Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer


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Background: We demonstrated that circulating tumor cell (CTC) number at baseline and follow-up is an independent prognostic factor in metastatic colorectal cancer (mCRC). This analysis was undertaken to explore whether patient and treatment characteristics impact the prognostic value of CTCs.

Patients and methods: CTCs were enumerated with immunomagnetic separation from the blood of 430 patients with mCRC at baseline and on therapy. Patients were stratified into unfavorable and favorable prognostic groups based on CTC levels of \( \leq 3 \) or \( < 3 \) CTCs/7.5 ml, respectively. Subgroups were analyzed by line of treatment, liver involvement, receipt of oxaliplatin, irinotecan, or bevacizumab, age, and Eastern Cooperative Oncology Group performance status (ECOG PS).

Results: Seventy-one percent of deaths have occurred. Median follow-up for living patients is 25.8 months. For all patients, progression-free survival (PFS) and overall survival (OS) for unfavorable compared with favorable baseline CTCs is shorter (4.4 versus 7.8 m, \( P = 0.004 \) for PFS; 9.4 versus 20.6 m, \( P < 0.0001 \) for OS). In all patient subgroups, unfavorable baseline CTC was associated with inferior OS (\( P < 0.001 \)). In patients receiving first- or second-line therapy (\( P = 0.003 \)), irinotecan (\( P = 0.0001 \)), having liver involvement (\( P = 0.002 \)), \( \geq 65 \) years (\( P = 0.0007 \)), and ECOG PS of zero (\( P = 0.04 \)), unfavorable baseline CTC was associated with inferior PFS.

Conclusion: Baseline CTC count is an important prognostic factor within specific subgroups defined by treatment or patient characteristics.

Key words: circulating tumor cells, colorectal cancer, metastatic

introduction

Colorectal cancer is the second leading cause of cancer death in the United States [1]. The last few years have seen a significant expansion in the number of available systemic therapies to treat metastatic colorectal cancer (mCRC) [2–4]. However, with increasing options comes greater complexity in decision making. A biomarker that could be obtained in a noninvasive manner to guide therapy would thus be of great potential clinical utility.

We recently demonstrated that circulating tumor cells (CTCs) can be isolated from the blood of patients with mCRC [5]. From this feasibility study, we designed a large, multicenter, international trial to evaluate whether CTCs could serve as a prognostic marker for patients with mCRC [6]. Patients beginning a new first-, second-, or third-line systemic therapy had peripheral blood obtained for enumeration of CTCs at baseline (pretreatment) and subsequent time points. We demonstrated that CTC count at baseline and during therapy was the strongest independent prognostic marker compared with other clinical factors for progression-free survival (PFS) and overall survival (OS). As the patient population was heterogeneous in this study, we carried out the current analysis with extended follow-up time to evaluate the prognostic significance of baseline CTC count by line of therapy, type of therapy, and important clinical characteristics. We hypothesized that baseline CTC count would remain an important prognostic factor within all patient subgroups.

patients and methods

study design

Details of patient selection and study design have been published previously [6]. Briefly, patients with mCRC beginning any new first- or second-line systemic therapy (or third-line with an inhibitor of the epidermal growth factor receptor) had peripheral blood obtained at baseline (pretreatment)
and follow-up time points for enumeration of CTCs. All CTC evaluations were carried out without the knowledge of patient clinical status in one of four central laboratories. The CellSearch®/C228 System (Veridex LLC, Raritan, NJ) was used for CTC enumeration, the technical details of which, including accuracy, precision, linearity, and reproducibility, have been previously described [7, 8]. Computed tomography or magnetic resonance imaging scans of the chest, abdomen, and pelvis were to be carried out at baseline and every 6–12 weeks after initiating treatment. All patients had an Eastern Cooperative Oncology Group performance status (ECOG PS) score of zero to two and hemoglobin ≥8 g/dl. The institutional review boards at each center approved the study protocol and all patients provided written informed consent.

