

Bevacizumab in the Adjuvant Treatment of Colorectal Cancer (QUASAR 2): A Randomised Phase III Trial

Rachel S Kerr FRCP(UK)¹, Sharon Love BSc¹, Eva Segelov FRACP², Elaine Johnstone PhD¹, Beverly Falcon PhD³, Peter Hewett FRACS⁴, Andrew Weaver MD⁵, David Church MRCP¹, Claire Scudder MSc¹, Sarah Pearson BSc¹, Patrick Julier MSc¹, *Francesco Pezzella MD¹, *Ian Tomlinson FRCPATH¹, Enric Domingo PhD¹ and *David J Kerr FRCP¹

Affiliations: 1. University of Oxford, UK; 2. University of New South Wales, Australia; 3. Eli Lilly & co, USA; 4. Queen Elizabeth Hospital, Adelaide, Australia; 5. Oxford University Hospitals Trust, UK

***Full Professor**

**Corresponding Author: To whom requests for reprints be addressed: Rachel Kerr,
Department of Oncology, Old Road Campus Research Building, University of Oxford,
OX3 7DQ**

Link to Protocol

<http://www.oncology.ox.ac.uk/trial/quasar-2>

Conflicts of Interest

There are no Conflicts of Interest to report with respect to this paper.

Ethics Statement

The study was undertaken in accordance with the protocol, good clinical practice, the EU Directive 2001/20/EC and 2005/28/EC, and the Declaration of Helsinki, and was approved by West Midlands Research Ethics Committee, United Kingdom (REC reference: 04/MRE/11/18). An independent data safety monitoring committee carried out annual safety reviews. This trial is registered, number ISRCTN 4513315.

RESEARCH IN CONTEXT

Evidence before this study

Before embarking on the study, we searched PubMed, Embase, MEDLINE, and the Cochrane Central Register of Controlled Trials databases without language restrictions for studies published between Jan 1, 1994, and December 31, 2005. Throughout the study and before we submitted the data for publication we repeated this exercise to allow us to put our findings into context with our final search on January 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 colorectal cancer survival after potentially curative surgery, chemotherapy can reduce the relative odds of dying of CRC by only about one third compared to surgery alone. When anti-angiogenic 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 six months is an established regime. The QUASAR2 trial therefore tested capecitabine alone versus capecitabine plus the anti-VEGF antibody, bevacizumab after primary resection. While the QUASAR2 was still recruiting, two other trials published which tested fluoropyrimidine/ oxaliplatin regimens with the addition of bevacizumab. The first showed no overall benefit in an unselected population but highlighted a possible benefit in the MSI-positive sub-population. The second suggested potential harm to patients randomised to bevacizumab and detailed translational analyses were not undertaken.

Added value of this study

QUASAR2 did not show benefit from the addition of bevacizumab to single agent capecitabine in an unselected CRC population. However through comprehensive biomarker analysis, we defined a bevacizumab-responder population (MSI+ or MSS with high expression of free CD31 – a vascular marker) who benefitted from the addition of bevacizumab; and demonstrated conversely that the population of patients with tumours that were MSS+ and expressed low levels of free CD31 suffered poorer disease free and overall survival when bevacizumab was added compared to receiving capecitabine alone. These were not pre-planned analyses but were driven by our knowledge of the biology of bevacizumab. The results therefore do need to be interpreted with caution.

Implications of all the available evidence

We performed a meta-analysis of all three adjuvant bevacizumab trials and we are able to state emphatically that this anti-angiogenic agent should not be used in the adjuvant setting in an

unselected CRC population. However the interesting finding of a potential responder population and, conversely, a potential adversely affected population could explain the perceived overall failure of this family of drugs in the adjuvant setting, and may open avenues for a possible future biomarker-selected prospective study, putting personalised cancer medicine into practice.

SUMMARY

Background

Anti-angiogenic agents have an established role in the treatment of metastatic colorectal cancer (CRC). It was hypothesised that their effectiveness may be augmented in earlier stage disease with a more malleable vasculature. The QUASAR 2 trial examined whether bevacizumab could improve disease-free survival (DFS) when added to single agent capecitabine in the adjuvant setting of colorectal cancer (registration ISRCTN 4513315).

Methods

Patients aged 18 or over with WHO Performance Status 0 or 1 from 170 centres across 6 countries, who had undergone potentially-curative surgery for histologically proven stage III/ high risk stage II CRC, were randomly assigned (1:1 ratio, non-blinded, minimisation with a random element stratified by age, stage, tumour subsite and country) to receive capecitabine alone (CAP), comprising a 3-week cycle of 1250mg/m² twice daily for 14 days followed by a 7 day break for a total of 8 cycles, or the same in combination with bevacizumab, 7.5mg/kg intravenous infusion over 90 minutes on day 1 of each 3-week cycle (CAPBEV). The primary end-point was 3-year disease-free survival (DFS) with overall survival (OS) a secondary endpoint. Both of these were intention-to-treat analyses. A comparative analysis of

toxicity included only patients who received at least one dose of their randomised treatment. Tumour tissue was used for DNA and vascular exploratory biomarker analysis.

Findings

The final results of the QUASAR2 trial are presented here. 1952 patients were entered onto the study between April 2005 and October 2010, with 1941 evaluable (968 CAP and 973 CAPBEV). Median follow-up was 4.92 years (95% CI 4.87-4.96). Overall, no significant difference was seen in 3-year DFS (primary endpoint) between CAPBEV (75.4%) and CAP (78.4%) (hazard ratio (HR) 1.06; 95% CI 0.89-1.25; $p=0.54$). Of 1922 patients analysed for toxicity, the most common grade 3 / 4 adverse events were hand foot syndrome ($n=201$ for CAP and $n=257$ for CAPBEV) and diarrhoea ($n=102$ for CAP and $n=104$ for CAPBEV). An expected increase in all-grade hypertension ($n=320$ versus 75), proteinuria ($n=197$ versus 49) and wound healing problems ($n=30$ versus 17) was observed with the addition of bevacizumab to capecitabine. 571 SAEs were reported (221 with CAP and 350 with CAPBEV).

Interpretation

This study shows that the addition of bevacizumab to capecitabine adjuvant therapy in CRC does not produce benefit in this patient population when considered as a whole and that it should not be used.

Source of Funding

Roche provided an unrestricted educational grant to the Principal Investigator for conduct of the trial and supplied capecitabine and bevacizumab.

