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ORIGINAL RESEARCH

Human epidermal growth factor receptor-2 overexpression and amplification in metastatic and recurrent high grade or type 2 endometrial carcinomas

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Introduction: Human epidermal growth factor receptor (HER)-2 overexpression or gene amplification is more common in high-grade or type 2 endometrial carcinomas. We assessed the discordance of HER-2 expression between primary and metastatic or recurrent endometrial carcinomas.

Materials and methods: Thirty-six primary, along with 14 metastatic and five recurrent tumors (matched to primaries), pathologically confirmed as high-grade or type 2 endometrial carcinomas, were submitted for immunohistochemistry (IHC) for HER-2. Fluorescence in situ hybridization was performed when the tumors showed HER-2 overexpression (≥2+ IHC score). The results of the IHC and fluorescence in situ hybridization assays were compared between the primary and metastatic or recurrent tumors. The relationships between HER-2 expression and clinicopathological factors or prognosis were investigated.

Results: HER-2 overexpression and HER-2 amplification (a ratio of HER-2 copies to chromosome 17 [CEP17] copies ≥2.2) were detected in 33.3% (twelve of 36 patients) and 5.6% (two of 36 patients) of primary tumors, respectively. HER-2 overexpression was not associated with clinicopathological factors or prognosis. In 19 tumor specimens obtained from metastatic or recurrent tumors, HER-2 overexpression and HER-2 amplification were detected in 57.9% (eleven patients) and 15.8% (three patients), respectively. HER-2 overexpression tended to predict a worse prognosis.

Conclusion: HER-2 expression in metastatic or recurrent tumors was more frequent than in matched primary high-grade or type 2 endometrial carcinomas. Trastuzumab in combination with cytotoxic chemotherapy may represent an alternative therapeutic option for these tumors.

Keywords: endometrial carcinoma, high grade, type 2, HER-2, metastatic or recurrent tumors

Introduction

The epidermal growth factor receptor (EGFR) family consists of four members – EGFR (human EGFR [HER]-1), HER-2, HER-3, and HER-4 – which have homologous extracellular domains and a cytoplasmic signal-transduction domain with tyrosine kinase activity.[1] HER-2 (also known as c-erbB-2), is a 185 kD receptor protein encoded by the HER-2/*neu* gene localized on chromosome 17q21. While no specific ligand has been identified for HER-2, it forms homodimers and heterodimers with the other HER receptors with subsequent ligand-independent activation. HER-2/*neu* amplification results in HER-2 overexpression with increased ligand-independent activation leading

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to mitogenic cell signaling and cell proliferation along with antiapoptotic and angiogenic effects.[2,3]

HER-2 overexpression has been associated with more aggressive biological behavior of various kinds of cancers including breast, gastric, ovarian, prostate, bladder, and lung.[4–9] In breast cancer, HER-2 gene amplification, which is present in 20% to 30% of cases, is an independent prognostic factor. This led to the development of trastuzumab (Herceptin®; Genentech Inc, San Francisco, CA, USA), a monoclonal antibody targeting HER-2,[10] which induces antibody-dependent cellular cytotoxicity, inhibits HER-2-mediated signaling, and prevents cleavage of the extracellular domain of HER-2.[11] Trastuzumab increases overall survival (OS) when coadministered with cytotoxic chemotherapy in patients with HER-2-positive, early and metastatic breast cancer,[12] and is now the standard of care. Similarly, in gastric cancers, in which HER-2 overexpression is present in 7% to 34% of cases, a Phase III trial has confirmed the effect of trastuzumab on OS.[13]

HER-2 overexpression and HER-2 gene amplification have also been demonstrated in endometrial cancers.[14–18] There are two distinct forms of endometrial cancer – type 1 and type 2 – distinguished based on epidemiologic, molecular, and clinical characteristics.[19] The aggressive, type 2 tumors include uterine papillary serous carcinoma (UPSC) and clear cell adenocarcinoma (CCC), and carry a poor prognosis.[20,21] HER-2 overexpression or amplification is more common in type 2 endometrial carcinomas than in type 1 tumors.[22–24] In endometrial cancers, HER-2 has been associated with other clinicopathological prognostic factors and a poor prognosis.[18,22]

