining [11,12]. Amplification of *ERBB2* in early stage ovarian neoplasms has been reported as infrequent at around 6.7% [13]. In advanced stage EOC, FISH analysis revealed that 22% to 71% of the cases exhibited

ERBB2 amplification [3,4]. Unfortunately, since their primary objective was to correlate immunohistochemical staining for *ERBB2* protein with amplification by FISH analysis, the majority of these studies suffered from small sample sizes ranging from 23 to 43 women [3,4]. In addition, these studies did not evaluate clinical correlates such as progression-free survival (PFS) or overall survival (OS).

High throughput techniques such as tissue microarray (TMA) have allowed analysis of large number of specimens for *ERBB2* amplification. FISH analysis by TMA of invasive ovarian cancers of all stages revealed a 24% amplification rate [14,15]. In a series of 173 invasive ovarian cancers of varying stages and histologies, only a 7.4% amplification rate was found [14]. While in a series of 103 high grade advanced stage EOC of various histologies, a 33.3% amplification rate of *ERBB2* was observed [15]. Both studies also revealed a lack of correlation between *ERBB2* overexpression by immunohistochemistry and *ERBB2* amplification by FISH analysis. Neither study demonstrated an association between *ERBB2* amplification or overexpression and PFS or OS [14,15].

To further elucidate the predictive and prognostic significance of *ERBB2* amplification in EOC, the Gynecologic Oncology Group (GOG) analyzed primary tumor from 133 women with suboptimally-resected, advanced stage, EOC who participated in GOG protocol 111 (GOG-111), a multi-center phase III randomized trial of cyclophosphamide and cisplatin vs. paclitaxel and cisplatin [16] and a companion protocol GOG-9404 which collected a formalin-fixed and paraffin-embedded (FFPE) primary tumor block from women enrolled in GOG-111 for translational research.

Materials and methods

Patients

The eligibility criteria for GOG-9404 were as follows. Women with previously-untreated, suboptimally-resected, advanced stage EOC who participated in the randomized phase III treatment protocol GOG-111 [16], completed the chemotherapy portion of the protocol, provided adequate follow-up information and a had representative FFPE primary tumor block from the initial surgical staging for submission for translational research. Patients provided written informed consent consistent with federal, state and local institutional requirements for GOG-111 protocol. In addition, GOG-111 and GOG-9404 were approved by the GOG, the Cancer Therapy Evaluation Program of the National Cancer Institute and the institutional review board at each of the participating GOG institutions in accordance with assurances filed with and approved by the Department of Health and Human Services. Histologic diagnosis was confirmed centrally by the GOG Pathology Committee.

FISH procedure

FISH was performed by Esoterix Oncology (4509 Freidrich Lane, Building 1, Suite 100, Austin, TX 78744) in FFPE primary tumor using a *ERBB2* gene (17q.11.2–17q12) probe and a centromeric a-satellite probe (D17Z1) specific for chromosome17 (CEP17). The *ERBB2* and CEP17 probe were simultaneously labeled with SpectrumOrange (peak excitation/emission = 559/588) and SpectrumGreen (peak excitation/emission = 509/538),

respectively. The slide-mounted specimens were immersed in 70% formamide/2× SSC, pH 7.0 for 5 min at 73 °C, then dehydrated in 70% ethanol for 1 min, 85% ethanol for 1 min, and 100% ethanol for 1 min. Probe mixture was prepared as directed by Vysis' protocol. Ten ml of probe mixture was applied onto pre-warmed (45 °C) slide-section. Hybridization was carried out in a humidified box for 14–18 h at 37 °C followed by post hybridization wash with Wash buffer I (0.4× SSC/0.3% NP-40) at 72 °C for 2 min, Wash Buffer II (2× SSC/0.1%NP-40) at room temperature for 1 min. Counter stain was performed with 15 ml of DAPI (4′,6-diamidino-2-phenylindole) (a mixture of DAPI I and DAPI II, Vysis).