INTRODUCTION

Approximately 30%-50% of all patients undergoing potentially curative surgery for colorectal cancer (CRC) ultimately relapse and die of metastatic disease. Adjuvant chemotherapy leads to improvement in overall survival (OS)¹ but the benefits are stage dependent and relatively small (4-12% absolute improvement in 5-year OS).²⁻⁴ Neo-angiogenesis is considered one of the hallmarks of cancer as a driver of tumour progression⁵ and a therapeutic target, thus we designed QUASAR 2, a randomised trial of adjuvant single agent capecitabine (CAP) plus or minus bevacizumab (BEV) in stage III/ high risk stage II CRC following curative resection.

Two trials reported whilst QUASAR 2 was still recruiting^{6,7} suggesting that addition of BEV to adjuvant combination chemotherapy was of no benefit. The NSABP C-08 study additionally reported that the subset of patients with microsatellite unstable (MSI) tumours did appear to benefit from the addition of BEV.⁸ We explored additional potential predictive biomarkers of BEV sensitivity, in particular the role of CD31⁹, also known as platelet endothelial cell adhesion molecule-1 and smooth muscle actin (SMA).

METHODS

Study Design and Participants

This was an international multicentre non-blinded phase III randomised controlled trial where CRC patients who had undergone potentially curative surgery were randomly assigned (1:1, non-blinded) to Arm A, capecitabine alone (CAP) or B, capecitabine plus bevacizumab (CAPBEV), with the primary aim of ascertaining whether CAPBEV would result in superior disease free survival (DFS) compared to CAP. The trial originally aimed to recruit 2240

patients from 170 hospitals spanning 6 countries (UK, Australia, Austria, Czech Republic, New Zealand, Slovenia).

The study was undertaken in accordance with the protocol, good clinical practice, the EU Directive 2001/20/EC and 2005/28/EC, and the Declaration of Helsinki, and was approved by West Midlands Research Ethics Committee, United Kingdom (REC reference: 04/MRE/11/18). An independent data safety monitoring committee carried out annual safety reviews. This trial is registered, number ISRCTN 4513315.

Patients were identified in oncology clinics and multidisciplinary meetings and recruited according to the following eligibility criteria. *Inclusion criteria:* patients aged 18 years or over with histologically proven (adenocarcinoma) R0 M0 stage III CRC or stage II CRC with one or more adverse prognostic features (stage T4, lymphatic or vascular invasion, peritoneal involvement, poor differentiation, preoperative obstruction or perforation of the primary tumour); primary resection more than 4 weeks but less than 10 weeks prior to randomisation; WHO Performance Status 0 or 1 and life expectancy (with co-morbidities but excluding cancer risk) greater than 5 years. *Exclusion criteria:* History of cancer (other than treated *in situ* carcinoma of the cervix, basal or squamous-cell carcinoma or if DFS interval was greater than 10 years); inflammatory bowel disease and / or active peptic ulcer requiring treatment in the last 2 years; lack of physical integrity of the upper gastrointestinal tract, malabsorption syndrome or inability to take oral medication; moderate or severe renal impairment (creatinine clearance <30ml/min); any of the following blood abnormalities - absolute neutrophil count <1.5 x 10⁹/L, platelet count < 100 x 10⁹/L, total bilirubin > 1.5 upper limit of normal (ULN), ALT/AST > 2.5 x ULN; alkaline phosphatase > 2.5 x ULN; proteinuria > 500 mg/24 hours; Previous chemotherapy, immunotherapy or infra-diaphragmatic radiotherapy (including neoadjuvant therapy to the rectum) or patients who are expected to require radiotherapy to these sites within the next 12 months; received any investigational

drug or agent/procedure within 4 weeks of randomisation; chronic use of full dose anticoagulants, high dose aspirin (>325mg/day), anti-platelet drugs or known bleeding diathesis (low dose aspirin was allowed); concomitant treatment with sorivudine or its chemically related analogues; history of uncontrolled seizures, central nervous system disorders or psychiatric precluding informed consent or interfering with compliance for oral drug intake; clinically significant cardiac disease (ie. active; or <12 months since e.g. cerebrovascular accident, myocardial infarction, unstable angina, New York Heart Association (NYHA) grade II or greater congestive heart failure, serious cardiac arrhythmia requiring medication; or uncontrolled hypertension) ; known coagulopathy; known allergy to Chinese hamster ovary cell proteins; women who were pregnant or lactating, or premenopausal women not using contraception. Written informed consent was obtained and a separate consent was obtained for use of tumour tissue and blood.

Randomisation

Patients were recruited by investigators at the sites and then randomisation was done by telephone with the central Trials Office (OCTO, Oxford, UK). Randomisation software ensured a balance of prognostic variables, using minimisation with a random element with the following strata: age (<50, 50-59, 60-69, 70+); stage (IIT3, IIT4, IIIT1-T3 and N1/ N2, IIIT4); site (colon or rectum); and country (UK, Australia, Austria, Czech Republic, New Zealand, Slovenia). The randomisation team had no ongoing role in the participants' treatment. Due to the use of the method of minimisation with several variables, and the fact that this was a multicentre study with 170 sites, no treating clinicians or trials office staff could know the allocation of the next randomised patient.

Neither treating clinician nor patients were blinded, as it was felt that it would be ethically unjustified to bring CAP patients to hospital for 8 extra treatment visits or to infuse intravenous placebo.

Procedures

After randomisation, patients commenced their randomised adjuvant treatment within fourteen days. In both arms, capecitabine was orally administered and comprised a three-week cycle of 1250mg/m² twice daily (capped at total daily dose 5000 mg or body surface area 2m²) for fourteen days followed by a seven day break, repeated for eight cycles. Patients over seventy years of age or with moderate renal impairment (calculated clearance 30-50ml/min) had a reduced starting dose of CAP1000mg/m² twice daily. Patients over seventy years who also had moderate renal impairment received a double-reduced starting dose of 750mg/m² twice daily. Bevacizumab (Arm B only) 7.5mg/kg was administered by intravenous infusion over 90 minutes on day 1 of each three-week cycle for a total of sixteen cycles. Standard laboratory monitoring tests (FBC, U and Es, LFTs) were performed prior each cycle of treatment. The protocol detailed dose delays and reductions required for toxicity. Briefly, for haematological toxicity grade 2 and above, chemotherapy was delayed until grade 0/1 before retreating. If haematological toxicity reached grade 4 then a 25% dose reduction in capecitabine was given for subsequent cycles. If grade 4 neutropenia persisted for more than 1 week, or was accompanied by fever, a 50% dose reduction was instigated.. For diarrhoea grade 2 to 4, the next cycle was held until recovered to grade 0-1, with subsequent cycles given at 75% (for grade 3) or 50% (for grade 4) diarrhoea. or the agent was 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. For mucositis or hand-foot syndrome, next capecitabine treatment was delayed until recovery to grade 0 / 1 and then was restarted at 75% dose for grade 3 and 50% dose for grade 4. In terms of bevacizumab related

toxicity, for pulmonary embolus, bevacizumab was delayed until therapeutic anticoagulation had been fully instituted (2 weeks delay) and for arterial embolus, bevacizumab was discontinued. For proteinuria of greater than 2g/day, bevacizumab was delayed until it was less than 1g/day. For grade 3 or 4 hemorrhage, for gastrointestinal perforation, or for grade 4 hypertension, bevacizumab was discontinued. A 24-hour medically staffed helpline was available for toxicity management and advice about dose reductions.

