Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents

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Background: Early predictive markers for response are needed for advanced colorectal cancer (ACC) patients. We assessed the value of circulating tumour cells (CTC) in ACC patients treated with chemotherapy plus targeted agents (CAIRO2 phase III trial) and compared the results with computed tomography (CT) imaging.

Materials and methods: CTC were determined at baseline and at different time points during treatment. Patients were stratified into low (less than three CTC per 7.5 ml of blood) or high CTC (three or more CTC per 7.5 ml of blood).

Results: A total of 467 patients were assessable for CTC analysis. Among them, 129 patients (29%) with high baseline CTC had a significantly decreased progression-free survival (PFS; hazard ratio (HR) 1.5) and overall survival (OS; HR 2.2) compared with 322 patients with low baseline CTC. This difference remained statistically significant during treatment. The sensitivity and specificity of high CTC at baseline for the prediction of progressive disease on CT imaging were 16.7% and 70.1%, respectively, and of high CTC at 1–2 weeks after the start of treatment 20.0% and 95.1%, respectively. The combined analysis of CTC and CT imaging provided a more accurate outcome assessment than either modality alone.

Conclusions: The CTC count before and during treatment independently predicts PFS and OS in ACC patients treated with chemotherapy plus targeted agents and provides additional information to CT imaging.

Key words: circulating tumour cells, colorectal cancer, CT imaging, predictive marker, prognostic marker, targeted therapy

Introduction

Colorectal cancer is the second most common cause of cancer deaths worldwide with 492 000 related deaths in 2000 [1]. Approximately 50% of the patients will eventually develop distant metastases, for which palliative systemic treatment is usually administered. Over the past decades, the treatment options for patients with advanced colorectal cancer (ACC) have changed considerably [2]. The standard chemotherapy for patients with ACC consists of a fluoropyrimidine, irinotecan, and oxaliplatin, which may be used either in combination or sequentially in the majority of patients [3, 4]. The prognosis for ACC patients has been further improved by the use of a new class of targeted agents that inhibit signal transduction pathways. Two types of targeted drugs are currently used in ACC: bevacizumab, an antibody against the vascular endothelial growth factor, and cetuximab and panitumumab, antibodies against the epidermal growth factor receptor (EGFR).

Despite the increased efficacy of treatment, only a subset of ACC patients will respond. The availability of early predictive markers for response could therefore prevent unnecessary toxicity in nonresponding patients and could also reduce the costs of treatment. KRAS mutation status was recently identified as a predictive marker for response to EGFR-targeted treatment [5]. Predictive tests with a broader applicability are still warranted.

Circulating tumour cells (CTC) can be detected in blood of patients with a variety of solid tumours [6] and have shown to be a predictor of progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast cancer [7, 8] and prostate cancer [9–11]. In ACC patients, CTC at baseline and during treatment were prognostic for OS and PFS [12, 13]. However, these studies included a heterogeneous population of...
both untreated and pretreated patients who received different schedules of systemic treatment. Therefore, it is still uncertain whether a large study in a more homogeneous population would yield similar results [12]. We assessed the prognostic and predictive role of CTC in ACC patients treated in a randomised phase III trial with first-line chemotherapy and targeted agents and compared the predictive value of CTC with conventional response assessment using computed tomography (CT) imaging.

**materials and methods**

**patient population**
All patients included in this study participated in the CAIRO2 trial (CKTO 2005-02) of the Dutch Colorectal Cancer Group [14, 15]. In this multicentre phase III trial, 755 ACC patients were randomly assigned to receive first-line treatment with capcitabine, oxaliplatin, and bevacizumab or the same schedule with the addition of weekly cetuximab. All cycles were given every 3 weeks. Inclusion criteria included a histologically proven ACC with irresectable distant metastases, the presence of at least one measurable disease parameter, World Health Organization performance status of 0 or 1, and adequate organ functions. The primary endpoint of the CAIRO2 study was PFS. Secondary end points were OS, response rate, toxicity, quality of life, and several translational research questions. Ineligible patients or patients withdrawing their informed consent were excluded from all analyses. Eligible patients were analysed according to the intention-to-treat principle. Patients alive without recurrence at the time of analysis were censored.

