Original Study

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 (≥ 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