Circulating Tumor Cells as a Biomarker Predictive of Sensitivity to Docetaxel Chemotherapy in Patients with Castration-resistant Prostate Cancer

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Abstract. *Aim: We examined whether Circulating Tumor Cells (CTCs) can be used to predict survival in patients with bone-metastatic castration-resistant-prostate cancer (mCRPC) treated with docetaxel chemotherapy. Patients and Methods: All patients with mCRPC who had experienced treatment failure with androgen deprivation therapy and had received docetaxel chemotherapy were eligible for study inclusion. CTCs in whole blood were enumerated with the CellSearch System. Results: The median CTC count at baseline before starting trial treatment was 7 (range=0-227) CTCs per 7.5 ml blood. Out of the 57 patients, 24 (42.1%) had a CTC count of less than 5, while 27 patients (47.4%) had a CTC count of 5-50 and six patients (10.5%) had a CTC count of more than 50. A threshold of 5 or more CTCs per 7.5 ml blood was used to assess the ability to predict survival. The patient charts were examined to determine the median overall survival time, which ranged from 6 to 37 months (mean=12.8±8.1 months, median=15.3 months). Thirty-three patients (57.9%) had 5 or more CTCs before docetaxel chemotherapy, with a median overall survival of 10.5 months compared to 25.0 months for 24 patients (42.1%) with fewer than 5 CTCs (p<0.001). CTC and alkaline phosphatase (ALP) were independent predictors of overall survival time (p=0.004, and p=0.023, respectively). In addition, poorer overall survival was predicted by a CTC count of 5 or more after three courses of docetaxel chemotherapy. Conclusion: The CTC count may be an independent predictor of overall survival in patients with mCRPC treated with docetaxel chemotherapy. The numbers of CTCs detected was important in assessing response to chemotherapy and predict disease outcome.*

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Most tumors in high-risk patients with prostate cancer ultimately progress to castration-resistant prostate cancer (CRPC). A regimen of docetaxel at 75 mg/m^2 once every three weeks with daily oral prednisone based on the TAX327 trial has conferred a significant survival advantage in patients with metastatic CRPC (mCRPC) compared to mitoxantolone-plus-prednisone (1). In Japan, docetaxel treatment has been established as standard chemotherapy for CRPC with bone metastasis. As Armstrong *et al*. reported, four independent risk factors were identified: pain, visceral metastases, anemia and bone scan progression by subgroup analysis of TAX327 (2). However, definitive prognostic factors at the initiation of docetaxel chemotherapy associated with disease progression and survival have not been identified.

Various groups have shown that the number and characteristics of circulating tumor cells (CTCs) in patients with cancer parallel tumor burden and response to therapy (3-6). CTCs are generally thought to detach from primary or secondary tumors of patients with advanced cancer prior to detection in the circulation. The CellSearch System (Veridex) was designed to detect CTCs in whole blood. This system was developed using an epithelial cell adhesion molecule (EpCAM) antibody-based immunomagnetic capture and automated staining methodology. With this system, it is possible to obtain highly reproducible quantitative results from different laboratories. Isolation and capture techniques of CTCs have been reported by several groups; however, only CellSearch has been analytically validated and is approved by the U.S. Food and Drug Administration (4, 5). The primary studies established that CTCs can be used in conjunction with other modalities for monitoring patients with various types of metastatic cancer (3, 4). Recent studies have shown that CTC counts may change over the course of therapy (7).

We, therefore, examined the prognostic and therapeutic value of the CTC count before and after docetaxel chemotherapy in a population of patients with bone-metastatic CRPC treated with docetaxel at the Kyorin University.

Patients and Methods

Patient characteristics. The clinical characteristics of the patients are shown in Table I. Fifty-seven patients with mCRPC who were treated at the Kyorin University Hospital between April 2008 and March 2012 were prospectively enrolled. All patients with mCRPC who had experienced treatment failure with androgen deprivation therapy and had received docetaxel-based chemotherapy were eligible. All patients received 4 mg zoledronic acid every four weeks in addition to androgen deprivation therapy. Disease progression was defined as documented prostate specific antigen (PSA) progression according to the Prostate-Specific Antigen Working Group 1 criteria and a PSA level of less than 5 ng/ml, or objective progression by Response Evaluation Criteria in Solid Tumors (RECIST) criteria for patients with measurable disease (8, 9). The Ethics Committee of the University approved the study protocol (NO.6) according to the Declaration of Helsinki. All patients provided written consent to participation in this study.

