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SHORT COMMUNICATION

Impact of circulating tumour cells on survival of eribulin-treated patients with metastatic breast cancer

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Abstract
Several clinical studies have examined circulating tumour cells (CTCs). However, the application of CTCs as a predictive/prognostic marker for breast cancer patients has yet to be established, particularly the selection of suitable markers for detecting CTCs. We recently investigated CTCs, including mesenchymal status, from metastatic breast cancer patients who had received eribulin-based treatment. We found that assessment of both mesenchymal and epithelial CTCs might be important for predicting eribulin responsiveness. In the current study, we followed up the outcomes of these patients after eribulin treatment and investigated the possibility of CTC analysis results serving as prognostic markers for this patient population. Twenty-one patients were enrolled and peripheral blood samples were collected before eribulin-based treatments. CTCs were then examined using a Microfluidic Chip device. CTCs positive for vimentin and pan-cytokeratin were defined as mesenchymal and epithelial CTCs, respectively. Overall survival (OS) was assessed in relation to the number of CTCs and clinicopathological factors. During the observation period, 13 patients (62%) died due to breast cancer and the median OS was 18 months. Patients with high-grade tumours and a high total number of CTCs showed significantly shorter OS than those with low-grade tumours and smaller CTC burdens ($p=0.026$ and 0.037, respectively). Patients who received eribulin as the first chemotherapy for metastatic disease showed longer OS ($p=0.006$). Our data suggest that determining numbers of both mesenchymal and epithelial CTCs might predict survival for patients receiving eribulin.

Keywords Breast cancer · Circulating tumour cell · Liquid biopsy · Eribulin · Epithelial mesenchymal transition

Background

Clinical studies focusing on a wide range of cancer types have introduced circulating tumour cells (CTCs) as a tool for predicting patient outcomes and treatment effects. High numbers of CTCs might reportedly portend poor outcomes in patients with both early and metastatic breast cancer (MBC) [1–3]. However, several problems persist, including standardisation of the analyses since laboratories employ different techniques, before CTC analysis can be introduced into routine clinical practice. The CellSearch® System, the most widely used CTC analysis technique [4], employs the epithelial cellular adhesion molecule (EpCAM) and several epithelial cell surface markers to capture CTCs. However, the inability of this system to detect CTCs with decreased levels of epithelial markers remains a major problem, since EpCAM is not expressed on all CTCs [5, 6]. The epithelial-to-mesenchymal transition (EMT) might be a major reason for this shortcoming of EpCAM [5, 7–11]. To overcome this problem, the EMT status of CTCs is now also assessed with more advanced technologies [10, 12, 13]. Several studies have shown improved CTC detection by employing EMT markers in addition to epithelial markers [14, 15]. Changes in EMT status during treatments have also been reported, as described in our prior publication [7, 16].

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Eribulin is a microtubule-depolymerising agent and an option for MBC patients. Interestingly, the suppression of EMT might reportedly be the mechanism by which eribulin regulates breast cancer progression [17, 18]. Recently, we investigated CTCs in MBC patients who had received eribulin and revealed that determinations of both mesenchymal and epithelial CTCs at baseline might be predictive markers for eribulin responsiveness [16].

In the current study, we followed up and examined patient outcomes after eribulin treatments. This study aimed to assess the possibility of CTC analysis serving as a tool for predicting patient survival.

Materials and methods

Patients and treatments

Twenty-one patients with metastatic/Stage IV breast cancer, who had started eribulin-based treatments and in whom CTCs had been examined at our department during the January through December 2017 period, were enrolled. Sixteen patients developed metastatic disease after undergoing curative surgery for primary breast cancer, while 5 had Stage IV disease. Clinicopathological features of the 21 patients are shown in Supplementary Table 1. Two-thirds of the patients were hormone receptor (HR)-positive, while 29% were HR-negative and human epidermal growth factor receptor 2 (HER2)-negative, i.e. they had triple negative tumours. Median disease-free-survival (DFS) after curative surgery was 75 months (range 8–125). Eribulin was administrated as the first, second and third/more line of chemotherapy for metastatic disease in 29%, 62% and 10% of patients, respectively.

