Circulating biomarkers during treatment in patients with advanced biliary tract cancer receiving cediranib in the UK ABC-03 trial

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BACKGROUND: Advanced biliary tract cancer (ABC) has a poor prognosis. Cediranib, in addition to cisplatin/gemcitabine [CisGem], improved the response rate, but did not improve the progression-free survival (PFS) in the ABC-03 study. Minimally invasive biomarkers predictive of cediranib benefit may improve patient outcomes.

METHODS: Changes in 15 circulating plasma angiogenesis or inflammatory-related proteins and cytokertatin-18 (CK18), measured at baseline and during therapy until disease progression, were correlated with overall survival (OS) using time-varying covariate Cox models (TVC).

RESULTS: Samples were available from n = 117/124 (94%) patients. Circulating Ang1&2, FGFb, PDGFBb, VEGFC, VEGFR1 and CK18 decreased as a result of the therapy, independent of treatment with cediranib. Circulating VEGFR2 and Tie2 were preferentially reduced by cediranib. Patients with increasing levels of VEGFA at any time had a worse PFS and OS; this detrimental effect was attenuated in patients receiving cediranib. TVC analysis revealed CK18 and VEGFR2 increases correlated with poorer OS in all patients (P < 0.001 and P = 0.02, respectively).

CONCLUSIONS: Rising circulating VEGFA levels in patients with ABC, treated with CisGem, are associated with worse PFS and OS, not seen in patients receiving cediranib. Rising levels of markers of tumour burden (CK18) and potential resistance (VEGFR2) are associated with worse outcomes and warrant validation.

INTRODUCTION

Novel therapeutic options, based on an improved understanding of underlying biology and response to therapy, are urgently needed for patients presenting with advanced biliary tract cancer (ABC). Whilst uncommon in the developed world, biliary tract cancer (BTC including cholangiocarcinoma [CCA], gallbladder and ampullary carcinoma) represent a significant global problem due to areas of high incidence, for instance of liver fluke-associated cholangiocarcinoma in Northern Thailand and of gallbladder cancer in Chile and India.

Surgery is the cornerstone of curative therapy for BTC; the use of adjuvant therapy has historically been based on meta-analyses of non-randomised series and prospective studies. The recently presented phase III, randomised, BiCap study has demonstrated an overall survival (OS) benefit from the use of adjuvant use of oral capecitabine following surgery versus surgery alone. Unfortunately, most patients are present with advanced (non-resectable or metastatic) disease and their survival is ≤3 months, with best supportive care alone. In the ABC-02 study, the combination chemotherapy with cisplatin and gemcitabine achieved a median survival of 11.7 months, compared to gemcitabine monotherapy (8.0 months; HR = 0.64, 95% confidence interval [CI] 0.52–0.80; P < 0.001), findings which were confirmed in the Japanese BT22 study. Although this is the international reference regimen, there is a pressing need to improve the efficacy, given these modest outcomes.

Angiogenesis is one of the hallmarks of neoplasia; the expression of vascular endothelial growth factor (VEGF) is associated with modest outcomes. Angiogenesis is one of the hallmarks of neoplasia; the expression of vascular endothelial growth factor (VEGF) is associated with...
adverse clinical features including the presence of liver metastases in intra-hepatic cholangiocarcinoma (iCCA)\(^9\) and increased micro-

vessel density (MVD) in both gallbladder cancer\(^{10}\) and CCA.\(^{11}\) In

patients undergoing curative resection, MVD has been identi-

fied as an independent prognostic risk factor for OS in lymph node-

negative iCCA\(^{12}\) and gallbladder cancer,\(^{13}\) as well as for disease-free

survival (DFS)\(^{13}\) and OS\(^{14}\) in patients with extrahepatic cholangio-

carcinoma (eCCA). These clinical observations are consistent with

the demonstration of receptors for VEGF (VEGFR1 and VEGFR2) in

tumour proximal endothelial cells\(^{15}\) along with the frequent (40–75\%)

expression of VEGF (particularly VEGFA) in BTC 9,\(^{11}\) particularly at the

invasive edge of the tumour.\(^{11}\)

Cediranib is an oral VEGFR1, VEGFR2 and VEGFR3 tyrosine kinase

inhibitor (TKI), with additional activity against platelet-derived

growth factor (PDGF) receptors and c-KIT.\(^{16}\) In the prospective

randomised double blind placebo-controlled phase II ABC-03

study, the cisplatin and gemcitabine combination was evaluated with either cediranib or placebo. Although an improved response

rate was observed (44\% vs. 19\% with placebo; \(P = 0.0036\)) along with an improved 6-month progression-free survival (PFS, 70.5\% vs.