Drug administration. Docetaxel at 70-75 mg/m^2 and dexamethasone a 8 mg were given by intravenous infusion every 3-4 weeks. Patients were simultaneously treated with hormonal therapy with an Luteinizing Hormone Releasing Hormone (LHRH) analog and daily oral dexamethasone (0.5-1.0 mg/day). Before this treatment, 43 (83%) patients had received estramustine and all developed resistance to this drug. Docetaxel treatment at 70-75 mg/m^2 was continued until the patient decided to stop, general health deteriorated due to disease progression, or unacceptable toxicity occurred.

Samples. Blood samples of patients diagnosed with mCRPC before and after treated with docetaxel chemotherapy were drawn into CellSave® Preservative Tubes (Immunicon, Huntingdon Valley, PA, USA), or an ethylene-diaminetetra-acetic acid (EDTA) Vacutainer®, an evacuated blood drawtube containing EDTA as an anticoagulant and a cellular preservative. All samples were maintained at ambient temperature, with those in EDTA tubes processed within 6 hours of collection and those in CellSave tubes processed within 72 h of collection.

Isolation and enumeration of CTCs (CellSearch system). The CellSearch System (Veridex LLC, Warren, NJ, USA) was used for the isolation and enumeration of CTCs. This system has been described elsewhere (10). In brief, samples were drawn into tubes containing cell preservatives, maintained at room temperature, incubated with EpCAM antibody-covered ferroparticles at room temperature, and processed on a CellTracks Autoprep (Immunicon). Enriched epithelial cells were identified by immunofluorescent staining with Cell Track Analyzer II (Immunicon). Cells were scored as CTCs when 4'6-diamidino-2-phenylindole-stained nucleated cells expressed cytokeratin, excluding white blood cell (WBC) contamination by negative selection with staining for CD45. Automatically selected images were reviewed by the operator for identification.

Statistical analysis. The time-to-death was defined as the time elapsed between the date on which blood was drawn and the date of death or last follow-up. Wilcoxon's rank sum test or Fisher's exact test was used to test for significant differences in the proportion of patients with CTCs greater than a particular threshold among the various patient characteristics. A threshold of 5 or more CTCs/7.5 ml, which has been shown to be prognostic in a number of prostate cancer trials, was used for overall survival (OS) analysis at each of the blood draw time points.

Table I. *Clinical characteristics of study patients*.

No. of patients	57
Mean age, years (range)	72 (61-82)
Mean PSA,ng/ml (range)	1485.3 (9.5-4276.7)
≤10.0	3 (5.3%)
10.1-20	12 (21.1%)
20.1-30	12 (21.1%)
30.1-40	10 (17.5%)
40.1-50	6 (10.5%)
50.1-100	11 (19.2%)
100.1-1000	3 (5.3%)
Gleason score	
7	8 (14.0%)
8	20 (35.1%)
9	18 (31.6%)
10	11 (19.3%)
EOD	
1	21 (36.8%)
2	18 (31.6%)
3	11 (19.3%)
4	7 (12.3%)
Disease involvement	
Only bone	29 (50.9%)
Bone plus node	28 (49.1%)

Number (and percentage) of patients is shown unless otherwise indicated. PSA: Prostate-specific antigen; EOD: extent of disease.