**imaging**
Tumour response was assessed every 9 weeks using CT imaging and evaluated according to RECIST [16]. For the current analysis, we used the response assessed at the first evaluation after 9 weeks of treatment. Response was defined as a complete response (CR; disappearance of all target lesions) or a partial response (PR; at least 30% decrease in the sum of the longest diameter of target lesions), and disease control was defined as CR, PR, or stable disease (SD; less than 30% reduction and less than 20% increase in the sum of longest diameter of the target lesions and no appearance of new lesions).

**collection and isolation of CTC**
All patients provided written informed consent for the collection of CTC. Peripheral blood was drawn for CTC evaluation before the start of treatment (baseline), after 1–2 weeks, after 3–5 weeks, after 6–12 weeks, and subsequently every 9 weeks (together with tumour evaluation by CT imaging) for up to 1 year. Blood samples were collected into 10-ml evacuated tubes containing a cellular preservative (CellSave tubes; Veridex, LLC, Raritan, NJ). Blinded samples were processed in a central laboratory (Veridex, LLC, Enschede, The Netherlands) within 96 h after sampling. CTC isolation and enumeration were carried out in 7.5 ml of whole blood using the CellSearch® System (Veridex, LLC, Raritan, NJ) as previously described [6]. CTC were defined as isolated and nucleated cells which were positive for the expression of epithelial cell adhesion molecule and cytokeratin but negative for CD45. Each analysis included a positive control sample provided by the manufacturer.

**statistical analysis**
The primary objective was to assess the prognostic and predictive value of CTC in ACC patients. Eligible patients were assessable for these analyses if at least one CTC sample was available. Patients were prospectively divided into two subgroups: low CTC count, defined as less than three CTC per 7.5 ml, and high CTC count, defined as three or more CTC per 7.5 ml. This cut-off level of three CTC was chosen on the basis of the results of a previous study [13]. The relationship between CT imaging and CTC levels was compared using Fisher’s exact test. In this analysis, PFS was defined as the interval from the date the CTC sample was drawn to the date of disease progression, death, or last follow-up, whichever occurred first. OS was determined from the date the CTC sample was drawn to the date of death or the date of last contact with the patient. Any CTC blood samples drawn after the date of progressive disease (PD) were excluded from the survival analyses. The prognostic value of CTC was defined as the correlation of baseline CTC and PFS and OS. The predictive role of CTC, defined as the ability of CTC to predict the response to treatment, was assessed as the change of CTC count during treatment compared with the baseline CTC count.

Survival curves were estimated using the Kaplan–Meier method and were compared using log-rank testing. Univariate and multivariate Cox proportional hazards models were built using prior adjuvant chemotherapy (yes versus no), the number of affected organs (one versus more than one), baseline serum lactate dehydrogenase (LDH) (normal versus abnormal), treatment arm (with or without cetuximab), and CTC count at baseline and at time points during follow-up (high versus low and as a continuous variable) as covariates for PFS and OS.

**results**

**patient characteristics**
The CTC count was determined in 477 patients enrolled from August 2005 to December 2006. A total of 467 patients were eligible: 235 (50%) in the arm treated with capcitabine, oxaliplatin, and bevacizumab and 232 (50%) in the arm treated with the same regimen plus weekly cetuximab. The baseline characteristics of patients in the CTC study subset are shown in Table 1 and were comparable with the total study population (data not shown). The median time span between randomisation and the baseline CTC sample was 5 days. The median duration of follow-up at the time of this analysis is 16.8 months.