Eribulin mesylate was administered at the standard dose of 1.4 mg/m^2 on days 1 and 8. During treatment, drug doses were gradually reduced as needed, such as in the event of neutropenia. Anti-HER2 drugs, such as trastuzumab and pertuzumab, were simultaneously administered according to the HER2 status of the patient's tumour. Peripheral blood (10 ml) samples were collected before eribulin treatment. The samples were sent to the Nihon Gene Research Laboratories (Japan) within 24 h for analysis of CTCs. CTCs were re-examined in some patients when treatment effects were evaluated (details presented in the "CTC analysis" section). Serum tumour markers, CEA and CA15-3, were also determined at baseline, along with the neutrophil–lymphocyte-rate (NLR).

After eribulin treatment, numbers of chemo-regimens administered were 0 in 38%, 1-2 in 57% and 3 or more in 5% of patients (Supplementary Table 1). In the current study, overall survival (OS) was defined as the time elapsed from the start of eribulin-based treatments until death.

This study was carried out with approval from the Ethics Committee of Juntendo University Hospital (No. 16-139) and all samples were collected after obtaining written informed consent from the patients.

CTC analysis

CTCs were examined using a Microfluidic Chip device at Nihon Gene Research Laboratories (Japan) and the methods were previously described in detail [12, 16]. Fluorescence images of cells, including EMT markers, can be obtained employing this device and CTCs positive for vimentin and pan-cytokeratin were defined as mesenchymal (mCTCs) and epithelial (eCTCs), respectively [16]. CTCs were detected in 20 of the 21 patients and the median numbers of total CTCs, mCTCs and eCTCs were 3.0, 1.0 and 2.0, respectively. Thus, we employed these numbers as cut-off values for dividing patients into two groups according to each of these CTC types.

Statistical analysis

Statistical analyses were performed using JMP 11.2.1 statistical software (SAS Institute, Inc., Cary, NC, USA). As to patient survival, a Cox proportional hazard model was used to evaluate any independent prognostic effects of the variables with a 95% confidence interval. Associations between two parameters were evaluated using the Pearson's χ^2 test. Kaplan–Meier curves were estimated and the log-rank test was applied for comparisons of the survival distributions of the two patient groups. A $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient outcomes after eribulin-based treatments

During the median 18-month observation period (range 2–25), 13 patients (62%) died due to breast cancer. The median OS was also 18 months.

Factors predicting OS

To identify possible predictors of OS, several clinicopathological factors were examined; age, histological type, tumour grade, oestrogen receptor (ER), progesterone receptor (PgR), HER2, DFS, presence of visceral metastasis, number of previous chemotherapies, CTCs, tumour markers (CEA, CA15-3), NLR at baseline, effect of eribulin and the duration of eribulin treatments (Table 1). The Cox proportional hazard model revealed that tumour grade, number of previous chemotherapies and total CTCs were

