# Circulating Tumor Cell Enumeration in a Phase II Trial of a Four-Drug Regimen in Advanced Colorectal Cancer

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## Abstract

**Circulating tumor cells (CTCs) are strongly prognostic in advanced colorectal cancer but have not yet been used to guide therapy. In the present phase II study of a 4-drug regimen, we sought to determine whether patients with high or low CTCs would benefit the most. Compared with historical controls, patients with high CTCs survived longer than expected; however, patients with low CTCs gained no extra benefit. Our data require validation from prospective CTC-guided randomized trials.**

**Background:** Multidrug regimens are active against advanced colorectal cancer (ACRC). However, the increased toxicity requires the use of biomarkers to select the patients who will derive the most benefit. We assessed circulating tumor cells (CTCs) as a prognostic biomarker in patients treated with a 4-drug regimen. **Patients and Methods:** A single-arm phase II trial (Erbitux Study of CPT11, Oxaliplatin, UFToral Targeted-therapy [eSCOUT]) was undertaken in patients with previously untreated *KRAS* wild-type ACRC using a regimen of irinotecan, oxaliplatin, and tegafur-uracil with leucovorin and cetuximab. Baseline CTCs were enumerated using CellSearch. The endpoints were an objective response rate (ORR) and overall survival (OS). We modeled our results and compared them with those modeled for the capecitabine, oxaliplatin, bevacizumab +/- cetuximab (CAIRO2) trial, stratifying patients a priori into low ($< 3$) and high ($\geq 3$) CTC groups. **Results:** For 48 eligible patients, the best ORR from the 4-drug regimen was 71%, with a disease control rate of 98%. The median OS for patients with a high and low CTC count was 18.7 and 22.3 months (log-rank test, $P = .038$), respectively. In our modeled data, for patients with a low CTC count, no differences were found between the median OS in the eSCOUT trial and that in the CAIRO2 trial (22.2 vs. 22.0 months). However, for the high CTC group, a clinically relevant improvement was seen in median OS (eSCOUT vs. CAIRO2, 18.7 vs. 13.7 months; $P = .001$). **Conclusion:** These data are hypothesis generating—for patients with ACRC, stratification by CTC count can identify those who might benefit the most from an intensive 4-drug regimen, avoiding high-toxicity regimens in low CTC groups. This hypothesis warrants validation in a phase III biomarker-driven trial.

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## Introduction

Colorectal cancer (CRC) is the second most common cause of cancer-related mortality in Europe, with approximately 150,000 deaths annually.[1] The mainstay of treatment of advanced colorectal cancer (ACRC) has been chemotherapy with oxaliplatin or irinotecan and 5-fluorouracil (5-FU).[2,3] The addition of targeted agents such as bevacizumab, an anti-vascular endothelial growth factor monoclonal antibody,[4-6] or cetuximab/panitumumab, monoclonal

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antibodies directed against the epidermal growth factor receptor (EGFR) receptor, in patients with wild-type *KRAS*/*BRAF*/*NRAS* tumors has led to improvement in overall survival (OS).[7-13]

One approach to improving outcomes for patients with ACRC is to administer multiple lines of therapy. However, in the clinical trial setting, only ~60% of patients with ACRC have been fit enough to receive second-line treatment, and this has probably been even less in routine clinical practice.[14,15] Thus, administering the most efficacious and tolerable treatment upfront to patients with only "one chance" of systemic therapy is an important strategy and the central theme of the present study. Falcone et al[16] reported improved efficacy using the multidrug regimen FOLFOXIRI (oxaliplatin/irinotecan/5-FU) compared with FOLFIRI (irinotecan/5-FU) in the first-line treatment setting. The triple chemotherapy with or without bevacizumab (TRIBE) trialists showed that FOLFOXIRI plus bevacizumab is efficacious with an improved response rate (RR) and progression-free survival (PFS) compared with FOLFIRI and bevacizumab.[17,18] The drawback to the use of multiple agents has been the greater toxicity rates.

