Molecular Detection and Prognostic Value of Circulating Cytokeratin-19 Messenger RNA–Positive and HER2 Messenger RNA–Positive Cells in the Peripheral Blood of Women with Early-Stage Breast Cancer

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Abstract

Purpose: The aim of this study was to evaluate the clinical relevance of the simultaneous detection of cytokeratin (CK)–19 messenger RNA (mRNA)– and HER2 mRNA–positive cells in peripheral blood of women with early-stage breast cancer. **Patients and Methods:** CK-19 mRNA– and HER2 mRNA–positive cells were detected using a real-time and a nested reverse-transcriptase polymerase chain reaction assay, respectively, in a cohort of 185 women with early-stage breast cancer before the initiation of any adjuvant systemic treatment. Detection of CK-19 mRNA– and HER2 mRNA–positive cells in the peripheral blood was correlated with clinical outcome. **Results:** Overall, 63 of the 185 patients (34%) had detectable CK-19 mRNA–positive cells, and 33 (52.3%) also had detectable HER2 mRNA–positive cells. Patients with CK-19/HER2 mRNA–negative cells showed a trend toward longer disease-free survival (DFS) compared with patients with CK-19 mRNA–positive/HER2 mRNA–negative cells ($P = .054$) and had longer DFS than patients with CK-19/HER2 mRNA–positive cells ($P < .001$). Similarly, overall survival (OS) was higher in patients with CK-19/HER2 mRNA–negative cells compared with patients with CK-19 mRNA–positive/HER2 mRNA–negative cells ($P = .039$) or CK-19/HER2 mRNA–positive cells ($P < .001$). Patients with CK-19/HER2 mRNA–positive cells had shorter DFS but not OS compared with patients with CK-19 mRNA–positive/HER2 mRNA–negative cells. In multivariate analysis, the simultaneous detection of CK-19 mRNA– and HER2 mRNA–positive cells was independently associated with early relapse. **Conclusion:** The simultaneous detection of CK-19 mRNA– and HER2 mRNA–positive cells in peripheral blood predicts poor clinical outcome for women with early-stage breast cancer.

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Introduction

Breast cancer is considered a systemic disease because early tumor dissemination can occur even in patients with small tumors. Micrometastases, which are undetectable by the classical imaging and laboratory studies, can contribute to disease relapse; therefore, their identification in patients with early-stage breast cancer could have substantial impact on determining prognosis and deciding treatment strategies for these patients. Using immunocytochemistry or molecular techniques, epithelial cells can be identified in the bone marrow (disseminated tumor cells [DTCs]) or the peripheral blood (circulating tumor cells [CTCs]) of otherwise

metastases-free patients with stage I-III breast cancer.[1-3] The clinical importance of the immunocytochemical detection of DTCs at the time of diagnosis was confirmed in a metaanalysis involving > 4500 patients with early-stage breast cancer, showing an independent association with poor outcome.[4] Recently, the detection of ≥ 5 CTCs using an automated system was shown to predict poor clinical outcome in patients with metastatic breast cancer (MBC).[5] Our group has previously demonstrated that the detection of cytokeratin-19 (CK-19) mRNA–positive CTCs by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) in patients with early-stage breast cancer before[6,7] and after[8,9] the initiation of adjuvant systemic treatment is also associated with poor clinical outcome. CK-19, mammoglobin, maspin, and carcinoembryonic antigen (CEA) are among the markers that have been used for the detection of CTCs and DTCs with different sensitivities and specificities,[10-14] with CK-19 being the most extensively studied.[2]

HER2/*neu* (p185/erbB2) oncoprotein is a transmembrane glycoprotein receptor sharing sequence homology with the epidermal growth factor receptor[15,16]; HER2/*neu* gene amplification in breast cancer cells has been shown to be associated with impaired survival and resistance to treatment.[16-19] Using a double-staining immunocytochemical assay, Braun et al demonstrated that patients with CK-18/HER2–positive DTCs had worse overall survival (OS) compared with patients with CK-18–positive/HER2-negative DTCs.[20] Our group has recently developed a nested RT-PCR assay for the detection of HER2 messenger RNA (mRNA)–positive cells in the peripheral blood[21]; in multivariate analysis, the detection of HER2 mRNA–positive cells after the administration of adjuvant chemotherapy was an independent prognostic factor for shorter disease-free survival (DFS).