FISH scoring criteria

A minimum of 100 cells were counted for *ERBB2* and CEP17 signals by coauthor (S.F.). Mean *ERBB2* copies were normalized by number of CEP17 copies in order to confirm whether increased number of *ERBB2* signal was result from gene amplification or aneusomy of chromosome 17. *ERBB2*/chromosome 17 copies were categorized as normal (0–2.0) and amplified (>2.0). Simultaneously, mean number of *ERBB2* copies per nucleus were calculated and categorized as normal (0–4.0) and amplified (>4.0).

Platinum-based chemotherapy

Women on GOG-111 were randomly allocated to receive six cycles of cisplatin and cyclophosphamide or six cycles of paclitaxel and cisplatin every three weeks as previously reported [16].

End points

Evaluation criteria and definitions for tumor response, disease status post-chemotherapy, PFS and OS were previously reported [16,17].

Statistical methods

Data were analyzed using SPSS versions 14.0 (SPSS Inc., Chicago, IL) and SAS® version 9.1 software (SAS Institute, Inc. Cary, NC). All tests were two-sided and the level of significance was set at 0.05. Associations between categorical variables were evaluated using Fisher's exact test [18,19]. PFS and OS were estimated using the Kaplan–Meier method [20] and the logrank test was used to compare the survival distributions between groups categorized by *ERBB2* amplification [21]. Unadjusted and adjusted Cox proportional hazards regression analyses were performed to model the association between *ERBB2* amplification and PFS or OS [22,23]. The multivariate analyses were performed with clinical covariates added during block 1 and *ERBB2* amplification added during block 2 of model development.

Results

GOG-9404 was a retrospective translational research protocol designed to examine p53 and *ERBB2* in advanced stage epithelial ovarian cancer and correlations with prognostic factors and treatment outcome. The results of the associations between p53 overexpression and clinical outcome were the subject of a separate publication [17]. The characteristics of 133 women who participated in this study are summarized in Table 1 and are representative of

that observed in the entire GOG-111 cohort [16]. At the time of the final analysis, seven women were alive with no evidence of the disease, five women were alive with disease progression and 121 women died. Among those who died, 97.5% of the deaths were attributed to disease progression, 0.8% were due to treatment, and 1.7% were caused by something other than disease or treatment. Median PFS and OS times for this cohort were 16.8 and 34.7 months, respectively.

ERBB2 amplification defined as >2 copies of ERBB2/CEP17

ERBB2 amplification was defined as >2 copies of *ERBB2*/CEP17, in accordance with previous studies in advanced epithelial ovarian cancer [15]. This was a rare event in EOC with only 7% (9/133) of women exhibiting between 2.2 and 33.7 copies of *ERBB2*/CEP17. Representative photomicrographs are provided that illustrate normal (Fig. 1A) and amplified *ERBB2* (Fig. 1B) in the GOG-9404 cohort. There was no evidence to suggest that *ERBB2* amplification, defined as >2 copies of *ERBB2*/CEP17, was associated with patient age, race/ethnicity, GOG performance status, stage, cell type, grade, measurable disease status or volume of ascites (Table 2), or with tumor response (Table 3), disease status post-chemotherapy (Table 3), PFS (Fig. 2A) or OS (Fig. 2B).

Women with >2 copies of *ERBB2*/CEP17 did not have a reduced risk of disease progression (hazard ratio [HR] = 0.56; 95% confidence interval [CI] = 0.27–1.16; p = 0.120) or death (HR = 0.57; 95% CI = 0.26–1.23; p = 0.152) compared with women with 2 copies of *ERBB2*/CEP17 (Table 4). After stratifying by patient age and adjusting for tumor stage, cell type, grade, measurable disease status, volume of ascites and treatment, *ERBB2* amplification was not an independent prognostic factor for PFS (HR = 0.56; 95% CI = 0.26–1.19; p = 0.132) or OS (HR = 0.53; 95% CI = 0.23–1.17; p = 0.115).