We previously evaluated the clinical efficacy and safety of a 3-drug chemotherapy combination regimen (alternating irinotecan and oxaliplatin plus tegafur-uracil [UFT]—known as the SCOUT trial) in the first-line setting.[15] The rationale for alternating oxaliplatin and irinotecan was to allow recovery from the toxicity of each agent, reducing cumulative toxicity, but harnessing the benefit of using multiple agents. The regimen was well tolerated, with a RR of 68% and median OS of 19.6 months. In the present phase II trial, known as eSCOUT, we extended the activity of the SCOUT regimen by the addition of cetuximab in patients with *KRAS* wild-type tumors.

Given the need to balance efficacy and toxicity in intensive drug combination regimens, we sought to determine whether a prognostic biomarker, measured at baseline, could identify those patients most likely to benefit from the eSCOUT regimen. We, and others, have shown that dichotomization of circulating tumor cell (CTC) numbers has strong prognostic discrimination in patients with advanced colorectal,[19-22] prostate,[23] breast,[24,25] and lung cancer[26,27] and cutaneous melanoma.[28] For patients with ACRC, studies enumerating CTCs using the Food and Drug Administration (FDA)—approved CellSearch platform (Janssen Diagnostics, Raritan, NJ) have already established the prognostic "cutoff" value of 3 CTCs/7.5 mL blood, independently of standard clinical prognostic variables on multivariate analysis.[20-22] Thus, we hypothesized that in patients with ACRC, the poor prognostic group, defined by a CTC of $\geq$ 3, would benefit the most from intensive first-line chemotherapy. To allow comparisons with a multidrug regimen used in patients with ACRC[29] and a study in which CTCs had been enumerated,[22] we modeled our results against those from the published CAIRO2 trial. In that trial, 755 patients had been randomly assigned to receive first-line treatment with capecitabine, oxaliplatin, and bevacizumab or the same schedule, with the addition of weekly cetuximab.[29]

If our hypothesis is upheld in the present phase II trial setting, the potential utility of CTC enumeration will inform the design of a prospective randomized trial for biomarker qualification.

## Patients and Methods

### *Patients*

We performed a prospective, multicenter, open-label, single-arm, phase II trial in 3 UK centers—The Christie Hospital (Manchester), The Royal Marsden Hospital (London); and the Glan Clwyd Hospital (North Wales)—from April 2009 to February 2012. Patients with inoperable locally advanced or metastatic CRC and World Health Organization performance status (PS) 0 to 1 were eligible. Tumor samples (from diagnostic biopsy or previous surgery) were tested by a clinical pathology-accredited laboratory for somatic mutations in *KRAS* (codons 12, 13, and 61) and *BRAF* (codon 600) using pyrosequencing. The patients with mutated *KRAS* were excluded. At the initiation of our study, molecular analysis of *NRAS* was not routine for selection of anti-EGFR therapy. Adequate bone marrow function and renal and liver function test results within the normal range were required for enrollment. The trial was performed with local ethical approval in accordance with the UK Clinical Trials regulations for compliance to Good Clinical Practice[30] and was EudraCT registered (no. 2007-002053-24).

### *Treatment*

The treatment was administered on a 28-day cycle with irinotecan 180 mg/m$^2$ (90-minute infusion) on day 1 and oxaliplatin 100 mg/m$^2$ (2-hour infusion) on day 15. UFT capsules 250 mg/m$^2$ with leucovorin 90 mg were administered on days 1 to 21 in 3 divided doses. Dosing was performed in accordance with the known maximum tolerated dose for this regimen.[15] Cetuximab 500 mg/m$^2$ was administered every 2 weeks. After treatment of the first 8 patients, the dose of cetuximab was reduced to 400 mg/m$^2$ because of an excess of National Cancer Institute Common Toxicity Criteria Adverse Events (NCI CTCAE, version 3.0) Grade 3 to 4 diarrhea and fatigue (see Supplemental Material in the online version). Patients were treated for $\geq$ 8 weeks (until the first radiologic assessment). Those with stable disease (SD) or a treatment response continued treatment until disease progression was found.

