Adjuvant capecitabine plus bevacizumab versus capecitabine alone in patients with colorectal cancer (QUASAR 2): an open-label, randomised phase 3 trial

Rachel S Kerr, Sharon Love, Eva Segelov, Elaine Johnstone, Beverly Falcon, Peter Hewett, Andrew Weaver, David Church, Claire Scudder, Sarah Pearson, Patrick Julier, Francesco Pezzella, Ian Tomlinson, Enric Domingo, David J Kerr

Summary

Background **Antiangiogenic agents have established efficacy in the treatment of metastatic colorectal cancer. We investigated whether bevacizumab could improve disease-free survival in the adjuvant setting after resection of the primary tumour.**

Methods **For the open-label, randomised, controlled QUASAR 2 trial, which was done at 170 hospitals in seven countries, we recruited patients aged 18 years or older with WHO performance status scores of 0 or 1 who had undergone potentially curative surgery for histologically proven stage III or high-risk stage II colorectal cancer. Patients were randomly assigned (1:1) to receive eight 3-week cycles of oral capecitabine alone (1250 mg/m² twice daily for 14 days followed by a break for 7 days) or the same regimen of oral capecitabine plus 16 cycles of 7·5 mg/kg bevacizumab by intravenous infusion over 90 min on day 1 of each cycle. Randomisation was done by a computer-generated schedule with use of minimisation with a random element stratified by age, disease stage, tumour site, and country. The study was open label and no-one was masked to treatment assignment. The primary endpoint was 3-year disease-free survival, assessed in the intention-to-treat population. Toxic effects were assessed in patients who received at least one dose of randomised treatment. This trial is registered with the ISRCTN registry, number ISRCTN45133151.**

Findings **Between April 25, 2005, and Oct 12, 2010, 1952 eligible patients were enrolled, of whom 1941 had assessable data (968 in the capecitabine alone group and 973 in the capecitabine and bevacizumab group). Median follow-up was 4·92 years (IQR 4·00–5·16). Disease-free survival at 3 years did not differ between the groups (75·4%, 95% CI 72·5–78·0 in the capecitabine and bevacizumab group *vs* 78·4%, 75·7–80·9 in the capecitabine alone group; hazard ratio 1·06, 95% CI 0·89–1·25, p=0·54). The most common grade 3–4 adverse events were hand–foot syndrome (201 [21%] of 963 in the capecitabine alone group *vs* 257 [27%] of 959 in the capecitabine and bevacizumab group) and diarrhoea (102 [11%] *vs* 104 [11%]), and, with the addition of bevacizumab, expected increases were recorded in all-grade hypertension (320 [33%] *vs* 75 [8%]), proteinuria (197 [21%] *vs* 49 [5%]), and wound healing problems (30 [3%] *vs* 17 [2%]). 571 serious adverse events were reported (221 with capecitabine alone and 350 with capecitabine and bevacizumab). Most of these were gastrointestinal (n=245) or cardiovascular (n=169). 23 deaths within 6 months of randomisation were classified as being related to treatment, eight in the capecitabine alone group and 15 in the capecitabine and bevacizumab group.**

Interpretation **The addition of bevacizumab to capecitabine in the adjuvant setting for colorectal cancer yielded no benefit in the treatment of an unselected population and should not be used.**

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Department of Oncology (R S Kerr FRCP(UK), S Love BSc, E Johnstone PhD, D Church MRCP, C Scudder MSc, S Pearson BSc, P Julier MSc, E Domingo PhD), **Radcliffe Department of Medicine** Prof F Pezzella MD, D J Kerr FRCP), **and Nuffield Department of Medicine** (Prof I Tomlinson FRCPath, E Domingo), **University of Oxford, Oxford, UK; Department of Medicine, University of New South Wales, Sydney, NSW, Australia** (E Segelov FRACP); **Eli Lilly, Indianna, IN, USA** (B Falcon PhD); **Queen Elizabeth Hospital, Adelaide, SA, Australia** (P Hewett FRACS); **and Oxford University Hospitals Trust, Oxford, UK** (A Weaver MD)

Correspondence to: Dr Rachel S Kerr, Department of Oncology, Old Road Campus Research Building, University of Oxford, Oxford OX3 7DQ, UK **rachel.kerr@oncology.ox.ac.uk**

Introduction

30–50% of patients who undergo potentially curative surgery for colorectal cancer relapse and die from metastatic disease.[1] Adjuvant chemotherapy leads to improvement in overall survival[2] but the benefits depend on stage and are small (4–12% absolute improvement in 5-year overall survival).[3,4] Neoangiogenesis is regarded as a hallmark of cancer progression[5] and is a therapeutic target.

For many patients with stage II and stage III colorectal cancer, capecitabine alone is an appropriate standard of care for postoperative adjuvant treatment because it avoids the chronic and disabling neuropathy associated with oxaliplatin. Bevacizumab is the most well established antiangiogenic treatment used in colorectal cancer and, in combination with capecitabine in advanced colorectal cancer, increased progression-free survival without significantly increasing toxicity.[6] Therefore we investigated whether this extra benefit from the addition of bevacizumab to capecitabine would translate into the adjuvant setting of colorectal cancer.

We designed the QUASAR 2 randomised, controlled, phase 3 trial to assess the efficacy of capecitabine alone versus capecitabine plus bevacizumab in the adjuvant treatment of patients with stage III or high-risk stage II colorectal cancer after potentially curative surgery.

For the **QUASAR 2 protocol** see http://www.oncology.ox.ac.uk/trial/quasar-2

Research in context

Evidence before this study

We searched PubMed, Embase, MEDLINE, and the Cochrane Central Register of Controlled Trials, without language restrictions, for studies published between Jan 1, 1994, and Dec 31, 2005. Throughout the study and before we submitted the data for publication we repeated this search to allow us to put our findings into context, with our final search being on Jan 31, 2016. We used the terms "colorectal cancer", "adjuvant treatment", "chemotherapy", "anti-angiogenic therapy", and "biomarkers". We also searched clinical trial registers (ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform) for ongoing randomised trials.

Although in the past 20 years there has been much interest in improving survival of patients with colorectal cancer after potentially curative surgery, chemotherapy can reduce the relative risk of dying from the disease by only about a third compared with surgery alone. When antiangiogenic agents proved promising in the setting of advanced colorectal cancer, it seemed appropriate to test them in the adjuvant setting. For stage II and low-risk stage III colorectal cancer, single-agent capecitabine for 6 months is an established treatment regimen. In the QUASAR 2 trial, therefore, we tested capecitabine alone versus capecitabine plus bevacizumab, an anti-VEGF antibody, after primary resection. During recruitment, two trials were published that had tested the addition of bevacizumab to combination fluoropyrimidine and oxaliplatin regimens. The first showed no overall benefit in an unselected population but highlighted a possible benefit in the patients with microsatellite unstable tumours. The second suggested potential harm to patients receiving bevacizumab and included no detailed translational analyses.

Added value of this study

QUASAR 2 showed no benefit from the addition of bevacizumab to single-agent capecitabine in an unselected population of patients with colorectal cancer. Through comprehensive biomarker analysis, however, we defined a bevacizumab-responder population of patients with microsatellite unstable tumours or with microsatellite stable tumours and high concentrations of free CD31, which is a vascular marker. Conversely, patients with microsatellite-stable tumours and low expression of free CD31 had poorer disease-free and overall survival when bevacizumab was added than those receiving capecitabine alone. These exploratory analyses were not preplanned, but rather were driven by our knowledge of the biology of bevacizumab and, therefore, the results need to be interpreted with caution.

Implications of all the available evidence

We did a meta-analysis of the findings from this study and the two previously published adjuvant bevacizumab trials (AVANT and NSABP C-08), which clearly showed that this antiangiogenic agent should not be used in the adjuvant setting in unselected patients with colorectal cancer. The biomarker results from QUASAR 2 and NSABP-C08 raise the question as to whether bevacizumab in the adjuvant setting of colorectal cancer should be tested in a properly powered prospective randomised trial in a specific biomarker-selected population.

Two trials that reported findings while QUASAR 2 was still recruiting[7,8] suggested that the addition of bevacizumab to adjuvant combination chemotherapy was of no benefit. The NSABP C-08 study,[9] however, showed that patients with microsatellite-unstable tumours did seem to benefit from adjuvant bevacizumab. Here we report the final results of QUASAR 2, plus exploratory analyses of potential predictive biomarkers.

Methods

Study design and participants

QUASAR 2 was an international, multicentre, open-label, phase 3, randomised, controlled trial done in 170 hospitals in seven countries (Australia, Austria, Czech Republic, New Zealand, Serbia, Slovenia, and the UK; appendix pp 1–5). Eligible patients were identified in oncology clinics and multidisciplinary meetings. Inclusion criteria were patients aged 18 years or older with colorectal cancer (adenocarcinoma) that was histologically proven to be R0 M0 stage III or high-risk stage II (ie, with one or more of the following adverse prognostic features: stage T4, lymphatic or vascular invasion, peritoneal involvement, poor differentiation, and preoperative obstruction or perforation of the primary tumour); primary resection between 4 and 10 weeks before randomisation; WHO performance status score 0 or 1; and life expectancy (with comorbidities but excluding cancer risk) of at least 5 years. Exclusion criteria were history of cancer (other than treated in-situ carcinoma of the cervix, basal or squamous-cell carcinoma, or previous cancer with a disease-free survival >10 years); inflammatory bowel disease, active peptic ulcer requiring treatment in the previous 2 years, or both; lack of physical integrity of the upper gastrointestinal tract, malabsorption syndrome, or inability to take oral medication; moderate or severe renal impairment (creatinine clearance <30 mL/min); absolute neutrophil count lower than $1·5\times10^9$ cells per L; platelet count lower than 100×10^9 cells per L; total bilirubin concentration higher than 1·5 times the upper limit of normal; alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase concentration greater than 2·5 times the upper limit of normal; and proteinuria worse than 500 mg per 24 h; previous chemotherapy, immunotherapy, or infradiaphragmatic radiotherapy (including neoadjuvant therapy to the rectum) or the need for radiotherapy to these sites expected within the next 12 months; use of any investigational drug, agent, or

See Online for appendix

procedure within 4 weeks of randomisation; chronic use of full-dose anticoagulants, high-dose aspirin (>325 mg daily), antiplatelet drugs, or known bleeding diathesis (low-dose aspirin was allowed); concomitant treatment with sorivudine or its chemically related analogues; history of uncontrolled seizures, CNS disorders or psychiatric disorders that precluded giving informed consent or interfered with adherence to oral drug intake; clinically important cardiac disease; known coagulopathy; known allergy to Chinese hamster ovary cell proteins; and pregnancy, lactation, or no use of contraception in premenopausal women.

