Baseline Circulating Tumor Cell Counts Significantly Enhance a Prognostic Score for Patients Participating in Phase I Oncology Trials

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Abstract

Background: High circulating tumor cell (CTC) counts are associated with poor prognosis in several cancers. Enrollment of patients on phase I oncology trials requires a careful assessment of the potential risks and benefits. Many patients enrolled on such trials using established eligibility criteria have a short life expectancy and are less likely to benefit from trial participation. We hypothesized that the incorporation of CTC counts might improve patient selection for phase I trials.

Methods: This retrospective analysis evaluated patients who had baseline CTCs enumerated prior to their starting on a phase I trial. CTCs were enumerated using the CellSearch System.

Results: Between January 2006 and December 2009 a total of 128 patients enrolled in phase I trials had CTC counts evaluated. Higher CTC counts as a continuous variable independently correlated with risk of death in this patient population ($P = 0.006$). A multivariate point-based risk model was generated using CTCs as a dichotomous variable ($\geq 3$ or $< 3$), and incorporated other established prognostic factors, including albumin $< 35$ g/L, lactate dehydrogenase greater than upper limit of normal, and $> 2$ metastatic sites. Comparison of receiver operating characteristic curves demonstrated that the addition of baseline CTC counts improved the performance of the prospectively validated Royal Marsden Hospital phase I prognostic score, which now identifies three risk groups ($P < 0.0001$): good prognosis [score 0–1, median overall survival (OS) 63.7 weeks], intermediate prognosis (score 2–3, median OS 37.3 weeks), and poor prognosis (score 4, median OS 13.4 weeks).

Conclusion: CTC enumeration improved the performance of a validated prognostic score to help select patients for phase I oncology trials. Clin Cancer Res; 17(15); 5188–96. ©2011 AACR.

Introduction

Phase I clinical oncology trials are dose-finding and toxicity-describing studies, designed primarily to establish a safe dose and schedule for a single agent or drug combination for use in phase II studies (1). These trials can pose a fine risk–benefit balance for oncology patients, with low average response rates and the potential for significant toxicity (2–7). Most conventional phase I trials therefore employ strict eligibility criteria to exclude those for whom this risk–benefit balance is least favorable, including Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 2, acceptable visceral organ function, and a projected life expectancy of greater than 3 months. However, the clinical outcome of patients enrolled on phase I trials using these criteria remains highly variable (2–11), and still permits the inclusion of unsuitable patients for whom inclusion on trial is unlikely to be either in their, or the trial’s, benefit. Thus, greater efforts are required to improve the screening of advanced cancer patients for phase I studies.

A high circulating tumor cell (CTC) count is associated with poor overall survival (OS) for patients with advanced breast, colorectal, and castration-resistant prostate cancer (CRPC [12–14]). The CTC cut off which optimally separates good from poor prognosis patients varies according to tumor type ($> 3$ for colorectal, $> 5$ for prostate, and breast cancer patients). Several technological platforms exist for the enumeration of CTCs, although only the CellSearch assay (Veridex) is Food and Drug Administration (FDA) cleared. This assay has been shown to be reproducible with minimal intrapatient and interlaboratory variability (15).

High baseline CTC counts have been shown to correlate with poorer prognosis in metastatic breast, colorectal, and prostate cancers. For example, the presence of 5 or more CTCs in 7.5 mL of blood is associated with poor OS in CRPC.
High circulating tumor cell (CTC) counts are associated with poor prognosis in several cancers. We hypothesized that CTC counts might improve patient selection for phase I trials, where careful assessment of potential risks and benefits of participation is required. This retrospective study evaluated 128 patients who had baseline CTCs enumerated as part of their enrollment onto phase I trials. CTC counts ≥3 independently correlated with risk of death (HR 1.59, 95% CI 1.01–2.52). Other significant, independent, prognostic factors included albumin <35 g/L, LDH > ULN, and >2 metastatic sites. The addition of baseline CTC counts enhanced the prospectively validated Royal Marsden Hospital phase I prognostic score (RMH2008), and established an improved prognostic score (RMH2010) with 3 risk categories (P < 0.0001): good (score 0–1, median OS 63.7 weeks), intermediate (score 2–3, median OS 37.3 weeks), and poor prognosis (score 4, median OS 13.4 weeks), which may be utilized for patient selection for phase I oncology trials.

In this analysis had given their informed consent to take part in phase I trials approved by our Institutional Review Boards, which also granted their approval for this analysis.