**Table 1.** PFS and OS by baseline CTC count and clinical and treatment characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>≥3 CTCs</th>
<th>&lt;3 CTCs</th>
<th>Median PFS from time of baseline draw (months)</th>
<th>Median OS from time of baseline draw (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>413</td>
<td>108 (26)</td>
<td>313 (74)</td>
<td>7.8 (7.0–8.5)</td>
<td>20.6 (18.5–23.1)</td>
</tr>
<tr>
<td>First-line Tx</td>
<td>295</td>
<td>71 (24)</td>
<td></td>
<td>8.6 (7.8–9.6)</td>
<td>23.6 (19.9–25.2)</td>
</tr>
<tr>
<td>Second- or third-line Tx</td>
<td>118</td>
<td>37 (31)</td>
<td></td>
<td>5.4 (4.6–6.8)</td>
<td>15.4 (12.6–19.5)</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>301</td>
<td>92 (31)</td>
<td></td>
<td>8.2 (7.3–9.2)</td>
<td>21.2 (17.5–23.6)</td>
</tr>
<tr>
<td>Oxaplatin</td>
<td>265</td>
<td>62 (24)</td>
<td></td>
<td>8.6 (7.8–9.7)</td>
<td>21.3 (18.6–24.7)</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>104</td>
<td>35 (32)</td>
<td></td>
<td>6.8 (4.9–8.1)</td>
<td>18.6 (14.5–23.1)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>248</td>
<td>65 (26)</td>
<td></td>
<td>8.6 (7.9–9.8)</td>
<td>21.5 (19.5–24.7)</td>
</tr>
<tr>
<td>Age ≥ 65</td>
<td>200</td>
<td>51 (26)</td>
<td></td>
<td>7.1 (5.9–7.9)</td>
<td>18.1 (14.5–20.1)</td>
</tr>
<tr>
<td>Age &lt; 65</td>
<td>213</td>
<td>57 (27)</td>
<td></td>
<td>8.7 (7.3–9.8)</td>
<td>23.7 (20.6–26.0)</td>
</tr>
<tr>
<td>ECOG = 0</td>
<td>187</td>
<td>41 (22)</td>
<td></td>
<td>8.5 (7.5–9.7)</td>
<td>24.7 (21.3–28.2)</td>
</tr>
<tr>
<td>ECOG = 1–2</td>
<td>210</td>
<td>65 (31)</td>
<td></td>
<td>6.9 (6.1–7.8)</td>
<td>17.5 (14.5–19.8)</td>
</tr>
</tbody>
</table>

PFS, progression-free survival; OS, overall survival; CTC, circulating tumor cell; Tx, treatment; ECOG, Eastern Cooperative Oncology Group.

**statistical analysis**

A threshold of CTCs to define unfavorable and favorable CTC levels was defined as ≥3 and <3/7.5 ml peripheral blood, respectively, based on previously published criteria [6]. PFS was defined as the time from blood collection to progression or death. OS was defined as the time from blood collection to death. Patients were censored at last follow-up if progression or death had not occurred. Kaplan–Meier survival plots were generated based on baseline CTC levels. Survival curves were compared using log-rank testing.

Subgroup categories were selected by characteristics which were predictive of PFS and/or OS in univariate analysis (line of therapy, age, ECOG PS, and type of therapy) or associated with higher baseline CTC yield (liver involvement) in the initial publication.

**results**

**patient characteristics**

From February 2004 to November 2006, a total of 481 patients were enrolled, 430 of whom met inclusion and exclusion criteria and were assessable for the primary and/or secondary objectives. Their characteristics and reasons for inclusion/exclusion have been published previously [6]. In the original analysis, the median follow-up for living patients was 11.0 months (range 0.8–30.0). At the time of the current updated analyses, death had occurred in 305 (71%) of the 430 patients, with a median follow-up time of 25.8 months (range 0.9–39.1) for the 125 (29%) patients still alive. Table 1 summarizes the

![Figure 1. Progression-free survival (panel A) and overall survival (panel B) for the entire patient population by favorable (<3) versus unfavorable (≥3) baseline circulating tumor cell (CTC) count.](image-url)
treatment and patient characteristics analyzed as part of this report. In all subgroups, the percentage of patients with baseline unfavorable CTCs is similar, ranging from 22% to 32%. For the patient population as a whole, PFS and OS were nearly twice as long in patients with favorable compared with unfavorable CTCs (Table 1 and Figure 1).

impact of baseline CTCs by treatment characteristics

Of 413 assessable patients, 295 received first-line therapy and 118 second- or third-line treatment. Table 1 lists PFS by treatment and patient characteristics. As expected, PFS was shorter in patients receiving second- or third-line therapy. A longer PFS was noted for patients with favorable compared with unfavorable baseline CTCs regardless of line of therapy received. However, this difference in PFS was only significant in second- or third-line therapy patients. PFS by CTC count is demonstrated for patients receiving first-line therapy in Figure 2A and second- or third-line therapy in Figure 2B. OS was also significantly longer for patients receiving first-line therapy compared with second- or third-line therapy. For patients receiving either first-line therapy or second- or third-line therapy, having favorable baseline CTCs resulted in an approximate doubling in median OS compared with patients with unfavorable CTCs (Table 1 and Figure 2C and D).