The study was done in accordance with the protocol, Good Clinical Practice, European Directives 2001/20/EC and 2005/28/EC, and the Declaration of Helsinki. Study approval was obtained from the West Midlands Research Ethics Committee (Edgbaston, Birmingham, UK; REC reference: 04/MRE/11/18). All participants provided written informed consent for treatment, and separate consent was obtained for use of tumour tissue and blood samples in the translational analyses.

Randomisation and masking

Randomisation was done at the central trials office, Oxford, UK, and allocations were obtained via telephone. Randomisation software ensured a balance of prognostic variables, with use of minimisation with a random element stratified according to age (<50 *vs* 50–59 *vs* 60–69 *vs* ≥70 years), stage (II [T3] *vs* II [T4] *vs* III [T2 and T3] *vs* III [T4]), disease site (colon or rectum), and country (Australia *vs* Austria *vs* Czech Republic *vs* New Zealand *vs* Serbia *vs* Slovenia *vs* the UK). The randomisation team had no role in the treatment of patients. The study was open label because it was felt that asking patients who were randomised to receive capecitabine alone for eight cycles to attend hospital for eight extra sham treatments or infusion of intravenous placebo would be unethical. Nevertheless, owing to minimisation with several variables and the inclusion of 170 study sites, it was not possible for treating clinicians or trials office staff to predict the allocation of the next randomised patient.

Procedures

Treatment was started within 14 days of randomisation in both groups. Capecitabine was administered orally to all patients in 3-week cycles of 1250 mg/m² twice daily for 14 days, followed by a break of 7 days, repeated for a total of eight cycles. Where a patient's surface area was greater than 2 m², the total dose was capped at a maximum of 2500 mg twice daily (total daily dose 5000 mg). Patients in the capecitabine and bevacizumab group received the same dosing schedule of capecitabine as the capecitabine alone group, plus 7·5 mg/kg bevacizumab by intravenous infusion over 90 min on day 1 of each cycle, repeated every 3 weeks for a total of 16 cycles (ie, for an extra eight cycles after the end of capecitabine treatment).

In patients aged 70 years or older or patients with moderate renal impairment (defined as a creatinine clearance of 30–50 mL/min) the starting dose was reduced to 1000 mg/m² twice daily. Patients who were aged 70 years and older and had moderate renal impairment received an even lower starting dose of 750 mg/m² twice daily. Laboratory monitoring with full blood counts, measurement of urea and electrolytes, and liver function testing was done before each cycle of treatment.

The first three patients in the study were initially randomised to a slightly different trial design, which set out to compare capecitabine alone versus capecitabine plus irinotecan versus capecitabine plus irinotecan plus bevacizumab. However, the trial design was changed by the chief investigator in May, 2005, after emerging data suggested no role for irinotecan in the adjuvant setting of colorectal cancer.[10,11] QUASAR 2 was changed to a two-arm trial design of capecitabine alone versus capecitabine plus bevacizumab.

Dose delays and reductions in response to toxic effects were prespecified. For haematological toxic effects of grade 2 or worse, chemotherapy was delayed until they resolved to grade 0 or 1. If haematological toxic effects (eg, neutropenia) reached grade 4, after recovery the dose of capecitabine was reduced by 25% for all subsequent cycles. If grade 4 neutropenia persisted for more than 1 week or was accompanied by fever, the dose was reduced by 50% for all remaining cycles, and clinicians could consider stopping capecitabine if neutropenia persisted for 3 weeks. If patients developed grade 2–4 diarrhoea, the next capecitabine cycle was delayed until recovery to grade 0 or 1 and resumed at the starting dose for grade 2 diarrhoea, or reduced in all subsequent cycles to 75% for grade 3 diarrhoea, and 50% for grade 4 diarrhoea, or capecitabine could be discontinued if that was felt to be in the patient's best interests. If grade 3 or 4 diarrhoea recurred despite dose reduction, capecitabine was discontinued. If mucositis or hand–foot syndrome developed, the next capecitabine treatment was delayed until recovery to grade 0 or 1 and was restarted at the starting dose for grade 2, or reduced to 75% of the starting dose for grade 3 and to 50% of the starting dose for grade 4.

In the capecitabine and bevacizumab group, if patients developed pulmonary embolus, bevacizumab was delayed until therapeutic anticoagulation had been given for a period of 2 weeks, and in cases of arterial embolus bevacizumab was discontinued. For proteinuria of greater than 2 g per day, bevacizumab was delayed until it fell to less than 1 g per day. Bevacizumab was discontinued if patients developed grade 3 or 4 haemorrhage, gastrointestinal perforation, or grade 4 hypertension. A 24 h medically staffed helpline was available for advice about management of toxic effects and dose reductions.

Data for disease-free survival and overall survival were collected annually on a follow-up case report form for

every patient entered into the study. Patients were followed up according to local practice, and investigators were not required to give the dates of negative investigations, only those when recurrence was confirmed. However, we asked investigators to consider following the available guidelines of clinical review and carcinoembryonic antigen testing every 3–6 months for 3 years then every 6–12 months for 2 years; colonoscopy at year 1 and then every 3–5 years; and CT scans of the chest, abdomen, and pelvis every 6–12 months for the first 3 years for the investigator to assess for recurrence as defined by the Response Evaluation Criteria in Solid Tumors (version 1). We asked that consistency in follow-up was maintained for all patients within a trial site.

All suspected colorectal cancer recurrences had to be confirmed or refuted by CT scanning or MRI, including those based on clinical symptoms, physical examination, or a raised concentration of carcinoembryonic antigen. Radiological data (including those for the primary endpoint) were assessed at the study sites, and were not assessed centrally. Patients with confirmed recurrence remained in the trial and were followed up to 3 years irrespective of trial group and other treatments received.

For our exploratory translational analyses, collection of blood samples and tumour samples from primary resections (formalin fixed and paraffin embedded) was encouraged but not mandated. Additionally, patients could consent to participate in the trial without giving consent for sample collection and participation in the translational study. Details of sample preparation have been reported previously.[12] Briefly, for DNA extraction, tumour sample blocks with more than 80% cancer cells were cut into scrolls of 40 µm and those with fewer cells were cut into 10 µm sections and needle-microdissected, with a haematoxylin and eosin section used as a guide. Tissues from scrolls and microdissections were digested with proteinase K, and DNA was extracted with the DNeasy Kit (Qiagen, Hilden, Germany). Standard direct DNA sequencing was done for mutations in *KRAS* (exon 2) and *BRAF* (exon 15). All reactions were visualised individually with Mutation Surveyor software (Softgenetics, State College, PA, USA) and mutations in codons 12 and 13 of *KRAS* and the $BRAF^{Val600Glu}$ mutation were annotated. All PCRs were done alongside negative controls, and a subset of mutated samples was resequenced in a second independent PCR for validation.

Microsatellite instability was investigated with all five so-called Bethesda markers (BAT25, BAT26, D2S123, D5346, and D17S250)[13] and BAT40, a mononucleotide repeat marker. Tumours were classified as having microsatellite instability if they had 40% or more unstable markers. Details of PCR primers and reaction conditions are available in the appendix (p 5).

For ploidy analysis, prepared cell monolayers were stained with the Feulgen-Schiff technique.[14] Nuclear DNA content was measured with the Ploidy Work Station Grabber software, version 1.4.12 (Room4, Crowborough, East Sussex, UK) and a Zeiss Axioplan microscope equipped with a 546 nm green filter and a black and white high-resolution digital camera (Axiocam MRM, Zeiss, Jena, Germany). Diploid histograms were classified as negative for chromosomal instability and aneuploid or tetraploid histograms as positive for chromosomal instability. Tumour vasculature was assessed with multiplexed immunohistochemistry, imaging, and quantification.[15] Tissue microarrays were stained with PECAM-1 (R&D Systems, Minneapolis, MN, USA) to detect CD31 to elucidate endothelial cells (AF806 at 1:50) and were also stained for α smooth-muscle actin (Sigma, St Louis, MO, USA) to detect myofibroplasts or pericytes (C6198 at 1:400). Stained sections were imaged and quantified with an iCys laser scanning cytometer (Thorlabs, Sterling, VA, USA). Percentages of cells expressing CD31 were classified as high (≥16% cells staining positive) or low (<16% positive).[16]

Outcomes

The primary outcome was 3-year disease-free survival, defined as the time from randomisation until confirmation of relapse or death from any cause. Secondary outcomes were overall survival (defined as the time from randomisation until death from any cause), disease-free survival (time from randomisation until confirmation of relapse of the cancer or of death from any cause) in the subgroup of patients with stage III colorectal cancer, and adverse events. The translational study and biomarker findings were exploratory endpoints. Biomarkers analysed included microsatellite status, chromosomal instability, *KRAS* or *BRAF* mutation status, and angiogenic marker assessment (expression of CD31, α smooth-muscle actin, or GLUT1, pericyte area, vessel area [small, medium, or large], and various combinations of these).