An annual follow-up case report form, confirming the presence or absence of recurrence and survival data for each year, was requested in real time from the sites for every patient entered on to the study. In order to make this investigator-led academic study as pragmatic as possible, the exact follow-up schema was according to local practice and investigators were not required to give the dates of negative investigations, only of positive affirmations of recurrence. However investigators were asked to consider the following recommended established guidelines: Clinical review and CEA determination every 3–6 months for 3 years then 6–12 monthly for 2 years; colonoscopy at year 1 and then every 3–5 years; CT scan chest/abdomen /pelvis every 6–12 months for the first 3 years looking for recurrence as defined by RECIST (v1) criteria; investigator assessed (no central review of scans): and consistency between all patients within the trial at their site was requested. All suspected recurrences had to be confirmed or refuted by imaging. Radiological data (including that for the primary endpoint) were assessed at each participant's site and were not centrally evaluated.

Where recurrence was suspected as a result of clinical symptoms, examination or a raised CEA, this was required to be confirmed by CT or MRI scan. As an intention to treat trial, patients remained in the trial and on follow-up irrespective of trial or other treatments received.

In terms of procedures for the translational analyses, collection of blood samples and formalin-fixed, paraffin-embedded (FFPE) blocks from primary resections was encouraged but not mandated and patients could consent to the trial without giving consent for sample collection and the translational study; and sites could contribute to the trial without committing to collecting any biological material. Details of sample preparation can be found in Domingo et al¹⁰. Briefly, for DNA extraction, blocks with >80% cancer cells were cut into scrolls. Other tumour blocks were cut into 10 µm sections and needle-microdissected, with an H&E section as a guide. Tissues from scrolls and microdissections were digested with proteinase K and DNA was extracted using the DNeasy Kit (Qiagen). Standard direct DNA sequencing was performed for KRAS (exon 2) and BRAF (exon 15). All reactions were visualised individually with Mutation Surveyor (Softgenetics) and mutations in codons 12 and 13 of KRAS and V600E in BRAF were annotated. All PCRs were performed alongside negative controls and, for validation, a subset of mutated samples was resequenced in a second independent PCR, giving concordant results.

MSI was determined using all five Bethesda markers (BAT25, BAT26, D2S123, D5346 and D17S250) and BAT40. Tumours were classified as MSI if they had 40% or more unstable markers, otherwise MSS. Details of PCR primers and reaction conditions are available from the authors.

For ploidy analysis, prepared monolayers were stained with Feulgen–Schiff as described.¹¹ Nuclear DNA content was measured using the Ploidy Work Station (PWS) Grabber version 1.4.12 (Room4 Ltd, 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 CIN negative and aneuploid/tetraploid as CIN positive. For assessment of tumour vasculature, multiplexed IHC, imaging and quantification were performed.¹² TMAs were stained for endothelial cells

using CD31 (PECAM-1; R&D Systems; AF806 at 1:50) and pericytes using smooth muscle actin (SMA) (Sigma; C6198 at 1:400). Stained sections were imaged and quantified using an iCys laser scanning cytometer (Thor labs). High (>16% cells staining +ve) and low (<16 % staining) CD31 levels were predefined.¹³

Outcomes

The primary outcome was disease-free survival (DFS), defined as the time from randomisation until confirmation of relapse or death from any cause. It was explicitly represented as 3-year DFS when comparing outcomes for the two arms of the study. Secondary outcomes were overall survival (OS; time from randomisation until death from any cause), DFS in stage III patients as a sub-population, side effect profiles and translational science and biomarker endpoints, largely exploratory.

Adverse Events (AE) including Serious Adverse Events (SAE) were collected for every treated patient for each cycle of treatment from enrolment until 30 days after their last dose of any study treatment. They were categorised using Common Terminology Criteria for Adverse Events (version 3.0). Specific toxicities including hypertension, wound healing problems and proteinuria required more intensive follow-up till resolution and specific advice was given in the protocol. An independent data safety monitoring committee carried out annual safety reviews as well as a defined safety analysis after the first 500 randomised patients had either completed 3 cycles of treatment or withdrawn from the trial.

The trial protocol defined the type of translational analyses that would be performed including those that would reveal information about potential prognostic and predictive markers, taking into account the nature of the experimental agent and the of the patient

population involved. However not all analyses were predefined prior to the trial and assessments of the predicted magnitude of effect of these markers were not used in the original power calculations for calculating sample size. These analyses are therefore exploratory and the results need to be interpreted with caution.

Statistical analyses

With an expected trial population comprising 70% stage III and 30% stage II patients, the combined 3-year DFS for the standard (CAP) arm patients was projected to be 66%. Assuming exponential survival, a calculated 1120 patients per arm (2240 patients and 766 events overall—ARTMENU in STATA 8) gave a 90% power to find a clinically important 6% absolute improvement in 3 year DFS (from 66% with CAP to 72% with CAPBEV) to be significant at the two-sided 5% level, 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 number of patients would also deliver 80% power to define a difference in DFS within stage III patients from 65% with CAP to 71% with CAPBEV. As recruitment subsequently took place over a longer period, with the agreement of the DSMC, the sample size was dropped to 1892 with the provision that recruitment could continue beyond this number to give sufficient stage III patients. All estimates remained as per the original sample size, but the recruitment period was changed to 5 years.

The primary DFS secondary OS analyses were conducted on the whole intention to treat (ITT) population. For the secondary endpoint of DFS in stage III patients, only the stage III patients were included, again on an ITT basis. The ITT population included every patient who could legally and ethically be retained within the analyses. Patients were only excluded from ITT analyses if either it was discovered that they had demonstrable metastatic disease already present at randomisation; or if the legal paperwork was not already complete and

current for the randomising country; or if the patients withdrew consent to use any data on withdrawing from the study. ; . The safety analysis was carried out on every patient receiving any amount of any trial drug.