ERBB2 amplification defined as >4 copies of ERBB2/nuclei

ERBB2 amplification was also defined as >4 copies of *ERBB2*/nuclei [15]. Using this definition, *ERBB2* amplification was still a rare event in EOC with only 9% (12/133) of women with levels ranging from 4.2 to 49.2 copies of *ERBB2*/nuclei. There was a direct association between *ERBB2* amplification and older age but not with race/ethnicity, GOG performance status, stage, cell type, grade or measurable disease status (Table 2). An inverse relationship was observed between *ERBB2* amplification and >100 ml of ascites (Table 2). There was no evidence to suggest that *ERBB2* amplification, defined as >4 copies of *ERBB2*/nuclei, was associated with tumor response (Table 3), disease status post-chemotherapy (Table 3), PFS (Fig. 3A) or OS (Fig. 3B).

Women with >4 copies of *ERBB2*/nuclei did not have a reduced risk of disease progression (HR = 0.90; 95% CI = 0.48–1.67; p = 0.730) or death (HR = 0.99; 95% CI = 0.53–1.85; p = 0.980) compared with women with 4 copies of *ERBB2*/nuclei (Table 4). After adjusting for the clinical covariates, *ERBB2* amplification was not an independent prognostic factor for PFS (HR = 0.71; 95% CI = 0.36–1.39; OS (HR = 0.76; 95% CI = 0.38–1.49; p = 0.420).

Discussion

ERBB2 amplification defined as >2 copies of *ERBB2*/CEP17, or as >4 copies of *ERBB2*/ nuclei, was a rare event and not associated with any of the usual clinical parameters such as race, GOG performance status, International Federation of Gynaecology and Obstetrics (FIGO) stage, histologic cell type, tumor grade, or gross residual disease. *ERBB2* amplification per chromosome 17 or per nuclei was not associated with tumor response, disease status post-chemotherapy, PFS or OS. A number of studies have suggested that overexpression of *ERBB2* indicates poor prognosis in ovarian, breast, endometrial, and colon cancers [5,24,27,28,9,30-35]. Tissue microarray analysis of 300 breast cancers by immunohistochemistry found that high expression of *ERBB2* was associated with a poor outcome [24]. Immunohistochemistry evaluation of 670 breast cancer specimens for *ERBB2* and EGFR expression revealed that the combination of epidermal growth factor receptor (EGFR) and *ERBB2* expression was an independently significant factor for disease free survival and OS [29]. *ERBB2* expression in endometrial cancer was associated with a more malignant phenotype and worse PFS [25,27], and was an independent prognostic factor for OS [27].

The association of *ERBB2* overexpression with prognosis in EOC has been less clear. The evaluation of 73 ovarian cancers by immunohistochemistry found a decreased median survival time of 15.7 months for women with *ERBB2* overexpression versus 32.8 months for women with normal *ERBB2* expression [5]. *ERBB2* overexpression has also been associated with a decreased total dose–response effect to cisplatin based chemotherapy in ovarian cancer [34]. Conversely, in an immunoperoxidase technique analysis of 40 early stage and 105 advanced stage ovarian cancer patients, *ERBB2* overexpression occurred infrequently, and upon multivariate analysis *ERBB2* expression was not a prognostic marker for OS [7,8].

It was hoped that gene amplification analysis would resolve some of these conflicting results, however associations between amplification of the *ERBB2* gene and prognosis in EOC have also had disparate results. Multicolor FISH allows the simultaneous study of individual cells for amplification. FISH also allows the quantification of the amount and distribution of the oncogene signals and the number of centromeres present in each cell [4]. FISH analysis in ovarian cancer has associated *ERBB2* amplification with more advanced stage [3,36,4,37]. These small studies however have found no specific clinical correlation between *ERBB2* amplification and the usual clinical prognostic factors, but the lack of correlation was possibly thought to be attributable to small sample size [4,37].

With the advent of tissue microarray (TMA) technology scientists have been able to solve the small sample size dilemma through the evaluation of large numbers of specimens by FISH analysis. A FISH analysis of 79 FIGO stage I and II EOC found a 6.7% rate of *ERBB2* amplification. No clinical correlation of survival was attempted in this study secondary to the limited number of informative cases in the sample set [13]. A FISH analysis of 103 advanced stage ovarian cancer specimens using a fluorescence ratio of 2.0 and 1.5 as cutoffs found *ERBB2* amplification rates of 10.7% and 33.3% respectively [15]. They found that immunohistochemical expression of *ERBB2* protein was not correlated with *ERBB2* amplification by FISH analysis. They also found *ERBB2* amplification defined as

>2.0 copies of *ERBB2*/CEP17 was not associated with PFS [15]. This study involved women with advanced stage disease but encompassed a variety of histologies and treatment regimens. Finally this was a retrospective cohort study in which the patients identified for analysis were obtained through a registry review of the British Columbia Cancer Agency. Thus the data analyzed are subject to the accuracy of the data entered into the registry. Operative reports, chemotherapy treatment cycles, and tissue histology were not specifically reviewed to ensure accuracy of data obtained [15].