Adverse events, including serious adverse events, were recorded at each treatment cycle for all patients receiving treatment, from enrolment until 30 days after the last dose of any study treatment was administered. Adverse events were categorised according to the Common Terminology Criteria for Adverse Events version 3.0. Specific toxic effects, including hypertension, wound healing problems, and proteinuria, required more intensive follow-up until resolution, as advised in the protocol. An independent data safety monitoring committee reviewed the safety data annually and did a predefined safety review after the first 500 randomised patients had completed three cycles of treatment or had withdrawn from the trial.

Statistical analyses

We expected 70% of the patients enrolled to have stage III and 30% to have high-risk stage II colorectal cancer and, therefore, we projected that the combined 3-year disease-free survival for patients in the capecitabine alone group would be 66%. Assuming

exponential survival, we calculated that we would need to recruit 1120 patients per study group (ie, 2240 in total) and would need 766 events overall to achieve 90% power to find a 6% absolute improvement in 3-year disease-free survival (from 66% with capecitabine alone to 72% with capecitabine and bevacizumab) with two-sided significance at the 5% level. These numbers allowed for 10% loss to follow-up, a 2-year recruitment period, and a period of 3 years from last recruitment to final analysis. Continuing to recruit only patients with stage III cancer beyond the 2240 required, until there were 1676 patients with stage III disease (predicted to take around 2 months), would also provide 80% power to define a 6% absolute difference in disease-free survival in patients with stage III colorectal cancer (from 65% with capecitabine alone to 71% with capecitabine and bevacizumab).

Survival analyses (disease-free and overall survival) were done by intention to treat. Patients were only excluded from these analyses if at the time of randomisation the legal paperwork was not completed and current for the randomising country, they were found to have had demonstrable metastatic disease, or if they left the study and withdrew consent for the use of data. The safety analysis included all patients who received any amount of any trial drug.

Median follow-up was calculated with the reverse Kaplan-Meier method. Disease-free survival and overall survival for the two treatment groups were analysed with unweighted log-rank analyses. We used a Cox analysis that included variables thought to be prognostic to calculate hazard ratios (HRs) and 95% CIs. We compared safety data between treatment groups with the χ^2 test and summarised results with relative risks (RRs) and 95% CIs. To allow comparison with the effect of bevacizumab seen in the NSABP C-08 study[8] we did a prespecified analysis considering a landmark (crossover) effect of the addition of bevacizumab at 1·25 years after randomisation. In March, 2010, the data safety monitoring committee suggested an extended analysis of possible treatment-related deaths that included deaths at any point in the follow-up period deemed to be related to a serious adverse event that had started during or within 30 days after the treatment period (with either drug). This decision was incorporated in August, 2010, and these were reported as serious adverse events.

For our multivariate analyses of the exploratory translational endpoints we used Fisher's exact test for categorical variables (T and N stages, sex, and cancer site) and Student's *t* test for the continuous variable (age; appendix pp 6–8). All analyses were done with Stata version 12.1. This trial is registered with the ISRCTN registry, number ISRCTN45133151.

Role of the funding source

The funder had no role in the study design, data collection, data analysis, data interpretation, or in the writing of the report. The corresponding author had access to all the data and had final responsibility for the decision to submit for publication.

Results

Between April 25, 2005, and Oct 12, 2010, 7475 patients were screened and 1952 eligible patients were recruited and enrolled into the trial. After the trial had accrued the target number of patients for the primary endpoint, the data safety monitoring committee closed recruitment because of the results of the AVANT trial[7] and the perceived potential risk of harm. After these

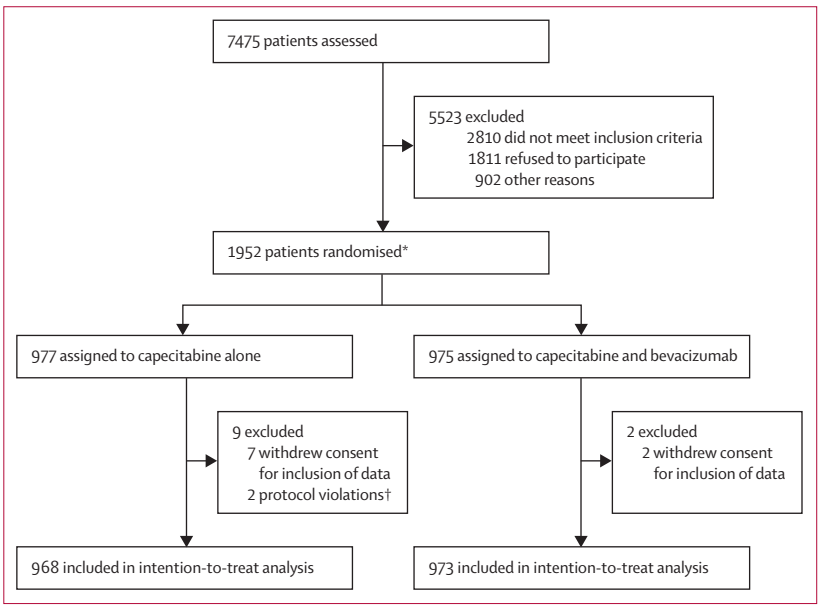

Figure 1: **Trial profile**

*Includes three patients who were randomly assigned to receive capecitabine plus irinotecan or capecitabine plus irinotecan and bevacizumab before a protocol change. †One patient mistakenly randomised with disease that had already relapsed and one patient before all legal documents were in place for the study country.

	Capecitabine (n=968)	Capecitabine and bevacizumab (n=973)
Disease stage		
II	373 (39%)	371 (38%)
III	595 (61%)	602 (62%)
Sex		
Women	414 (43%)	418 (43%)
Men	554 (57%)	555 (57%)
Primary cancer site		
Colon	854 (88%)	861 (88%)
Rectum	114 (12%)	112 (12%)
Age (years)		
Median (IQR)	65 (58–71)	6 (58–71)
<50	93 (10%)	96 (10%)
50–59	197 (20%)	192 (20%)
60–69	394 (41%)	388 (40%)
≥70	284 (29%)	297 (30%)

Table 1: **Baseline characteristics**

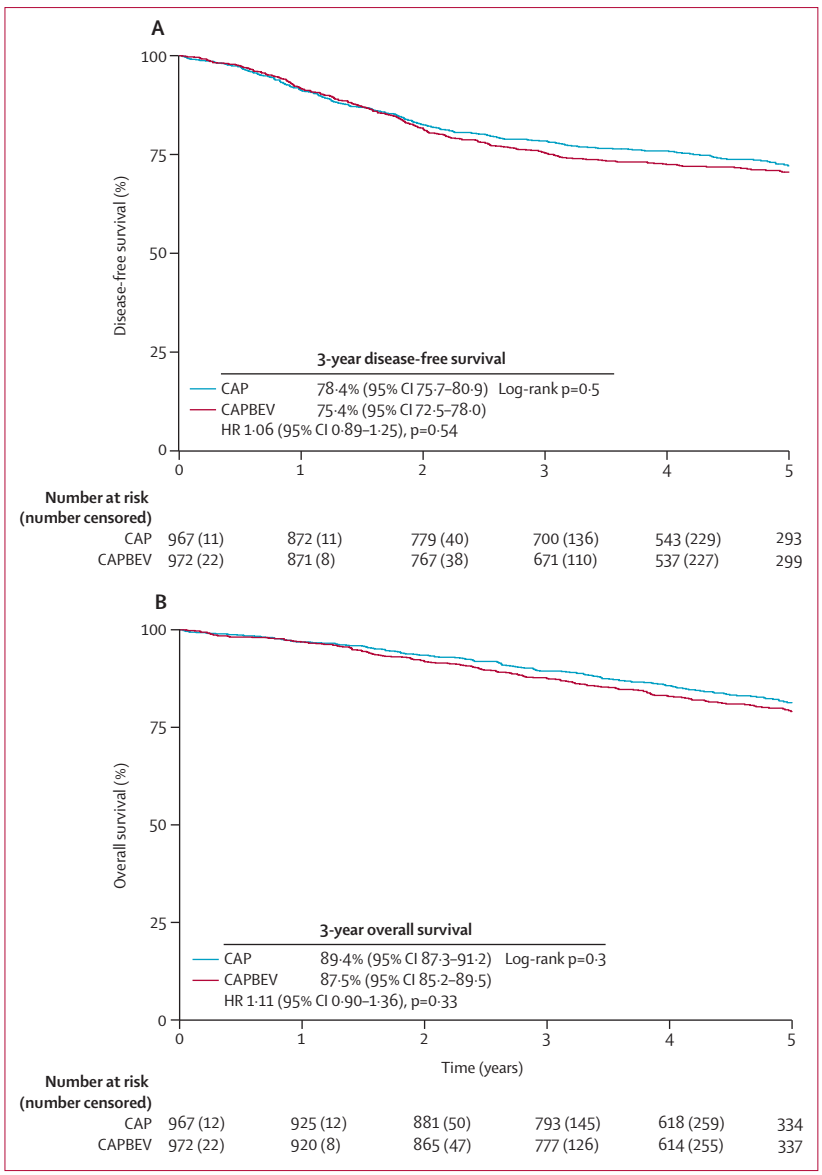

***Figure 2*: Survival outcomes in the intention-to-treat population**
(A) Disease-free survival. (B) Overall survival. Numbers in brackets correspond to censored patients in the intervals between timepoints. CAP=capecitabine alone. CAPBEV=capecitabine and bevacizumab. HR=hazard ratio.

1952 patients had been enrolled and randomised to capecitabine alone (n=977) or capecitabine and bevacizumab (n=975), 11 were excluded from all analyses, one because of a legal issue (randomised before all legal documents were in place for the randomising country), one because of an ethical issue (they had metastatic disease at the time of randomisation), and nine because they withdrew consent for their data to be used. Therefore, 1941 patients were included in the intention-to-treat analyses (968 in the capecitabine alone group and 973 in the capecitabine and bevacizumab group; figure 1). At the beginning of recruitment, of the three people already enrolled when the original three-arm trial design was changed to the two-arm design by excluding irinotecan, one who was initially assigned to receive capecitabine and irinotecan continued with capecitabine alone and was analysed within that group, and two who had initially been assigned to capecitabine plus irinotecan plus bevacizumab were analysed within the capecitabine plus bevacizumab group. One of these two patients received one cycle of irinotecan. The other patient did not wish to stop irinotecan and completed the full course of eight cycles along with the other treatments. Among recruiting countries (appendix pp 1–5), the UK enrolled the majority of analysed patients (1525 [80%] of 1941), followed by Australia (229 [12%]).