In the present study, we intended to evaluate the clinical significance of the molecular detection of CK-19 mRNA–positive and HER2 mRNA–positive cells in the peripheral blood before the administration of any adjuvant systemic treatment in a cohort of 185 patients with early-stage breast cancer. We found that the simultaneous detection of circulating CK-19/HER2 mRNA–positive cells was an independent prognostic factor for early disease relapse.

Patients and Methods

Patients and Clinical Samples

Peripheral blood (20 mL in ethylenediamine tetraacetic acid) was obtained from 185 patients with early-stage (I-III) breast cancer after the resection of the primary tumor and before the initiation of any adjuvant systemic treatment. All blood samples were obtained at the middle of vein puncture after the first 5 mL of blood were discarded. This precaution was undertaken to avoid contamination of blood with epithelial cells from the skin during sample collection. Surgical treatment was mastectomy or lumpectomy with axillary lymph node dissection. Radiation treatment was delivered to patients with lumpectomy and those with ≥ 4 involved axillary lymph nodes. The administration of adjuvant chemotherapy and hormonal treatment was decided independently of the CK-19 mRNA and HER2 mRNA detection. All patients included in this study received adjuvant chemotherapy, and most of them were treated in the context of research protocols of the Hellenic Oncology Research Group. Adjuvant chemotherapy regimens consisted of FEC (5-fluorouracil [5-FU] 700 mg/m^2 on day 1, epirubicin 75 mg/m^2 on day 1, and cyclophosphamide 700 mg/m^2 on day 1, every 3 weeks for 6 cycles), EC-T (epirubicin 75 mg/m^2 on day 1, cyclophosphamide 700 mg/m^2 on day 1, every 3 weeks for 4 cycles, followed by docetaxel 100 mg/m^2 on day 1 every 3 weeks for 4 additional cycles), or classical CMF (cyclophosphamide 100 mg/m^2 orally on days 1-14, methotrexate 40 mg/m^2 on days 1 and 8, 5-FU 600 mg/m^2 on days 1 and 8, every 4 weeks for 6 cycles). There were no subgroups of patients who received hormone therapy only or no systemic therapy at all. Patients with HER2-positive tumors did not receive adjuvant trastuzumab, because all patients were enrolled before the positive results from the adjuvant trastuzumab trials were reported.[22,23] All patients with estrogen receptor (ER)– and/or progesterone receptor (PgR)–positive tumors received tamoxifen 20 mg daily for 5 years or tamoxifen for 2-3 years followed by an aromatase inhibitor for 2-3 years; premenopausal women also received luteinizing hormone-releasing hormone analogues for 2 years. Patient follow-up consisted of clinical examination with laboratory and imaging studies every 3 months for the first 2 years, every 6 months for the next 3 years, and yearly thereafter. All patients gave written informed consent to participate in the study, which was approved by the ethics and scientific committee of our institution.

Immunohistochemistry for HER2 in the Primary Tumor

HER2 expression of the primary tumors was detected by immunohistochemistry (IHC) with the monoclonal antibody (MoAb) CB11. HER2 scoring was performed using the criteria recommended by DACO A/S for the HercepTest™ (membrane staining, IHC 0-3+ intensity scale). Fluorescence in situ hybridization (FISH) was not performed for tumors HER2 IHC 2+.

