Circulating tumour cell (CTC) counts as intermediate end points in castration-resistant prostate cancer (CRPC): a single-centre experience


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Background: The purpose of this study was to evaluate the association of circulating tumour cell (CTC) counts, before and after commencing treatment, with overall survival (OS) in patients with castration-resistant prostate cancer (CRPC).

Experimental design: A 7.5 ml of blood was collected before and after treatment in 119 patients with CRPC. CTCs were enumerated using the CellSearch System.

Results: Higher CTC counts associated with baseline characteristics portending aggressive disease. Multivariate analyses indicated that a CTC \( \geq 5 \) was an independent prognostic factor at all time points evaluated. Patients with baseline CTC \( \geq 5 \) had shorter OS than those with \( <5 \) [median OS 19.5 versus \( >30 \) months, hazard ratio (HR) 3.25, \( P = 0.012 \)]; patients with CTC \( >50 \) had a poorer OS than those with CTCs \( 5-50 \) [median OS 6.3 versus 21.1 months, HR 4.1, \( P < 0.001 \)]. Patients whose CTC counts reduced from \( \geq 5 \) at baseline to \( <5 \) following treatment had a better OS compared with those who did not. CTC counts showed a similar, but earlier and independent, ability to time to disease progression to predict OS.

Conclusion: CTC counts predict OS and provide independent prognostic information to time to disease progression; CTC dynamics following therapy need to be evaluated as an intermediate end point of outcome in randomised phase III trials.

Key words: biomarkers, castration-resistant prostate cancer, circulating tumour cell

introduction

Castration-resistant prostate cancer (CRPC) is a heterogeneous disease with prognoses varying significantly between patients. In addition, the assessment of treatment response in CRPC remains a major challenge impacting not only routine clinical care but also anticancer drug development. Post-therapy declines in prostate-specific antigen (PSA) have been commonly utilised to identify antitumour activity [1] as conventional radiological assessments have severe limitations. First, commonly used criteria such as response evaluation criteria in solid tumours (RECIST) [2] are not useful in a large proportion of patients [3]. Secondly, changes in radionuclide bone scans are difficult to quantify objectively and reproducibly [4]. However, it has long been recognised that changes in PSA often do not accurately reflect disease activity [4, 5], and PSA decline posttreatment is not a reliable intermediate end point of overall survival (OS).

There is an urgent need to develop biomarkers for this disease and to assess antitumour activity of novel therapies.

The isolation, separation and enumeration of circulating tumour cells (CTCs) have been reported by several groups, but controversy exists on the optimal approach to enumerate them, although only the CellSearch™ assay (Immucor, Huntington, PA) is Food and Drug Administration (FDA) cleared. In particular, a recent report, by Nagrath et al. [6], has suggested a new highly sensible assay for CTC isolation. However, different methodologies have also used different criteria (presence of 4',6-diaminidine-2-phenylindole (DAPI) staining, size and morphology, antibodies used for identification) to measure these CTC counts and have led to substantial variations in the numbers of cells; it remains difficult to directly compare these methodologies at this time. Nonetheless, the CellSearch™ assay (Immucor) has been

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shown to be reproducible with low intrapatient and interlaboratory variability [7]. Using this technique, high CTC counts have been detected only in patients with cancer and may represent the haematogenous spread of tumours. CTC counts have additionally been found to have prognostic utility in different tumour types including advanced breast and colorectal cancer with high CTC counts correlating with poorer prognosis [7–11]. Specifically, the presence of more than five CTCs in 7.5 ml of blood has been associated with poor OS in patients with metastatic CRPC [12]. Furthermore, a fall in CTC count early after treatment for breast cancer correlates with longer progression-free survival and OS [8–10]. In fact, the correlation of CTC counts with OS was superior to that between time to radiological progression with OS in advanced breast cancer [13]. We therefore elected to examine the prognostic and therapeutic values of CTC counts before and after treatment in the population of CRPC patients treated on clinical trials of novel anticancer drugs at the Royal Marsden Hospital.