Assignment of study treatment was well balanced by cancer stage, sex, disease site, and age (table 1). Follow-up was completed on Oct 12, 2013. Median follow-up was 4·92 years (IQR 4·00–5·16) and 130 (6·7%) of 1941 patients were lost to follow-up by 3 years.

Analysis took place 3 years after the last patient was recruited as specified in the protocol; the criterion of requiring a total of at least 766 events was not met due to a lower than expected observed number of events and better than expected outcomes in the control population. The final analysis of disease-free survival was based on 525 events (256 in the capecitabine alone group and 269 in the capecitabine and bevacizumab group; 442 recurrences and 83 deaths without documented recurrence), meaning power to assess the primary endpoint was 77%. Disease-free survival was similar in the two groups (figure 2). In the 1197 patients with stage III colorectal cancer, 3-year disease-free survival was 74·5% (95% CI 70·8–77·9) with capecitabine alone and 71·3% (67·4–74·8%) with capecitabine and bevacizumab (HR 1·07, 95% CI 0·87–1·31, p=0·52). No significant variation in effect was seen related to known prognostic factors (stage, age, or sex; figure 3). Hazard ratios for disease recurrence in the whole population at 1, 2, and 3 years were 0·83 (95% CI 0·61–1·13), 0·87 (0·65–1·17), and 1·32 (0·90–1·98), respectively. No significant variation was found as a function of time, and there was no evidence of a change in effect from benefit to disadvantage at 1·25 years with the addition of bevacizumab, as was noted in the NSABP C-08 study.

At 3 years, 169 (17%) of 977 patients in the capecitabine alone group died, compared with 186 (19%) of 975 in the capecitabine and bevacizumab group (figure 2). 110 (65·1%) deaths in the capecitabine alone group and 136 (73·1%) in the capecitabine and bevacizumab group were specifically attributed to colorectal cancer. The numbers of deaths from any other causes, including other cancers (non-colorectal) and non-cancer or non-treatment-related deaths, were very similar in the two groups.

Doses were reduced in 1178 patients overall (507 [52%] of 968 receiving capecitabine alone and 671 [69%] of 973 receiving capecitabine and bevacizumab). Dose reduction decisions were based on adverse event

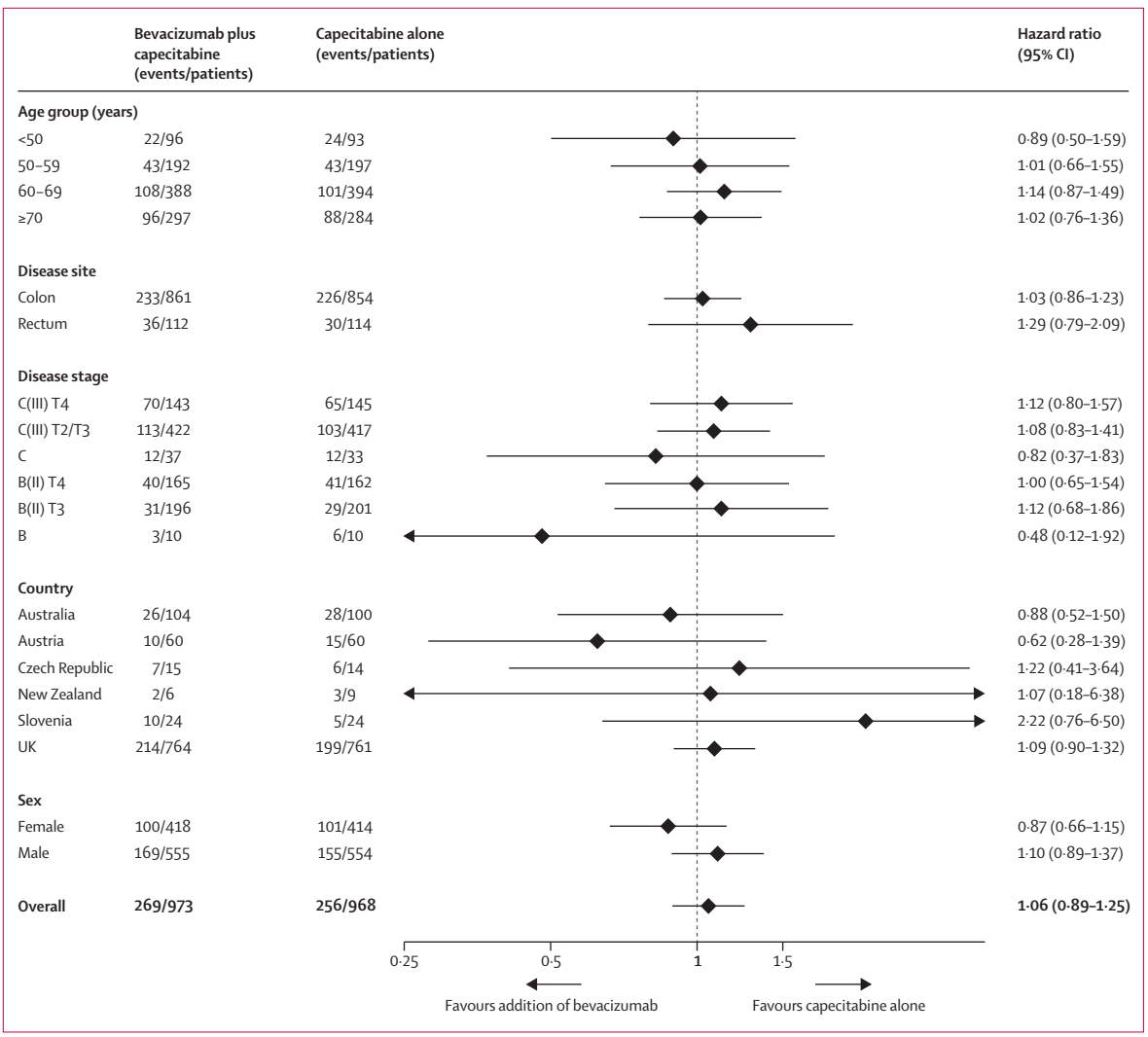

Figure 3: **Subgroup analysis of disease-free survival**
Only one patient was enrolled from Serbia and, therefore, this country is not included in this figure. HR=hazard ratio.

profiles in individual patients. The most frequent reason for reduction in capecitabine dose was diarrhoea, and those for bevacizumab were hypertension and proteinuria. Patients in the capecitabine and bevacizumab group received, on average, 85·5% of the total protocolised dose of capecitabine, which did not differ significantly from the average capecitabine dose in the capecitabine alone group (87·3%). This similarity suggests that the addition of bevacizumab did not substantially compromise the delivery of capecitabine. 746 (77%) of 968 patients in the capecitabine alone group and 743 (76%) of 973 in the capecitabine and bevacizumab group received eight cycles of capecitabine with no difference in the frequency of dose delays. The average dose intensity for bevacizumab was 75·4% of the total, and 699 (72%) of patients received more than eight cycles of bevacizumab, with 536 (55%) receiving all 16 cycles.

As expected, we noted significantly more hypertension and proteinuria with capecitabine and bevacizumab than with capecitabine alone (table 2). The frequency of wound healing problems was also raised in the capecitabine and bevacizumab group but did not reach significance (table 2). Epistaxis was reported significantly more frequently with the addition of bevacizumab as were stomatitis and hand–foot syndrome, although increases were seen with all grades of stomatitis, whereas the frequency of grade 3 or 4 hand–foot syndrome was increased but not grade 1 or 2 (table 2). Rates of neutropenia, thrombocytopenia, and diarrhoea did not differ between treatment groups (table 2).

571 serious adverse events were reported overall: 221 in the capecitabine alone group and 350 in the capecitabine and bevacizumab group. Most were gastrointestinal (n=245) or cardiovascular (n=169). During cycles one to eight, the frequency of serious adverse events was

	Capecitabine alone (n=963)		Capecitabine and bevacizumab (n=959)		Relative risk (95% CI)		p value	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade ≥1	Grade ≥3	Grade ≥1	Grade ≥3
Hypertension*	69 (7%)	6 (1%)	284 (30%)	36 (4%)	4·3 (3·4–5·4)	6·0 (2·6–14·2)	<0·0001	<0·0001
Proteinuria*	48 (5%)	1 (<1%)	188 (20%)	9 (1%)	4·0 (3·0–5·4)	9·0 (1·1–71·2)	<0·0001	0·011
Poor wound healing*	17 (2%)	0	28 (2%)	2 (<1%)	1·8 (1·0–3·2)†	Not available	0·056	0·16
Diarrhoea*	476 (49%)	102 (11%)	484 (51%)	104 (11%)	1·0 (0·95–1·1)	1·0 (0·8–1·3)	0·56	0·86
Hand–foot syndrome*	555 (58%)	201 (21%)	526 (55%)	257 (27%)	1·0 (1·0–1·1)	1·3 (1·1–1·5)	0·085	0·0024
Epistaxis†	13 (1%)	NA	132 (14%)	NA	10·2 (5·8–17·9)	NA	<0·0001	NA
Stomatitis*	201 (21%)	8 (1%)	281(29%)	14 (2%)	1·4 (1·2–1·7)	1·7 (0·7–4·2)	<0·0001	0·20
Neutropenia*	71 (7%)	26 (3%)	93 (10%)	16 (2%)	1·1 (0·9–1·5)	0·62 (0·3–1·1)	0·36	0·12
Thrombocytopenia*	48 (5%)	4 (<1%)	68 (7%)	3 (<1%)	1·4 (1·0–1·9)	0·75 (0·2–3·4)	0·073	0·71
Vomiting*	146 (15%)	24 (3%)	173 (18%)	19 (2%)	1·1 (0·9–1·4)	0·79 (0·4–1·4)	0·18	0·45

Adverse events defined according to Common Terminology Criteria for Adverse Events version 3.0. The worst grade reported for each patient is listed. N/A=not applicable. *Specifically inquired about during consultations with patients and specified on the case report form. †All grades.