Reverse Transcriptase-Polymerase Chain Reaction Assays for the Detection of CK-19 mRNA– and HER2 mRNA–Positive Cells

The procedures of RNA extraction and complementary DNA (cDNA) synthesis have already been described elsewhere.[6,24] The real-time RT-PCR assay for the detection of CK-19 mRNA–positive cells has been previously reported in detail. The nested RT-PCR assay for the detection of HER2 mRNA–positive cells has also been previously described.[21] Real-time RT-PCR for the housekeeping gene *GAPDH* was performed in all of the clinical samples to evaluate the quality of the cDNA used in the study.

Table 1 Patient Characteristics

Characteristic	N (%)	CK-19/HER2 Positive, N (%)	CK-19 Positive/HER2 Negative, N (%)	CK-19/HER2 Negative, N (%)	*P* Value
Patients Enrolled	185	33 (17.8)	30 (16.2)	122 (66)	–
Median Age, Years (Range)	55 (28/80)	55 (28-74)	56 (32-72)	53.5 (30-80)	.143
Menopausal Status					
Premenopausal	68 (36.8)	11 (16.2)	13 (19.1)	44 (64.7)	.687
Postmenopausal	117 (63.2)	22 (18.8)	17 (14.5)	78 (66.7)	
Tumor Size					
≤ 2 cm	64 (34.6)	7 (10.9)	11 (17.2)	46 (71.9)	.203
> 2 cm	121 (65.4)	26 (21.5)	19 (15.7)	76 (62.8)	
Histologic Grade					
I/II	90 (48.6)	13 (14.4)	15 (16.7)	62 (68.9)	.244
III	85 (45.9)	18 (21.2)	13 (15.3)	54 (63.5)	
Unknown	10 (5.4)	2	2	6	
Axillary Lymph Nodes					
0	64 (34.6)	10 (15.6)	12 (18.8)	42 (65.6)	.475
1-3	71 (38.4)	17 (23.9)	10 (14.1)	44 (62)	
≥ 4	50 (27)	6 (12)	8 (16)	36 (72)	
ER					
Positive	107 (57.8)	19 (17.8)	17 (15.9)	71 (66.3)	.889
Negative	70 (37.8)	14 (20)	12 (17.1)	44 (62.9)	
Unknown	8 (4.4)	–	1	7	
PgR					
Positive	65 (35.1)	14 (21.5)	11 (17)	40 (61.5)	.644
Negative	111 (60)	18 (16.2)	18 (16.2)	75 (67.6)	
Unknown	9 (4.9)	1	1	7	
HER2/*neu* Primary Tumor					
Positive (+2,+3 by IHC)	21 (11.3)	5 (23.8)	5 (23.8)	11 (52.4)	.377
Negative (0, +1 by IHC)	160 (86.5)	28 (17.5)	24 (15)	108 (67.5)	
Unknown	4 (2.2)	–	1	3	
Vessel Infiltration					
Yes	89 (48.1)	19 (21.3)	15 (16.8)	55 (61.8)	.458
No	95 (51.4)	14 (14.7)	15 (15.8)	66 (69.5)	
Unknown	1 (0.5)	–	–	1	
Adjuvant Chemotherapy					
CMF	31 (16.8)	6 (19.3)	4 (13)	21 (67.7)	.853
FEC or T-EC	154 (83.2)	27 (17.5)	26 (16.9)	101 (65.6)	

Statistical Analysis

Continuous variables were compared between the 2 groups with unpaired *t* tests and categoric data with the χ^2 or Fisher exact test, as appropriate. The time from study entry until the day of the first evidence of disease recurrence (locoregional or distant; DFS) and the time from study entry to death (OS) were the main dependent variables of the study. The DFS and OS curves for subgroups of patients were constructed using the Kaplan-Meier product limit estimate method[25] and were compared by the log-rank test to provide a univariate assessment of the prognostic value of selected clinical risk-factors measured at study entry. Clinicopathologic factors known to be associated with prognosis, like menopausal status (premenopausal vs. postmenopausal), tumor size (T2-3 vs T1), nodal infiltration (≥ 4 vs. 0-3), histologic grade (III vs. I/II), ER status (negative vs. positive), PgR status (negative vs. positive), HER2 status (positive vs. negative), chemotherapy regimen (FEC or EC-T vs. CMF), and additionally, the detection of CK-19 mRNA–positive cells (yes vs. no) and the simultaneous detection of CK-19 mRNA– and HER2 mRNA–positive cells (yes vs. no), were tested in univariate analysis. Variables that were found to be significant at the univariate screen were then entered in a stepwise multivariate Cox proportional hazard regression model to identify those with independent prognostic information.[26]