Table 1 Relationships between clinicopathological factors OS

Variables	Univariate			Multivariate		
	HR	(95% CI)	*p* values	HR	(95% CI)	*p* values
Age[a] (> 55 vs. ≤ 55)	0.65	(0.2–2.1)	0.452			
Histology [IDC (NST) vs. special type]	1.72	(0.4–11.2)	0.460			
Tumour grade (high vs. low/mod)	4.75	(1.2–19.9)	0.026*	2.49	(0.6–11.0)	0.201
ER (positive vs. negative)	0.36	(0.1–1.1)	0.077			
PgR (positive vs. negative)	0.83	(0.3–2.8)	0.760			
HER2[b] (positive vs. negative)	0.29	(< 0.1–1.5)	0.164			
DFS[c] (> 60 vs. ≤ 60 months)	0.74	(0.2–3.1)	0.668			
Visceral metastasis[a] (yes vs. no)	1.51	(0.5–6.8)	0.523			
Number of previous chemotherapies for metastatic disease (0 vs. ≥ 1)	0.11	(< 0.1–0.6)	0.006*	0.13	(< 0.1–0.8)	0.030*
CTCs[a]						
Total CTCs (≥ 3 vs. < 3)	4.18	(1.1–27.7)	0.037*	1.21	(0.3–8.6)	0.816
mCTCs (≥ 1 vs. 0)	2.62	(0.8–12.0)	0.124			
eCTCs (≥ 2 vs. < 2)	2.23	(0.7–8.3)	0.166			
Tumour markers[a]						
CEA (> 5 vs. ≤ 5)	2.08	(0.7–6.5)	0.190			
CA15-3 (> 30 vs. ≤ 30)	0.64	(0.2–2.0)	0.430			
NLR[a] (> 3.0 vs. ≤ 3.0)	1.34	(0.4–4.1)	0.611			
Effect of eribulin (PR/long SD vs. PD)	0.83	(0.2–2.6)	0.759			
PFS (> 18 vs. ≤ 18 weeks)	0.81	(0.3–2.5)	0.707			

IDC invasive ductal carcinoma, *NST* non-special type, *DFS* disease-free survival, *NLR* neutrophil–lymphocyte-rate, *PFS* progression-free survival, *HR* hazard ratio, *CI* confidential interval

*$p < 0.05$

[a]At the time of starting eribulin

[b]HER2 overexpression was defined as IHC (3+) or FISH (+)

[c]For 16 patients who underwent curative surgery for the primary tumour

factors predicting OS. Patients with high-grade tumours and high total numbers of CTCs showed significantly shorter OS ($p = 0.026$ and 0.037, respectively). Patients who received eribulin as the first chemotherapy for metastatic disease had longer OS ($p = 0.006$). Multivariate analysis revealed that only the number of previous chemotherapies, among these three variables, was an independent predictive factor for OS ($p = 0.030$). We further examined the relationships among these three factors employing the Pearson's χ^2 test but all showed impacts that were statistically independent of each other. As to the 6 patients who received eribulin as the first chemotherapy, we compared clinicopathological factors with those of 15 other patients, to examine whether any drug selection bias existed. There were no differences in tumour characteristics such as intrinsic subtype and presence of visceral metastasis, while mean age was slightly higher ($p = 0.088$) in the 6 patients (Supplementary Table 2).

Kaplan–Meier curves for OS according to CTC types are presented in Fig. 1. Patients with higher total numbers of CTCs had significantly shorter OS than those with lower CTC numbers ($p = 0.045$). The same trend was observed for eCTCs but without a statistically significant difference ($p = 0.161$). On the other hand, there was no difference according to the numbers of mCTCs.

Similarly, Kaplan–Meier curves for tumour grade and number of previous chemotherapies (eribulin as first chemo-regimen vs. second/later) are shown in Supplementary Fig. 1. Patients with high-grade tumours and who had received eribulin as the second or later regimen had shorter OS ($p = 0.013$ and 0.011, respectively). Moreover, among factors showing no significant difference according to the Cox hazard model, ER status tended to be associated with longer OS, but the relationship did not reach statistical significance ($p = 0.052$). Patients with ER-negative tumours showed shorter OS and the difference was judged to be significant when the generalised Wilcoxon test was employed ($p = 0.022$).