<table>
<thead>
<tr>
<th>Table 1. Patients’ characteristics at baseline (N = 467)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
</tr>
<tr>
<td><strong>Median age, years (range)</strong></td>
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<tr>
<td><strong>Treatment arm</strong></td>
</tr>
<tr>
<td>Without cetuximab</td>
</tr>
<tr>
<td>With cetuximab</td>
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<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td><strong>Tumour localisation</strong></td>
</tr>
<tr>
<td>Colon</td>
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<tr>
<td>Rectum</td>
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<tr>
<td>Rectosigmoid</td>
</tr>
<tr>
<td><strong>Serum LDH</strong></td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Abnormal</td>
</tr>
<tr>
<td><strong>Prior adjuvant chemotherapy</strong></td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td><strong>Number of affected organs</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>&gt;1</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase.
prognostic value of CTC

Of the 451 patients with evaluable baseline CTC results, 129 patients (29%) had high CTC. The CTC count was not evaluable in 11 samples (2.4%), mainly due to problems in blood draw procedure and transport of blood samples and due to analytical errors. The median number of CTC was zero (range 0–312, mean 6.96) at baseline and zero (range 0–197, mean 1.39) after 1–2 weeks of treatment. In the group with high baseline CTC, more patients had an abnormal serum LDH compared with the group with low baseline CTC (76% versus 29%, respectively; \(P < 0.001\)), less patients received prior adjuvant chemotherapy (9% versus 17%; \(P = 0.019\)), and more patients presented with stage IV disease (77% versus 60%; \(P = 0.001\)). No difference in the number of affected organs was observed (61% versus 55% of patients with more than one organ affected; \(P = 0.293\)). We did not observe a difference in the CTC value as to the site of metastases, except that patients with liver metastases more frequently had high CTC values (33% versus 12% in patients without liver involvement; \(P < 0.001\)). However, within the group of patients with liver metastases, the CTC count did not discriminate between patients with liver-only metastases and patients with liver plus other metastases. When patients in both treatment arms were considered together, the median PFS was 8.1 months in patients with high baseline CTC and 10.5 months in patients with low baseline CTC \(\{P = 0.0003; \text{hazard ratio (HR) 1.5 [95% confidence interval (CI) 1.2–1.9]}\}\) (Figure 1A and Table 2). The median OS was significantly lower in patients with high baseline CTC compared with patients with low baseline CTC \(\{13.7 \text{ versus 22.0 months, respectively; } P < 0.0001; \text{HR 2.2 [95% CI 1.7–2.9]}\}\) (Figure 1B and Table 3).

Within each treatment arm, the median PFS and the median OS were also significantly lower in patients with high baseline CTC compared with low baseline CTC. In the treatment arm without cetuximab, the median PFS was 8.3 versus 11.4 months, respectively \(\{P = 0.0034\}\), and the median OS was 13.7 versus 24.2 months, respectively \(\{P < 0.0001\}\). In the treatment arm with cetuximab, the median PFS was 7.7 versus 10.2 months, respectively \(\{P = 0.0364\}\), and the median OS was 13.3 versus 21.5 months, respectively \(\{P = 0.0001\}\).

predictive value of CTC

At all time points tested during treatment, the median PFS and OS were significantly decreased for patients with high CTC compared with patients with low CTC (Tables 2 and 3). The predictive value of CTC was defined as the effect on PFS and OS of change in CTC count after 1–2 weeks of treatment. In 360 patients, both baseline CTC and CTC after 1–2 weeks were drawn, on the basis of which three groups of patients were identified: group I consisted of 250 patients (69%) with low CTC both at baseline and after 1–2 weeks and had a median PFS of 10.5 months (95% CI 9.8–12.0 months). Group II consisted of 89 patients (25%) who had high CTC at baseline which converted to low CTC after 1–2 weeks of treatment and had a median PFS of 7.9 months (95% CI 7.0–9.9 months).