Responses to trastuzumab of advanced or recurrent type 2 endometrial carcinomas (including UPSC) with HER-2 overexpression have been described in case reports.[25–27] However, a recent Phase II trial (GOG 181B) of trastuzumab as a single agent failed to demonstrate activity against advanced or recurrent endometrial carcinomas harboring HER-2 overexpression or HER-2 gene amplification.[28] The interpretation of the results of this trial is still under debate.[29] It is possible that the results may have been affected by changes in HER-2 expression between primary and recurrent tumors. Recurrent tumors are often genetically different from primary tumors. In breast cancer, HER-2 amplification is significantly more frequent in distant metastatic tumors than in primary tumors.[30] In metastatic breast cancer, a high level of HER-2 protein expression and amplification predict the response to trastuzumab.[10] Whether these facts are consistent in endometrial cancers is not well known. Herein, we assess the discordance of HER-2 overexpression or amplification between primary tumors and metastatic or recurrent tumors of high-grade or type 2 endometrial carcinomas.

Materials and methods

Thirty-six patients, all of whom were treated at our institute from 2000 to 2010 with pathologically confirmed high-grade or type 2 endometrial carcinomas, were enrolled in this study. All of the patients underwent comprehensive surgical staging including total abdominal hysterectomy, bilateral salpingo–oophorectomy, and omentectomy with pelvic and/or paraaortic lymph node dissection. Staging, defined according to the International Federation of Gynecology and Obstetrics (FIGO) staging system: 16 patients were classified as stage 1; 13 patients as stage 3; and seven patients as stage 4. Pathologically confirmed histological subtypes included grade 3 endometrioid adenocarcinoma (EMC-G3; n = 19), papillary serous adenocarcinoma (UPSC; n = 5), and clear cell adenocarcinoma (CCC; n = 12). Tumor samples were obtained from primary uterine endometrial tumors of all 36 patients and metastatic tumors from 14 out of 36 patients (EMC-G3, eight patients; UPSC, four patients; and CCC, two patients) at the staging surgery for each patient. Metastatic lesions were as follows: lymph node, eight samples; omentum, three samples; ovary, two samples; and colon, one sample. All of the patients received six cycles of TC chemotherapy (paclitaxel 175 mg/m^2 and carboplatin area under the blood concentration time curve, 6.0) as postoperative adjuvant therapy. There was no patient who received radiotherapy. After completion of adjuvant chemotherapy, six patients showed persistent or progressive disease and ten patients had recurred. Five of ten patients with recurrent disease, two patients with EMC-G3, and three patients with CCC, underwent cytoreductive surgery. The recurrent lesions were as follows: colon, two samples; spleen, one sample; adrenal gland, one sample; and vaginal wall, one sample. The patients with persistent, progressive, or recurrent disease were treated with second-line chemotherapy with doxorubicin and cisplatin, docetaxel and carboplatin, or irinotecan.

All tumor samples (36 primary tumors, 14 metastatic tumors, and five recurrent tumors) were submitted for immunostaining. The current study was approved by our institutional review board.

IHC assay

Immunohistochemical (IHC) staining was performed on formalin-fixed, paraffin-embedded specimens using Pathway HER-2 (Clone CB11) on the BenchMark XT automated

system (Ventana Medical Systems, Inc, Tucson, AZ) according to the manufacturer's recommended protocol. Each formalin-fixed, paraffin-embedded specimen was selected after careful inspection in matched hematoxylin and eosin-stained slides, which represented the typical morphological features for each case. Evaluation of staining followed the breast cancer guideline [Clinical Oncology/ College of American Pathologists (ASCO/CAP) guideline].[31] Briefly, a 3+ score is defined as intense membrane staining in more than 30% of invasive cells; 2+ is defined as moderate membrane staining in more than 10% of cells, or intense membrane staining in 10% to 30% of cells; and a score of 1+ or 0 is defined as light or no membrane staining. Cases with an IHC score of 1+ or 0 were categorized as negative, and an IHC score ≥2+ was defined as HER-2 overexpression. Evaluation by visual inspection was performed by one gynecologic oncologist (KH) and one expert cytopathologist (HH). Both were blinded to all clinical information. Disagreements were resolved by the joint review of two investigators. The cases with an IHC score ≥ 2+ were confirmed with the fluorescence in situ hybridization (FISH) assay.