Median follow-up was calculated using the reverse Kaplan-Meier method. Event outcomes were analysed using unweighted logrank analyses of DFS / OS for CAPBEV vs CAP alone on an intention to treat basis. A Cox analysis including variables thought to be prognostic was then performed. Safety data were summarised and compared using a chi-square test. All analyses were pre-defined and were undertaken using Stata version 12.1 (StataCorp, College Station, TX). For comparison with the NSABP C-08 study, we conducted a pre-specified analysis considering a landmark (crossover) effect of the addition of bevacizumab at 1.25 years post randomisation.

For the statistical analyses of the translational endpoints, which were exploratory in nature, Fisher's test was used for categorical variables and t-test was used for the continuous variable, age. Translational analyses were multivariate, adjusted by T, N, age, gender and location (colon/rectum) (see web appendix page 3 for more details).

Role of Funding Source

Roche provided an unrestricted educational grant to the Principal Investigator for conduct of the trial and supplied capecitabine and bevacizumab. The company had no role in the study design, data collection, data analysis, data interpretation, or in the writing of the report. The listed authors (none of whom are affiliated to the funding source) held access to all the data and drafted and had final responsibility to submit the manuscript. All authors reviewed the manuscript before submission.

RESULTS

QUASAR 2 commenced recruitment on the 25th April 2005 and ceased on October 12th 2010 with follow-up continuing until 12th October 2013. At closure to recruitment, the trial had accrued the target number of patients for the primary aim but was closed by the DSMC whilst recruitment was ongoing and aiming to accrue sufficient patients to satisfy the secondary aims. This closure was a consequence of the results of the AVANT trial⁶ and the perceived potential risk of harm. Overall, 1952 patients were entered in the CAP versus CAPBEV randomisation; however 11 were excluded from all analyses due to either a legal issue (one patient was randomised before all legal documents were in place for that country), an ethical issue (one patient who was randomised despite having metastatic disease) or because there was withdrawal of consent to use their data (9 patients), leaving 1941 patients analysed here (Consort flow diagram: Figure 1). Of this ITT analysed population of 1941, nine hundred and sixty eight were randomised to CAP and nine hundred and seventy three to CAPBEV. The analysis includes two patients who were initially randomised to capecitabine plus irinotecan plus bevacizumab and one who was randomised capecitabine plus irinotecan using the Protocol v5.0 three arm design. Their randomisation occurred before irinotecan was removed from the trial. They were analysed in the CAPBEV and CAP arms respectively. The UK enrolled almost 80% (1525/1941) of the patients.

The population analysed for treatment outcome comprised 1197 (1197/1941=61.7%) patients with a stage III primary CRC and 744 (744/1941=38.3%) with a high risk stage II primary CRC. Most of the primary cancers were colonic (1715/1941=88.4%). The median age of the study population was 65 (range 21-88), with a slight preponderance of males (1109/1941=57.1%). Study treatment assignment to the two arms was well balanced on the basis of gender, disease

site, stage and age (Table 1). Median follow up was 4.92 years (IQR 4.00-5.16) with 130/2491 (6.7%) of patients lost to follow-up by 3 years.

Analysis took place 3 years after the last patient recruitment 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 event rate and better than expected outcomes in the control (CAP) population. The final analysis includes 525 DFS events comprising 442 recurrences and 83 deaths without documented recurrence, and gives a power of 77%. There were 269 DFS events with CAPBEV compared to 256 with CAP alone. The 3-year DFS rates were 75.4% (95% CI 72.5-78.0) for CAPBEV versus 78.4% (95% CI 75.7 to 80.9) for CAP (Figure 2a) giving a DFS HR of 1.06 (95% CI 0.89-1.25; $p=0.54$). For the pre-specified analysis of stage III patients ($N=1197$), 3-year DFS was CAPBEV 71.3% (95% CI 67.4 to 74.8%) and CAP 74.5% (70.8 to 77.9) giving a HR of 1.07 (95% CI 0.87 to 1.31); $p=0.52$. Across both analyses, there was no evidence of statistically significant variation according to known prognostic factors (stage, age, gender) (Figure 3). Hazard ratios for recurrence in the whole analysed population (CAPBEV compared to CAP alone) at 1, 2 and 3 years post randomisation were 0.83, 0.87 and 1.32 respectively. However statistical analysis did not prove any significant variation as a function of time. In addition there was no evidence of crossover in terms of initial benefit followed by deleterious effect (landmark analysis).

With respect to overall survival, there were 186 deaths in patients randomised to CAPBEV versus 169 deaths with CAP (Figure 2b). The absolute 3-year OS rates (CAPBEV versus CAP) were 87.5% (95% CI 85.2 to 89.5) versus 89.4% (95% CI 87.3 to 91.2), giving an OS HR of 1.11 (95% CI 0.90 to 1.36; $p=0.33$). Deaths specifically attributed to CRC were 136 in the CAPBEV arm compared to 110 in the CAP arm, with the number of deaths from any other categorised cause being very similar between the two arms.

Overall 1178 patients underwent some dose reduction; 507 of 968 patients on CAP (52.4%) and 671 of 973 patients on CAPBEV (69.0%). However, the average dose intensity for capecitabine as a percentage of total protocolised dose was 85.5% of expected in the CAPBEV arm, compared to 87.3% in CAP arm, suggesting that the addition of bevacizumab did not significantly compromise ability to deliver capecitabine. 746/968 (77.1%) of patients in the CAP arm and 743/973 (76.4%) of patients in the CAPBEV arm received all 8 cycles of capecitabine and there was no difference in the frequency of dose delays. The average dose intensity as a percentage of total protocolised dose for bevacizumab was 75.4%: 699/973 (71.8%) of CAPBEV patients received more than 8 cycles of bevacizumab and 536/973 (55.1%) received all 16 cycles.

With respect to toxicity, analysis of the 1922 patients who received any of the designated treatment was performed. Table 2 lists adverse events by diagnosis or system and grade, for CAP versus CAPBEV over the first 8 cycles of either treatment arm to allow direct comparison, and also details numbers for CAPBEV for the whole duration of treatment (16 cycles). The upper half of table 2 (shaded) describes AEs that were specifically prompted within the case report form. Comparing the first eight cycles of the two randomised arms, the most common grade 3 / 4 adverse events were hand foot syndrome (n=201 for CAP and n=257 for CAPBEV; RR 1.3 (95% CI 1.1-1.5); p=0.0024) and diarrhoea (n=102 for CAP and n=104 for CAPBEV; RR 1.0 (95% CI 0.8-1.3); p=0.86).