Table 2: **Adverse events related to treatment in cycles one to eight**

	Capecitabine alone (n=963)	Capecitabine and bevacizumab (n=959)	Relative risk (95% CI)	p value
Arterial thromboembolism	6 (1%)	11 (1%)	1·8 (0·7–5·0%)	0·23
Venous thromboembolism	22 (2%)	41 (4%)	1·9 (1·1–3·1%)	0·014
Gastrointestinal perforation	1 (<1%)	4 (<1%)	4·0 (0·5–35·9%)	0·18

Table 3: **Serious adverse events related to treatment in cycles one to eight**

similar in the two groups, but arterial and venous thromboembolism occurred almost twice as frequently with the addition of bevacizumab. However, the difference was only significant for venous thromboembolism (table 3). Gastrointestinal perforation, which is regarded as a particular problem with bevacizumab therapy, was reported in one patient (<1%) receiving capecitabine alone and four (<1%) receiving capecitabine and bevacizumab (table 3). 118 patients withdrew from treatment in the capecitabine alone group because of adverse events (43 patients) and serious adverse events (75 patients), whereas 149 patients withdrew from treatment in the capecitabine and bevacizumab group because of adverse events (62 patients) or serious adverse events (87 patients; RR 1·27, 95% CI 1·01–1·59, p=0·043). Data for all grade 3 or 4 adverse events or grade 1 or 2 adverse events in more than 10% of the population are shown in table 4. Complete adverse event data are available in the appendix (pp 9–18).

Of the 23 treatment-related deaths that occurred within 6 months of randomisation, eight were in the capecitabine alone group (one cardiac arrhythmia, one cardiac failure, one myocardial infarction, three gastrointestinal perforation or haemorrhage, one neutropenic infection, and one respiratory failure due to fibrosis) and 15 in the capecitabine and bevacizumab group (one cardiac arrhythmia, one myocardial infarction, one aortic dissection, two arterial thromboembolism, three venous thromboembolism, three gastrointestinal perforation or haemorrhage, three neutropenic infection, and one cerebral haemorrhage; overall RR 1·88, 95% CI 0·8–4·4, p=0·11). The numbers of each individual cause of death were too small to infer any differences between study groups. When assessed according to the extended definition of possible treatment-related deaths within the whole follow-up period, including deaths related to a serious adverse event that had commenced during the treatment period with either drug or within 30 days of cessation, eight patients in the capecitabine alone group and 18 in the capecitabine and bevacizumab group died (0·9% *vs* 1·9%; RR 2·3, 95% CI 1·0–5·2, p=0·047). Among the three additional deaths in the capecitabine and bevacizumab group, one was due to cardiac failure, one to neutropenic infection, and one to non-neutropenic infection. We also did a meta-analysis of the AVANT, NSABP C-08, and QUASAR 2 data to assess the effect of the addition of bevacizumab and found an overall HR for death of 1·03 (95% CI 0·88–1·21, p=0·72). Further details are provided in the appendix (p 5).

Tumour DNA was available for analysis from 1187 patients (61% of the intention-to-treat population), and clinicopathological characteristics showed that the samples were representative of the tumours across the whole trial population (appendix p 6). Specific molecular markers were positive in 156 (13%) of 1166 patients for microsatellite instability, 740 (65%) of 1134 for chromosomal instability, 366 (33%) of 1114 for *KRAS* mutation, and 145 (13%) of 1121 for *BRAF* mutation. The frequencies of all mutational characteristics were balanced across the two trial groups (appendix p 6). We found the expected significant positive and inverse correlations between molecular markers and clinicopathological features[12] (appendix p 7).

We noted a possible correlation between microsatellite status (alone or in combination with high free CD31 expression) and the effect of bevacizumab on clinical outcome. We found no significant difference in outcome (disease-free or overall survival) with the addition of bevacizumab to capecitabine in patients with microsatellite-unstable tumours, but for patients with

	Capecitabine cycles 1–8 (n=963)			Capecitabine and bevacizumab cycles 1–8 (n=959)			Capecitabine and bevacizumab cycles 1–16 (n=959)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Blood									
Anaemia	128 (13%)	0	0	86 (9%)	1 (<1%)	1 (<1%)	91 (9%)	1 (<1%)	1 (<1%)
Coagulopathy	0	2 (<1%)	0	0	1 (<1%)	0	0	1 (<1%)	0
Febrile neutropenia	0	1 (<1%)	0	0	0	0	0	0	0
Leucopenia	17 (2%)	0	0	15 (2%)	1 (<1%)	0	16 (2%)	1 (<1%)	0
Pancytopenia	0	0	1 (<1%)	0	0	0	0	0	0
Cardiovascular									
Angina	40 (4%)	17 (2%)	3 (<1%)	33 (3%)	25 (3%)	1 (<1%)	41 (4%)	26 (3%)	1 (<1%)
Arterial thromboembolism	0	0	0	2 (<1%)	3 (<1%)	1 (<1%)	3 (<1%)	3 (<1%)	1 (<1%)
Atrial arrhythmia	2 (<1%)	1 (<1%)	0	4 (<1%)	3 (<1%)	0	5 (1%)	4 (<1%)	0
Cardiac arrest	0	0	3 (<1%)	0	0	0	0	0	0
Heart failure	0	0	0	1 (<1%)	1 (<1%)	0	2 (<1%)	1 (<1%)	0
Hypotension	4 (<1%)	0	1 (<1%)	1 (<1%)	2 (<1%)	1 (<1%)	3 (<1%)	2 (<1%)	1 (<1%)
Cranial haemorrhage	1 (<1%)	0	1 (<1%)	0	0	0	0	0	0
Claudication	0	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Cranial ischaemia	0	0	0	0	1 (<1%)	0	0	3 (<1%)	0
Myocardial infarction	0	0	1 (<1%)	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)
Palpitations	8 (1%)	0	0	2 (<1%)	0	0	5 (1%)	1 (<1%)	0
Pericarditis	0	0	0	0	0	0	0	1 (<1%)	0
Sinus tachycardia	0	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Stroke	0	0	1 (<1%)	0	0	0	0	0	0
Swollen ankles	28 (3%)	1 (<1%)	0	21 (2%)	0	0	31 (3%)	0	0
Venous thromboembolism	10 (1%)	12 (1%)	7 (1%)	13 (1%)	17 (2%)	8 (1%)	13 (1%)	18 (2%)	8 (1%)
Eye disorders									
Blurred vision	9 (1%)	1 (<1%)	0	10 (1%)	1 (<1%)	0	19 (2%)	1 (<1%)	0
Dry eyes	126 (13%)	0	0	87 (9%)	3 (<1%)	0	103 (11%)	3 (<1%)	0
Hemianopia	0	0	1 (<1%)	0	0	0	0	0	0
Impaired vision	1 (<1%)	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Gastrointestinal									
Abdominal distension	6 (1%)	2 (<1%)	1 (<1%)	8 (1%)	0	0	12 (1%)	0	0
Abdominal pain	104 (11%)	19 (2%)	2 (<1%)	125 (13%)	26 (3%)	1 (<1%)	151 (16%)	28 (3%)	1 (<1%)
Anorexia	64 (7%)	9 (1%)	0	82 (9%)	2 (<1%)	1 (<1%)	93 (10%)	2 (<1%)	2 (<1%)
Bleeding per rectum	8 (1%)	1 (<1%)	0	21 (2%)	2 (<1%)	0	35 (4%)	3 (<1%)	0
Bowel ischaemia	0	0	0	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)
Bowel obstruction	0	1 (<1%)	0	2 (<1%)	1 (<1%)	0	2 (<1%)	2 (<1%)	0
Bowel perforation	0	0	1 (<1%)	0	1 (<1%)	0	0	1 (<1%)	0
Cheilitis	9 (1%)	0	0	11 (1%)	1 (<1%)	0	13 (1%)	1 (<1%)	0
Colitis	2 (<1%)	0	0	0	2 (<1%)	0	0	2 (<1%)	0
Constipation	91 (9%)	4 (<1%)	0	106 (11%)	2 (<1%)	1 (<1%)	132 (14%)	3 (<1%)	1 (<1%)
Dehydration	6 (1%)	8 (1%)	0	8 (1%)	12 (1%)	2 (<1%)	9 (1%)	12 (1%)	2 (<1%)
Dental problems	7 (1%)	0	0	26 (3%)	1 (<1%)	0	32 (3%)	1 (<1%)	0
Dyspepsia	65 (17%)	3 (<1%)	0	68 (7%)	0	0	83 (9%)	0	0
Dysphagia	2 (<1%)	1 (<1%)	0	1 (<1%)	0	0	4 (<1%)	0	0
Fistula	0	0	0	0	0	1 (<1%)	0	0	1 (<1%)
Hernia	0	0	0	3 (<1%)	0	0	10 (1%)	1 (<1%)	0
Ileitis	0	2 (<1%)	0	1 (<1%)	2 (<1%)	0	1 (<1%)	2 (<1%)	0
Nausea	301 (31%)	8 (1%)	0	299 (31%)	12 (1%)	0	312 (33%)	12 (1%)	0
Pancreatitis	0	0	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)	0
Tenesmus	2 (<1%)	0	0	3 (<1%)	1 (<1%)	0	3 (<1%)	1 (<1%)	0

(Table 4 continues on next page)