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Time} & \text{High CTC, } n (\%) & \text{Median PFS, months (95% CI)} & \text{Hazard ratio (95% CI)} \\
\hline
\text{Baseline} & 451 & 129 (29) & 10.5 (9.6–11.9) & 8.1 (7.2–9.1) & 0.0003 & 1.5 (1.2–1.9) \\
\text{1–2 weeks} & 368 & 21 (6) & 10.0 (8.8–11.2) & 3.9 (1.7–5.4) & 0.0000 & 3.2 (2.1–5.0) \\
\text{3–5 weeks} & 320 & 17 (5) & 9.8 (8.5–10.8) & 3.5 (1.3–6.7) & 0.0015 & 2.2 (1.3–3.7) \\
\text{6–12 weeks} & 336 & 18 (5) & 9.3 (8.4–10.5) & 2.8 (1.7–7.2) & 0.0004 & 2.4 (1.4–4.0) \\
\text{13–20 weeks} & 254 & 16 (6) & 7.7 (6.3–8.9) & 2.2 (1.2–7.2) & 0.0000 & 2.9 (1.7–4.8) \\
\hline
\end{array}
\]

PFS, progression-free survival; CTC, circulating tumour cells; CI, confidence interval.

Table 2. Median PFS in patients with low and high CTC at different time points
Group III consisted of 21 patients with high CTC after 1–2 weeks of treatment irrespective of the CTC level at baseline and had a median PFS of 3.9 months (95% CI 1.7–5.4 months) (Table 4). The differences in PFS between these three groups were statistically significant (group I versus group II: \(P = 0.0031\); group I versus group III: \(P < 0.0001\); and group II versus group III: \(P = 0.0003\)) (Figure 3A). The median OS was 21.9 months (95% CI 20.2–25.0 months) in group I, 14.5 months (95% CI 12.2–16.6 months) in group II, and 6.3 months (95% CI 3.3–10.5 months) group III (all \(P < 0.0001\); Figure 3B).

correlation of CTC with response on CT imaging
We assessed the correlation between CT imaging results at first evaluation and CTC both at baseline and after 1–2 weeks of treatment.

First, of the 372 patients with available baseline CTC counts and an evaluable CT imaging study at 9 weeks, a response (CR or PR) was observed in 141 patients (38%), SD in 213 patients (57%), and PD in 18 patients (5%). The disease control rate (CR + PR + SD) in these patients was 95% (354 patients) (Table 5). The response rate was significantly higher in patients with low baseline CTC compared with high baseline CTC (41% versus 29%, respectively; \(P = 0.034\)). However, the disease control rate did not significantly differ between patients with high and low baseline CTC (94% and 97%, respectively; \(P = 0.295\)). Only 3 of 18 patients (17%) with PD at first evaluation by CT imaging had high baseline CTC. Assuming CT imaging as the standard resulted in a sensitivity of high baseline CTC for the detection of PD of 17% (95% CI 4% to 41%) and a specificity of 70% (65% to 75%). The positive and negative predictive values of high baseline CTC for PD are 3% (1% to 8%) and 94% (91% to 97%), respectively.

Next, we correlated CT imaging result to the CTC count after 1–2 weeks of treatment. Of the 307 patients with available CTC counts after 1–2 weeks of treatment and evaluable imaging studies, 117 patients had CR or PR (38%), SD in 213 patients (55%), and PD in 20 patients (7%) (Table 6). Also in this group, the response rate was significantly higher in patients with low versus high CTC at 1–2 weeks (40% versus 11%, respectively; \(P = 0.022\)). The disease control rate was also significantly higher in patients with low compared with high CTC at 1–2 weeks (94% versus 78%, respectively; \(P = 0.022\)). Only 4 of 20 patients (20%) with PD had high CTC at 1–2 weeks. The sensitivity and specificity of high CTC at 1–2 weeks to detect PD are 20% (6% to 44%) and 95% (92% to 97%), and its positive and negative predictive values are 22% (6% to 48%) and 95% (91% to 97%), respectively.

To assess whether CTC count has an additional value to CT imaging, we combined the CTC count at 1–2 weeks with the CT imaging results at first evaluation, by which four groups were identified: 273 patients with low CTC at 1–2 weeks and disease control had a median OS of 21.6 months (95% CI 20.1–25.5 months); 16 patients with low CTC count and PD...
had a median OS of 7.1 months (95% CI 6.1–14.4 months); 14 patients with high CTC and disease control had a median OS of 9.4 months (95% CI 5.0–11.6 months); and 4 patients with high CTC and PD had a median OS of 3.4 months (95% CI 1.8–N/A) (Figure 4). The difference between the patients with low CTC at 1–2 weeks and disease control and patients in the three other groups is statistically significant (P < 0.0001 for all three comparisons).