Table 3 gives further detail of specific toxicities (AEs and SAEs) relevant to treatment with capecitabine and bevacizumab. Analysis confirmed the expected increase in all-grade hypertension (n=320 versus 75; RR 6.0 (95% CI 2.6-4.2); p<0.0001), proteinuria (n=197 versus 49; RR 4.0 (95% CI 3.0-5.4); p<0.0001) and wound healing problems (n=30 versus 17; RR 1.8 (95% CI 2.6-14.2); p=0.056) with the addition of bevacizumab compared to capecitabine alone. In terms of toxicities traditionally ascribed to capecitabine, the only one

that was exacerbated significantly by the addition of bevacizumab was hand-foot syndrome. There were 571 SAEs reported overall (221 with CAP and 350 with CAPBEV) of which the majority were either gastrointestinal (n=245) or cardiovascular (n=169). Comparing SAE reports between the two arms during the first six months of drug administration, both arterial and venous thrombo-embolism rates were approximately doubled with the addition of bevacizumab; arterial frequency 11 versus 6 (CAPBEV vs CAP; RR 1.8 (95% CI 0.7-5.0); p=0.23) and venous frequency 41 versus 22 (CAPBEV vs CAP; RR 1.9 (95% CI 1.1-3.1); p=0.014). Gastrointestinal perforations were suffered by 4 patients receiving CAPBEV and 1 patient receiving CAP, RR 4.0 (95% CI 0.5-35.9); p=0.19. Numbers of patients prematurely withdrawing from treatment due to SAE/AE were 149 (62+87) in the CAPBEV arm compared to 118 (43+75) in the CAP arm; RR= 1.27 (95% CI 1.01-1.59); p=0.043.

Of the 23 treatment related deaths that occurred within *6 months* of randomisation, 15 were in the CAPBEV arm compared to 8 in CAP alone arm, giving a relative risk with the addition of BEV of 1.88 (95% CI 0.8-4.4); p=0.11). In order to explore this further, especially as the BEV treatment continued for up to 16 cycles, the independent DSMC extensively assessed all deaths within the whole follow-up period and applied a broader definition of 'possible-treatment-related deaths', which included not only deaths during trial treatment and for up to 30 days after finishing treatment (either drug), which is the standard definition of a potentially drug related death, but also deaths at any point in the follow up period which were judged to be related to an SAE that had commenced during the treatment period (either drug) or within 30 days after cessation. The reasons for doing this unusual analysis were two-fold: firstly the bevacizumab treatment according to protocol continued for up to 12 months and therefore censoring deaths at 6 months plus 30 days could miss potential harm caused by long term treatment; secondly because the DSMC took the view that if a catastrophic event such as

a myocardial infarction or cerebral vascular accident happened on treatment, and if this eventually led to death even if it were more than 30 days later, this should be considered a possible-treatment-related death'. In the latter calculation, with 18 deaths in the CAPBEV arm and 8 in the CAP arm, there was an excess of 'possible-treatment-related deaths' in patients receiving bevacizumab (1.9% vs. 0.9%: RR 2.3: CI 1.0-5.2; p=0.047).

For translational analyses and biomarker assessment, tumour DNA was extracted from FFPE tumour samples of 1187 patients (61% of total ITT analysed population); clinicopathological characteristics showed these were representative of the trial population as a whole (Web Appendix page 1). Rates of positivity for specific molecular markers were MSI 156/1166 (13%), chromosomal instability (CIN+ 740/1134 (65%)), *KRAS* mutation (KRASMut+ 366/1114 (33%)) and BRAF mutation (BRAFMut+ 145/1121 (13%)); and for each of these mutational characteristics, there was balance between the CAP and CAPBEV groups assessed in these analyses (Web Appendix page 1). Relationships between each of the molecular markers and correlations with clinicopathological features are presented in Web Appendix page 2. For comparisons in the exploratory biomarker analyses, Fisher's test was used for all categorical variables and t-test was used for age.

We demonstrated that microsatellite status (alone or in combination with high free CD31 levels) may influence bevacizumab effect. Thus in the MSI population there was no statistically significant difference in outcome with the addition of bevacizumab (CAPBEV versus CAP 5-year DFS 80.1% (95% CI 69.2%-87.5%) versus 75.6% (95% CI 63.7%-84.1%); HR 0.56 (95% CI 0.27-1.17); p=0.12. However in the MSS population 5-year DFS was worse with the addition of bevacizumab; 66.5% (95% CI 62.1%-70.6%) versus 71.8% (95% CI 67.3%-75.8%); HR 1.36 (95% CI 1.08-1.73); p=0.01; suggesting detriment (Figures 4a and b); p for interaction for the differential effect of the addition of bevacizumab on the two groups was 0.064. OS followed a similar pattern (Figures 4c and d). In the MSI group

the addition of bevacizumab gave a 5-year OS rate of 82.4% (95% CI 71.5%-89.4%) compared to 79.3% (95% CI 67.7%-87.2%) with capecitabine alone; HR 0.69 (95% CI 0.32-1.5); $p=0.35$. In contrast the 5-year OS rates in the MSS population with the addition of bevacizumab was impaired at 75.6% (95% CI 71.3%-79.4%) compared to 81.1% (95% CI 77.0%-84.6%) with capecitabine alone; HR 1.43; 95% CI 1.07-1.93; $p=0.017$; p for interaction was not found to be significant ($p=0.13$). All of these analyses were multivariate, adjusting for T stage, N stage, age, gender and location (colon/rectum), See Web Appendix page 3 for details.

A further exploratory analysis was then performed, with the same translational patient population, assessing the impact of bevacizumab on disease free survival, according to both the tumour MSI/MSS status, and also to the levels of free CD31 (CD31 not associated with SMA) in the tumour. Those patients who had a tumour that was either MSI (any free CD31 level; $n=95$) or MSS with high levels of free CD31 ($n=143$), together comprising 29.1% of analysed population, appeared to benefit from the addition of bevacizumab; 5-year DFS rates 79.2% (95% CI 70.2%-85.4%) versus 71.1% (95% CI 61.2% - 78.9%) with capecitabine alone; HR 0.44 (CI 0.23-0.86); $p=0.017$). In the group who had MSS tumours with low levels of free CD31 ($n=580$) the converse was true, with a significantly worse 5-year DFS in the CAPBEV arm, 61.3% (95% CI 55.2%-66.9%) versus 70.4% (64.2%-75.5%) with capecitabine alone; HR 1.45 (CI 1.04-2.02); $p=0.03$; figures 5a and b. The p for interaction was significant ($p=0.002$). These analyses were also multivariate (see Web Appendix page 3 for more detail).