FISH assay

FISH was performed when the tumors showed 2+ or 3+ positivity for HER-2 by IHC. FISH for HER-2 amplification was performed manually on tissue sections adjacent to those analyzed by IHC using the PathVysion HER-2 DNA Probe kit (Vysis, Inc, Dover, IL, USA) according to the manufacturer's protocol. Tumor cells displaying at least two centromeric chromosome-17 (CEP17) signals and multiple HER-2 signals, with a HER-2/CEP17 ratio ≥2.2 were considered to have HER-2 amplification in accordance with the breast cancer ASCO/CAP guideline.[31] The results of the IHC and FISH assay were compared between the primary tumors and the matched metastatic or recurrent tumors.

Correlation between HER-2 expression, clinicopathological factors, and prognosis

The relationships between HER-2 expression and clinicopathological factors, including age, histological subtypes, International Federation of Gynecology and Obstetrics stage, myometrial invasion, vascular invasion, peritoneal cytology, and lymph node metastases were investigated in all 36 patients, as well as in 19 patients whose metastatic or recurrent tumors could be obtained. Moreover, the relationships between HER-2 expression and progression-free survival rates (PFS) and OS rates were also investigated.

Statistical analysis

Statistical analysis of the relationship between clinicopathological factors and HER-2 expression was performed using Fisher's exact test. The Kaplan–Meier method was used to evaluate the PFS and OS between the HER-2-negative and overexpression groups, and the log-rank test was used to assess the differences between groups. A value of $P < 0.05$ was defined as statistically significant.

Results

HER-2 overexpression (≥2+ IHC score) was detected in 33.3% (twelve of 36 patients) in primary uterine endometrial tumors (Table 1). HER-2 IHC expression was not associated with any clinicopathological factor in all 36 high-grade or type 2 endometrial carcinoma patients (Table 2). The PFS for the HER-2-negative and overexpression groups were 61.9% and 41.7%, with observation periods of 6 months to 110 months (median 40 months) and 5 months to 120 months (median 39 months), respectively; this was not a significant finding ($P = 0.21$) (Figure 1). The OS for the HER-2-negative and overexpression groups were 68.5% and 64.3%, respectively; this was not significant ($P = 0.78$) (data not shown). In the 19 tumor tissues obtained from metastatic (n = 14) or recurrent (n = 5) tumors, HER-2 overexpression was detected in 57.9% of patients (eleven of 19 patients) (Table 3).

HER-2 amplification (a ratio of HER-2 copies to CEP17 copies ≥2.2) was detected in 5.6% (two of 36 patients) of primary endometrial tumors (EMC-G3 and CCC) (Table 4). On the other hand, HER-2 amplification was detected in 15.8% (three of 19 patients) in metastatic or recurrent tumors (EMC-G3, UPSC, and CCC). HER-2 expression (IHC scores) or HER-2/CEP17 ratios by FISH were increased in nine of 14 metastatic tumors compared with the matched primary uterine endometrial tumors for each patient (Table 4). Furthermore, IHC scores or HER-2/CEP17 ratios were increased in all five recurrent tumors compared with the matched primary uterine endometrial tumors (Table 4).

Table 1 HER-2 expression in primary tumors (IHC)

Histological subtypes	IHC scores				HER-2 overexpression
	0	1+	2+	3+	
EMC-G3	4	7	7	1	8/19 (42.1%)
UPSC	2	1	2	0	2/5 (40.0%)
CCC	4	6	1	1	2/12 (16.7%)
Total	10	14	10	2	12/36 (33.3%)

Abbreviations: HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; EMC-G3, grade 3 endometrioid adenocarcinoma; UPSC, uterine papillary serous carcinoma; CCC, clear cell adenocarcinoma.