**Table 4.** Outcome parameters for patient groups on the basis of the change in CTC count from baseline to 1–2 weeks of treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline CTC</th>
<th>CTC 1–2 weeks</th>
<th>Number of patients</th>
<th>Median PFS</th>
<th>Median OS</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Low</td>
<td>Low</td>
<td>250</td>
<td>10.5(^a)</td>
<td>21.9(^b)</td>
<td>43</td>
</tr>
<tr>
<td>II</td>
<td>High</td>
<td>Low</td>
<td>89</td>
<td>7.9(^a)</td>
<td>14.5(^b)</td>
<td>31</td>
</tr>
<tr>
<td>III</td>
<td>Low or high</td>
<td>High</td>
<td>21</td>
<td>3.9(^a)</td>
<td>6.3(^b)</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^a\)Difference in median PFS: group I versus group II, \(P = 0.0031\); group I versus group III, \(P < 0.0001\); and group II versus group III, \(P = 0.0003\).

\(^b\)Difference in median OS: group I versus group II, group I versus group III, and group II versus group III; \(P < 0.0001\).

CTC, circulating tumour cells; PFS, progression-free survival; OS, overall survival.

**Table 5.** CTC at baseline and response by computed tomography imaging after 9 weeks of treatment

<table>
<thead>
<tr>
<th>Baseline CTC result</th>
<th>Imaging response, n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response</td>
<td>SD</td>
</tr>
<tr>
<td>High CTC</td>
<td>32 (29)(^a)</td>
<td>74 (68)(^b)</td>
</tr>
<tr>
<td>Low CTC</td>
<td>109 (41)(^b)</td>
<td>139 (53)(^b)</td>
</tr>
<tr>
<td>Total</td>
<td>141 (38)</td>
<td>213 (57)</td>
</tr>
</tbody>
</table>

\(^a\)The response rate (CR or PR) was significantly higher in patients with low baseline CTC compared with high baseline CTC (\(P = 0.034\)).

\(^b\)The disease control rate (CR + PR + SD) did not significantly differ between patients with high and low baseline CTC (94% and 97%, respectively; \(P = 0.295\)).

CTC, circulating tumour cells; SD, stable disease; PD, progressive disease; CR, complete response; PR, partial response.

**Table 6.** CTC after 1–2 weeks of treatment and response by computed tomography imaging after 9 weeks of treatment

<table>
<thead>
<tr>
<th>Week 1–2 CTC result</th>
<th>Imaging response, n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Response</td>
<td>SD</td>
</tr>
<tr>
<td>High CTC</td>
<td>2 (11)(^a)</td>
<td>12 (67)(^b)</td>
</tr>
<tr>
<td>Low CTC</td>
<td>115 (40)(^b)</td>
<td>158 (55)(^b)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (38)</td>
<td>170 (55)</td>
</tr>
</tbody>
</table>

\(^a\)The response rate was significantly higher in patients with low versus high CTC at 1–2 weeks (\(P = 0.022\)).

\(^b\)The disease control rate (CR + PR + SD) was significantly higher in patients with low compared with high CTC at 1–2 weeks (94% versus 78%; \(P = 0.022\)).

CTC, circulating tumour cells; SD, stable disease; PD, progressive disease; CR, complete response; PR, partial response.