### *Response and Toxicity Evaluation*

Computed tomography (CT) imaging was performed at 8 weeks (after 2 cycles of therapy) and every 2 months thereafter. The images were assessed for response using the Response Evaluation Criteria in Solid Tumours (RECIST), version 1.0.[31] For the present analysis, the data have been reported as the "best response" on serial CT scans within the first 6 months. Only patients receiving 2 full 28-day cycles were assessable for the objective response rate (ORR). Patients who discontinued treatment before 8 weeks were ineligible for the response assessment but were followed up for survival on an intent-to-treat basis. The patients were assessed clinically every 2 weeks, and toxicities were recorded in accordance with the NCI CTCAE, version 3.0.

### *CTC Analysis*

The baseline number of CTCs was determined from a peripheral blood sample collected $\leq$ 1 hour before the patient began their first chemotherapy cycle. Blood (10 mL) was collected in a CellSave preservative tube (Janssen Diagnostics), stored at room temperature, and processed within 96 hours of collection. The CTCs were

enumerated using the CellSearch platform, according to previously published protocols.[27,32] The sensitivity, accuracy, linearity, and reproducibility of the CellSearch platform have been previously deemed "fit for purpose" in clinical trials.[32,33] Because of the critical need to enumerate the cells at low numbers (owing to a prognostic cutoff of just 3 CTCs/7.5 mL blood in ACRC), we performed method validation using statistical techniques, including β-expectation tolerance intervals and β-content γ-confidence tolerance intervals to optimize the analytical accuracy.[34] We dichotomized a priori our CTC numbers into low (< 3 CTCs) and high (≥ 3 CTCs) groups.

### *Statistical Analysis*

For the eSCOUT trial, the primary endpoint was ORR according to RECIST (version 1.0) and a sample size of 48 was estimated accordingly (see Supplemental Material in the online version). For CTC enumeration, to allow comparison with the reported data,[20-22] the primary and secondary endpoint was OS and PFS, respectively. All time-to-event analyses were performed using the Kaplan-Meier method, taking time 0 as the date of administration of the first cycle of chemotherapy and the events as the date of confirmed radiologic progression or death. Comparisons were performed using log-rank tests. Because of the relatively small sample size, we did not seek to determine statistical independence using multivariate methods.

### *Statistical Modeling*

To allow a comparison with a less-intensive cetuximab-containing multidrug regimen performed in a study in which CTCs were enumerated,[22] we modeled our results against the baseline CTC numbers reported from the CAIRO2 trial. We restricted our modeling to OS and generated a Weibull distribution parameterized to estimate the key outcomes (median and 1- and 2-year survival rates) for the CAIRO2 trial by CTC category at baseline. We similarly modeled the eSCOUT data and tested the fit against the observed values. All statistical analyses and modeling were performed using STATA, version 11.1 (StataCorp, College Station, TX).

## Results

### *Phase II Trial Response and Toxicity*

Of the 82 patients initially screened, 48 were eligible for inclusion. Of the ineligible patients, 26 had a *KRAS* mutation, the PS of 2 patients had deteriorated before beginning treatment, 1 patient withdrew, 1 had received previous chemotherapy, and in 4 patients screening had revealed biochemical parameters that rendered them ineligible for the present study. The baseline characteristics for all eligible patients are listed in Table 1.

A total of 44 patients were evaluable for response. Of the 4 nonevaluable patients, 3 had died (2 likely of their disease and 1 of an unrelated myocardial infarction) and 1 patient had discontinued therapy because of toxicity before the first evaluation. For the 44 evaluable patients, the best ORR was 71% (2 patients [5%] had a complete response [CR] and 29 (66%) a partial response [PR]), with a disease control rate of 98% (PR/CR and SD). All 48 patients were evaluable for the survival endpoint analyses. The median OS and PFS was 21.2 months (95% confidence interval [CI], 20-22) and 8.4 months (95% CI, 7.5-9.1), respectively. The 1- and 2-year OS rate was 79% and 31%, respectively.