We also evaluated the relationship of baseline CTCs to PFS and OS in subgroups of patients who received oxaliplatin, irinotecan, or bevacizumab. The majority of patients received first-line therapy with bevacizumab and oxaliplatin. PFS was significantly longer for patients with favorable compared with unfavorable baseline CTCs who were receiving irinotecan (Figure 3C), while the increase in PFS did not reach statistical significance for patients receiving oxaliplatin or bevacizumab (Table 1 and Figure 3A and E). OS was significantly increased in patients with favorable compared with unfavorable baseline CTCs whether they received oxaliplatin, irinotecan, or bevacizumab (Table 1 and Figure 3B, D and F).

impact of baseline CTCs by patient characteristics

Approximately half of the patients in this clinical trial were 65 years of age or older. In patients ≥65 years of age, unfavorable CTCs at baseline were predictive of inferior PFS and OS compared with patients with favorable CTCs (Table 1 and Figure 4A and C). For patients <65 years of age, unfavorable baseline CTCs predicted inferior OS but PFS was not significantly different (Table 1 and Figure 4B and D). When

![Figure 2](image-url). Progression-free survival (panels A and B) and overall survival (panels C and D) by favorable (<3) versus unfavorable (≥3) baseline circulating tumor cell (CTC) count for first-line or second- or third-line treatment patients with metastatic colorectal cancer.
ECOG PS was taken into account, patients with unfavorable CTCs and ECOG PS of zero had significantly worse PFS and OS compared with patients with PS of zero and favorable CTCs (Table 1 and Figure 5A and C). For patients with ECOG PS of one to two, unfavorable baseline CTCs resulted in significantly worse OS than patients with favorable CTCs but did not reach statistical significance when PFS was evaluated (Table 1 and Figure 5B and D).

As liver involvement was associated with increased CTC yield in this trial, we evaluated the impact of unfavorable baseline CTCs in the subgroup of patients with liver metastases. Patients with unfavorable CTCs in this group had significantly shorter
PFS and OS compared with patients with favorable CTCs (Table 1).

multivariate analysis

In an updated univariate Cox regression analysis, baseline CTC level, age, line of therapy, type of therapy, and ECOG PS were significantly associated with both PFS and OS. For multivariate Cox regression analyses, only the univariately significant clinical factors were included in the multivariate model. After adjusting for these clinically significant factors, CTCs at baseline, age, line of therapy, and ECOG PS remained strong independent predictors of PFS and OS (Table 2). Receipt of bevacizumab and oxaliplatin was an independent predictor of PFS but not OS.

discussion

Our initial report of this clinical trial with 47% of patient deaths recorded and a median follow-up for living patients of 11.0 months demonstrated that CTCs at baseline and follow-up time points are a strong independent predictor of PFS and OS [6]. The current report extends these analyses with additional follow-up, with 71% of patients having died and a median follow-up for living patients of 25.8 months. The results are remarkably consistent, with a near-doubling in PFS and OS for patients with favorable compared with unfavorable baseline CTCs. We also conducted an analysis of the impact of CTC baseline levels within patient and clinical subgroups and demonstrated that elevated baseline CTC count is associated with poorer OS in all patient subgroups. PFS was also generally inferior in patients with elevated baseline CTCs, but this finding did not reach statistical significance in all subgroups. However, each subgroup demonstrated at least a trend toward inferior PFS with unfavorable baseline CTCs. An updated multivariate analysis confirmed that baseline CTC count remains an independent predictor of PFS and OS.

These data indicate that CTCs should be considered as a stratification factor for OS in future mCRC clinical trials regardless of line of therapy. In addition, choice of a particular drug does not affect the impact of CTCs for predicting OS. For all treatment- and patient-related characteristics evaluated, the percent of patients with baseline unfavorable CTCs was similar, and the impact on OS statistically significant. Given the large difference in survival noted between unfavorable and favorable CTC groups for all patient subgroups, it is critical that this

Figure 4. Progression-free (panels A and B) and overall survival (panels C and D) by favorable (<3) versus unfavorable (≥3) baseline circulating tumor cell (CTC) count for patients with metastatic colorectal cancer by age <65 versus ≥65.
factor be balanced when evaluating patient outcome in subsequent clinical trials.