Table 2 Incidence of Recurrences and Deaths in Patient Groups Based on Detection of CK-19 mRNA–Positive and HER2 mRNA–Positive Cells

Patient Group	Relapse, N (%)	*P* Value	Death, N (%)	*P* Value
Entire Cohort (N = 185)	44 (23.8)	–	23 (12.4)	–
CK-19/HER2 mRNA–positive cells (n = 33)	19 (57.5)	< .001	10 (30.3)	< .001
CK-19 mRNA–positive/HER2 mRNA–negative cells (n = 30)	9 (30)		6 (20)	
CK-19/HER2 mRNA–negative cells (n = 122)	16 (13.1)		7 (5.7)	

Entry into and removal from the model were set at 5% and 10%, respectively. All statistical tests were performed at the 5% level of significance.

Results

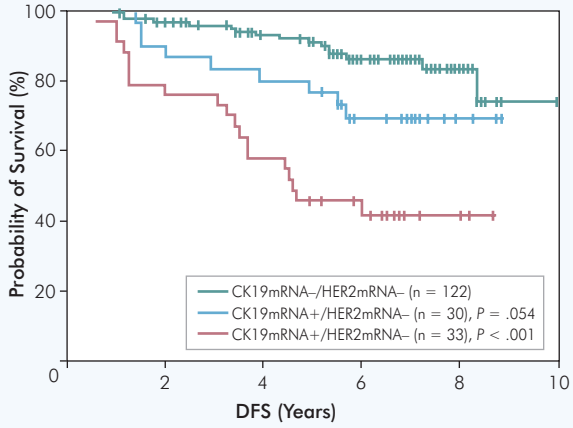
Patient Characteristics and Detection of CK-19 mRNA– and HER2 mRNA–Positive Cells

The median age of the 185 patients was 55 years, and 68 (36.8%) were premenopausal; in 121 patients (65.4%), the tumor size was > 2 cm, 85 patients (48.6%) had grade III tumors, 50 (27%) had ≥ 4 involved axillary lymph nodes, and 70 (37.8%) had ER-negative tumors (Table 1). Based on the detection of CK-19 mRNA– and HER2 mRNA–positive cells in the peripheral blood, patients in our cohort had 1 of the following 3 molecular profiles: CK-19/HER2 mRNA–positive cells, CK-19 mRNA–positive/HER2 mRNA–negative cells, or CK-19/HER2 mRNA–negative cells. CK-19/HER2 mRNA–positive and CK-19 mRNA–positive/HER2 mRNA–negative profiles were present in 33 (17.8%) and 30 (16.2%) of the 185 patients, respectively, before the initiation of any adjuvant systemic treatment. Overall, 63 of the 185 patients (34%) had detectable CK-19 mRNA–positive cells and 33 (52.3%) also had detectable HER2 mRNA–positive cells. The CK-19/HER2 mRNA–positive or CK-19 mRNA–positive/HER2 mRNA–negative profiles were not associated with any of the known tumor or patient characteristics.