**Table 1 Baseline Characteristics of 48 Patients in eSCOUT Phase II Trial**

| Characteristic | n (%) |
|---|---|
| Age (years) | |
| Median | 61 |
| Range | 30-78 |
| Sex | |
| Male | 35 (73) |
| Female | 13 (27) |
| Stage | |
| Locally advanced | 3 (6) |
| Metastatic | 45 (94) |
| WHO performance status | |
| 0 | 27 (56) |
| 1 | 21 (44) |
| Adjuvant chemotherapy | |
| Yes | 5 (10) |
| No | 43 (90) |
| Liver metastases | 30 (63) |
| Liver only | 18 (38) |
| Liver plus other sites | 12 (25) |

Abbreviations: eSCOUT = Erbitux Study of CPT11, Oxaliplatin, UFToral Targeted-therapy; WHO = World Health Organization.

The full list of hematologic and nonhematologic toxicities is presented in Table 2. The most common grade 3 to 4 toxicities were fatigue (19%), diarrhea (29%), and neutropenia (35%). After the first 8 patients were treated, an excess of grade 3 diarrhea (38%) had occurred and was attributed to the addition of cetuximab to the SCOUT regimen. Thus, the cetuximab dose was reduced to 400 mg/m$^2$ every 2 weeks for all subsequent eSCOUT patients, reducing the diarrhea incidence (see Supplemental Material and Supplemental Table 1 in the online version). The incidence of grade 3 peripheral neuropathy (6%) and grade 2 alopecia (0%) was very low, likely owing to the alternation of oxaliplatin and irinotecan, which reduced the cumulative toxicity of either agent alone. Only 1 patient developed a grade 4 hematologic toxicity, and only 2 patients developed palmar-plantar erythema at grade 2 or greater.

All patients entered into the present trial had inoperable disease. However, 4 patients had a treatment response sufficient to allow for resection of locally advanced colonic tumors and 1 patient underwent curative-intent liver resection. All 5 patients were alive at the last follow-up visit.

### *Circulating Tumor Cells*

A total of 42 patients had evaluable baseline blood samples for CTC enumeration. The other 6 patients had an insufficient blood volume available for analysis. The range of CTCs was 0 to 90/7.5 mL blood, and 22 patients were categorized into the high CTC group (≥ 3 CTCs). A positive association was found between the high CTC number and patients with PS 1 versus 0. The median OS for patients with < 3 CTCs and ≥ 3 CTCs was 22.2 and 18.7 months ($P$ = .034), respectively (Figure 1A). No difference was found in PFS between the 2 groups (Figure 1B).