The median survival of patients with values greater than or equal to various PSA thresholds was evaluated to establish a PSA threshold (cut-off=35 ng/ml) to stratify the patients into two groups by receiver operating characteristic (ROC) analysis. The extent of bone metastasis was classified by the extent of disease (EOD) grade according to the method of Soloway *et al*. (11). The PSA doubling time (PSADT) was calculated using the formula described by Shulman *et al*. (12). Patients were divided into two groups: with a PSADT more than 3 months, and less than 3 months. The criteria for anemia and development of bone metastases were modified to hemoglobin (Hb) <11.5 g/dl and alkaline phosphatase (ALP) above the upper normal limit (UNL) at our hospital. Median OS was determined for patients with 5 or more CTCs per 7.5 ml blood at baseline and specified intervals. The patient charts were examined retrospectively to determine the OS time. The correlation of CTCs with OS on Kaplan–Meier survival curves was examined using the log-rank test. Cox logistic regression analysis was performed with nine categorical variables: PSA, Gleason score, EOD, PSADT, Hb, ALP, lactate dehydrogenase (LDH), albumin, and CTC count.

Results

CTC count. The median CTC count at baseline before starting trial treatment was 7 (range=0-227) CTCs per 7.5 ml of blood. Overall, 24 patients (42.1%) had a CTC count of less than 5, while 27 patients (47.4%) had a CTC count of 5-50 and six patients (10.5%) had a CTC count greater than

Table II. *Association between CTC and baseline characteristics.*

	N	CTC/7.5 ml blood		*p*-Value
		Mean	Range	
CTC count at baseline	57	7	0-227	
PSA (ng/ml)				0.032
<35	28	1	0-7	
≥35	29	14	0-227	
Biopsy Gleason score				0.025
7-8	28	3	0-18	
9-10	29	21	0-227	
EOD				0.006
1-2	20	1	0-16	
3-4	37	15	0-227	
Hemoglobin (g/dl)				0.021
<11.5	31	14	0-227	
≥11.5	26	4	0-63	
Serum albumin (g/dl)				0.067
<3.7	32	15	0-227	
≥3.7	25	3	0-116	
Alkaline phosphatase (IU/l)				0.003
<313	26	4	0-63	
≥313	31	16	0-227	
Lactate dehydrogenase (IU/l)				0.075
<226	27	3	0-84	
≥226	30	15	0-227	
Disease involvement				0.007
Only bone	29	2	0-63	
Bone plus node	28	15	0-227	

Table III. *Baseline prognostic factors for overall survival.*

	Univariate analysis	Hazard ratio	95% CI	*p*-Value
CTC count/7.5 ml blood at baseline				
≥5 *vs.* <5	<0.001	3.13	1.3-6.3	0.004
PSA (ng/ml)				
≥35 *vs.* <35	0.089	1.20	0.4-2.3	0.563
PSA doubling time (months)				
<3 *vs.* ≥3	0.035	1.43	0.6-2.7	0.145
Biopsy Gleason score				
9-10 *vs.* 7-8	0.004	1.67	0.6-3.5	0.12
EOD				
3-4 *vs.* 1-2	0.012	1.49	0.5-6.8	0.265
Hemoglobin (g/dl)				
<11.5 *vs.* ≥11.5	0.001	1.32	0.7-3.3	0.147
Serum albumin (g/dl)				
<3.7 *vs.* ≥3.7	0.026	1.28	0.8-3.6	0.334
Alkaline phosphatase (IU/l)				
≥313 *vs.* <313	0.001	2.45	1.2-5.1	0.023
Lactate dehydrogenase (IU/l)				
≥226 *vs.* <226	0.007	1.50	0.6-2.8	0.104
Disease involvement				
Bone plus node *vs.* only bone	0.068	1.08	0.5-3.7	0.327

PSA: Prostate-specific antigen; EOD: extent of disease.

50. All patients had CTC counts measured following the three courses of treatment at 12-15 weeks. The median CTC count after three courses of treatment was 3 (range=0-1317).

Correlation of baseline CTC count with patient characteristics. The correlation of CTC count and baseline characteristics is shown in Table II. Multivariate analysis revealed that higher CTC counts were associated with: ALP >UNL (p=0.003), hemoglobin level <11.5 g/dl (p=0.021), PSA >35 ng/ml (p=0.032) EOD>3 (p=0.006) and Gleason score >9 (p=0.025). Patients with bone and lymph node metastases had a higher median CTC count than patients with only bone metastases (p=0.007).