HER2 Expression on Primary Tumor and Detection of Peripheral Blood CK-19 mRNA– and HER2 mRNA–Positive Cells

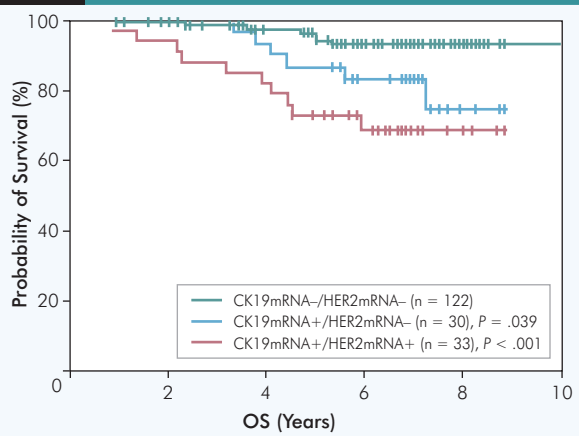
In the cohort of 185 patients, CK-19/HER2 mRNA–positive cells were detected in 5 of 21 patients (23.8%) with HER2-positive primary tumors and in 28 of 160 patients (17.5%) with HER2-negative primary tumors. Similarly for the entire cohort, the CK-19 mRNA–positive/HER2 mRNA–negative profile was detected in 5 of 21 patients (23.8%) with HER2-positive primary tumors and in 24 of 160 patients (15%) with HER2-negative primary tumors. Among the 62 patients with detectable CK-19 mRNA–positive cells and known primary tumor HER2 status, HER2 mRNA–positive cells were detected in 5 of 10 patients (50%) with HER2-positive primary tumors and in 28 of 52 patients (53.8%) with HER2-negative primary tumors.

Figure 1 Disease-free Survival in Groups of Patients with Early-Stage Breast Cancer *



*Based on the molecular detection of peripheral blood circulating CK-19mRNA- and HER2mRNA-positive cells.

Figure 2 Disease-free Survival in Groups of Patients with Early-Stage Breast Cancer *



*Based on the molecular detection of peripheral blood circulating CK-19mRNA- and HER2mRNA-positive cells.

Clinical Relevance of the Detection of Peripheral Blood CK-19 mRNA– and HER2 mRNA–Positive Cells in Early-Stage Breast Cancer

Relapse. After a median follow-up period of 65 months (range, 10-120 months), 44 of 185 patients (23.8%) experienced a distant (n = 35) or a locoregional relapse (n = 9). The recurrence rates were 13.1%, 30%, and 57.5% for patients with CK-19/HER2 mRNA–negative, CK-19 mRNA–positive/HER2 mRNA–negative, and CK-19/HER2 mRNA–positive cells, respectively ($P < .001$; Table 2). The estimated median DFS was 55 months (95% CI, 25.6-84.4 months) for

patients with CK-19/HER2 mRNA–positive cells, whereas it has not yet been reached for patients with CK-19/HER2 mRNA– negative and CK-19 mRNA–positive/HER2 mRNA–negative cells (Figure 1). However, patients with CK-19/HER2 mRNA–negative cells had higher DFS compared with patients with CK-19 mRNA–positive/HER2 mRNA–negative (P = .054) or CK-19/HER2 mRNA–positive cells (P < .001). Furthermore, patients with CK-19/HER2 mRNA–positive cells had shorter DFS than patients with CK-19 mRNA–positive/HER2 mRNA–negative cells (P = .021). The 5-year DFS rates of patients with CK-19/HER2 mRNA–negative, CK-19 mRNA–positive/HER2 mRNA–negative, and CK-19/HER2 mRNA–positive cells were 91%, 76.7%, and 45.5%, respectively.

Survival. During the same follow-up period, 23 of 185 patients (12.4%) died of disease progression. The incidence of death was 5.7%, 20%, and 30.3% for patients with CK-19/HER2 mRNA–negative, CK-19 mRNA–positive/HER2 mRNA–negative, and CK-19/HER2 mRNA–positive cells, respectively (P < .001; Table 2). The median OS has not yet been reached for any of these 3 groups. However, OS was significantly higher in patients with CK-19/HER2 mRNA–negative cells compared with patients with CK-19 mRNA–positive/HER2 mRNA–negative cells (P = .039; Figure 2) or CK-19/HER2 mRNA–positive cells (P < .001). Moreover, OS was not significantly different between patients with CK-19/HER2 mRNA–positive and CK-19 mRNA–positive/HER2 mRNA–negative cells (P = .247). The 5-year OS rates of patients with CK-19/HER2 mRNA–negative, CK-19 mRNA–positive/HER2 mRNA–negative, and CK-19/HER2 mRNA–positive cells were 94.3%, 86.7%, and 72.7%, respectively.