**Table 2** All Grade Toxicities According to NCI CTCAE, Version 3.0, Criteria

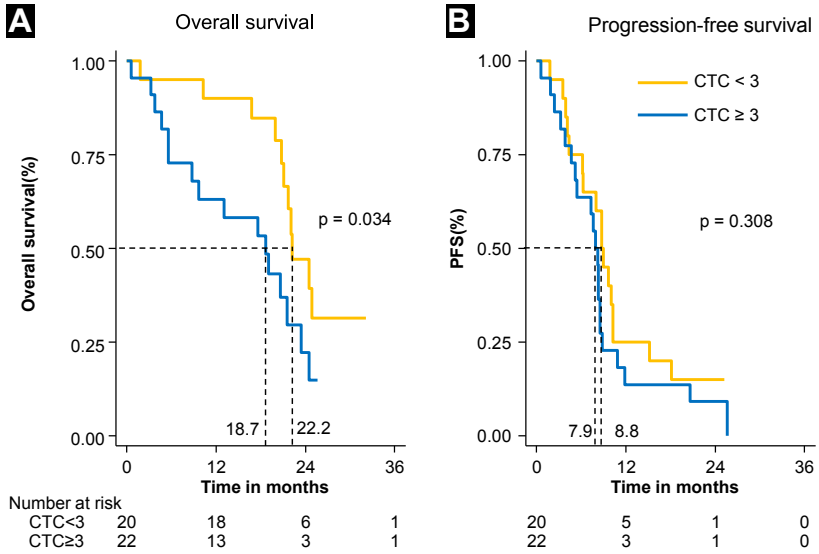
| Toxicity | Toxicity Grade | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Hematologic | | | | |
| Anemia | 17 (35) | 4 (8) | 0 | 0 |
| Leukopenia | 3 (6) | 0 | 1 (2) | 0 |
| Neutropenia | 12 (25) | 0 | 17 (35) | 1 (2) |
| Thrombocytopenia | 0 | 0 | 0 | 0 |
| Nonhematologic | | | | |
| Nausea | 12 (25) | 5 (10) | 2 (4) | 0 |
| Vomiting | 9 (19) | 0 | 4 (8) | 0 |
| Constipation | 10 (21) | 5 (10) | 2 (4) | 0 |
| Diarrhea | 14 (29) | 9 (19) | 14 (29) | 0 |
| Lethargy | 12 (25) | 17 (35) | 9 (19) | 0 |
| Mucositis | 10 (21) | 0 | 0 | 0 |
| Sensory neuropathy | 26 (54) | 8 (17) | 3 (6) | 0 |
| Palmar-plantar erythema | 3 (6) | 1 (2) | 1 (2) | 0 |
| Acneiform rash | 17 (35) | 16 (33) | 1 (2) | 0 |
| Cetuximab allergy | 4 (8) | 0 | 0 | 1 (2) |
| Alopecia | 18 (38) | 0 | 0 | 0 |
| Anorexia | 8 (17) | 1 (2) | 1 (2) | 0 |
| Abdominal pain | 13 (27) | 7 (15) | 4 (8) | 0 |
| Bowel obstruction | 0 | 0 | 1 (2) | 1 (2) |

Data presented as n (%).
Abbreviations: CTCAEs = Common Toxicity Criteria Adverse Events; NCI = National Cancer Institute.

### *Modeled Comparisons*

We modeled the time-to-event analyses for OS for our data and compared them with the larger CAIRO2 trial data, both categorized by low- and high-CTC count groups (Figure 2). For patients with a low CTC count, no difference was found between the median OS for the eSCOUT versus CAIRO2 data (22.2 vs. 22.0 months).

**Figure 1** Time-to-event Analysis by Low and High Circulating Tumor Cell (CTC) Categories for (A) Overall Survival and (B) Progression-Free Survival



However, for the high CTC group, a clinically relevant improvement in median OS (eSCOUT vs. CAIRO II, 18.7 vs. 13.7 months) was seen ($P = .001$).

### *BRAF* Status

*BRAF* mutation data were available from the tumor biopsy analysis for 27 of 42 patients (64%) with evaluable CTCs. Only 1 patient exhibited a *BRAF* mutation (CTC number, 4/7.5 mL blood). Thus, no association was identified between *BRAF* status and the number of CTCs. Because the 15 patients with unknown *BRAF* status were split equally between the high (n = 8) and low (n = 7) CTC groups, it is unlikely that a *BRAF* mutation was a confounding factor for prognosis in the present study.