Multivariate analyses indicate that CTC count at baseline is an independent predictor of OS. The survival rates were calculated from the time of the baseline blood draw. The patient charts were examined to determine OS, which ranged from 6.0 to 37.0 months (mean=12.8±8.1 months, median=15.3 months). Multivariate analysis demonstrated that patients with a CTC count of 5 or more at baseline had a shorter OS (10.5 months) than patients with a CTC count of less than 5 (25 months) (Figure 1A). Apart from CTC count, ALP above the UNL was also independently associated with a poor OS (Table III).

CTC count dynamics predicts OS. On evaluation of the CTC count and PSA change after three courses of treatment, we were able to demonstrate that a drop of ≥30% from baseline PSA was not associated with improved OS (p=0.064) and a CTC count of less than 5 after chemotherapy was associated with improved OS (p<0.001) (Figure 1B and 1C). To investigate whether a change in CTC count from baseline predicts a change in the initial prognosis of survival, we compared changes in the count between baseline and after three cycles of therapy (Figure 1D). Four different groups of patients were compared: group 1 (n=12), patients with fewer than 5 CTCs at baseline and after three cycles; group 2 (n=18), patients with 5 or more CTCs at baseline but fewer than 5 after three cycles; group 3 (n=17), patients with fewer than 5 CTCs at baseline but with 5 or more CTCs after three cycles; group 4 (n=10), patients with more than 5 CTCs at baseline and after three cycles. The survival rates were calculated from the time of the baseline blood draw. Patients of group 4 had a shorter median OS (7.25 months), significantly different from the median OS time of group 1 (30.5 months; p<0.001), and group 2 (25 months; p<0.001). Patients of group 3 had a shorter median OS (11.5 months), significantly different from the median OS of group 1 (p<0.001), and group 2 (p=0.003). Differences between survivaI curves for groups 1 and 2 (p>0.05), and groups 3 and 4 (p>0.05) were not significant.