A comprehensive FISH analysis of 173 invasive ovarian cancers of all stages however, revealed that *ERBB2* amplification was associated with immunohistochemical expression of *ERBB2*, but not with tumor stage, histologic cell type, grade or prognosis [14]. In this study, seventy-nine percent of the invasive EOC specimens analyzed were of serous histology. The treatment period however spanned 17 years from 1985 to 2002, and as a result probably included a variety of adjuvant chemotherapy regimens. Although the authors conclude that *ERBB2* amplification did not correlate with FIGO stage, the authors did not specifically give the percentages of early and advanced stage cancers analyzed. They also did not clarify any inclusion of borderline or germ cell tumors, which were evaluated for *ERBB2* amplification, and may have been included in the analysis [14]. The current study is truly comprehensive and solves the pitfalls of the previous studies of the predictive and prognosis associations of *ERBB2* amplification in EOC by examining a large cohort (*N*= 133) of women who were uniformly staged and treated. Only 71% of the women with suboptimally-resected, advanced stage disease had serous adenocarcinomas which is representative of that observed for this patient population [16,38].

As previously stated the lack of correlation between *ERBB2* amplification and survival in the current study contradicts evaluation of this marker in other cancers including breast and endometrial cancer [4,5,9,24-30-35]. The disparity in findings does not reflect the use of a different cut point for *ERBB2* amplification between disease sites. Using the criteria established for breast cancer in 2007 [39], *ERBB2* amplification defined as >2.2 copies of *ERBB2*/CEP17 or >4 copies of *ERBB2*/nuclei was a rare event in EOC occurring in 7% (9/133) or 4% (5/133) of the women in this cohort, respectively, and was not associated with any measure of clinical outcome including tumor response, disease status post-chemotherapy, PFS or OS (data not show). Moreover, our finding, is consistent with some clinical observations in ovarian cancer. In a phase II evaluation of Trastuzumab by the Gynecologic Oncology group in 837 patients with recurrent ovarian cancer, 95 tumors (11%) exhibited overexpression of *ERBB2* by immunohistochemistry [10]. Treatment of 41 patients with Trastuzumab by weekly infusion revealed only a 7% response rate. They also found no evidence to suggest that tumor expression of *ERBB2* was associated with tumor response, PFS or OS [10].

The current study is comprehensive and as a result, solves the pitfalls of the previous studies regarding the predictive and prognostic associations of *ERBB2* amplification in EOC. We report a large FISH analysis of advanced stage, uniformly treated, suboptimally debulked, ovarian cancer patients who were treated on GOG protocol 111. To date this is the largest analysis of a homogeneously treated patient population. It should be noted however that patients randomized to the cyclophosphamide + cisplatin arm were found to have an inferior

treatment when comparing progression-free and overall survival compared to the paclitaxel + cisplatin arm which was adjusted for in the statistical analysis and no difference was noted. Our analysis demonstrated that *ERBB2* amplification using either definition was a rare event, was not associated with tumor response, disease status post-chemotherapy, PFS or OS, and was not associated with patient race/ethnicity, tumor stage, cell type, grade or measurable disease status. *ERBB2* amplification defined as >4 copies of *ERBB2*/nuclei, but not as >2 copies of *ERBB2*/CEP17, was directly associated with older age and inversely associated with >100 ml ascites. The lack of association between *ERBB2* amplification defined as >4 and PFS and OS could be due to the relatively small power of our study and the low incidence (9%) of this event. The ratio of *ERBB2* copies per nuclei is likely a more accurate assessment of true *ERBB2* amplification because it could be possible to amplify a complete diploid chromosome 17 many times and still have a *ERBB2* to chromosome 17 ratio that is unity. FISH analysis does provide a powerful accurate method for the evaluation of oncogene amplification among tumor samples. In conclusion, *ERBB2* amplification is a rare event and has no predictive or prognostic value in suboptimally-resected, advanced stage EOC treated with platinum-based combination chemotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