**CTC enumeration**

Blood samples were drawn from patients into CellSave tubes (Veridex) just before starting trial medication. To assess the baseline intrapatient variability, a second baseline CTC enumeration was performed within 2 weeks before starting study drug when possible. All samples were kept at room temperature and processed within 72 to 96 hours of collection. CTC isolation and enumeration were carried out using the CellSearch system (Veridex) as described previously (15). Results were expressed as the number of cells per 7.5 mL of blood.

**Statistical considerations**

Associations between baseline CTC count and patient characteristics were analyzed using Mann–Whitney U and Kruskal–Wallis tests. For ordinal variables, Spearman rank correlation was assessed. For patients with 2 separate baseline CTC counts available, regression analysis was used to assess intrapatient variability.

OS was defined as the time between the pretreatment blood draw and either the date of death or the last follow-up (if death was not observed during the follow-up period). Multivariate analysis (MVA) for OS using a stepwise Cox-regression model was carried out to identify baseline characteristics with independent prognostic value. Baseline characteristics examined included age, gender, PS, tumor type, number of metastatic sites, hemoglobin, white cell count, platelets, albumin, and LDH. Continuous variables were converted to binary (high/low) variables using the following cut-offs: LDH ≤ or > ULN, albumin <35 or ≥35 g/L, and ≤2 or ≥2 sites of metastasis. For CTCs, the effects of choosing different thresholds between 3 and 10 CTC/7.5 mL were systematically explored to identify groups with different prognostic median OS and the 95% CI were determined with the Kaplan–Meier method and OS curves were compared using the log-rank test.

As previously described (2, 3), the following 3 poor prognostic factors were combined with equal weight to calculate the RMH2008 prognostic score: LDH > ULN, albumin <35 g/L, and ≥2 sites of metastasis. Patients were then defined as having either a good risk score (0–1) or poor risk score (2–3) according to the number of factors present. Subsequently, we derived a similar score based on LDH, albumin, number of metastatic sites,
and additionally CTC count as a 4th factor (hereafter called the RMH2010 prognostic score). In addition, we applied to our series 5 previously reported phase I prognostic scores (Supplementary Table S1): Chicago (8), Oxford (5), Lyon (4), Lille (9, 24), and MD Anderson (10). We compared the RMH2008 and RMH2010 prognostic scores with the other scores, and evaluated their performance in predicting 90-day mortality using receiver operating characteristic (ROC) curves and comparing their areas under the curves (AUCs) using a nonparametric statistical method based on the Mann–Whitney U-statistic (25, 26). All P values reported are 2 sided, and use a significance threshold of 0.05. Data analysis was performed using SPSS 15.0 (SPSS, Inc.).

Results

Patient characteristics

We identified 128 eligible patients of 813 patients treated on phase I trials at our institution between January 1, 2006 and December 31, 2009 with CTC counts tested before starting on trial. Patient characteristics are shown in Table 1. The median age was 61 years (range 18–79 years), with 52% of patients being male. ECOG PS was 0 in 24%, 1 in 71%, and 2 in 5% of patients. Overall, patients had a median of 2 prior systemic therapies (range 0–8); 42% of patients had more than 2 sites of metastatic disease (median 2, range 0–6). The most common sites of metastasis were lymph nodes (52%), lung (43%), liver (41%), and bone (31%). Baseline biochemistry showed decreased albumin levels (<35 g/L) in 46% of patients, and LDH levels were greater than the ULN (>192 U/L) in 42% of patients. Baseline full blood counts showed a median hemoglobin of 11.8 g/dL (range 9.1–15.6), a median white cell count of 6,800/mm³ (range 3,000–16,900), and a median platelet count of 263,000/mm³.

Trial characteristics

During the 4-year period under study, 52 phase I oncology trials were open for recruitment at the RMH Drug Development Unit. The 128 patients who had baseline CTC counts were treated within 10 of these 52 phase I trials. One hundred and twelve patients (87.5%) were treated on trial. Patient characteristics are shown in Table 1.