There was no suggestion of an interaction between CIN+ status or KRASMut+ status and the effect of bevacizumab on outcome.

DISCUSSION

The QUASAR 2 study was designed as a pragmatic investigator-led academic study investigating the impact of adding bevacizumab to single agent capecitabine in the adjuvant treatment of stage III / high risk stage II CRC and we found that its addition is not beneficial for this unselected whole population of patients. This is the first and only phase III trial adding bevacizumab to single agent capecitabine in the adjuvant setting. The capecitabine / bevacizumab combination has been used with much promise in advanced disease, and the exclusion of oxaliplatin has reaped great benefit in terms of preventing long term neurotoxicity, which is especially important in the adjuvant setting. Single agent capecitabine is still a commonly used treatment in the adjuvant setting of stage II and lower risk stage III CRC. Therefore it was imperative to test the capecitabine / bevacizumab combination in early stage disease and the lack of overall efficacy is an extremely important negative finding, which adds to the current evidence surrounding the lack of benefit of bevacizumab in the adjuvant setting in combination with doublet agent chemotherapy. The results demonstrate again that extrapolation of treatment with biological agents from the advanced disease setting in to earlier disease is not straightforward.

Although QUASAR2 did recruit to the sample size we set to fulfil the primary objective, and did fulfil the minimum of three years follow up on all patients, the trial did not reach the pre-specified number of events and therefore the resultant power was less than the 90% expected from the sample size calculation (overall final power 77%). The stage distribution (III versus II) was different to that expected. However, because of the similar event rates in high risk stage II and low risk stage III patients, this in itself had very little impact upon the power and the results. An assessment of the ongoing rate of accumulation of events was performed and it was clear that because of the slowing of the rate of accumulation of events, that even with much longer follow up, few extra events would be registered, and therefore the power would

not significantly increase. 9 patients (0.46%) were not used in the analysis because of 'withdrawal of consent to use data'. We believe this relatively high number was the result of an initially poorly phrased 'withdrawal of consent to treatment' form signed by 9 patients who only wanted to stop treatment, but whose data, because of ambiguity in the form, we could not use. However this small percentage of patients would have been adequately covered by our calculations for loss to follow-up had the event rate been higher as described above.

Two large randomised adjuvant trials both reported negative results towards the end of the QUASAR 2 recruitment period. The NSABP C-08 trial (n=2673)⁷ demonstrated that the addition of bevacizumab to infusional FOLFOX6 in the adjuvant setting of CRC did not improve DFS (HR 0.93, 95% CI 0.81-1.08; p=0.35) or OS (HR 0.95, 95% CI 0.79-1.13; p=0.56). The authors subsequently reported that patients with MSI+ primary tumours derived statistically significant survival benefit from the addition of bevacizumab (OS HR= 0.52; 95% CI 0.29 to 0.94; P = 0.02) but no benefit was observed in patients with MSS+ tumours (HR = 1.03; 95% CI 0.84 to 1.27; p = 0.78).⁸ The phase III AVANT study⁶ (n=2867) also failed to show an improvement in DFS / OS when bevacizumab was added to FOLFOX4 or CAPOX in the adjuvant treatment of stage III colon cancer. After a minimum of 5 years follow up, a harmful effect was detected, with an excess of relapses and deaths due to progression in the arms incorporating bevacizumab. We have performed a meta-analysis of the AVANT, NSABP- C-08 and QUASAR2 trials with respect to the effect of the addition of bevacizumab which reveals an overall HR for death of 1.03 (95% CI 0.88 to 1.21; p=0.72), confirming that there is no role for the use of bevacizumab in the adjuvant management of CRC in an unselected population.

Exploratory subgroup analysis in this study has highlighted a potential patient population (around 30%) with either an MSI+ tumour, or MSS+ in combination with high levels of free

CD31, who benefit from the addition of bevacizumab to capecitabine (DFS HR 0.44; $P=0.017$). Conversely, the population of patients whose tumours exhibit MSS with low free CD31 suffer a significantly poorer disease free survival when bevacizumab is added to capecitabine (DFS HR 1.45; CI 1.04-2.02; $p=0.03$). This divergence of effect has not been thoroughly explored in previous publications, but perhaps explains why the C-08 and AVANT studies gave somewhat conflicting results in terms of whether benefit was gained at all, whether any benefit showed a time-dependent course (C-08), or whether the bevacizumab may produce harm (suspected in AVANT). The QUASAR2 trial therefore adds significantly to our knowledge in this area.

As the translational analyses are exploratory, the results do need to be interpreted with caution, but there are potential biological mechanisms for the effects observed. One explanation for the differential impact on these subgroups is that high free CD31 levels can reflect the presence of more immature non-pericyte covered vessels which tend to be associated with enhanced susceptibility to bevacizumab therapy¹³. This population of blood vessels may have similarities to the ‘tumour vessel phenotype’ described in preclinical tumour models by Smith et al, 2013, which also demonstrated sensitivity to anti-VEGF antibody treatment.¹⁴ The alternative explanation for the observed differential effect is a consequence of some positive interaction between bevacizumab and immunological effectors, many of which express CD31. However we did not identify individual leukocytes in great quantities in these specific tumour specimens suggesting that the latter explanation is less likely.

MSI tumours tend to have greater microvessel density than their MSS counterparts, which may be the basis of some element of differential sensitivity to bevacizumab¹⁵ and there are data to suggest that bevacizumab treatment can reduce regulatory T cells in colon cancer

patients, potentially altering the immune microenvironment in hypermutated MSI tumours, where there is a greater frequency of neo-antigens, thereby favouring immune rejection¹⁶.

Although the selection of translational analyses to be performed was based on a mechanistic understanding of the agents and patient populations involved, because the results are findings from a retrospective analysis without an *a priori* hypothesis stated in the statistical analysis plan, then the results presented should be regarded only as hypothesis generating.