61.3\%; \(P < 0.05\)) in cediranib-treated patients, the magnitude of this

effect did not reach the pre-defined level of statistical significance

(hazard ratio [HR] for PFS: 0.93, 80\% CI 0.74–1.19; \(P = 0.72\)) for the

primary endpoint. This may have been due to lack of efficacy, or

alternatively, underpowering of the statistical plan, or because cediranib was not well tolerated in this combination.\(^{17}\)

Recognising the challenge of serial tumour biopsy, an

exploratory translational endpoint of the ABC-03 study was the

prospective longitudinal profiling of circulating biomarkers associated withangiogen-

es. We now present the findings of this work, set the findings in

context and evaluate the implications for future clinical trials.

MATERIALS AND METHODS

Patients and treatment

ABC-03 (clinicaltrials.gov NCT09398484) was an investigator-initiated,

multi-centre (15 UK sites), double-blind, placebo-controlled, ran-

domised phase II study of cediranib added to the standard-of-care

chemotherapy regimen (cisplatin and gemcitabine), the details of

which have been described previously.\(^{11}\) Permission for this trial was

awarded by the North West 5 Research Ethics Committee, Haydock

Park on 23 August 2010 (10/H1010/42). All patients provided written

informed consent before randomisation.

Material collection and analysis

Blood samples were collected from the patients for biomarker

studies into EDTA tubes and processed into plasma at up to 11
timepoints; two pre-treatment baseline samples and then on the

first day of cycles 2–8, at the end of chemotherapy and 1-month

after the end of chemotherapy. The circulating markers of

angiogenesis (VEGFA, VEGFC, VEGFR1, VEGFR2, angiopoietins 1

and 2 [Ang1, Ang2], fibroblast growth factor b [FGFb], hepatocyte

growth factor [HGF], PDGFbb, keratinocyte growth factor [KGF],

placental growth factor [PFG], tyrosine kinase with Ig and EGF

homology domains 2 [Tie2], stromal-derived growth factor 1b

[SDF1b]) and inflammation (interleukin 6 and interleukin 8 [IL6

and IL8]) were measured with a validated\(^{18}\) multiplex enzyme-

linked immune-sorbent assay (ELISA) platform (Aushon BioSys-
tems, Billerica, Massachusetts, USA), according to the Good Clinical

Practice (GCP) standards at the Cancer Research UK Manchester

Institute (Manchester, UK). Concentrations of the circulating total

cytokeratin18 (CK18),\(^{19}\) released from epithelial cells during death

(apoptosis and necrosis), were measured with an M65 ELISA

(Peviva, Nacka, Sweden), also previously validated and implemen-
ted to GCP as previously described.\(^{20}\)

Whole-blood (10 mL) was collected in CellSave Preservative

Tubes at up to four time points (pre-treatment baseline sample, on
day 1 of cycles two and five, and 1-month after the end of

chemotherapy) for the enumeration of circulating tumour cells

(CTCs) with the CellSearch platform (Janssen Diagnostics, South

Raritan, New Jersey, USA) within 4 days of blood draw.\(^{21}\) Briefly,

after immunomagnetic capture of EpCAM-positive cells, immuno-

phenotyping of cells with an intact (4′,6-diamidino-2-phenylindole

[DAPI] stained) nucleus using antibodies cytokeratin (CK) and

CD45 allowed the classification of circulating tumour cells as

EpCAM\(^+\), CK\(^+\), DAPI\(^+\) and CD45\(^-\).

All collected samples were analysed, unless the samples were

not available for clinical reasons or patient discontinuation from

the study (per protocol).

Statistical methods

Two aliquots of each plasma sample were analysed to determine the

biomarker levels. The mean was calculated and used in

statistical analyses. Two pre-treatment baseline samples (collected

on separate days) were used to establish a mean pre-treatment

value. This concentration was assigned to the date that patient

was randomised in the trial and used as a reference point to

compare with the longitudinal sampling data. So as to retain as

much data as possible for analysis, samples which were analysed

and found to be above the upper limit of assay detection (ULOD)

were assigned a numerical value of 1 pg/mL above the ULOD.