Table 2 The relationship between HER-2 overexpression and clinicopathological factors

	HER-2 negative (n = 24)	HER-2 overexpression (n = 12)	*P*-value
Median of age (range)	39 (36–81)	64 (47–88)	0.24
Histological subtype			
EMC-G3	11	8	0.32
UPSC	3	2	
CCC	10	2	
FIGO stage			
1	11	5	0.30
3	10	3	
4	3	4	
Myometrial invasion			
<1/2	17	8	0.80
≥1/2	7	4	
Vascular invasion			
Negative	7	5	0.45
Positive	17	7	
Peritoneal cytology			
Negative	17	9	0.79
Positive	7	3	
Lymph node metastases			
Negative	18	8	0.60
Positive	6	4	

Abbreviations: HER-2, human epidermal growth factor receptor 2; n, number; EMC-G3, grade 3 endometrioid adenocarcinoma; UPSC, uterine papillary serous carcinoma; CCC, clear cell adenocarcinoma; FIGO, International Federation of Gynecology and Obstetrics.

The actual results of the IHC and FISH assay of a case with metastatic tumors are shown in Figure 2. This case was a stage 4 UPSC where the IHC scores and HER-2/CEP17 ratios were 2+ and 1.2 for the primary tumor, and 3+ and 2.2 for the metastatic tumor (ovary), respectively (Figure 2 and Table 4).

As for the 19 patients with metastatic or recurrent tumors, the PFS for the HER-2-negative and overexpression groups were 50.0% and 10.0%, with observation periods of 6 months to 110 months (median 42 months) and 6 months to 110 months (median 21 months), respectively. Eleven patients with HER-2 overexpression tended to have a worse prognosis; however, the difference was not significant ($P = 0.08$) (Figure 3). The OS for the HER-2-negative and overexpression groups were 68.0% and 48.0%, respectively; there was no significant difference ($P = 0.50$) (data not shown).

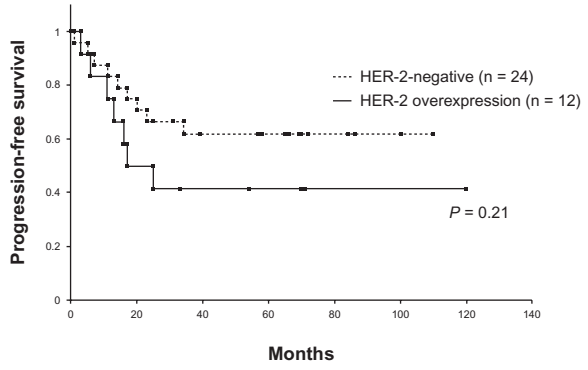

Figure 1 HER-2 expression and progression-free survival in all 36 patients.
Notes: The progression-free survival for the HER-2-negative and overexpression groups were 73.8% and 57.6%, respectively; this was not a significant finding ($P = 0.21$).
Abbreviation: HER-2, human epidermal growth factor receptor 2.

Table 3 HER-2 expression in metastatic or recurrent tumors (IHC)

Histological subtypes	IHC scores				HER-2 overexpression
	0	1+	2+	3+	
Metastatic tumors					
EMC-G3	2	2	3	1	4/8 (50%)
UPSC	0	3	0	1	1/4 (25%)
CCC	0	0	1	1	2/2 (100%)
Recurrent tumors					
EMC-G3	0	0	2	0	2/2 (100%)
CCC	0	1	2	0	2/3 (66.7%)
Total	2	6	8	3	11/19 (57.9%)

Abbreviations: HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; EMC-G3, grade 3 endometrioid adenocarcinoma; UPSC, uterine papillary serous carcinoma; CCC, clear cell adenocarcinoma.

Discussion

HER-2 overexpression and HER-2 gene amplification have been detected in 44% and 12% of endometrial carcinomas, respectively.[23] In one study, HER-2 amplification was identified in 3% of grade 1 endometrioid carcinomas, 4% of grade 2 endometrioid carcinomas, 21% of grade 3 endometrioid carcinomas, 21.4% of UPSC, and 50.0% of CCC, conclusively supporting an association of HER-2 gene amplification with type 2 endometrial carcinomas.[23] In another study, HER-2 overexpression and HER-2 gene amplification were recognized in 3% and 1% of grade 1 EMC, 7% and 3% of grade 2 tumors, 29% and 8% of grade 3 tumors, 43% and 29% of UPSC, and 33% and 22% of CCC, respectively.[22]