	Capecitabine cycles 1–8 (n=963)			Capecitabine and bevacizumab cycles 1–8 (n=959)			Capecitabine and bevacizumab cycles 1–16 (n=959)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
(Continued from previous page)									
Upper gastrointestinal haemorrhage	2 (<1%)	1 (<1%)	0	2 (<1%)	1 (<1%)	1 (<1%)	3	1 (<1%)	1 (<1%)
Weight gain	7 (1%)	0	0	3 (<1%)	1 (<1%)	0	3 (<1%)	1 (<1%)	0
General									
Decreased performance status	0	1 (<1%)	0	0	1 (<1%)	0	0	1 (<1%)	0
Fatigue/lethargy	486 (50%)	38 (4%)	0	500 (52%)	32 (3%)	2 (<1%)	525 (55%)	34 (4%)	2 (<1%)
Hypothermia	0	1 (<1%)	0	0	0	0	0	0	0
Infection									
Gastrointestinal	3 (<1%)	0	0	4 (<1%)	0	0	9 (1%)	1 (<1%)	0
Chest	15 (2%)	2 (<1%)	0	27 (3%)	2 (<1%)	0	42 (4%)	3 (<1%)	0
Ear	8 (1%)	0	0	0	1 (<1%)	0	4 (<1%)	1 (<1%)	0
Herpes	14 (1%)	0	0	15 (2%)	1 (<1%)	0	16 (2%)	1 (<1%)	0
Neutropenic sepsis	0	4 (<1%)	2 (<1%)	0	1 (<1%)	0	0	1 (<1%)	0
Non-specific	42 (4%)	7 (1%)	1 (<1%)	63 (7%)	7 (1%)	0	73 (8%)	7 (1%)	1 (<1%)
Skin	17 (2%)	2 (<1%)	0	22 (2%)	1 (<1%)	0	31 (3%)	1 (<1%)	0
Upper respiratory tract	37 (4%)	1 (<1%)	0	48 (5%)	1 (<1%)	0	78 (8%)	1 (<1%)	0
Urinary	15 (2%)	1 (<1%)	0	29 (3%)	3 (<1%)	0	45 (5%)	4 (<1%)	0
Investigations									
High cholesterol	1 (<1%)	0	1 (<1%)	2 (<1%)	0	1 (<1%)	2 (<1%)	0	1 (<1%)
Hyperglycaemia	1 (<1%)	2 (<1%)	0	5 (1%)	2 (<1%)	2 (<1%)	12 (1%)	2 (<1%)	2 (<1%)
Hyperkalaemia	3 (<1%)	0	0	0	2 (<1%)	0	4 (<1%)	2 (<1%)	0
Hypernatraemia	0	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Hyperuricaemia	3 (<1%)	1 (<1%)	1 (<1%)	10 (1%)	0	0	12 (1%)	0	1 (<1%)
Hypoalbuminaemia	9 (1%)	2 (<1%)	0	3 (<1%)	1 (<1%)	0	5 (1%)	1 (<1%)	0
Hypocalcaemia	3 (<1%)	0	0	0	0	1 (<1%)	0	0	1 (<1%)
Hypoglycaemia	0	1 (<1%)	0	1 (<1%)	0	0	2 (<1%)	1 (<1%)	0
Hypokalaemia	11 (1%)	4 (<1%)	1 (<1%)	2 (<1%)	3 (<1%)	1 (<1%)	3 (<1%)	3 (<1%)	1 (<1%)
Hypomagnesaemia	1 (<1%)	0	0	3 (<1%)	0	1 (<1%)	3 (<1%)	0	1 (<1%)
Hyponatraemia	8 (1%)	0	0	2 (<1%)	1 (<1%)	0	4 (<1%)	3 (<1%)	0
Hypophosphataemia	1 (<1%)	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Isolated bilirubin rise	40 (4%)	6 (1%)	0	54 (6%)	4 (<1%)	1 (<1%)	58 (6%)	5 (1%)	1 (<1%)
Raised liver function tests	46 (5%)	5 (1%)	0	65 (7%)	6 (1%)	0	81 (8%)	8 (1%)	0
Triglycerides high	1 (<1%)	0	0	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)
Musculoskeletal									
Back pain	15 (2%)	1 (<1%)	0	29 (3%)	2 (<1%)	0	47 (5%)	3 (<1%)	0
Cramp	4 (<1%)	2 (<1%)	0	8 (1%)	0	1 (<1%)	12 (1%)	0	1 (<1%)
Joint pain	15 (2%)	3 (<1%)	0	98 (10%)	6 (1%)	0	156 (16%)	7 (1%)	0
Joint swelling	4 (<1%)	1 (<1%)	0	2 (<1%)	0	0	5 (1%)	0	0
Limb pain	23 (2%)	1 (<1%)	0	44 (5%)	3 (<1%)	0	65 (7%)	4 (<1%)	0
Myalgia	9 (1%)	0	0	23 (2%)	1 (<1%)	0	37 (4%)	2 (<1%)	0
Myopathy	0	0	0	0	1 (<1%)	0	1 (<1%)	1 (<1%)	0
Non-specific pain	28 (3%)	2 (<1%)	0	53 (6%)	8 (1%)	1 (<1%)	67 (7%)	11 (1%)	1 (<1%)
Nervous system									
Amnesia	0	0	0	1 (<1%)	0	0	1 (<1%)	1 (<1%)	0
Ataxia	2 (<1%)	0	0	2 (<1%)	1 (<1%)	0	4 (<1%)	1 (<1%)	0
Confusion	3 (<1%)	1 (<1%)	0 (<1%)	1 (<1%)	2 (<1%)	0	2 (<1%)	1 (<1%)	0
Decreased consciousness	2 (<1%)	2 (<1%)	1 (<1%)	2 (<1%)	1 (<1%)	0	2 (<1%)	2 (<1%)	0
Depression	26 (3%)	1 (<1%)	0	23 (2%)	0	0	34 (4%)	0	0
Dizziness	35 (4%)	3 (<1%)	0	43 (4%)	1 (<1%)	0	53 (6%)	3 (<1%)	0

(Table 4 continues on next page)

	Capecitabine cycles 1–8 (n=963)			Capecitabine and bevacizumab cycles 1–8 (n=959)			Capecitabine and bevacizumab cycles 1–16 (n=959)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
(Continued from previous page)									
Dysarthria	1 (<1%)	0	1 (<1%)	1 (<1%)	2 (<1%)	0	2 (<1%)	2 (<1%)	0
Dysphasia	0	0	0	1 (<1%)	2 (<1%)	0	1 (<1%)	2 (<1%)	0
Facial nerve palsy	1 (<1%)	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Headache	42 (4%)	1 (<1%)	0	87 (9%)	7 (1%)	1 (<1%)	129	8 (1%)	1 (<1%)
Hearing impaired	0	0	0	1 (<1%)	1 (<1%)	0	13 (1%)	2 (<1%)	0
Leg weakness	1 (<1%)	0	0	1 (<1%)	1 (<1%)	0	3 (<1%)	2 (<1%)	0
Memory impairment	3 (<1%)	0	0	3 (<1%)	1 (<1%)	0	5 (1%)	1 (<1%)	0
Neuralgia	1 (<1%)	0	0	1 (<1%)	1 (<1%)	0	3 (<1%)	1 (<1%)	0
Paraesthesia	81 (8%)	5 (1%)	0	107 (11%)	6 (1%)	0	133 (14%)	6 (1%)	0
Seizure	0	0	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)	0
Sensory neuropathy	0	0	0	1 (<1%)	1 (<1%)	0	1 (<1%)	1 (<1%)	0
Suicidal ideation	0	0	1 (<1%)	0	0	0	0	0	0
Syncope	4 (<1%)	4 (<1%)	0	3 (<1%)	2 (<1%)	0	4 (<1%)	2 (<1%)	0
Vertigo	4 (<1%)	1 (<1%)	0	9 (1%)	1 (<1%)	0	10 (1%)	1 (<1%)	0
Genitourinary									
Acute kidney injury	30 (3%)	2 (<1%)	2 (<1%)	13 (1%)	3 (<1%)	2 (<1%)	25 (3%)	3 (<1%)	2 (<1%)
Cystitis	7 (1%)	0	0	17 (2%)	2 (<1%)	0	21 (2%)	2 (<1%)	0
Haematuria	8 (1%)	0	0	12 (1%)	0	0	17 (2%)	1 (<1%)	0
Penile pain	0	1 (<1%)	0	0	0	0	0	0	0
Renal calculus	0	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Sexual dysfunction	1 (<1%)	0	0	0	1 (<1%)	0	0	1 (<1%)	0
Ureteric spasm	0	1 (<1%)	0	1 (<1%)	0	0	1 (<1%)	0	0
Respiratory									
Chest tightness	5 (1%)	0	0	10 (1%)	0	1 (<1%)	15 (2%)	0	1 (<1%)
Dyspnoea	49 (5%)	6 (1%)	0	59 (6%)	11 (1%)	2 (<1%)	72 (8%)	15 (2%)	2 (<1%)
Haemoptysis	1 (<1%)	1 (<1%)	0	2 (<1%)	1 (<1%)	0	4 (<1%)	1 (<1%)	0
Pleuritic pain	3 (<1%)	0	0	6 (1%)	1 (<1%)	0	9 (1%)	1 (<1%)	0
Skin and nails									
Acne dermatitis	80 (8%)	0	0	8 (1%)	2 (<1%)	0	12 (1%)	2 (<1%)	0
Dry/irritated skin	100 (10%)	0	0	85 (9%)	3 (<1%)	0	125 (13%)	3 (<1%)	0
Nail conditions	37 (4%)	1 (<1%)	0	29 (3%)	1 (<1%)	0	72 (8%)	1 (<1%)	0
Photosensitivity	2 (<1%)	0	0	2 (<1%)	1 (<1%)	0	3 (<1%)	1 (<1%)	0
Psoriasis	1 (<1%)	1 (<1%)	0	0	0	0	0	0	0
Rash	92 (10%)	9 (1%)	0	89 (9%)	3 (<1%)	0	112 (12%)	3 (<1%)	0
Skin ulceration	0	1 (<1%)	0	2 (<1%)	0	0	1 (<1%)	1 (<1%)	0

Grade 1–2 adverse events occurring in ≥10% of patients and all grade 3 and 4 adverse events are reported. Adverse events defined according to Common Terminology Criteria for Adverse Events version 3.0. Listed events were not specifically prompted on the case report form but were reported in free text by investigators. The worst grade reported for each patient is listed. 15 deaths in the capecitabine plus bevacizumab group and eight in the capecitabine alone group were reported within 6 months of randomisation.