The number of useful stratification factors in advanced colorectal cancer is limited. While an elevated carcinoembryonic antigen (CEA) may be a poor prognostic factor for resectable colorectal cancer [9], no data support its prognostic value for patients initiating chemotherapy for metastatic disease. In the last reported gastrointestinal intergroup mCRC study, CEA was not utilized as a stratification factor [10]. Other stratification factors

Table 2. Multivariate Cox regression analysis for prediction of PFS and OS among univariately significant parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>PFS risk from baseline draw$^a$</th>
<th>OS risk from baseline draw$^a$</th>
<th>Number of patients</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>HR (95% CI)</td>
<td>$P$ value$^b$</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Baseline CTC number</td>
<td>≥3</td>
<td>&lt;3</td>
<td>1.45 (1.15–1.85)</td>
<td>0.002</td>
<td>2.22 (1.70–2.89)</td>
</tr>
<tr>
<td>Line of therapy</td>
<td>Second or third</td>
<td>First</td>
<td>1.58 (1.23–2.03)</td>
<td>0.000</td>
<td>1.52 (1.15–2.02)</td>
</tr>
<tr>
<td>Age at baseline blood draw (year)</td>
<td>≥65</td>
<td>&lt;65</td>
<td>1.41 (1.14–1.74)</td>
<td>0.002</td>
<td>1.54 (1.21–1.97)</td>
</tr>
<tr>
<td>ECOG status at study entry</td>
<td>2 versus 1 versus 0</td>
<td></td>
<td>1.28 (1.08–1.51)</td>
<td>0.004</td>
<td>1.37 (1.13–1.66)</td>
</tr>
<tr>
<td>Bevacizumab used in Tx regimen?</td>
<td>Yes</td>
<td>No</td>
<td>0.68 (0.55–0.85)</td>
<td>0.001</td>
<td>0.78 (0.61–1.02)</td>
</tr>
<tr>
<td>Irinotecan used in Tx regimen?</td>
<td>Yes</td>
<td>No</td>
<td>0.77 (0.53–1.12)</td>
<td>0.17</td>
<td>1.20 (0.79–1.80)</td>
</tr>
<tr>
<td>Oxaliplatin used in Tx regimen?</td>
<td>Yes</td>
<td>No</td>
<td>0.53 (0.38–0.76)</td>
<td>0.000</td>
<td>0.94 (0.64–1.38)</td>
</tr>
</tbody>
</table>

$^a$PFS and OS times calculated from baseline blood draw.

$^b$P value from Wald test of Z statistic.
PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; CTC, circulating tumor cell; ECOG, Eastern Cooperative Oncology Group.
included age (<65 versus ≥65), prior therapy, PS, and treatment location [10]. Our analysis clearly shows that CTCs remain a prognostic factor regardless of age, prior therapy, or PS. Thus, one can argue that the next large mCRC trial evaluating a new systemic therapy should utilize CTCs as a stratification factor for OS.

While OS was statistically inferior in all subgroups with unfavorable CTCs, PFS was statistically inferior in many but not all subgroups with unfavorable CTCs. However, the trend in all subgroups was in the expected direction. This is not surprising when considering the utility of baseline CTCs as a prognostic versus a predictive marker. Prognostic markers will yield information about clinical outcome regardless of therapy. Predictive markers will yield information about the differential impact of a selected therapy on clinical outcome [11]. At the current time, the weight of evidence supports the utility of CTCs as a prognostic marker. Thus, baseline CTCs may reveal relatively less information regarding PFS, depending on which regimen is initially selected. However, over a longer period of clinical follow-up, they are ultimately a strong predictor of survival. In support of this are data from our prior publication demonstrating that among those patients beginning with unfavorable CTCs, conversion to favorable CTCs on treatment results in significantly longer PFS and OS than patients who remain with unfavorable CTCs on treatment. Baseline CTCs would therefore have less impact on PFS than OS as they are dependent upon treatment selection. Further studies specifically powered to evaluate the impact of unfavorable CTCs on disease progression in the context of specific therapies are necessary to validate CTCs as a predictive marker.

The current report has several limitations. It was a retrospective subgroup analysis of a prospective trial. Thus, while it is supportive of the hypothesis that the prognostic impact of CTCs is similar within each treatment and patient subgroup, it cannot assure with absolute certainty that large studies focused on each patient subgroup would yield similar results. However, additional data should be forthcoming from a large European study evaluating serial CTC measurements in patients receiving only first-line therapy [12].

In conclusion, the current report with additional follow-up demonstrates that baseline CTC number remains an important prognostic factor for OS regardless of treatment and patient characteristics. PFS was also shorter for patients with unfavorable CTCs in all patient subgroups, reaching statistical significance in many. This can be used as supportive evidence for future evaluations of CTCs in specific patient subgroups as a marker of outcome and treatment effect. Baseline CTC levels should be considered as a stratification factor in future large mCRC clinical trials.

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references