## Discussion

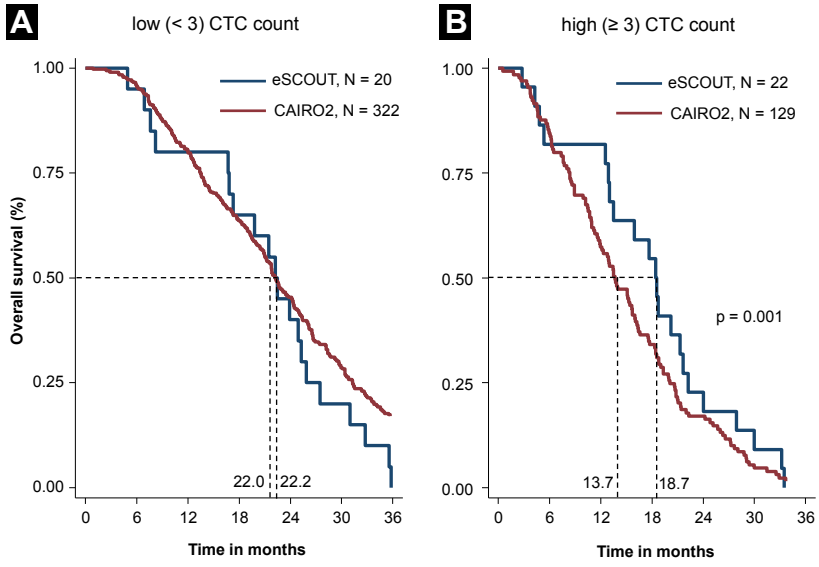
### *Main Findings*

In the present phase II study, we exploited the opportunity to enumerate baseline CTCs, a known prognostic biomarker in ACRC, to test whether a subgroup of patients could be identified that would benefit most from an intensive chemotherapy regimen. We confirmed that stratification of CTCs at a cutoff of 3 is prognostic for OS in patients with ACRC and showed that patients with high CTC counts could be more likely to benefit from an intensive regimen. Compared with the published data, we found no additional benefit for patients with a low CTC count; thus, intensive, high-toxicity regimens can be avoided for these patients. The present findings are hypothesis-generating and warrant additional validation as a part of a randomized controlled trial of treatment intensification versus the standard-of-care for patients with a poor prognosis with ≥ 3 CTCs/7.5 mL blood.

### *Context of Other Data*

The eSCOUT regimen demonstrated a high response rate (71%) and disease control rate (98%). The main toxicities were diarrhea (overlapping toxicity from cetuximab/irinotecan and UFT) and fatigue, although these toxicities improved after the dose of cetuximab was reduced by 20% (to 400 mg/m$^2$). Our rationale for reducing cetuximab was the previously reported pharmacokinetic data[35] showing that bi-weekly cetuximab at 400 mg/m$^2$ resulted in serum cetuximab concentrations similar to that with 250 mg/m$^2$ weekly (used in the Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer [CRYSTAL] study[10]) and the knowledge that the previous SCOUT chemotherapy regimen[15] was well-tolerated and efficacious. By alternating oxaliplatin and irinotecan, the incidence of peripheral neuropathy and alopecia was low compared with previous studies of either FOLFOX[36] or FOLFIRI.[37] Furthermore, the rates of diarrhea (29%) and neutropenia (35%) were less than those reported by a recent study using FOLFOXIRI and panitumumab (diarrhea 35% and neutropenia 48%) in which all agents were given simultaneously.[38] The rationale for adopting UFT in the present study was the comparable clinical efficacy data with (the now more commonly used) capecitabine but with a better tolerability profile.[39-41] The incidence of palmar-plantar erythema was low; however, we acknowledge that this agent is no longer in routine use, and future studies will need to incorporate an alternative 5-FU as a backbone of the therapy.

**Figure 2** **Comparison of eSCOUT Trial With CAIRO2 Study for Overall Survival by (A) Low and (B) High Circulating Tumor Cell (CTC) Categories. We Parameterized the 2 CTC Groups (< 3 and ≥ 3 CTCs) for Survival According to the Data Reported in the CAIRO2 Study, Assuming a Weibull Distribution. The eSCOUT Data Equally Informed the Parameterization of the 2 CTC Groups Undergoing Intensive 4-drug Combination Therapy, Again Assuming a Weibull Distribution**




Abbreviations: CAIRO2, capecitabine, oxaliplatin, bevacizumab +/- cetuximab; eSCOUT, Erbitux Study of CPT11, Oxaliplatin, UFToral Targeted-therapy.