**methods**

**study design and patient selection**

Patients with biochemically or histologically confirmed progressive metastatic CRPC and castrate (<50 ng/dl) levels of testosterone treated on phase I or II clinical trials at the Royal Marsden NHS Foundation Trust were eligible. Patients gave informed consent for the collection and analysis of CTC. A medical history was taken from all patients, including details of the initial treatments and all subsequent therapeutic interventions. A physical examination, including Eastern Collaborative Oncology Group (ECOG) performance status (PS) [14] and laboratory studies, including full blood count, routine biochemistry, albumin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and PSA, were carried out at baseline. Patients were deemed to have progressive disease if they had either a rise in PSA with a minimum of three rising levels at least 1 week apart or radiological progression by RECIST criteria. All clinical trials were approved by the Royal Marsden NHS Foundation Trust Research Ethics Committee and deliberately included trials in both the pre- and postdocetaxel space with novel molecularly targeted drugs.

**CTC enumeration**

CTC isolation and enumeration were carried out using the CellSearch™ system (Immucor) as described previously [7]. Blood samples were drawn into CellSave™ tubes (Immucor) at baseline before patients were started on trial medication. Further samples were collected before each cycle whenever possible. All samples were kept at room temperature and processed within 72 h of collection. To calculate the CTC count, 7.5 ml of blood was mixed with magnetic particles coated with anti-EpCAM antibodies. After immunomagnetic enrichment, cells were fluorescently labelled and individually captured using a four-colour semiautomated fluorescence microscope. The captured images were then presented to trained operators, blinded to patient outcome, who selected cells that met the definition of CTC. Criteria used to define a CTC include round to oval morphology, size >5 μm, a visible nucleus (4’6-diamidino-2-phenylindole positive), positive staining for cytokeratins 8,18 and/or 19 (phycocerythrin) and negative staining for CD45 (allophycocyanin). Results were expressed as the number of cells per 7.5 ml of blood.

**statistical analysis**

This analysis was carried out using SPSS 14.0 (SPSS Inc., Chicago, IL). OS was defined as the time between the blood draw and either the date of death or the last follow-up (if death was not observed during the follow-up period). Time to disease progression was defined as the time between the start of treatment and progression of disease on the basis of a composite end point comprising (i) PSA progression [1], (ii) radiological progression [1; 2], (iii) clinical progression requiring a new therapeutic intervention and/or (iv) death of any cause. Associations of CTC count with baseline patient characteristics were analysed using Mann–Whitney U or Kruskal–Wallis tests; for ordinal variables, Spearman rank correlation was assessed. Subsequently, significant characteristics associated (P value <0.05) with higher CTC counts in the univariable analysis were analysed in multivariate analyses using a logistic regression model. A threshold of ≥5 CTC/7.5 ml, which has been shown to be prognostic in a number of breast cancer trials [8, 9] and in a recent communication of a prostate cancer trial [15], was used for OS analysis at each of the blood draw time points. Moreover, thresholds of 10–100 were systematically correlated in our series in an attempt to identify groups with different prognosis for OS. Median OS and the 95% confidence intervals (CIs) were determined with the Kaplan–Meier method and OS curves were compared using the log-rank test. A multivariate analysis for OS using a Cox regression model was carried out to determine the subset of baseline characteristics that provided independent prognostic information in our series. In addition, a Cox regression model with a time-dependent covariate was used to compare the independent prognostic value of CTC count at different time points. All P values reported were two sided. Time to disease progression and CTC counts were compared by calculating the proportion of the variation in OS that could be explained by these parameters using the method of Royston [16] employing the Stata 9.0 programme (StataCorp, College Station, TX). CTC counts were treated as a log-transformed continuous variable in this context.

**results**

**patient characteristics**

In all, 119 CRPC patients were treated in 14 different phase I and II trials from January 2005 to July 2007 (Table 1). The median age of patients included in this analysis was 67.5 years (range 48.2–85.5). A total of 94.1% of patients had ECOG PS of zero to one at baseline. The pattern of metastatic spread included disease limited to the lymph nodes without bone metastases in 11 patients (9.2%) and bone metastases in 108 patients (90.8%). In all, 87 patients (73%) were chemotherapynaive (previous hormone therapies median 2, range 2–4). Overall, 32 patients (27%) received previous chemotherapy (median range 1–4). A total of 76 patients were treated with a molecularly targeted drug only, 35 patients received docetaxel plus/minus a targeted agent. Eight patients received single-agent mitoxantrone. After a median follow-up time of 15.3 months (range 1.3–33.9), the median OS was 26.4 months (95% CI 20.0–32.7).