Precision medicine is a rapidly evolving field across all specialties from cancer to heart disease and diabetes. Precision or personalisation can be delivered either through exclusion of patients unlikely to benefit from therapy or positive selection of those with a response-favourable phenotype. This current study reveals populations on both sides of this same coin, increasing the biological plausibility of this observation. It is clear that bevacizumab should not be given to an unselected population of colorectal cancer patients in the adjuvant setting. It is also clear, given the excess deaths with prolonged treatment, that if bevacizumab is to be tested again in the adjuvant setting, serious consideration should be given to what is the appropriate duration of treatment to maximise effect but minimise toxicity.

We have identified limitations of our study: a lower than expected event rate reducing power; a small number of patients whose data we could not use in the ITT analysis; a loss to follow-up of approximately 6%; exploratory biomarker analyses that were not pre-specified.

However, despite these cautions, the data presented here with respect to patient selection and adjuvant bevacizumab are thought provoking and hypothesis generating and, in the absence of any new drugs being introduced into the colorectal cancer adjuvant arena for more than a decade, perhaps worth investigating in clearly defined prospective studies.

Acknowledgements

Roche provided an unrestricted educational grant to the Principal Investigator for conduct of the trial and supplied capecitabine and bevacizumab. We also acknowledge the kind support of the Oxford NIHR Comprehensive Biomedical Research Centre (all UK authors) and core funding to the Wellcome Trust Centre for Human Genetics from the Wellcome Trust (090532/Z/09/Z) (IT, DC, ED) and finally we acknowledge support from the Department of Health with respect to personal fellowships (RK). Finally we thank Haitao Wang for her hard work processing and preparing all the translational samples ready for research use. Pages 4 to 12 of the Web Appendix give a full list of sites and PIs to whom we are extremely grateful for the success of this study.

Author Contributions

RSK: Co-CI, trial design, protocol development, patient recruitment, safety review, data analysis, data interpretation, writing

SL: statistical lead, trial design, protocol development, data analysis, writing

ES: Australian CI, patient recruitment, data interpretation, writing

EJ: Tissue coordinator, data interpretation

BF: Translational scientist, data analysis (translational)

PH: Australian PI, patient recruitment

AW: UK PI, patient recruitment, safety review

DC: Patient recruitment, data analysis (translational)

CS: Data collection, trial coordination

SP: Data collection, trial coordination

PJ: Database development

FP: Lead pathologist, tissue review

IT: Chief scientist translational, data interpretation

ED: Translational scientist, data analysis, data interpretation, writing

DJK: Co-CI, trial design, protocol development, patient recruitment, safety review, data analysis, data interpretation, writing

Conflict of Interest Statement

Dr Falcon reports and Beverly L Falcon is an employee of Eli Lilly and co. The other authors declared no conflicts of interest.

REFERENCES

1. Gray, R, Barnwell J, McConkey C, Williams N and Kerr, DJ. QUASAR: a randomised study of adjuvant chemotherapy versus observation including 3239 colorectal cancer patients QUASAR Collaborative Group. *Lancet* 2007; 370(9604): 2020-9.
2. Andre T, Boni C, Navarro M et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncology* 2009; 27: 3109-3116.
3. Schmoll HJ, Tabernero J, Maroun J et al. Capecitabine plus oxaliplatin compared with fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: Final results of the NO16968 randomized controlled phase III trial. *J Clin Oncol* 2015; 33(32): 3733-40.
4. Yothers G, O'Connell MJ, Allegra CJ et al. Oxaliplatin as adjuvant therapy for colon cancer: updated results of NSABP C-07 trial, including survival and subset analyses. *J Clin Oncol* 2011; 29(28): 3768-74.
5. Hanahan D and Weinberg R. Hallmarks of cancer: The next generation. *Cell* 2011; 144: 644-9.
6. De Gramont A, Van Cutsem E, Schmoll HJ et al. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial. *Lancet Oncol* 2012; 12: 1225-33.
7. Allegra CJ, Yothers G, O'Connell MJ et al. Bevacizumab in stage II-III colon cancer: 5-year update of the National Surgical Adjuvant Breast and Bowel Project C-08 trial. *J Clin Oncol* 2013; 31(3): 359-64.
8. Pogue-Geile K, Yothers G, Taniyama Y et al. Defective mismatch repair and benefit from bevacizumab for colon cancer: findings from NSABP C-08. *J Natl Cancer Inst* 2013; 105(13): 989-92.

9. Wang D, Stockard C, Harkins L et al. Immunohistochemistry in the evaluation of neovascularization in tumor xenografts. *Biotech Histochem* 2008; 83(0): 179–189.
10. Domingo E, Ramamoorthy R, Oukrif D et al. Use of multivariate analysis to suggest a new molecular classification of colorectal cancer. *J Pathol* 2013; 229(3): 441-448.
11. Danielsen, H.E., Pradhan, M. & Novelli, M. Revisiting tumour aneuploidy - the place of ploidy assessment in the molecular era. *Nat Rev Clin Oncol* 2015; Nov 24. doi: 10.1038/nrclinonc.2015.208. [Epub ahead of print] Review.
12. Falcon BL, Stewart J, Ezell S et al. High-content multiplexed tissue imaging and quantification for cancer drug discovery. *Drug Discovery Today* 2013; 18(11-12): 510-22.
13. Ciocâlțeu A, Săftoiu A, Cârțână T et al. Evaluation of new morphometric parameters of neoangiogenesis in human colorectal cancer using confocal laser endomicroscopy (CLE) and targeted panendothelial markers. *PLoS One* 2014; 9(3): e91084.
14. Smith NR, Baker D, Farren M et al. Tumor Stromal Architecture Can Define the Intrinsic Tumor Response to VEGF-Targeted Therapy. *Clin Cancer Res* 2013; 19: 6943.
15. Xavier Sagaert, Eric Van Cutsem, Sabine Tejpar, Hans Prenen, Gert De Hertogh. MSI versus MSS sporadic colorectal cancers: Morphology, inflammation, and angiogenesis revisited. *J Clin Oncol* 32, 2014 (suppl 3; abstr 495).
16. Terme M, Pernot S, Marcheteau E, et al. VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. *Cancer Res.* 2013;73(2):539–549.

List of Tables and Figures

Table 1: Baseline demographics and disease characteristics of the whole analysed population

Table 2: Summary of all adverse events for CAP versus CAPBEV including prompted (shaded) and non-prompted (non-shaded) AEs.