AOC	advanced ovarian cancer
FISH	fluorescence *in situ* hybridization
FIGO	International Federation of Gynecologic Oncologists
GOG	Gynecologic Oncology Group

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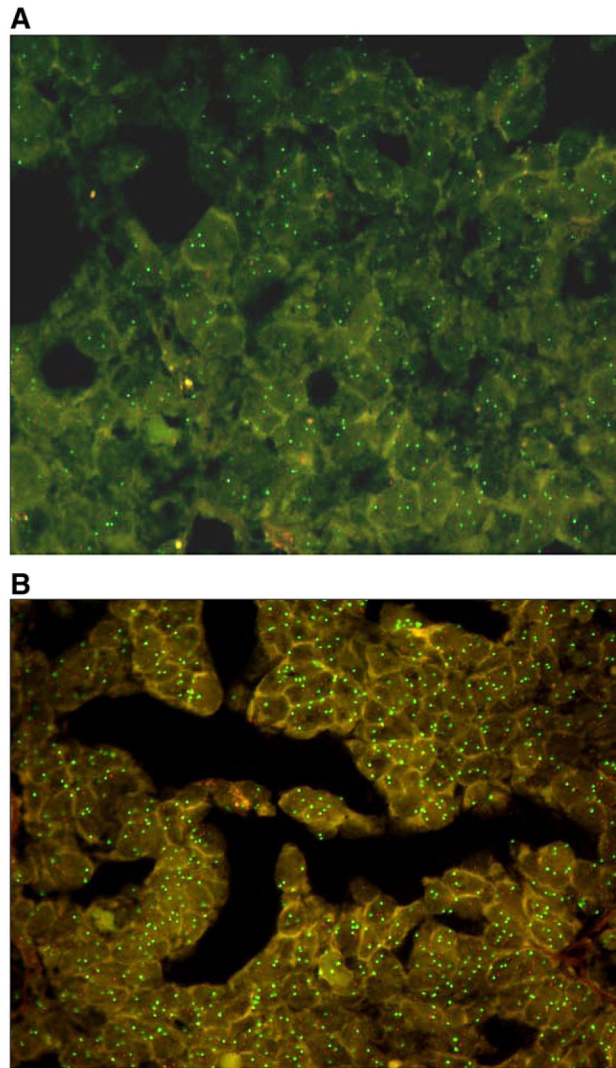
Fig. 1.
FISH analysis. Representative FISH analysis of *ERBB2* labeled with SpectrumOrange and CEP17 labeled with SpectrumGreen illustrating a normal ratio (A), and amplification of *ERBB2* (B).
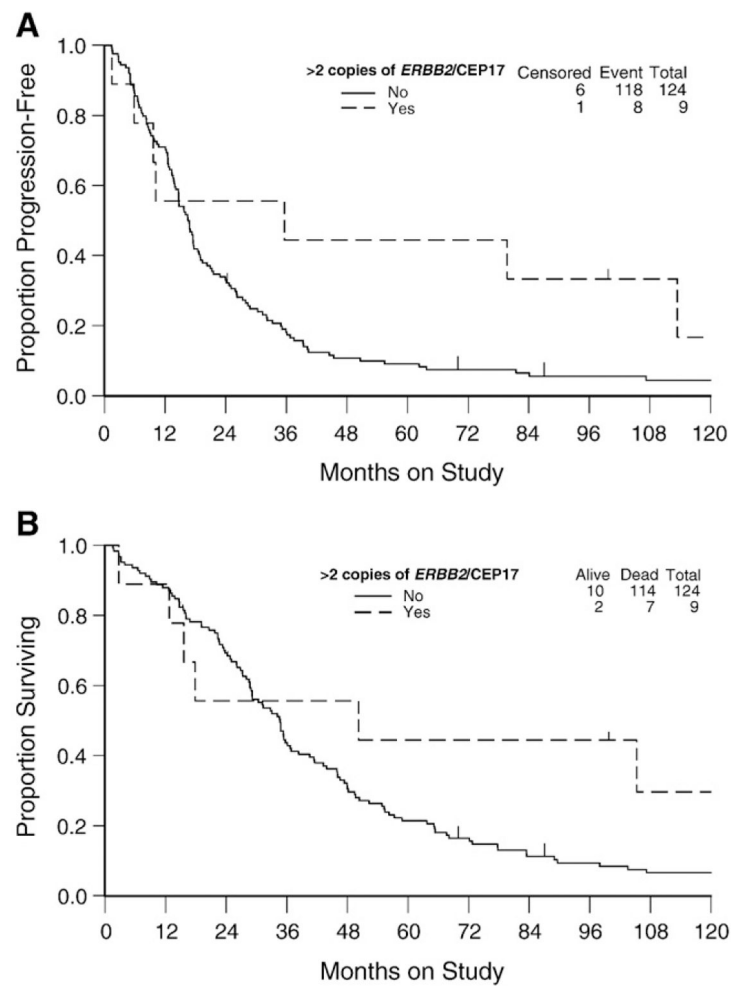
Fig. 2.
Kaplan–Meier estimate of progression-free survival (A) and overall survival (B) for women with or without *ERBB2* amplification defined as >2 copies of *ERBB2*/CEP17. Censored indicates patients who were alive with no evidence