**CTC counts**

The median CTC count at baseline before starting trial treatment was six CTC per 7.5 ml of blood (range 0–545). In all, 59 patients (49.6%) had a CTC count <5, while 38 patients (31.9%) had a CTC count 5–50 and 22 patients (18.5%) had a CTC count >50. Overall, 101 patients had CTC counts measured following the first course of treatment at 3–4 weeks, and 98 patients had CTC counts measured following the second course of treatment at 6–8 weeks. The median CTC counts following the first and second courses of treatment were three CTC (range 0–1317) and one CTC (range 0–1144), respectively,
The correlation of CTC count distribution and baseline characteristics are shown in Table 2. Multivariate analysis revealed that higher CTC counts were associated with: ALP > upper normal limit (P = 0.0004), haemoglobin level < 12 g/dl (P = 0.027), PSA > 150 ng/ml (P < 0.035) and prior cytotoxic chemotherapy administration (P = 0.008). The univariate analysis revealed that presence of bone metastases was correlated with higher CTC counts (P = 0.013). Patients with metastatic disease confined exclusively to lymph nodes had
a median of zero CTC at baseline and all time points on study. Patients with bone involvement and concomitant lymph node metastases had a higher median CTC count than patients with solely bone metastases (10 CTC versus seven CTC, $P = 0.012$).

**Multivariate analyses indicate that CTC counts at baseline are an independent predictor of OS**

Multivariate analysis demonstrated that patients with a CTC count ≥5 at baseline had a shorter OS [19.5 months, 95% CI 8.9–30.1, hazard ratio (HR) 3.25, $P = 0.005$] compared with patients with a CTC count <5 (≥30 months; Table 3). Apart from CTC count ≥5, LDH >UNL was also independently associated with a poor OS (HR 2.44, 95% CI 1.2–4.9, $P = 0.012$). Additionally, we categorised patients into three groups of CTC counts (<5, 5–50 and >50). This categorisation enabled us to show that patients with a CTC count >50 had a poorer OS compared with patients with 5–50 CTCs and CTCs <5 at baseline (6.3 versus 21.1 versus >30 months, HR 4.1, $P < 0.001$). These categories continued to show significant different outcomes at all CTC time points following treatment (Figure 1).

**CTC count dynamics predict OS**

To investigate whether a change in CTC level from baseline predicts a change in the initial prognosis for survival, we compared changes in the level between baseline and after the first cycle and the second cycle (Figure 2). Four different groups of patients were compared—group 1: patients with <5 CTC/7.5 ml at every blood drawn time points; group 2: patients with ≥5 CTC/7.5 ml before the initiation of therapy who decreased to <5 CTC/7.5 ml after therapy; group 3: patients with <5 CTC/7.5 ml who increased to ≥5 after treatment and group 4: patients with ≥5 CTC/7.5 ml at all blood drawn time points. After treatment, 114 patients were eligible for this analysis. Patients with ≥5 CTC/7.5 ml at all time points (group 4, $n = 29$) had the shortest median OS of 9.2 months (95% CI 19.6–25.0), which was significantly worse compared with group 1 ($n = 51$, median OS >30 months, $P < 0.0001$) and with group 2 ($n = 28$, median OS 21.4 months, 95% CI 19.6–25.0, $P = 0.0055$). Furthermore, patients in group 3 ($n = 6$) showed a shorter OS (11.2 months, 95% CI 5.6–16.8), and this also differed from both group 1 ($P < 0.0001$) and group 2 ($P = 0.048$). However, there was no significant difference between group 3 and group 4 ($P = 0.49$).

To evaluate CTC dynamics as a continuous variable, we were able to demonstrate that a drop of ≥30% from baseline CTC count during the first two treatment cycles was associated with improved OS in all patients with a CTC count ≥5 (Figure 3). Among the 48 patients eligible for this analysis, patients with a drop of <30% from baseline had a poorer OS...