Table 3 Unvariate Analysis (Unadjusted Relative Risks) for DFS and OS of Patients with Early-Stage Breast Cancer

Parameter	Hazard Ratio (95% CI)	P Value
Disease-Free Survival		
Menopausal status (pre vs. post)	0.755 (0.4-1.424)	.385
Tumor size (T2-3 vs. T1)	2.139 (1.028-4.452)	.042
Histology grade (III vs. I/II)	1.87 (0.99-3.534)	.054
Lymph nodes (≥ 4 vs. 0-3)	1.637 (0.873-3.069)	.124
ER status (negative vs. positive)	1.614 (0.881-2.959)	.121
PgR status (negative vs. positive)	1.153 (0.614-2.162)	.658
HER2 primary tumor (positive vs. negative)	1.597 (0.711-3.591)	.257
Vessel infiltration (yes vs. no)	1.1 (0.608-1.991)	.753
Adjuvant chemotherapy (FEC or T-EC vs. CMF)	1.528 (0.602-3.88)	.373
CK19 mRNA–positive cells (yes vs. no)	3.76 (2.034-6.954)	< .001
CK19/HER2 mRNA–positive cells (yes vs. no)	4.565 (2.507-8.313)	< .001
Overall Survival		
Menopausal status (pre vs. post)	0.869 (0.368-2.051)	.748
Tumor size (T2-3 vs. T1)	3.489 (1.037-11.743)	.044
Histology grade (III vs. I/II)	1.732 (0.717-4.186)	.222
Lymph nodes (≥ 4 vs. 0-3)	2.24 (0.981-5.117)	.056
ER status (negative vs. positive)	1.757 (0.761-4.054)	.187
PgR status (negative vs. positive)	1.456 (0.564-3.764)	.438
HER2 primary tumor (positive vs. negative)	0.699 (0.164-2.985)	.629
Vessel infiltration (yes vs. no)	1.827 (0.774-4.313)	.169
Adjuvant chemotherapy (FEC or T-EC vs. CMF)	1.323 (0.393-4.454)	.651
CK19 mRNA–positive cells (yes vs. no)	4.251 (1.748-10.334)	.001
CK19/HER2 mRNA–positive cells (yes vs. no)	3.835 (1.68-8.754)	.001

Univariate and Multivariate Analyses

Univariate analysis revealed that tumor size > 2 cm, the detection of CK-19 mRNA–positive cells, and the simultaneous detection of CK-19 mRNA–positive and HER2 mRNA–positive cells were significantly associated with decreased DFS and OS (Table 3). The multivariate analysis demonstrated that the simultaneous detection of CK-19 mRNA–positive and HER2 mRNA–positive cells was the only independent factor associated with early disease relapse (P = .029; Table 4). Finally, the detection of CK-19 mRNA–positive cells was the only independent factor associated with decreased OS (P = .002).

Discussion

The early dissemination of malignant cells from the primary tumor and the inability of adjuvant treatment[8,9,27] to completely eliminate them might account for the failure of the TNM classification system to accurately identify the subgroup of patients with early-stage breast cancer who might be at high risk for relapse. We have previously reported that the detection of CK19 mRNA–positive CTCs by a real-time RT-PCR assay in patients with early-stage breast cancer before the initiation of any adjuvant systemic treatment was associated with shorter DFS and OS.[6,7] Furthermore, we have already demonstrated the malignant origin of CK-19 mRNA–positive cells by performing FISH analysis for HER2/*neu* gene amplification and aneusomy detection for chromosomes 1, 8, 11, and 17.[28] More recently, we reported that the detection of HER2 mRNA–positive cells by nested RT-PCR after the administration of adjuvant chemotherapy was an independent prognostic factor for reduced DFS.[21]