Similarly, measurements which fell below the lower limit of assay

detection (LLOD) were assigned a numerical value of half of the

assay LLOD.

In order to explore the ability of the biomarkers to predict OS

based on greatest change from baseline, the percentage change

from baseline was calculated. Patients were ranked in order and
divided into three groups (tertiles) for comparison. The middle
tertile was set to 0, as this represented the ‘least change’ group.
The two extremes were compared with this.

The longitudinal sampling data were analysed using time-

varying covariate Cox models (TVC), this is a model that considers

the proportionality of hazards at any point in time. The HR is

obtained by integrating the longitudinal sampling data. All

statistical analysis was carried out at the Cancer Research UK,

and University College London (UCL) Cancer Trials Centre, London.

The means and 95\% CI for each marker were plotted over time

by the treatment group to assess the change over time and the
difference between the treatment groups. In order to assess

whether a change in the marker at 3 months (the time point at

which the efficacy evaluation took place) is associated with survival

outcomes, patients were grouped in terms of their percentage change at 3 months, from baseline, into three groups

based on the distribution of the data (tertiles): lower, mid and

higher groups. The mid tertile group was used as the reference
group and represented the group of patients with the least

percentage change at 3 months from baseline. The lower tertile
group included the group of patients with a percentage change
decrease at 3 months from baseline. The higher tertile group

included the group of patients with a percentage increase at

3 months from baseline. For CTC count, a different approach was

used by grouping patients into no detectable CTCs at baseline and

at cycle 3 or any detectable CTCs at baseline and at cycle 3. These

groups were also compared using standard Cox model for PFS and

OS. Considering that the biomarkers were evaluated at different
timepoints and were variable over time, and that the aim of this

study was to evaluate the effect of the changing biomarkers over
time on the time-to-event outcomes, a TVC approach was

performed. The time-to-event endpoints considered were PFS and

OS. The TVC models were fitted separately for each biomarker at

a time adjusting for treatment. Also, TVC models were fitted

separately for each biomarker and the interaction between the
treatment and the marker were evaluated.

Considering that there were multiple biomarkers, backward

selection was applied to a Cox model including all biomarkers to
Fig. 1  Changes in key biomarkers during treatment, split by treatment arm. The mean log of pg/ml of each biomarker, by treatment arm, is shown at baseline (BL), during treatment cycles (C2-8), at the end of chemotherapy (End) and 1 month after the end of all treatment (+1m) which equates to 1 month after disease progression has been documented. Panels a-g indicate markers that change similarly in both arms; the cause may be CisGem chemotherapy or tumour burden (rather than Cediranib). Panels h and i show markers that occur at lower levels in the circulation as a result of treatment with Cediranib. Panels j and k show markers that appear to be shed into the circulation as a result of Cediranib. Error bars indicate 95% confidence intervals. Number of patients at each time-point: BL = 114, C2 = 96, C3 = 92, C4 = 90, C5 = 79, C6 = 73, C7 = 71, C8 = 59, End = 55, +1m = 44.
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05 April 2011 and 28 Sept 2012. Details of the patient population have been described previously.16 In summary, 104 (84%) patients had metastatic disease (the remainder had locally-advanced disease). The primary disease site was cholangiocarcinoma in 77 patients (62%), gallbladder cancer in 39 (31%) and ampulla of Vater in 8 (6%). The median PFS was 8 months (95% CI 6.5–9.3) in the cediranib group and 7.4 months (5.7–8.5) in the placebo group (HR 0.93, 80% CI 0.74–1.19, P = 0.72). The median OS was 14.1 months (95% CI 10.2–16.4) in the cediranib group and 11.9 months (9.2–14.3) in the placebo group (HR 0.86, 80% CI 0.58–1.27, P = 0.44).

Dynamic biomarker changes in response to chemotherapy and cediranib
Figure 1 describes the changing levels of the multiple biomarkers in each arm over time and demonstrates that there were some differences between the treatment groups. Panels a–g demonstrate a decrease in circulating Ang1 and 2, FGFb, PDGFbb, VEGFC, VEGFR1 and CK18 from the second time-point (prior to cycle 2, i.e. post-cycle 1 of systemic treatment) and that this effect was lost at the time-of-disease progression. This was independent of cediranib, and is likely to be related to chemotheraphy and/or disease load. In contrast, panels h and i demonstrate a differential effect of cediranib, preferentially reducing VEGFR2 and Tie2. This difference was again lost at disease progression. Panels j and k demonstrate a reverse effect, whereby cediranib was associated with the preserved levels of circulating VEGFA and PlGF. The effect-neutral biomarkers measured are shown in Supplementary Figure S1 (HGF, IL6, IL8, KGF and SDF1b).