CTC counts

For all 128 patients, the median baseline CTC count was 1 CTC per 7.5 mL of blood (range 0–134). Seventy-two had a second baseline CTC count taken on a different day within 14 days of starting study drug. The median value for this latter group was 1 CTC per 7.5 mL of blood (range 0–137). Regression analysis demonstrated a correlation coefficient (R²) for both baseline samples of 0.915 (P < 0.0001) and a best-fit line with a slope of 0.96 (95% CI 0.89–1.03), and an intercept of 1.02 (95% CI 0.61 to 2.69, Supplementary Fig S1). In all, 75 patients (59%) had a CTC count ≤2, whereas 28 patients (22%)

Table 1. Patient baseline characteristics

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male vs Female</td>
</tr>
<tr>
<td>ECOG status</td>
<td>ECOG 0 vs ECOG 1, 2</td>
</tr>
<tr>
<td>Tumor category</td>
<td>Genitourinary cancer vs Prostate cancer, Gastrointestinal cancer, Breast cancer, Gynecological cancer, Malignant melanoma, Sarcoma, Lung cancer, Other tumours</td>
</tr>
<tr>
<td>Number previous systemic treatment lines</td>
<td>Median (range)</td>
</tr>
<tr>
<td>White cell count (/mm³)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Platelets (&gt;10⁹/mm³)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Serum lactate dehydrogenase (times ULN)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Bone metastasis</td>
<td>Median (range)</td>
</tr>
<tr>
<td>RMH2008 prognostic score</td>
<td>Good prognosis (Score 0–1), Poor prognosis (Score 2–3)</td>
</tr>
<tr>
<td>Drug targets</td>
<td>c-MET inhibitors, CCL2 inhibitors, PI3K-AKT-mTOR inhibitors, IGF-1R inhibitors, Androgen synthesis inhibitor, Mitotic kinase inhibitor</td>
</tr>
</tbody>
</table>
had a CTC count between 3 and 10, and 25 patients (19%) had a CTC count >10. Univariate analysis revealed that higher median CTC counts were associated with a worse PS ($P = 0.003$) and bone metastases ($P < 0.001$). CTC counts were associated with tumor type ($P = 0.007$); higher median CTC count was associated with prostate cancer ($P = 0.005$) and below median CTC counts were found in patients with nonepithelial tumors such as melanoma ($P = 0.008$) and sarcoma ($P = 0.042$). CTC count distribution for each tumor group is illustrated in Figure 1.

**Outcomes: response, progression-free survival, and overall survival**

One hundred and sixteen of the 128 patients analyzed were evaluable on the first planned and subsequent tumor assessments by RECIST criteria (22). Overall, partial responses (PR) were observed in 3 (3%) patients. Nonprogression rates at 3 and 6 months were 30% (95% CI 22–39) and 9% (95% CI 4–14), respectively. After a median follow-up of 34 weeks (range 2–245), the median PFS was 8 weeks (95% CI 7–9); the estimated median OS was 46 weeks for the entire study cohort (95% CI 33–58 weeks), which was not significantly different ($P = 0.65$) than the OS for the 685 patients not included in this study (median OS 42 weeks, 95% CI 34–49). The 90-day mortality rate was 15% (95% CI 9–21), and similar ($P = 0.779$) to the 16% (95% CI 13–19) observed in the remaining 685 patients. No treatment-related deaths were observed in this series. Cox-regression MVA (Table 2) revealed that a worse outcome was associated with >2 metastatic sites (HR 2.12, $P = 0.003$), LDH > ULN (HR 1.91, $P = 0.006$), albumin <35 g/L (HR 1.69, $P = 0.014$), and CTC count ≥3 (HR 1.59, $P = 0.048$). Baseline CTC counts as a continuous variable strongly associated with OS ($P < 0.006$).

Kaplan–Meier analysis was used to further explore the prognostic value of these factors in our series (Fig. 2). Patients with a low albumin (<35 g/L) had a shorter median OS compared to patients with normal albumin levels (34 vs. 55 weeks, $P = 0.025$; Fig. 2A). Those patients who had an elevated LDH (>ULN) had a shorter median OS compared to patients with normal LDH (35 vs. 62 weeks, $P = 0.001$; Fig. 2B). Patients with >2 metastatic organs and/or sites had a worse OS compared to patients with ≤2 metastatic sites (36 vs. 64 weeks, $P < 0.001$; Fig. 2C). A CTC threshold of 3 was identified as the cut point that separated patients into 2 groups with the greatest difference in median OS. Patients with high CTC counts (≥3 CTCs/7.5 mL) had a significantly shorter median OS compared to those with low CTC counts (<3 CTCs/mL) at baseline (36 vs. 55 weeks, $P = 0.048$; Fig. 2D).