Table 3: Detailed comparison of specific relevant toxicities (AEs and SAEs), CAPBEV versus CAP

Figure 1: Consort diagram

Figure 2: Survival curves CAPBEV versus CAP a) DFS and b) OS

Figure 3: Forest plot of hazard ratios for DFS for CAPBEV versus CAP, plotted according to standard demographic and pathological variables

Figure 4: Survival curves for CAPBEV versus CAP a) DFS for MSI population b) DFS for MSS population c) OS for MSI population and d) OS for MSS population

Figure 5: DFS survival curves CAPBEV versus CAP for a) MSI / MSS with high free CD31 and b) MSS with low free CD31

QUASAR2 Tables

		Arm A 968	Arm B 973	Total 1941
Stage	II	373	371	744 (38·3%)
	III	595	602	1197 (61·7%)
Gender	Female	414	418	832 (42·9%)
	Male	554	555	1109 (57·1%)
Primary site	Colon	854	861	1715 (88·4%)
	Rectum	114	112	226 (11·6%)
Age Band	<50	93	96	189 (9·7%)
	50-59	197	192	389 (20·0%)
	60-69	394	388	782 (40·3%)
	70 or above	284	297	581 (30%)

**Table 1: Baseline demographics and disease characteristics
of the whole analysed population**

Reported adverse event symptom / category	CAP cycles 1-8					CAPBEV Cycles 1-8					CAPBEV Cycles 1-16				
	Worst CTCAE grade					Worst CTCAE grade					Worst CTCAE grade				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Handfoot syndrome	293	262	198	3	0	231	295	254	3	0	229	306	257	3	0
Diarrhoea	311	165	95	7	0	346	138	96	8	0	356	152	99	8	0
Hypertension	53	16	6	0	0	164	120	36	0	0	203	166	70	0	0
Proteinuria	39	9	1	0	0	159	29	8	1	0	197	64	19	1	0
Wound Healing	12	5	0	0	0	16	12	2	0	0	22	12	4	0	0
Stomatitis	151	50	7	1	0	223	58	14	0	0	233	61	14	0	0
Neutropenia	50	21	18	8	0	60	33	12	3	1	63	33	13	3	1
Thrombocytopenia	43	5	1	3	0	59	9	1	2	0	72	10	1	2	0
Vomiting	103	43	22	2	0	111	62	17	2	0	131	72	18	2	0
Biochemistry	89	21	18	5	0	78	30	19	6	0	89	38	24	7	0
Cardiovascular	55	31	32	10	0	37	35	48	7	1	57	39	52	7	1
Endocrine	1	1	3	0	0	3	5	3	3	0	6	8	3	3	0
Gastrointestinal	341	108	42	3	1	348	144	58	7	0	364	164	63	8	0
Genito-urological	12	11	2	0	0	22	9	4	0	0	29	12	5	0	0
Haematology	101	23	2	1	0	74	20	2	1	0	80	21	3	1	0
Infection	83	70	14	7	0	100	101	17	6	0	134	131	21	7	0
Musculoskeletal	59	18	6	0	0	129	48	10	1	0	183	84	15	1	0
Neurological	163	43	14	3	1	209	51	25	1	0	253	72	32	1	0
Respiratory	74	15	7	1	0	174	29	12	3	0	216	39	16	3	0
Skin/ Hair	255	83	14	0	0	250	58	16	0	0	302	77	17	0	0
Miscellaneous	333	160	44	1	0	351	166	45	3	0	353	184	50	3	0

Table 2: Summary of all adverse events for CAP versus CAPBEV including prompted (shaded) and non-prompted (non-shaded) AEs.

For CAPBEV, AEs for the first 8 cycles only are given for direct comparison with the CAP arm, as well as the total number experienced for all cycles of CAPBEV (16 cycles)

	CAP (963) N (%)	CAPBEV (959) N (%)	RR CAPBEV:CAP (95% CI)	P value
AEs worst grade reported Cycles 1 to 8				
Hypertension <i>Grade 1 or 2</i> <i>Grade 3 or 4</i>	69 (7.2) 6 (0.6)	284 (29.6) 36 (3.8)	All grades 4.3 (3.4-5.4) Grade 3 and 4 6.0 (2.6-14.2)	<0.001 <0.001
Proteinuria <i>Grade 1 or 2</i> <i>Grade 3 or 4</i>	48 (5.0) 1 (0.1)	188 (19.6) 9 (0.9)	All grades 4.0 (3.0-5.4)	<0.001
Poor Wound Healing <i>Grade 1 or 2</i> <i>Grade 3 or 4</i>	17 (1.8) 0 (0.0)	28 (2.9) 2 (0.2)	All grades 1.8 (1.0-3.2)	0.05
Diarrhoea <i>Grade 1 or 2</i> <i>Grade 3 or 4</i>	476 (49.4) 102 (10.6)	484 (50.5) 104 (10.8)	Grade 3 and 4 1.0 (0.8-1.3)	0.9
Hand-foot syndrome <i>Grade 1 or 2</i> <i>Grade 3 or 4</i>	555 (57.6) 201 (20.9)	526 (54.8) 257 (26.8)	Grade 3 and 4 1.3 (1.1-1.5)	0.002
Epistaxis <i>All Grades</i>	13 (1.3)	132 (13.8)	All grades 10.2 (5.8-17.9)	<0.001
SAEs reported Cycles 1 to 8				
Arterial thromboembolism	11 (1.1)	6 (0.6)	1.8 (0.7-5.0)	0.2
Venous thromboembolism	41 (4.3)	22 (2.3)	1.9 (1.1-3.1)	0.01
Gastrointestinal perforation	4 (0.4)	1 (0.1)	4.0 (0.5-35.9)	0.2

Table 3: Detailed comparison of specific relevant toxicities (AEs and SAEs), CAPBEV versus CAP

Figure Legends for QUASAR2 paper (please see separate file for figures)

IN PUBLICATION

Figure 1: Consort Flow diagram

Figure 2: Survival curves for CAPBEV versus CAP, whole analysed population

a) Disease-free survival b) Overall survival

CAPBEV  CAP 

Figure 3: Forest plot of hazard ratios for disease-free survival for CAPBEV versus CAP, plotted according to standard demographic and pathological variables

Figure 4: Survival curves CAPBEV versus CAP for populations separated according to

MSI+/MSS+ status: a) DFS MSI+ b) DFS MSS+ c) OS MSI+ d) OS MSS+

CAPBEV  CAP 

Figure 5: Disease-free survival curves for CAPBEV versus CAP, according to

MSI+/MSS+ status and free CD31+ levels: a) MSI+ (any level free CD31) or MSS+ (high free CD31) b) MSS+ (low levels free CD31)

CAPBEV  CAP 