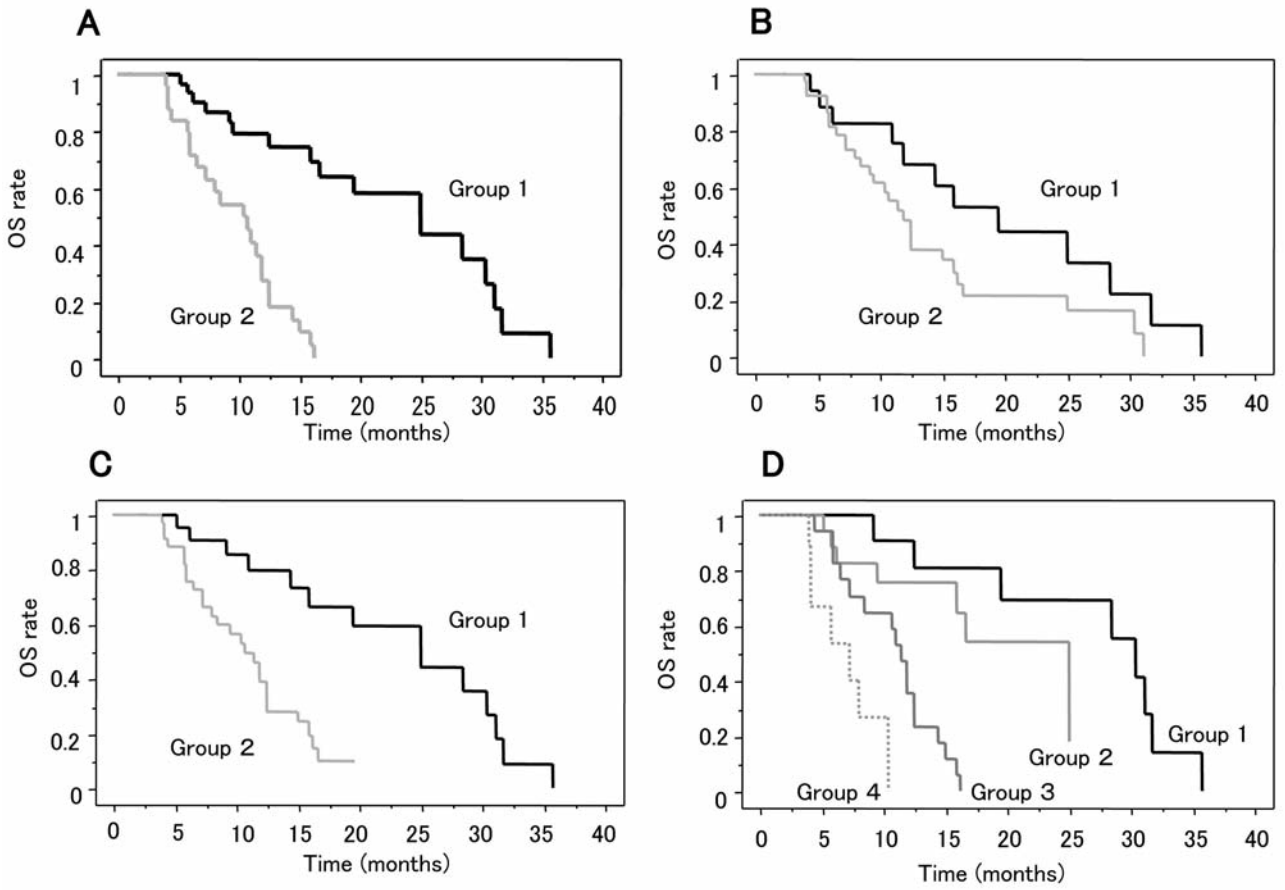

Figure 1. *A: Kaplan–Meier analysis of baseline Circulating Tumor Cells (CTC) count to predict overall survival (OS) duration in patients with bone-metastatic castration-resistant-prostate cancer (mCRPC). The duration of OS was significantly shorter in patients with 5 or more CTCs/7.5 ml of blood. Twenty-four patients (42.1%) with fewer than 5 CTCs/7.5 ml blood (group 1) had a median OS duration of 25.0 months compared with 10.5 months in 33 patients (57.9%) with 5 or more CTCs/7.5 ml of blood (group 2) (p<0.001, log-rank test). B: Kaplan–Meier analysis of docetaxel, a decline in PSA of 30% or more from baseline was not significantly associated with improved OS. Seventeen patients (29.9%) with such a decline (group 1) had a median OS duration of 19.1 months compared with 12.0 months in 40 patients (70.1%) with a decline of less than 30% (group 2) (p=0.064). C: Kaplan–Meier analysis using CTC count after three courses of docetaxel to predict OS duration in patients with mCRPC. After three courses of docetaxel, the OS duration was significantly shorter in patients with 5 or more CTCs/7-5 ml of blood. Twenty-two patients (38.6%) with fewer than 5 CTCs/7.5 ml of blood (group 1) had a median OS duration of 25.0 months compared to 10.5 months in 35 patients (61.4%) with 5 or more CTCs/7.5 ml of blood (group 2) (p=0.003). D: Kaplan–Meier curves for OS duration using the change in CTC count from baseline. Four different groups of patients were compared: group 1: patients with fewer than 5 CTCs/7.5 ml of blood at baseline and after three cycles; group 2: patients with 5 or more CTCs at baseline but fewer than 5 CTCs after three cycles of therapy; group 3: patients with fewer than 5 CTCs at baseline but 5 or more CTCs after three cycles; group 4: patients with 5 or more CTCs at baseline and after three cycles of therapy. The survival rates were calculated from the time of the baseline blood draw. Group-4 patients had a significantly shorter median OS (7.25 months) than did group 1 (30.5 months; p<0.001), and group 2 (25 months; p<0.001). Patients of group 3 had a significant shorter median OS (11.5 months) than those of group 1 (p<0.001), and group 2 (p=0.003). Differences between survival curves for group 1 and 2 (p>0.05), and group 3 and 4 (p>0.05) were not significant.*