of disease progression. Logrank test was used to evaluate the equality in progression-free survival ($p = 0.115$) and survival ($p = 0.147$) distributions between women with or without *ERBB2* amplification defined as >2 copies of *ERBB2*/CEP17.
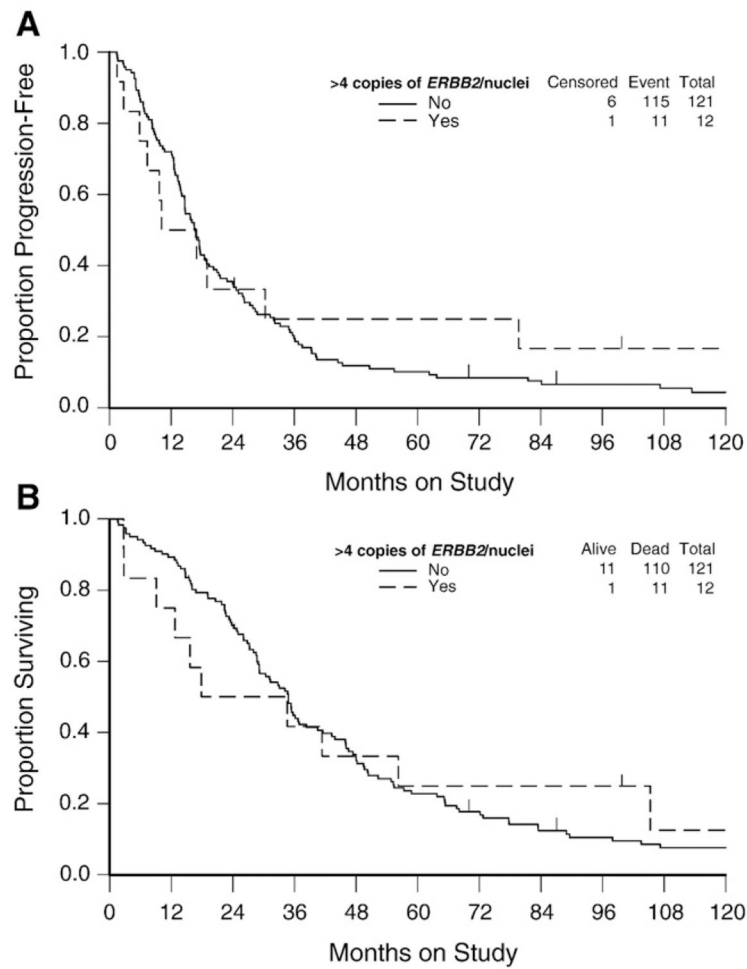
Fig. 3.
Kaplan–Meier estimate of progression-free survival (A) and overall survival (B) for women with normal, borderline or amplified *ERBB2*/nuclei copies. Censored indicates patients who were alive with no evidence of disease progression. Logrank test was used to evaluate the equality in progression-free survival ($p = 0.730$) and survival ($p = 0.980$) distributions between women with normal and amplified *ERBB2*/nuclei copies.