In the present study, we evaluated the clinical relevance of the simultaneous detection of CK-19 mRNA–positive and HER2 mRNA–positive cells using a real-time and a nested RT-PCR assay, respectively, in a cohort of 185 patients with early-stage breast cancer before the initiation of any adjuvant systemic treatment. CK-19 mRNA–positive cells were detected in 63 patients, and 33 (52.3%) of them also had detectable HER2 mRNA–positive cells. Patients with CK-19/HER2 mRNA–positive cells had shorter DFS but not OS compared with patients with CK-19 mRNA–positive/HER2 mRNA–nega-

Table 4 Independent Predictive and Prognostic Factors by Multivariate Analysis for DFS and OS of Patients with Early-Stage Breast Cancer

Parameter	Hazard Ratio (95% CI)	*P* Value
Disease-Free Survival		
CK19 mRNA–positive cells (yes vs. no)	2.237 (0.988-5.064)	.053
CK19/HER2 mRNA–positive cells (yes vs. no)	2.432 (1.095-5.4)	.029
Tumor size (T2-3 vs. T1)	1.963 (0.94-4.098)	.073
Overall Survival		
CK19 mRNA–positive cells (yes vs. no)	4.132 (1.699-10.048)	.002
Tumor size (T2-3 vs. T1)	3.33 (0.989-11.212)	.052

tive cells. Moreover, in multivariate analysis, the simultaneous detection of CK-19 mRNA–positive and HER2 mRNA–positive cells was independently associated with early disease relapse. Based on these results, it seems that the detection of HER2 mRNA–positive cells might have additional prognostic value (at least for DFS) beyond that of CK-19 mRNA–positive cells. However, this needs to be confirmed in future studies.

Similarly, Braun et al used double immunocytochemical staining for CK-18 and HER2 and reported that HER2 was expressed in 31 of 52 patients (60%) with detectable DTCs.[20] They found that patients with HER2-positive DTCs had significantly shorter OS compared with patients with HER2-negative micrometastatic cells. Solomayer et al used a double immunofluoresence staining procedure with an antibody against pancytokeratin and the HER2 antibody CB11 and reported HER2 positivity in 20 of 46 patients (43%) with CK-positive DTCs.[29] Although the cited studies have used different methods for the detection and characterization of DTC HER2 status, they are in agreement with the current report that HER2-positive cells are detected in approximately half of patients with early-stage breast cancer presenting CTCs and/or DTCs. Furthermore, all studies agree that patients with HER2-positive cells have worse prognosis than their counterparts with CTCs and/or DTCs not expressing HER2. Indeed, HER2 positivity on occult micrometastatic cells might define a subpopulation of cells with aggressive metastatic potential. Furthermore, in vitro extravasation experiments have shown that most of the transendothelial invasive cells with metastatic potential displayed HER2 expression independently of the HER2 status of the primary tumor.[30] Our results are also in agreement with the results of Wulfing et al, who used double immunocytochemical staining after an initial enrichment step combined with immunomagnetic separation and detected CK/HER2 double positive cells in 17 of 35 patients (48.6%) with stage I-III breast cancer.[31] The detection of CK/HER2–positive CTCs was predictive of reduced DFS and OS in these patients. Based on all the aforementioned studies, our report is the largest series in which the simultaneous molecular detection of CK-19 mRNA–positive and HER2 mRNA–positive cells was shown to have independent prognostic value in early-stage breast cancer.