***Table 4*: Adverse events**

microsatellite-stable tumours, 5-year disease-free and overall survival were shorter with the addition of bevacizumab (figure 4). The test for interaction for the differential effect of the addition of bevacizumab on the two molecular groups (microsatellite unstable *vs* microsatellite stable), however, was not significant ($p_{interaction}$=0·064 for disease-free survival and $p_{interaction}$=0·13 for overall survival). All these analyses were multivariate analyses, adjusted for T stage, N stage, age, sex, and tumour location (appendix p 8).

In a further exploratory analysis assessing the effect of bevacizumab on disease-free survival according to both tumour microsatellite status and free CD31 expression (ie, expression of CD31 not associated with expression of α smooth-muscle actin), 95 patients with microsatellite-unstable tumours (irrespective of whether they had high or low free CD31 expression) and 143 with microsatellite-stable tumours with high expression of free CD31 (together accounting for 238 [29%] of the analysed population) had longer 5-year disease-free survival with

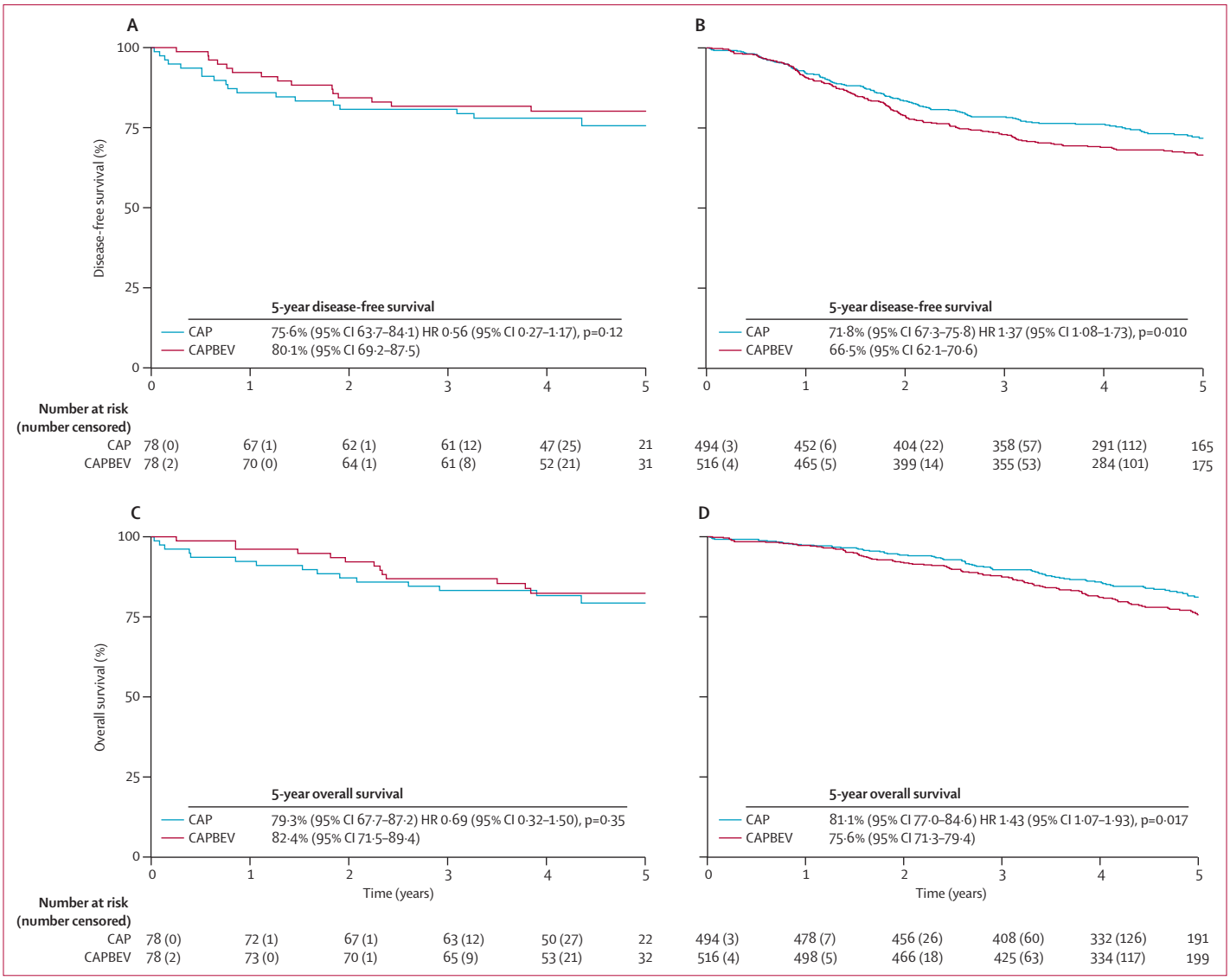

***Figure 4*: Survival outcome according to microsatellite instability status**

(A) Disease-free survival in patients with microsatellite-unstable tumours. (B) Disease-free survival in patients with microsatellite-stable tumours. (C) Overall survival in patients with microsatellite-unstable tumours. (D) Overall survival in patients with microsatellite-stable tumours. Numbers in brackets correspond to censored patients in the intervals between timepoints. CAP=capecitabine alone. CAPBEV=capecitabine and bevacizumab. HR=hazard ratio.

the addition of bevacizumab (79·2%, 95% CI 70·7–85·4 *vs* 71·1%, 61·2–78·9; HR 0·44, 95% CI 0·23–0·86, p=0·016; figure 5). By contrast, in the 580 patients who had microsatellite-stable tumours with low expression of free CD31, the converse was true, with a significantly shorter 5-year disease-free survival in the capecitabine and bevacizumab group than in the capecitabine alone group (figure 5). The interaction for the differential effect of the addition of bevacizumab was significant ($p_{interaction}$=0·0014). We also did multivariate analysis and adjusted for T stage, N stage, age, sex, and tumour location (appendix p 8). We found no interaction between chromosomal instability, *KRAS*, or *BRAF* status and the effect of bevacizumab on outcome (data not shown).

Discussion

The QUASAR 2 study showed no benefits from the addition of bevacizumab to capecitabine in the adjuvant setting in a biomarker-unselected population of patients with stage III or high-risk stage II colorectal cancer. The capecitabine and bevacizumab combination has shown promise in the advanced setting of colorectal cancer, which has allowed the exclusion of oxaliplatin and led to great benefits in terms of preventing long-term neurotoxic effects.[6] Reducing the risk of this complication is especially important in the adjuvant setting, where a high proportion of patients achieve cure after resection.[3,4] Capecitabine alone is widely used as an adjuvant treatment for stage II and low-risk stage III colorectal

cancers. To test the combination capecitabine and bevacizumab in early-stage disease was, therefore, imperative. The lack of overall efficacy is an extremely important negative finding, which supports existing evidence for a lack of benefit in the adjuvant setting with bevacizumab in combination with double-agent chemotherapy.[7,8] Thus, extrapolation of treatment with biological agents from the advanced disease setting into treatment of earlier disease is not straightforward.

The QUASAR 2 trial was ultimately underpowered. The initial power calculations suggested that 1120 patients would be needed per group to yield 766 events overall and give 90% power to find a clinically important 6% absolute improvement in 3-year disease-free survival (from 66% with capecitabine alone to 72% with capecitabine plus bevacizumab), allowing for a 10% loss to follow-up, a 2-year recruitment period, and a period of 3 years from last recruitment to analysis. This recruitment plan would also provide 80% power to define a difference in disease-free survival between patients with stage III disease in the two groups, from 65% with capecitabine to 71% with capecitabine plus bevacizumab. Because recruitment was subsequently slower than expected, with the agreement of the data safety monitoring committee in March, 2009, the recruitment period was extended to 5 years, and in March, 2010, the overall target sample size was dropped to 1892. The expectation was that, with the extended recruitment period (and with the provision that recruitment could continue beyond this number to give sufficient stage III patients for the secondary analysis), this sample size would maintain power of 90% for the primary endpoint and 80% for the secondary endpoint. All estimates remained the same as those for the original sample size. Although QUASAR 2 did recruit beyond this modified sample size (final population n=1941) and did fulfil the minimum of 3 years of follow-up on all patients, the prespecified number of events was not reached and, therefore, the resultant power was less than the 90% expected (final power 77%). There was a higher percentage of patients with stage II disease enrolled than predicted (38·3% instead of the expected 30%), but, because of the similar numbers of event in patients with high-risk stage II disease and those with low-risk stage III disease, this change from protocol in itself had very little effect on the power and the results. Estimates indicated that, because of the slowed accumulation of events, even with much longer follow-up, few extra events would accumulate, and, therefore, the power would not significantly increase. Nine (0·46%) patients were not included in the analysis because of withdrawal of consent to use data. We believe that these patients were omitted because of an initially poorly phrased and ambiguous withdrawal of consent to treatment form, which meant that although these patients had only wanted to stop treatment, their data also became inadmissible. This small percentage of patients (<1%), however, would have been adequately covered by our calculations for loss to follow-up had the numbers of events been higher.