Table 1

Clinical characteristics

Characteristics	Cases	%
Age (in years) median (range) 59.5 (21.7–78.6)		
<50	36	27.1
50–59	33	24.8
60–69	46	34.6
70	18	13.5
Performance		
Asymptomatic	41	30.8
Symptomatic	92	69.2
Tumor stage		
III	84	63.2
IV	49	36.8
Histologic cell type		
Serous	94	70.7
Endometrioid	12	9.0
Mucinous	5	3.8
Clear cell	3	2.3
Other	19	14.3
Tumor grade		
1 Well differentiated	6	4.5
2 Moderately differentiated	58	43.6
3 Poorly differentiated/not specified	69	51.9
Gross residual disease		
Measurable	80	60.2
Non-measurable	53	39.8
Ascites		
<100 ml	47	35.3
100 ml	86	64.7
Treatment		
Cyclophosphamide + cisplatin	68	51.1
Paclitaxel + cisplatin	65	48.9

Table 2

Association between *ERBB2* amplification and clinical characteristics

| | >2 *ERBB2*/CEP17 copies | | | | | >4 Copies of *ERBB2*/nuclei | | | | |
| | No | | Yes | | *p*-value[a] | No | | Yes | | *p*-value[a] |
	Cases	%	Cases	%		Cases	%	Cases	%	
Age (in years)					NS					0.010
<50	34	94.4	2	5.6		36	100.0	0	0.0	
50-59	32	97.0	1	3.0		30	90.9	3	9.1	
60-69	44	95.7	2	4.3		42	91.3	4	8.7	
70-79	14	77.8	4	22.2		13	72.2	5	27.8	
Race and ethnicity					NS					NS
Caucasian	112	94.1	7	5.9		109	91.6	10	8.4	
African American	8	88.9	1	11.1		8	88.9	1	11.1	
Hispanic	2	100.0	0	0.0		2	100.0	0	0.0	
Other	2	66.7	1	33.32		2	66.7	1	33.3	
Performance status					NS					NS
Asymptomatic	39	95.1	2	4.9		37	90.2	4	9.8	
Symptomatic	85	92.4	7	7.6		84	91.3	8	8.7	
Tumor stage					NS					NS
III	79	94.0	5	6.0		75	89.3	9	10.7	
IV	45	91.8	4	8.2		46	93.9	3	6.1	
Histologic cell type					NS					NS
Serous	87	92.6	7	7.4		85	90.4	9	9.6	
Endometrioid	12	100.0	0	0		11	91.7	1	8.3	
Mucinous	4	80.0	1	20.0		4	80.0	1	20.0	
Clear cell	3	100.0	0	0		3	100.0	0	0	
Other	18	94.7	1	5.3		18	94.7	1	5.3	
Tumor grade					NS					NS
1 Well differentiated	5	83.3	1	16.7		5	83.3	1	16.7	
2 Moderately differentiated	52	89.7	6	10.3		53	91.4	5	8.6	
3 Poorly differentiated/not specified	67	97.1	2	2.9		63	91.3	6	8.7	

	>2 *ERBB2*/CEP17 copies					>4 Copies of *ERBB2*/nuclei				
	No		Yes		p-value [a]	No		Yes		p-value [a]
	Cases	%	Cases	%		Cases	%	Cases	%	
Gross residual disease					NS					NS
Measurable	74	92.5	6	7.5		70	87.5	10	12.5	
Non-Measurable	50	94.3	3	5.7		51	96.2	2	3.8	
Ascites					NS					0.004
<100 ml	43	91.5	4	8.5		38	80.9	9	19.1	
100 ml	81	94.2	5	5.8		83	96.5	3	3.5	

NS: *p*-value >0.05.

[a]Fisher's exact test.