It is important to note that complete datasets are available only for patients who were originally benefitting from the treatment, as, in patients whose disease progressed early, provision of further research samples was discontinued.

Cediranib attenuates the detrimental outcome associated with rising VEGFA
Changes at cycle 3 (C3) compared to the baseline for patients in both treatment arms combined (i.e. chemotherapy with placebo and chemotherapy with cediranib) are shown in Fig. 2a. Increased levels of seven biomarkers at C3 describe a group of patients who may benefit from the treatment (with the exception of PDGFbb, in whom, patients with decreased levels at C3 may benefit less from the treatment). The remaining biomarkers measured are shown in Supplementary Figure S2A (CK18, HGF, IL6, IL8, PlGF, SDF1b, VEGFR1 and VEGFR2).

In keeping with the effect seen at cycle 3 (above), patients with increasing levels of VEGFA at any time in the TVC had a worse outcome for both PFS and OS (Fig. 2b). However, in patients who received cediranib, this detrimental outcome was attenuated. All other biomarkers measured are shown in Supplementary Figure S2B.

Multivariable models for biomarkers predictive of the outcome
Table 1 details multivariable models using the principle of backward selection for biomarkers at baseline and when assessed longitudinally using PFS and OS as outcomes. For the predictive capacity of biomarkers at baseline, two different models are generated for PFS and OS, respectively. These differences are likely to be due to the short-term, rather than the longer-term, biological impact. When considering the change in biomarkers over time, rising levels of Ang2 are associated with longer OS (HR 0.77 [0.64–0.93] P = 0.007), but conversely increasing levels of CK18 (a surrogate measure of disease burden/cell death) and VEGFR2 (potentially a mechanism of resistance to therapy) are associated with shorter OS (HR 1.07 [1.04–1.10] P < 0.001 and HR 1.12 [1.01–1.23] P = 0.02). PDGFbb does not feature in these models as it did when previously described,16 primarily because PDGFbb was previously analysed in two subsets (dichotomised at

| Biomarker | % change in tertiles | Forest plot | HR (95% CI) | N | P-value |
|-----------|----------------------|-------------|-------------|---|---------|
| Ang1      | < 53                 |             | 1.06 (0.62, 1.82) | 32 | 0.025   |
|           | ≥ 53 – 117           |             | 1.00 (1.00, 1.00) | 30 | 0.999   |
|           | > 118                |             | 0.56 (0.31, 1.01) | 30 | 0.060   |
| Ang2      | < 58                 |             | 1.19 (0.69, 2.04) | 32 | 0.504   |
|           | ≥ 58 – 88            |             | 1.00 (1.00, 1.00) | 31 | 0.999   |
|           | > 89                 |             | 0.61 (0.34, 1.12) | 29 | 0.219   |
| FGFb      | < 41                 |             | 1.30 (0.75, 2.22) | 32 | 0.253   |
|           | ≥ 41 – 92            |             | 1.00 (1.00, 1.00) | 30 | 0.999   |
|           | > 93                 |             | 0.53 (0.29, 0.96) | 30 | 0.049   |
| KGF       | < 71                 |             | 0.91 (0.54, 1.54) | 31 | 0.758   |
|           | ≥ 71 – 109           |             | 1.00 (1.00, 1.00) | 32 | 0.999   |
|           | > 110                |             | 0.49 (0.27, 0.69) | 29 | 0.028   |
| PDGFbb    | < 58                 |             | 1.51 (0.86, 2.64) | 31 | 0.420   |
|           | ≥ 58 – 119           |             | 1.00 (1.00, 1.00) | 32 | 0.999   |
|           | > 120                |             | 0.97 (0.54, 1.74) | 29 | 0.020   |
| Tie2      | < 89                 |             | 1.06 (0.59, 1.89) | 31 | 0.866   |
|           | ≥ 89 – 113           |             | 1.00 (1.00, 1.00) | 32 | 0.999   |
|           | > 114                |             | 0.78 (0.44, 1.39) | 29 | 0.411   |
| VEGFA     | < 71                 |             | 1.04 (0.61, 1.78) | 32 | 0.904   |
|           | ≥ 71 – 113           |             | 1.00 (1.00, 1.00) | 31 | 0.999   |
|           | > 114                |             | 0.53 (0.29, 0.97) | 29 | 0.214   |
| VEGFC     | < 37                 |             | 1.02 (0.60, 1.75) | 32 | 0.959   |
|           | ≥ 37 – 95            |             | 1.00 (1.00, 1.00) | 31 | 0.999   |
|           | > 96                 |             | 0.58 (0.33, 0.93) | 29 | 0.019   |