### Table 3. Baseline prognostic factors for OS

<table>
<thead>
<tr>
<th>Factor</th>
<th>N</th>
<th>OS 24 months (%)</th>
<th>Median OS (months)</th>
<th>95% CI</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>Hazard ratio</th>
<th>95% CI</th>
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<td>Normal</td>
<td>58</td>
<td>66</td>
<td>29.4</td>
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<td>0.360</td>
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<td>0.7–2.9</td>
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<td>20.5</td>
<td>15.2–25.7</td>
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<td>Normal</td>
<td>58</td>
<td>65</td>
<td>28.3</td>
<td>24.5–32.1</td>
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<td>0.012</td>
<td>2.44</td>
<td>1.2–4.9</td>
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<td>Chemo naive</td>
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<td>21.0–35.4</td>
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<td>25.8–30.7</td>
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<td>20.5</td>
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</table>

OS, overall survival; CI, confidence interval; CTC, circulating tumor cell.
time to disease progression and CTC counts

After a median follow-up time of 15.3 months (range 1.3–33.9), the median time to disease progression, on the basis of a composite of PSA, radiology and symptoms, was 5.3 months (95% CI 4.5–6.1). In our analysis, we applied time-dependent covariates to evaluate the use of time to disease progression. In this model, \( R^2 \) values were used to estimate the proportion of variation in OS explained by variation in the intermediate end points (in this case CTC counts and time to disease progression). CTC counts at baseline and after one or two treatments (after 3–8 weeks) showed \( R^2 \) values of 52%, 55% and 55%, meanwhile time to disease progression showed a \( R^2 \) of 39%. The \( R^2 \) values were compared by observing that the simulation-based 95% confidence interval for the first \( R^2 \) value was 52% (95% CI 32% to 69%), supporting the fact that CTC was independent but not necessarily superior to time to disease progression in predicting OS, although the CTC counts are earlier predictors of outcome.

discussion

Several methods to isolate and evaluate CTC counts have been reported [6, 17, 18]. A noncytometric methodological approach, on the basis of the detection of telomerase activity in by real time polymerase chain reaction, has been reported to be very sensitive for CTC detection in the context of prostate cancer; however, this kind of molecular approach does not allow the individual CTC identification. Previous reported
Multivariate analysis was carried out using a Cox regression model with the described above, following first cycle, second cycle and at any time.

Figure 3. Kaplan–Meier plot for overall survival (OS) of patients with ≥ 5 circulating tumour cell (CTC) at baseline and percentage of CTC fall using a threshold ≥30% at any time point following the first or second cycle of therapy. Group 1 (red) patients with a baseline CTC count ≥ 5 which drop ≥30%; group 2 (light blue) patients with a baseline CTC count ≥ 5 which drop <30%, remained stable or increased. Table inside figure shows 6-month, 12-month and 24-month OS rates and median for groups described above, following first cycle, second cycle and at any time. Multivariate analysis was carried out using a Cox regression model with a time-dependant covariable.

This is one of the first studies in this disease to examine early changes in CTC counts as a reflection of treatment benefit. We showed that changes in CTC counts from one category to another could have a role in predicting clinical outcome. As shown in Figure 2, patients who remained below the threshold of five CTCs during the initial two cycles of treatment had the best clinical outcome, whereas patients in whom the CTC count decreased from ≥5 to <5 had a better outcome compared with patient who remained in the category ≥5 throughout treatment. Interestingly, patients in whom the CTC count increased from <5 to ≥5 had a similar outcome as those patients whose count remained ≥5, suggesting the presence of treatment-resistant disease.

In addition, we demonstrated that other ways of characterising CTC changes are also useful. For instance, patients with a proportional fall in CTC count of ≥30% had an improved prognosis compared with those whose CTC count remained stable or increased. This supports the prospective exploration of CTC changes as a proportion of change and possibly as a continuous variable.

Moreover, in this series, prediction of OS using CTC counts was as effective as that on the basis of time to progression, but CTC levels are available earlier and could decrease the use of ineffective treatments. Changes in levels of LDH may also offer additional prognostic information to that offered by CTC counts because they have been shown to have independent prognostic relevance in our series. Change in CTC count, perhaps in combination with other parameters in a composite model, is therefore a strong candidate for use as an intermediate end point. Further exploration of CTC counts in this context should be supported.

The present series is heterogeneous, but multivariate analysis revealed CTC counts to be an independent factor. Significantly, we maintain that this is a true reflection of the CRPC setting in daily clinical practice.

In conclusion, our data suggest that CTC may provide a more sensitive marker in monitoring disease status during treatment, especially in early-stage disease, and give an important indication of long-term outcome. In order to validate these techniques prospectively, large randomised trials...
are under way, in which predictive models on the basis of CTC counts would be validated. This method may additionally have potential advantages in accessing tissue for molecular analysis. CTCs thus hold immense potential as improved biomarker of response and to accelerate evaluation of emerging novel therapies.

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**references**