**Impact of CTC counts on the RMH2008 prognostic score**

In this series our previously derived RMH2008 prognostic score (Table 3) classified patients in 2 groups with significant differences in OS (Fig. 3A): good prognosis (score 0–1, median OS 59 weeks) versus poor prognosis (score 2–3, median OS 31 weeks, $P = 0.0001$). The addition of baseline CTC counts(<3 or ≥3 /7.5 mL) as a 4th prognostic factor to the RMH2008 prognostic score...
enabled the identification of a third category of patients with significantly worse outcome than either score 0 to 1 or score 2 to 3 patients (Fig. 3B). Thus the RMH2010 score incorporating CTC counts (Table 3) identifies 3 groups of patients: good prognosis (score 0–1, median OS 64 weeks), intermediate prognosis (score 2–3, median OS 37 weeks), and poor prognosis (score 4, median OS 13 weeks, \( P = 0.000001 \)).

We also tested the prognostic value of 5 other scoring systems: Chicago (ref. 8; \( P = 0.006 \)), and Oxford (ref. 5; \( P = 0.0001 \)) scores were validated in our series, with calculated median OS for the poor prognosis groups defined by these 2 scoring system of 23 weeks (95% CI 12–24), and 35 weeks (95% CI 25–48), respectively. We were unable to confirm the prognostic value of the remaining scores in our series, and found overlapping survival curves for their prognostic groups: Lille (refs. 9 and 24; \( P = 0.230 \)), MD Anderson (ref. 11; \( P = 0.13 \)) and Lyon (ref. 4; \( P = 0.06 \)).

Comparative performance of RMH2008 and RMH2010 scores in predicting 90-day mortality

To evaluate this further, we compared the ability of the RMH2010 score to the RMH2008 score to predict death within 90 days of the phase I study entry (90-day mortality). The area under the ROC curve for 90-day mortality was 83% for the RMH2008 prognostic score and 89% for RMH2010 prognostic score (Fig. 3C). The overall predictive performance of the RMH2010 score was better than our previous model, although the paired comparison of both models was of borderline statistical significance (\( P = 0.0623 \)). We also compare these ROC curve AUCs with 5 different prognostic scores. The RMH2010 score was significantly
The performance of the RMH2008 score was not significantly different to that of the University of Chicago (ref. 8; \( P = 0.4421 \)) and Oxford University scores (ref. 5; \( P = 0.1001 \)), but was superior to the rest (all other \( P < 0.035 \)).

**Discussion**

Conventional methods for the selection of patients for phase I clinical trials exclude those patients with poor PS and deteriorating organ function, as well as those with an estimated prognosis of less than 3 months. We have previously reported that for patients taking part in phase I trials using these conventional criteria, we observed an objective response rate (ORR) of 8% to 9%; a combined clinical benefit rate of 25% to 53%; median PFS of 11 weeks and median OS of 27 to 43 weeks; 90-day mortality rates were 15% to 22%, and the treatment-related mortality rate was <1% (2, 3, 27). These findings were comparable to those reported in other series (6, 9, 10, 24, 28). Here we report on the outcome of 128 patients for whom baseline CTC counts were available. Most of these patients were treated after June 2007, and thus represent the latest cohort of patients from our unit for whom outcomes have been analyzed. We found ORR 3%, nonprogression rate at 3 months of 30% (95% CI 22–39) and median OS 46 weeks overall (95% CI 33–58), consistent with our previously reported results.

The number of poor prognostic variables present in patients has previously been expressed as a score from 0 to 3 (2, 3). This scoring system robustly identifies 2 groups of patients with significantly different outcomes: those with a good prognostic score (0–1); and those with a poor prognostic score (2–3). Previously, other groups have similarly described prognostic scoring system for patients with phase I (4, 5, 7–9, 11, 29). The series reported by Janisch (1987–1991; ref. 8), Bachelor (1986–1993; ref. 4), and Han (1991–2000; ref. 5) are 10 to 25 years old now, and differ from our series in the following significant ways: greater proportion of PS 2 patients; phase I trials of predominantly cytotoxic chemotherapy rather than modern molecular targeted agents; fewer prior systemic treatments as well as shorter median OS. In addition, the series reported by Italiano and colleagues (7) excluded patients who discontinued their trial early, and the series from Yamamoto and colleagues (29) included only lung cancer patients, therefore these results were not comparable.