Discussion

Several investigators have shown that the CTC count predicts survival pre-therapy and changes post-therapy are predictive of both progression-free survival (PFS) and OS in patients with CRPC (4, 5, 13-17). de Bono *et al*. reported that a comparison of the reduction in CTCs *versus* a reduction in PSA at earlier time-points revealed the limitations of PSA as a biomarker for survival and response to chemotherapy (4). The persistence of CTCs after the initiation of therapy suggests that patients derive a less than optimal benefit from treatment. Scher *et al*. showed CTC count to be a prognostic factor for survival in patients with progressive, metastatic, CRPC receiving first-line chemotherapy (5). At 4, 8, and 12 weeks after treatment, changes in CTC numbers were strongly associated with risk,

whereas changes in the PSA titer were weakly or not associated with risk. The most predictive factors for survival were the LDH concentration and the CTC count. Olmos *et al*. evaluated the association of the CTC count before and after commencing treatment with OS in patients CRPC. Patients whose CTC count reduced from 5 or more per 7.5 ml of blood at baseline to less than 5 CTCs/7.5 ml blood following treatment had a better OS than those in whom it did not (16). Our previous report also showed changes in CTC counts as a reflection of treatment benefit in patients with CRPC under several treatments (17).

Armstrong *et al*. investigated pre-treatment factors that predicted PSA decline and OS in men treated with docetaxel chemotherapy by subgroup analysis of TAX327 (2). Consequently, they reported that four independent risk factors (pain, visceral metastases, anemia and bone scan progression) predicted PSA decline and OS. In our study, all patients were treated with zoledronic acid after diagnosis of mCRPC. Therefore, Cox logistic regression analysis was performed with 10 categorical variables: CTC, PSA, Gleason score, EOD, Hb, albumin, ALP, LDH, and disease involvement. ALP and CTC were independent predictors of OS (p=0.023, and p=0.004, respectively).

We found CTCs prior to docetaxel therapy in 62% of patients with CRPC using a cut-off of 5 cells per 7.5 ml of blood. A threshold of 5 CTCs per 7.5 ml blood was used to evaluate the suitability of CTC count to predict survival. We examined the usefulness of CTCs for predicting survival in 57 patients with CRPC treated with docetaxel chemotherapy. Patients with fewer than 5 CTCs per 7.5 ml of blood had a median OS time greater than 25.0 months compared with 10.5 months in patients with 5 or more CTCs (p<0.001). The results showed that the assessment of CTC levels accurately and reproducibly predicts clinical outcome, as previously reported (13). Apart from a CTC count of 5 or more, ALP above the UNL was also independently associated with a poor OS. Such changes may also offer additional prognostic information to those offered by CTC count because they were shown to have independent prognostic relevance in our study.

As a response indicator of docetaxel efficacy, a change in CTC is more associated with survival than is a decline in PSA measured after three cycles. The prognostic factor for OS was a CTC count of 5 or more after three cycles. These findings suggest that monitoring CTC early post-chemotherpy may add clinical data that assist in treatment decisions, but this has not been confirmed. Recently, Goldkorn *et al*. analyzed CTCs in patients with CRPC treated with first-line docetaxel-based therapy in the Southwest Oncology Group (SWOG) S0421 trial (18). The median OS was 26 months for those with fewer than 5 CTCs per 7.5 ml pre-docetaxel therapy *versus* 13 months for those with 5 or more CTCs per 7.5 ml and an increasing CTC count at three weeks heralded significantly worse OS, as in this study. The CTC count at baseline is a strong, independent prognostic biomarker prior to therapy with docetaxel. In addition, measuring the CTC count after three cycles of docetaxel therapy predicts response to therapy.

Phase I/II trials using CTC monitoring as an embedded end-point studied patients with CRPC progressing post-docetaxel being treated with abiraterone acetate or enzalutamide (19-23). Two trials of abiraterone acetate demonstrated CTC conversion (≥5 CTCs at baseline but <5 CTCs at the final blood draw) rates of 34% and 41%, respectively (21.22). Phase I/II trials of enzalutamide demonstrated a CTC conversion rate of 49% (23).

Conclusion

These findings suggest the high risk and aggressiveness of tumors in patients with 5 or more CTCs prior to docetaxel chemotherapy, which resulted in the systemic spread of tumor cells and treatment failure. The CTC count after three courses of docetaxel chemotherapy was important in assessing the response to chemotherapy and in predicting disease outcome.

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