The evidence is conflicting regarding HER-2 as a prognostic factor in endometrial cancer. Grushko et al[23] showed no correlation between HER-2 overexpression or amplification and OS in endometrial carcinomas. In contrast, Morrison et al[22] reported that HER-2 overexpression or amplification was an independent prognostic factor, and OS was significantly shorter in patients who showed HER-2 overexpression and/or HER-2 amplification compared with those who did not. Results regarding the association between

Table 4 HER-2 expression in primary and metastatic or recurrent tumors

Histological subtypes	Primary tumors		Metastatic or recurrent tumors		
	IHC scores	HER-2/CEP17 ratios	Lesions	IHC scores	HER-2/CEP17 ratios
Metastatic lesions					
EMC-G3	1+	–	LND	2+	0.9
EMC-G3	0	–	LND	1+	–
EMC-G3	1+	–	LND	2+	1.0
EMC-G3	1+	–	LND	0	–
EMC-G3	1+	–	LND	1+	–
EMC-G3	3+	3.3	LND	3+	4.6
EMC-G3	1+	–	Ovary	0	–
EMC-G3	2+	1.0	Colon	2+	1.3
UPSC	2+	0.8	LND	1+	–
UPSC	0	–	Ovary	1+	–
UPSC	0	–	LND	1+	–
UPSC*	2+	1.2	Ovary	3+	2.2
CCC	1+	–	OMT	2+	1.0
CCC	3+	4.5	OMT	3+	2.5
Recurrent lesions					
EMC-G3	2+	1.0	Colon	2+	1.3
EMC-G3	2+	1.1	Spleen	2+	1.4
CCC	0	–	AD	1+	–
CCC	1+	–	VW	2+	1.1
CCC	1+	–	Colon	2+	1.3

Note: *The actual results of the IHC and FISH are shown in Figure 2.
Abbreviations: HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; CEP17, chromosome 17; EMC-G3, grade 3 endometrioid adenocarcinoma; LND, lymph node; UPSC, uterine papillary serous carcinoma; CCC, clear cell adenocarcinoma; OMT, omentum; AD, adrenal gland, VW, vaginal wall; FISH, fluorescence in situ hybridization.

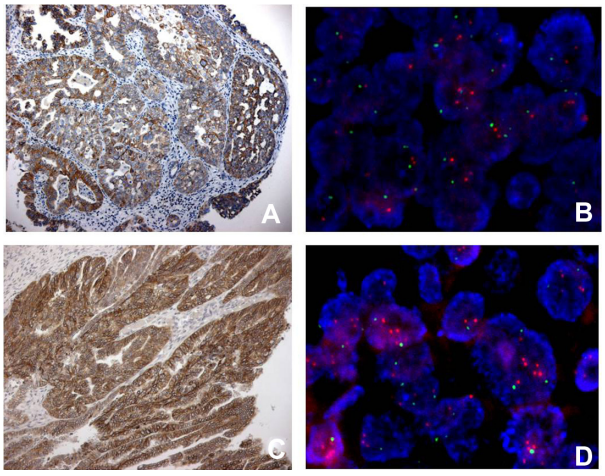

Figure 2 HER-2 overexpression and amplification.
Notes: A case of a 59-year-old with stage 4 papillary serous carcinoma. (**A**) IHC assay and (**B**) FISH assay of HER-2 for the primary tumor (original magnification, ×100 and ×1,000, respectively). (**C**) IHC assay and (**D**) FISH assay of HER-2 for the metastatic tumor (ovary) (original magnification, ×100 and ×1,000, respectively). The *HER-2* gene is localized by red fluorescent signals, and the CEP17 centromere (CEP17) is localized by green fluorescent signals. The cells were counterstained with 4′,6-diamino-2-phenilindole (blue). IHC scores and HER-2/CEP17 ratios were 2+ and 1.2 for the original tumor and 3+ and 2.2 for the metastatic tumor (ovary).
Abbreviations: HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; CEP17, chromosome 17.