Table 3

Association between *ERBB2* amplification and clinical outcome[a]

ERBB2 Amplification	Progression-free survival						Overall survival					
	Unadjusted model			Adjusted model[b]			Unadjusted model			Adjusted model[b]		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
>2 Copies ERBB2/CEP17												
No (*N* = 124)	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
Yes (*N* = 9)	0.56	0.27–1.16	0.120	0.56	0.26–1.19	0.132[c]	0.57	0.26–1.23	0.152	0.53	0.23–1.17	0.115[d]
>4 Copies ERBB2/nucleus												
No												
Yes	0.90	0.48–1.67	0.730	0.71	0.36–1.39	0.314[e]	0.99	0.53–1.85	0.980	0.76	0.38–1.49	0.420[f]

HR: hazard ratio estimated per 0.1 U change in copies of *ERBB2*/CEP17 or *ERBB2*/nuclei; 95% CI: 95% confidence interval.

[a] Cox regression analysis for modeling the relative risk of disease progression or death. The goodness of fit of the overall model was evaluated using the likelihood ratio test and the association between the *ERBB2* gene amplification and outcome was assessed using the Wald test.

[b] Modeling was adjusted for patient age and stratified by tumor stage (III vs. IV), histologic subtype (clear cell or mucinous vs. other histologic subtypes), tumor grade (well differentiated vs. moderately differentiated vs. poorly differentiated), measurable disease (no vs. yes), ascites (<100 ml vs. 100 ml) and treatment regimen (cyclophosphamide plus cisplatin vs. paclitaxel plus cisplatin).

[c] Inclusion of *ERBB2* gene amplification defined as >2 copies of *ERBB2*/CEP17 into the Cox model for PFS reduced the −2 log likelihood from 991.773 to 989.168 and did not add significant prognostic information over the clinical covariates added during the first stage of model development (*p*=0.107).

[d] Inclusion of *ERBB2* gene amplification defined as >2 copies of *ERBB2*/CEP17 into the Cox model for OS reduced the −2 log likelihood from 955.613 to 952.696 and did not add significant prognostic information over the clinical covariates added during the first stage of model development (*p*=0.088).

[e] Inclusion of *ERBB2* gene amplification defined as >4 copies of *ERBB2*/nuclei into the Cox model for PFS reduced the −2 log likelihood from 991.773 to 990.693 and did not add significant prognostic information over the clinical covariates added during the first stage of model development (*p*=0.299).

[f] Inclusion of *ERBB2* gene amplification defined as >4 copies of *ERBB2*/nuclei into the Cox model for OS reduced the −2 log likelihood from 955.613 to 954.929 and did not add significant prognostic information over the clinical covariates added during the first stage of model development (*p*=0.408).

Table 4

Association between *ERBB2* amplification and clinical response or results of second look laparotomy[a]

	Tumor response				Evidence of disease/progression after chemotherapy			
	Cases	No	Yes	p-value[†]	Cases	No	Yes	p-value[‡]
>2 copies *ERBB2*/CEP17				NS				NS
No	72	19 (26.4)	53 (73.6)	20 PR 33 CR	115	25 (21.7)	90 (78.3)	55+RL 35+DP
Yes	6	2 (33.3)	4 (66.7)	3 PR 1 CR	8	2 (25.0)	6 (75.0)	3+RL 3+DP
>4 copies *ERBB2*/nuclei				NS				NS
No	68	16 (23.5)	52 (76.5)	19 PR 33 CR	112	26 (23.2)	86 (76.8)	54+RL 32+DP
Yes	10	5 (50.0)	5 (50.0)	4 PR 1 CR	11	1 (9.1)	10 (90.9)	4+RL 6+DP

[†] Response includes a complete response (CR) or a partial response (PR). Of the 80 women with measurable disease, only 78 were evaluated for tumor response. An additional 53 women had non-measurable disease and were not evaluable for tumor response.

[‡] Evidence of disease/progression after chemotherapy includes women with microscopic or gross disease at reassessment laparotomy (+RL) or with clinical evidence of disease progression documented during first-line treatment (+DP). Ten women did not undergo reassessment laparotomy as specified in the protocol due to patient refusal or medical contraindications.

[a] Fisher's exact test; NS: *p*-value>0.05.