Discussion

In the current study, we found that total CTCs, comprised of mCTCs and eCTCs measured at the time of initiating eribulin administration, might predict patient survival. To

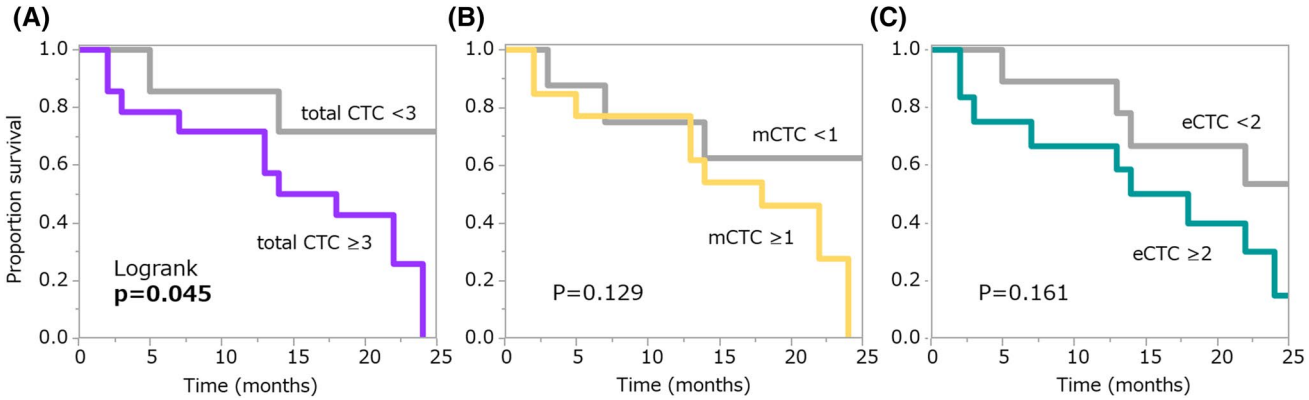

Fig. 1 Kaplan–Meier curves of OS according to CTC types. Kaplan–Meier curves for OS in 21 patients according to each CTC type are shown. **a** Total CTCs, **b** mCTCs and **c** eCTCs are indicated in purple, yellow and green, respectively. The log-rank test was applied for comparisons of the survival distributions of the two groups

our knowledge, this is the first study to examine the possibility of baseline CTCs serving as a prognostic marker specifically for patients receiving eribulin-based treatment. We focused on OS because our previous analysis indicated a relationship between CTCs and eribulin efficacy [16]. Herein, we evaluated OS in patients with a median 18-month observation period.

It can be readily understood that the first administration of eribulin was a factor independent of the number of subsequent systemic therapies. It is, however, noteworthy that while tumour characteristics, such as tumour grade and ER status, reflect OS, neither the presence of visceral metastasis nor the effect/duration of eribulin treatment showed any association with OS.

Patients with more total CTCs had shorter OS. Our data correlate with those in a number of previous studies examining a variety of chemotherapeutic regimens, though most prior investigations employed EpCAM-based CTC analysis [2, 19, 20]. In our previous study, mCTC was a factor correlating with PFS, along with total CTCs. In contrast, in this study, eCTCs appeared to be more important determinants of OS than mCTCs. Although the difference did not reach statistical significance, patients having fewer eCTCs tended to show longer OS (Fig. 1c). We speculate that the survival difference according to the number of eCTCs might reflect good responsiveness to endocrine therapies after eribulin. Seven patients in our cohort received endocrine therapy after eribulin. Three patients belonged to the eCTC-low (<2) and 4 to the eCTC-high (≥ 2) group. However, mean durations of endocrine treatment in total were 4.5 and 6.3 months, respectively, and there were no statistically significant differences between the two groups. Nevertheless, we confirmed that assessing total CTCs, based on mCTC and eCTC, might be the optimal approach for predicting the OS of patients given eribulin-based treatments.

Limitations of the current study include the lack of a control group and the small number of cases. Assessments of mCTCs and eCTCs in randomised studies, comparing eribulin with other forms of chemotherapy, with a larger number of patients are needed.

Our data suggest that determinations of total CTCs, based on mCTCs and eCTCs combined at baseline, along with tumour grade and ER status, might predict survival in patients receiving eribulin-based treatment.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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