HER-2 overexpression or amplification and prognosis in endometrial carcinomas have been conflicting, likely due to differences in the sample population or treatment modalities between the patients in each study. Coronado et al[32] reported that the prognostic value of HER-2 overexpression

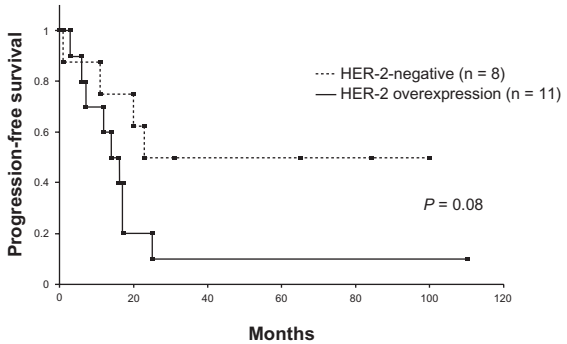

Figure 3 HER-2 expression and progression-free survival of the patients with metastatic or recurrent tumors.
Notes: The progression-free survival for the HER-2-negative and overexpression groups were 56.9% and 22.7%, respectively. Eleven patients with HER-2 overexpression tended to have a worse prognosis ($P = 0.08$).
Abbreviation: HER-2, human epidermal growth factor receptor 2.

was higher in early-stage than in advanced-stage tumors. It should be noted that the sample population of the study by Grushko et al[23] only included patients with recurrent or advanced measurable disease in the setting of uniform first-line chemotherapy. The authors also found higher expression of HER-2 in grade 3 tumors or UPSC, in which HER-2 overexpression was a poor prognostic indicator in early-stage disease.[23] In contrast, in the GOG trial, overexpression was only a weak prognostic factor and did not predict the response to chemotherapy.[20] HER-2 may be more relevant in the setting of UPSCs. Singh et al[33] have demonstrated HER-2 gene amplification in 40% of UPSC cases, and associated it with worse prognosis in stage 1/2 disease.

In the current study, there was no association between HER-2 expression and PFS or OS regardless of stage. For the 19 patients with metastatic or recurrent tumors, the PFS of the eleven patients with HER-2 overexpression tended towards a worse prognosis ($P = 0.08$). It is likely that this study lacked sufficient power to show a difference; therefore, it would be worthwhile to investigate this question further in a larger trial.

In breast cancer, HER-2 amplification is significantly more frequent in distant metastatic tumors than in primary tumors.[30] In metastatic breast cancer, a high level of HER-2 protein expression and amplification predicts the response to trastuzumab.[10] Moreover, in bladder cancer, HER-2 amplification is significantly more frequent in lymph node metastases than in the matched primary tumor.[8] In endometrial carcinoma, the reports that demonstrate the biological characteristics change during metastasis or recurrence are very limited. There have been a few studies concerning the difference of HER-2 expression between primary tumors and metastatic or recurrent tumors.[34,35] Vandenput et al[34] reported that a change in immunostaining for HER-2 expression between primary and recurrent tumors was encountered in 7% of paired biopsies from primary and recurrent endometrial cancer tumors (n = 85).[34] In addition, Tangjitgamol et al reported observing higher HER-2 expression in extracorporeal lesions than in primary tumors (20.7% versus 13.9%); however, the difference was not significant ($P = 0.262$).[35] In the current study, the frequencies of HER-2 overexpression and amplification were increased in the metastatic and recurrent tumors compared with matched primary tumors. Moreover, IHC scores or HER-2/CEP17 ratios by FISH were increased in 14 of 19 metastatic or recurrent tumors compared with the matched primary tumors.

Unlike in breast and gastric cancers, trastuzumab did not show significant activity against endometrial carcinomas with HER-2 overexpression or gene amplification in advanced or recurrent disease.[28] This trial tested trastuzumab as a single agent, and the percentage of patients with high-grade or type 2 carcinomas enrolled in this trial was relatively small.[29] Therefore, additional trials are warranted in which trastuzumab is tested in combination with cytotoxic chemotherapy for high-risk (high grade/type 2) endometrial cancers in both the primary and recurrent settings.

Conclusion

This study suggests that HER-2 expression in metastatic lesions as well as primary tumors predicts prognosis; the study also identifies patients who may benefit from targeted therapy. HER-2 overexpression or amplification in metastatic or recurrent tumors occurred more frequently than in primary tumors of high-grade or type 2 endometrial carcinomas. Finally, the role of trastuzumab combined with other chemotherapeutic agents should be investigated further in this population.

Disclosure

The authors report no conflicts of interest in this work.

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