**univariate and multivariate analyses**

Prior adjuvant chemotherapy, the number of affected organs, treatment arm, baseline serum LDH, and CTC counts (at baseline and follow-up) were assessed for their ability to predict PFS and OS in univariate Cox regression analyses. Serum LDH and CTC levels (at baseline and all follow-up) were significantly associated with both PFS and OS. The HR (95% CI) of progression for serum LDH and CTC count were 1.4 (1.2–1.7) and 1.5 (1.2–1.9) at baseline and 1.5 (1.2–1.9) and 3.2 (2.1–5.0) after 1–2 weeks of treatment, respectively. The HR (95% CI) of death for serum LDH and CTC count at baseline were 1.7 (1.4–2.2) and 2.2 (1.7–2.9), respectively.
and for serum LDH and CTC count at 1–2 weeks 1.9 (1.4–2.4) and 5.0 (3.1–7.9), respectively. When the baseline CTC count was analysed as a continuous variable instead of high versus low, the HR was 1.005 (95% CI 1.003–1.008) for PFS and 1.007 (1.004–1.010) for OS (both \( P < 0.001 \)). The CTC count after 1–2 weeks of treatment when analysed as a continuous variable resulted in an HR of 1.066 (95% CI 1.045–1.088) for PFS and 1.082 (1.059–1.105) for OS (both \( P < 0.001 \)).

In the multivariate Cox regression analysis, CTC at baseline and all follow-up time points remained the strongest predictors of PFS (HR 1.4 and 2.7 for CTC at baseline and at 1–2 weeks, respectively) and OS (HR 1.9 and 3.9 for CTC at baseline and at 1–2 weeks, respectively). As patients with high baseline CTC more frequently had an abnormal baseline serum LDH, we added the interaction term of baseline serum LDH and baseline CTC to the multivariate model. This term was insignificant (\( P = 0.901 \) for PFS and \( P = 0.725 \) for OS), indicating that baseline CTC predicts PFS and OS independent of baseline serum LDH.

**discussion**

This is the first large randomised study in patients with ACC treated with first-line chemotherapy and targeted agents in which the prognostic and predictive values of CTC counts are investigated. The CTC counts before and during treatment were shown to be a strong independent prognostic factor for PFS and OS. The change of CTC counts between baseline and early during treatment is predictive for outcome. Patients in whom CTC converted from high to low counts during the first treatment cycle had better outcome parameters than the patients with persisting high CTC counts. We found a statistically significant correlation between CTC counts and tumour response as determined by CT imaging. CTC counts provide relevant information on the outcome of treatment in addition to CT imaging, which makes CTC combined with CT imaging a promising noninvasive, fast, validated, and accurate response marker. However, since 80% of 20 patients with PD on CT imaging had low CTC counts at 1–2 weeks and 11% of 18 patients with high CTC counts had an objective response on CT imaging, CTC counts alone cannot accurately predict the clinical outcome. A limited sensitivity of CTC compared with radiographic imaging has also been demonstrated in a much more heterogeneous group of ACC patients treated with different regimens in different lines of treatment [13]. There are several possible explanations for this poor correlation. Clinical efficacy is not always correlated to a decrease in tumour size, such as for targeted agents that induce central necrosis and/or cavitation without tumour shrinkage [17]. Another explanation may be the length of the period between CTC draw (1–2 weeks after start of treatment) and CT imaging (9 weeks after start treatment). However, since only one of the 17 patients with PD at first tumour evaluation and low CTC showed an increase in CTC count at a later stage, we consider this cause unlikely.

Lastly, in ACC patients a poor correlation between objective response rate and survival has been demonstrated [18]. We assessed the prognostic role of CTC by defining two groups (high and low CTC). A recent study in prostate cancer showed that, rather than using a cut-off level, the change in CTC number is a strong prognostic marker [19]. However, this approach is not feasible in our ACC study population due to the low number of patients with positive CTC during treatment.

We demonstrate that CTC counts identify a small group of patients with unfavourable outcome early during treatment. However, whether a change in treatment on the basis of CTC will result in a better survival for this group is yet unknown, and this issue should be addressed in a prospective trial. In such a design, it will also be worthwhile to investigate the cost-effectiveness of CTC testing.

In conclusion, CTC counts are an early prognostic and predictive marker in ACC patients treated with chemotherapy and targeted agents and provide additional information to CT imaging. The consequences of medical decision making on the basis of CTC counts should be further explored.

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