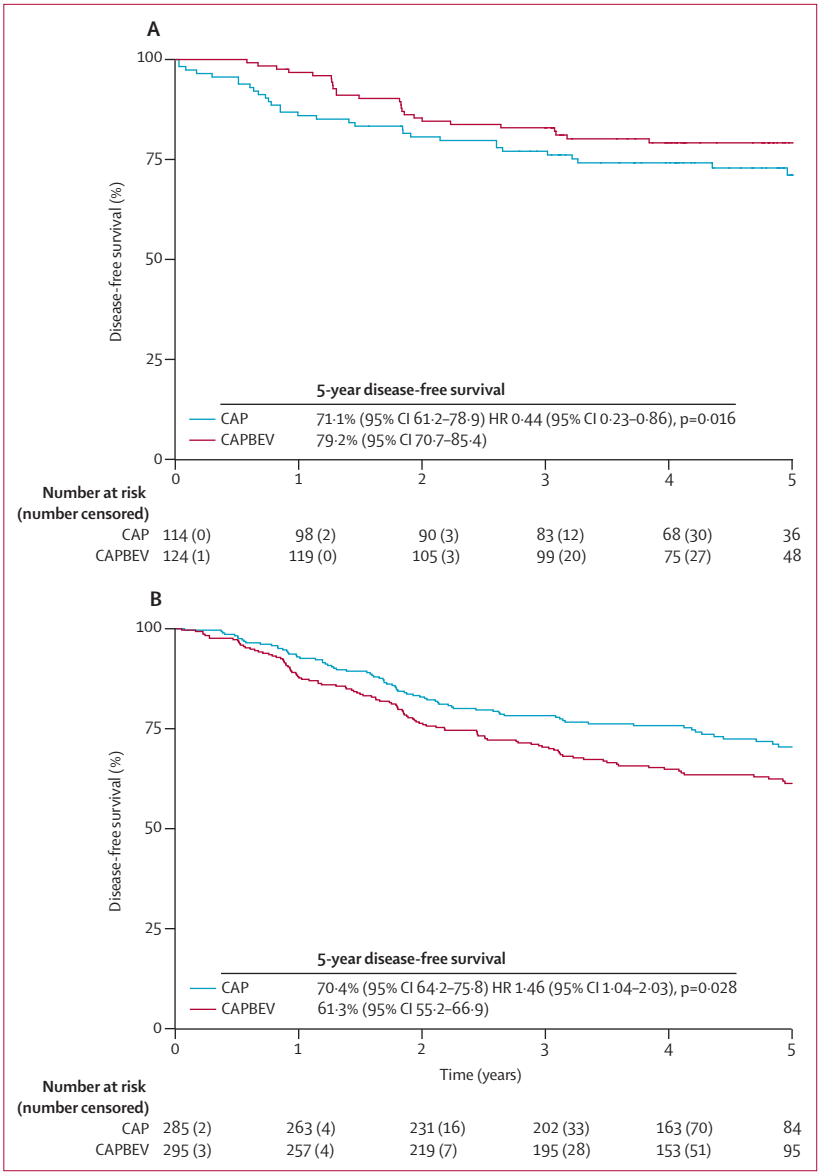

***Figure 5*: Survival outcomes according to microsatellite instability status and free CD31 expression**
(A) Disease-free survival in patients with microsatellite-unstable tumours and any free CD31 expression, combined with patients with microsatellite-stable tumours and high free CD31 expression. (B) Disease-free survival in patients with microsatellite-stable tumours and low free CD31 expression. Numbers in brackets correspond to censored patients in the intervals between timepoints. CAP=capecitabine. CAPBEV=capecitabine and bevacizumab. HR=hazard ratio.

Towards the end of the QUASAR 2 recruitment period, two large randomised adjuvant trials of bevacizumab reported negative results. The NSABP C-08 trial of 2673 patients,[8] which added bevacizumab to infusional FOLFOX6 (oxaliplatin, leucovorin, and 5-fluorouracil) in adjuvant treatment of colorectal cancer, showed no improvement in disease-free survival (HR 0·93, 95% CI 0·81–1·08, p=0·35) or overall survival (0·95, 0·79–1·13, p=0·56). A later report by the same authors showed that patients with microsatellite-unstable primary tumours derived a significant survival benefit from the addition of

bevacizumab (overall survival 0·52, 0·29–0·94, p=0·02) but those with microsatellite-stable tumours did not (1·03, 0·84–1·27, p=0·78).[9] The phase 3 AVANT study of 2867 patients[7] also showed no improvement in disease-free or overall survival when bevacizumab was added to FOLFOX4 or capecitabine and oxaliplatin in the adjuvant treatment of stage III colon cancer. After at least 5 years follow-up, a harmful effect was detected in the bevacizumab groups, with an excess of relapses and deaths due to progression. In conjunction with the results of the AVANT and NSABP C-08 trials, the findings from the QUASAR 2 trial confirm that there is no role for bevacizumab in the adjuvant management of colorectal cancer in unselected patients.

To assess extensively all deaths in the whole follow-up period of QUASAR 2, the data safety monitoring committee broadened the definition of possible treatment-related deaths to include those that occurred at any point in the follow-up period and were judged to be related to a serious adverse event that had started in the treatment period or within 30 days after cessation of treatment for either drug. There were two reasons for doing this unusual analysis. First, bevacizumab treatment could continue for up to 12 months (16 cycles) and, therefore, censoring deaths at 6 months plus 30 days could miss potential harm. Second, because the data safety monitoring committee took the view that if a catastrophic event, such as a myocardial infarction or cerebral vascular accident, happened during treatment and the patient died, even if more than 30 days later, the death might be related to treatment. This additional analysis suggested an excess of possibly treatment-related deaths of 18 in the capecitabine plus bevacizumab group versus eight in the capecitabine alone group (1·9% *vs* 0·9%; RR 2·3, 95% CI 1·0–5·2; p=0·047), indicating that even if the proportion of patients with colorectal cancer recurrence had been decreased with the combination treatment, the potential increase in deaths possibly related to treatment would need to be considered carefully in terms of determining the overall clinical usefulness of bevacizumab in this setting.

Translational analyses showed that patients with microsatellite-unstable tumours or microsatellite-stable tumours in combination with high free CD31 expression who received bevacizumab had a disease-free survival benefit. Conversely, patients with microsatellite-stable tumours in combination with low free CD31 expression had reduced disease-free survival when bevacizumab was added to capecitabine. This divergence of effect has not been thoroughly explored in previous publications, but perhaps explains why the NSABP C-08 and AVANT studies showed somewhat conflicting results in terms of benefit. Our findings, therefore, add important information.

One potential explanation for the differential effect on these subgroups is that high free CD31 expression is indicative of increased numbers of immature non-pericyte-covered vessels, which have been associated with enhanced sensitivity to bevacizumab.[16] This blood vessel population could have similarities to the tumour vessel phenotype described in preclinical tumour models by Smith and colleagues,[17] which are sensitive to treatment with antibodies against VEGF. Another explanation is a positive interaction between bevacizumab and immunological effectors, many of which express CD31, although we did not identify individual leucocytes in large quantities in the tumour specimens. Microsatellite-unstable tumours generally have greater microvessel densities than microsatellite-stable tumours, which might contribute to their differential sensitivity to bevacizumab.[18] Finally, bevacizumab treatment seems to reduce the numbers of regulatory T cells in patients with colon cancer, which could potentially change the immune microenvironment in hypermutated microsatellite-unstable tumours, where the frequency of neoantigens is raised, thereby favouring immune rejection.[19]

The trial protocol defined the broad type of translational analyses that would be done, including those that would reveal information about potential prognostic and predictive markers, taking into account the antiangiogenic nature of the experimental agent and the patients involved. However, the specifics of these analyses were not outlined a priori, and the predicted sizes of their effects were not included in the original power calculations. Therefore, these analyses can only be viewed as exploratory and the results need to be interpreted with caution and viewed only as hypothesis generating.

Precision or personalised medicine is a rapidly evolving field across all specialties from cancer to heart disease to diabetes. Personalisation may be delivered either through the exclusion of patients unlikely to benefit from treatments or by positive selection of those with biomarker profiles favourable to response. Our study revealed subpopulations in these two groups, which increases the biological plausibility of both approaches. Bevacizumab should clearly not be given as an adjuvant therapy to an unselected population of patients with colorectal cancer. Additionally, given the excess of deaths associated with longer-term treatment, if bevacizumab were to be tested again in the adjuvant setting, the appropriate duration of treatment to maximise effect but minimise toxic effects must be considered.

Our study has several limitations. The number of events was lower than expected, which reduced power. We were unable to use the data from nine patients in the intention-to-treat analysis, and loss to follow-up was around 6%, which might also have weakened the study's power. Some of the exploratory biomarker analyses were not prespecified and, therefore, must be interpreted with caution. Despite these limitations, the data are thought provoking and hypothesis generating in relation to selecting patients for adjuvant use of bevacizumab. Since no new drugs have been introduced for the adjuvant treatment of colorectal cancer for more than a decade, perhaps bevacizumab will be worth investigating

in prospective studies with clearly defined populations of patients who have been specifically selected on the basis of biomarker profiles.

Contributors

RSK, SL, and DJK designed the trial and developed the protocol. CS and SP were the trial coordinators. ES was the Australian chief investigator and PH was the Australian principal investigator. AW was the UK principal investigator. DJK was the co-chief investigator. SL led the statistical analyses. Recruitment of patients was overseen by RSK, ES, PH, AW, DC, and DJK. Data were collected by CS and SP, data analysis was done by RSK, SL, BF, DC, ED, and DJK and data interpretation by RSK, ES, EJ, IT, ED, and DJK. RSK, AW, and DJK did the safety review. EJ coordinated the tissue samples and FP did the tissue review. The translational scientists were BF, IT, and ED. PJ developed the study database. RSK, SL, ES, ED, and DJK wrote the report.

Declaration of interests

BF is an employee of Eli Lilly. The other authors declare no competing interests.

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