Our CTC data confirmed that enumeration of CTCs at baseline is prognostic for OS in patients with ACRC, consistent with data from previous reports.[20-22] However, we noted that the median OS of 18.7 months in the eSCOUT "poor" prognostic group (≥ 3 CTCs) was longer than that reported by Tol et al[22] (13.7 months) and Cohen et al[21] (11.6 months) in the first-line treatment setting. In contrast, the median OS rate for patients with a "good" prognosis (< 3 CTCs) was similar among the studies (22.3 months for eSCOUT; 22.0 months for CAIRO2[22]; and 23.6 months for the study by Cohen et al[21]). We found no differences in PFS between the patients categorized by a low and high CTC count, consistent with previous data that reported only modest differences in PFS.[21,22]

### *Study Strengths and Limitations*

The present study had methodologic strengths. First, we demonstrated that the enumeration of CTCs is portable across several recruitment centers with assays centralized to 1 research laboratory. Second, despite the small sample size, our data mirrored that of the CTC counts and prognostic modeling in other studies of patients with ACRC, in both the trial setting[22] and the "real-world" setting,[21] giving us the confidence to model our data with that of published data.

The potential limitations were first that the study sample size was initially estimated according to the anticipated response rates of the phase II trial. We addressed this in our modeling, which allowed us to use larger numbers in our comparator set. Second, potential residual confounders were present in the relationship between CTC categorization and prognosis. We did not model for these on multivariate analysis, because the numbers were too small. Although an association was found between the CTC number and PS, differentiating between a PS 0 or 1 clinically would have little influence in guiding therapy. Future studies are needed to model multivariate prognostic factors before drawing any firm conclusions.

At the initiation of our study, molecular analysis of *NRAS*/*BRAF* was not routine for the selection of anti-EGFR therapy. The *BRAF* status was known for 64% of our patients (only 1 patient had a positive finding). Because the patients with "unknown" *BRAF* status were split equally between the high and low CTC groups, it is unlikely that *BRAF* influenced the prognosis of our patient cohorts. However, again, this should be considered in multivariate models in future studies.

### *Clinical Implications and Future Research*

The results we have presented suggest that baseline CTC enumeration and categorization as a prognostic biomarker can be used to select for patient treatment. Currently, CTCs are rarely used in clinical practice because they have not yet been proved to help guide therapy. One drawback to the FDA–approved CellSearch system is that CTCs are present in only one third to one half of patients with ACRC[21,22] and usually in small numbers. We have previously shown the importance of optimizing analytical accuracy in providing confidence of CTC enumeration at these low levels.[34] Nonetheless, newer CTC technologies, such as the CTC iCHIP (Massachusetts General Hospital & Janssen Diagnostics, New Jersey),[42] are emerging that promise greater sensitivity for CTC detection. Alongside the prognostic evaluation capability of these technologies is the highly anticipated application of CTCs as a liquid biopsy to determine the *KRAS*/*NRAS*/*BRAF* mutation status (and, ultimately, larger panels of genes) in real-time. This will be invaluable in terms of obtaining an up-to-date status of tumor characteristics and monitoring for tumor resistance mechanisms. An increasing number of studies are evaluating CTCs for this purpose.[43]

Circulating tumor DNA (ctDNA) is relatively easy to extract from blood and promises to become a useful tool for tumor characterization. Thierry et al[44] recently showed 98% specificity and 92% sensitivity for detection of *KRAS* status and 100% specificity and sensitivity for detection of *BRAF* status in ctDNA. This sets a precedent for beginning to introduce these tools to the clinic. Furthermore, the emergence of a *KRAS* mutation has been detected in ctDNA in a cohort of patients who became resistant to anti-EGFR–directed therapy.[45,46] CTCs will arguably be more informative for tumor characterization than ctDNA, because they are intact cells ultimately responsible for distant metastases. Also, parallel aberrations in the same signaling pathways of individual cells might inform on dependent-resistance mechanisms. The origin of ctDNA is less understood, and it is unknown whether it derives from primary or secondary tumor or apoptosing cells within the blood.

If CTCs are to progress to use in the clinic, CTC-guided prospective trials are essential to confirm their utility. Furthermore, the molecular characterization of CTCs will inform on the predictive, prognostic, and treatment resistance biomarkers to guide future drug development.

## Conclusion

In patients with ACRC, enumeration of CTCs will identify those patients with a high CTC count who might benefit the most from treatment intensification, avoiding high-toxicity regimens in the low CTC group. These data require validation in future phase III trials.