One potential criticism for all these scores, including the RMH2008 score, is that the group of patients defined as being “poor prognosis” still has a very wide range of outcomes. For example, patients with an RMH2008 score of 2 to 3 have a median OS of 31 weeks. This group therefore contains both patients suitable to go on trial, and patients who should probably be excluded. The poor prognostic groups defined by the older scoring systems (4, 5, 8, 9, 11) suffered from the same flaw. In contrast, the addition of baseline CTC counts enabled us to identify a group (RMH2010 score 4) who had a median OS of 13 weeks.
We believe that this latter group of patients in the final stages of their illness should be excluded from phase I trials because they are unlikely to benefit, and more likely to suffer from adverse events and unplanned hospital admissions (27, 30). Still, about a third of the patients with RMH\textsuperscript{2010} score 4 in the present series survive longer than 24 weeks, which many phase I trialists would consider adequate for participating in these studies.

We have demonstrated that the RMH\textsuperscript{2010} score predicts 90-day mortality more accurately than any of the other scores tested. This paper describes the first direct comparison of different scoring systems in this setting. Notwithstanding the current results, a larger multi-institutional series would be helpful to confirm whether indeed the RMH prognostic score is a more accurate tool to help select patients for contemporary phase I clinical trials.

In our series 41% of patients have a median CTC count \(\leq 3\) per 7.5 mL blood, a figure similar to the 36% previously reported in a pooled analysis of all metastatic patients (15). We converted CTC counts from a continuous to a dichotomous variable so that they could be incorporated into a simple risk score. A threshold of \(\geq 3\) or \(< 3\) CTCs/7.5 mL was found to be the optimal cut point for separating patients into 2 groups with the greatest difference in median OS. This is consistent with 2 studies examining CTC counts in patients with cancer as well as healthy volunteers, all of the healthy individuals had a CTC count \(\leq 2\) (15, 31). In addition, Maestro and colleagues (31) reported that breast, colorectal, and prostate cancer patients with CTC counts \(\geq 3\) tend to have poorer prognosis.

Better patient selection for phase I clinical trials could reduce the costs incurred with the enrollment of extra patients to replace those who are considered nonevaluable when they come off trial during the screening process or during the first few weeks of therapy. In this way, use of the improved RMH\textsuperscript{2010} prognostic score incorporating baseline CTC counts has the potential to reduce the costs and time taken to complete phase I clinical trials. Minimizing the inclusion of patients at high risk of adverse events will help minimize risk to both patients and trials, and could help improve the efficiency of the drug development process (30, 32).

The prognostic value of baseline CTC counts in phase I clinical trials is one example of a number of promising novel blood-borne biomarkers, which include circulating free plasma DNA and microRNAs (33, 34). CTC studies have additional uses in drug development, which include monitoring CTC counts in pharmacodynamic and/or intermediate endpoint studies (17) and molecular characterization to help optimize treatment selection (35, 36). A prospective confirmatory study of the value of adding CTC counts to the RMH\textsuperscript{2010} prognostic score is currently underway for patients entering phase I trials in our unit.

### Table 2. Multivariate analysis of prognostic variables

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum LDH: (&gt;\text{ULN}) vs. (&lt;\text{ULN})</td>
<td>1.91 (1.21–3.05)</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of metastatic sites: (\geq 3) vs. (&lt;3)</td>
<td>2.12 (1.28–3.60)</td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline CTC count (\geq 3) vs. (&lt;3)</td>
<td>1.56 (1.01–2.52)</td>
<td>0.048</td>
</tr>
<tr>
<td>Serum albumin: (&lt;35) vs. (\geq 35) g/L</td>
<td>1.69 (1.07–2.69)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

### Table 3. RMH\textsuperscript{2008} score (without CTCs) and RMH\textsuperscript{2010} score (with CTCs)

<table>
<thead>
<tr>
<th>Variable</th>
<th>RMH\textsuperscript{2008} score (without CTCs)</th>
<th>RMH\textsuperscript{2010} score (with CTCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low albumin (&lt;35) g/dL</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>Number metastatic sites  (\geq 3)</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>Elevated LDH (\geq 1\times\text{UNL})</td>
<td>+1</td>
<td>+1</td>
</tr>
<tr>
<td>Categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good prognosis</td>
<td>Score 0–1</td>
<td>Good prognosis Score 0–1</td>
</tr>
<tr>
<td>Poor prognosis</td>
<td>Score 2–3</td>
<td>Intermediate prognosis Score 2–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor prognosis Score 4</td>
</tr>
</tbody>
</table>
Disclosure of Potential Conflicts of Interest

The research team lead by J.S. de Bono has received commercial research support from Immunocim and Veridex for investigation on CTC in Prostate Cancer.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked

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