Currently, the decision of whether to offer adjuvant trastuzumab is solely based on the HER2 status of the primary tumor. The administration of adjuvant trastuzumab for 1 year[22,23] or for a short 9-week course[32] significantly improved outcome of women with HER2-positive primary breast cancer. The significant benefits from adjuvant trastuzumab and the associated high cost of treatment and risk for cardiac toxicity necessitate accurate HER2 testing of the primary tumor. However, approximately 20% of current primary tumor HER2 testing could be inaccurate, and guidelines have recently been issued to help improve the accuracy of HER2 testing.[33] Moreover, intriguing preliminary results from a subset analysis of the National Surgical Adjuvant Breast and Bowel Project B-31 trial have suggested that benefit from adjuvant trastuzumab might not only be confined to patients with IHC 3+ or FISH-positive primary tumors.[34] In accordance with the previously mentioned clinical data, preclinical studies have shown that trastuzumab enhanced the cytotoxic effects of chemotherapy and induced antibody-dependent cell-mediated cytotoxicity even in HER2 IHC 1+ cells, like the MCF-7 cells. Alternatively, the benefit of trastuzumab could be related to effective targeting of HER2–positive CTCs.[28] In our study, CK-19 mRNA–positive and HER2 mRNA–positive cells were detected in 28 of 160 patients (17.5%) with HER2-negative primary tumors, and these women would not be eligible for adjuvant trastuzumab. Similarly, Wulfing et al and Solomayer et al have reported CK/HER2–positive CTCs in 12 of 24 patients and DTCs in 12 of 106 patients with HER2-negative primary tumors.[29,31] Furthermore, we and others have shown that trastuzumab can effectively eliminate CTCs and DTCs overexpressing HER2 regardless of the HER2 status of the primary tumor in patients with early-stage or MBC.[28,35] The results from these pilot studies suggest that even patients with HER2-negative primary tumors who have HER2-positive CTCs and/or DTCs could benefit from adjuvant trastuzumab. Well-designed clinical trials are urgently needed to validate this hypothesis. Moreover, it is not clear whether patients with HER2-positive primary tumors and without detectable HER2-positive CTCs and/or DTCs benefit less from adjuvant trastuzumab because the presence of micrometastatic cells was not studied in the adjuvant trastuzumab trials.[22,23,32]

Because blood can be easily obtained at different times during patients' follow-up, the simultaneous detection of CK-19 mRNA– and HER2 mRNA–positive cells might serve as a tool to assess the "real-time" HER2 status of a patient. This assay might allow testing of the value of secondary adjuvant treatment strategies (eg, to assess the benefit of a short course of adjuvant trastuzumab if CK-19 mRNA–positive and HER2 mRNA–positive cells are detected during the follow-up of patients with early-stage breast cancer). However, issues of toxicity, eg, the cardiac toxicity of trastuzumab, need to be carefully evaluated when these experimental treatment strategies are tested in clinical trials.

Targeting occult tumor cells using other MoAbs, like the

murine MoAb 17-1A (edrecolomab), have also been reported. This antibody is directed against the epithelial adhesion molecule (EpCAM) and resulted in a marked reduction or complete elimination of EpCAM-positive/CK-positive DTCs in patients with advanced breast cancer.[36,37] Nevertheless, as we have shown herein, the detection of HER2-positive cells might define a particularly aggressive phenotype, and thus, strategies targeting HER2 instead of EpCAM might be preferable. Finally, the relative resistance of micrometastatic cells to chemotherapy,[8,9,27] probably a result of their low proliferative potential,[38] provides further rationale for targeting them using MoAbs like trastuzumab. If the results from the aforementioned studies are confirmed, they will represent a paradigm shift in the adjuvant treatment of breast cancer from the current one, which is based only on the primary tumor, to one that also takes into account the characteristics of micrometastatic cells.

Conclusion

The simultaneous detection of CK-19 mRNA–positive and HER2 mRNA–positive cells in the blood by RT-PCR defined a subpopulation of cells with aggressive metastatic potential. Their presence was shown to be an independent prognostic factor for early relapse. The detection of CK-19 mRNA–positive and HER2 mRNA–positive cells offers the opportunity for secondary adjuvant treatment using anti-HER2 targeted therapies, even for patients whose primary tumors do not overexpress HER2. This hypothesis should be tested in future clinical trials.

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