### *Clinical Practice Points*

- Multidrug regimens are tolerable and effective in patients with ACRC; however, the increased toxicity means that careful patient selection is vital to avoid toxicity in those unlikely to derive a benefit.
- The CTC number measured using CellSearch is an FDA–approved, independent prognostic biomarker in ACRC but has rarely been used in clinical decision-making.
- The eSCOUT regimen was tolerable but with overlapping toxicity of diarrhea and fatigue.
- Our data suggest that patients with a high CTC count, in particular, will benefit most from treatment intensification.
- Patients with low CTC counts can be adequately treated with standard-of-care therapy and thus avoid the unnecessary toxicity associated with multidrug regimens.
- These data require validation in a prospective study with randomization between intensive treatment and standard-of-care therapy for both high and low CTC groups with potential practice-changing implications for selecting intensive therapy.

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## Disclosure

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## Supplemental Data

Supplemental materials and tables accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clcc.2014.12.006.

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## Supplemental Material

### Methods

*Dose Modifications.* If the hematologic parameters were not acceptable or nonhematologic toxicity occurred that was greater than grade 2 at day 1 or day 15, the entire treatment was delayed by 1 week until the parameters had improved. If the scheduled day 15 treatment was delayed, the patient received both oxaliplatin and cetuximab, with the remaining week of UFT given, once the parameters were acceptable. If a subject experienced grade 3 toxicity with cetuximab, the therapy was delayed for $\leq 2$ consecutive weeks without changing the dose level. With the second or third occurrence of grade 3 toxicity, cetuximab was delayed for up to an additional 2 weeks with concomitant dose reduction to 300 mg/m$^2$ and 200 mg/m$^2$, respectively.

*Sample Size Estimation for Phase II Trial.* The primary objective was the ORR according to the RECIST, version 1.0. The trial was designed to demonstrate at least a 50% and at best a 70% ORR with the combination of cetuximab and chemotherapy. Using Simon's 2-stage optimal design, the sample size was calculated as 43 subjects, with 15 accrued in the first stage and in the absence of futility (at least an 8-patient response needed), an additional 28 patients would be recruited. The $\alpha$ level of the design was 0.05 and the power was 0.8. To allow for patients who were not assessable for response, a total of 48 patients were recruited to the present study.

### Results

*Cetuximab Dose Modification.* After the first 8 patients were recruited, it was apparent that an excess of grade 3 diarrhea (38%) had occurred and was attributed to the addition of cetuximab to SCOUT. Of these 8 patients, 5 required chemotherapy dose reductions. The trial management group concurred that the addition of cetuximab to the SCOUT regimen caused the excess diarrhea. In the original SCOUT study, only 3 of 29 patients (10%) treated at the maximum tolerated dose developed grade 3 to 4 diarrhea.

Thus, the dose of cetuximab was reduced to 400 mg/m$^2$ every 2 weeks for all subsequent eSCOUT patients, which led to a reduced incidence of diarrhea (Supplemental Table 1). Four patients required dose reductions of cetuximab to $< 400$ mg/m$^2$.

*Chemotherapy Dose Modifications.* The median number of cycles administered was 6 (range, 1-26), and 29 patients completed $\geq 6$ cycles. Dose reductions (oxaliplatin/irinotecan/UFT) were required in 18 patients (38%) during the first 6 cycles, mainly because of diarrhea and lethargy. A total of 40 patients (89%) experienced dose delays in their treatment.

**Supplemental Table 1** Toxicity Before and After Cetuximab Reduction

| Pt. No. (Dosage) | Diarrhea | | | | Lethargy | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| 1-8 (cetuximab 500 mg/m$^2$) | 2 (25) | 3 (38) | 3 (38) | 0 | 3 (38) | 4 (50) | 1 (12) | 0 |
| 9-48 (cetuximab 400 mg/m$^2$) | 12 (30) | 6 (15) | 11 (28) | 0 | 9 (23) | 13 (33) | 8 (20) | 0 |

Data presented as n (%).
Abbreviations: G = grade; Pt. No. = patient number.