| Forest plot | HR (95% CI) | N | Events | P-value |
|-------------|-------------|---|--------|---------|
| Placebo     | 1.23 (1.04, 1.46) | 59 | 54 | 0.025   |
| Cediranib   | 0.96 (0.83, 1.11) | 59 | 57 |         |
| PFS         |             |   |        |         |
| Placebo     | 1.31 (1.06, 1.62) | 59 | 48 | 0.033   |
| Cediranib   | 0.99 (0.86, 1.15) | 59 | 48 |        |
| OS          |             |   |        |         |

RESULTS
Patient information
A total of 124 patients (62 each in the cediranib and placebo groups) with a median age of 65.1 years were recruited between
the median), rather than as a continuous variable. The tumour markers CEA, CA19-9 and CA125 are not described, as no longitudinal data was available for them. Supplementary Figure S3 shows the median and the range of all baseline circulating biomarkers by the treatment arm.

CellSearch-detected circulating tumour cells are not predictive of the benefit from cediranib

Changes in CellSearch (CS)-detected CTCs do not predict for patient outcomes for either PFS or OS, as illustrated in Fig. 3. Given the low absolute numbers of CTCs, combined analysis of baseline and cycle 3 CTC numbers did not improve the discrimination over baseline counts alone. As such, assessment of on-treatment CTCs did not predict the outcome.

Figure 4 summarises the data presented in Figs. 1–3.

DISCUSSION

The ABC-03 clinical trial assessed the effect of adding cediranib (an oral VEGFR1, VEGFR2 and VEGFR3 receptor tyrosine kinase inhibitor, with additional activity against PDGF receptors and c-KIT) to cisplatin and gemcitabine chemotherapy in a double-blind, placebo-controlled manner. The study did not meet its primary endpoint (improvement in PFS); however, signals were observed that would support further anti-angiogenesis approaches. Elevated baseline levels of the tumour markers CEA and CA125 in addition to CA19-9, and total cytokeratin 18 and VEGFR2, as well as CTCs were shown to be prognostic in ABC. Baseline plasma PDGFb concentrations might predict for the cediranib activity.

The second paper considers the change in circulating biomarkers during the treatment; decrease in circulating Ang1, Ang2, CK18, FGFb, PDGFb, VEGFC and VEGFR1 was observed in patient samples independent of treatment with cediranib. Cediananb has previously been proposed to be causally linked with a reduction in circulating VEGFR1, both in hepatocellular carcinoma (HCC) and glioblastoma. However, both were uncontrolled single-arm studies. In this prospective double-blind placebo-controlled study, we have shown that this observation is not, in fact, due to cediranib, rather due to the chemotherapy or disease load. Similarly, a reduction in circulating plasma Ang2 has been reported in uncontrolled studies in glioblastoma and HCC. We demonstrated that this is also independent of cediranib treatment and highlights the importance of a prospective randomized study design in evaluating a potential biomarker.

Our observation of cediananb-induced reduction in VEGFR2 is in keeping with previously published studies and its known mechanism of action. This has been described following cediranib monotherapy in solid tumours; phase I study4 acute myeloid leukaemia,5 glioblastoma,33 HCC,32 gastrointestinal stromal tumour and in combination with lomustine in glioblastoma,27 carboplatin and paclitaxel in cervical cancer28 and gefitinib in solid tumours.29 Placebo-controlled studies in colorectal cancer,30 renal cell cancer,31 and breast cancer32 confirmed that a reduction in VEGFR2 was due to cediranib and was independent of the companion therapies (primarily a combination with chemotherapy, as in this study). We also observed that cediananb-induced reduction in circulating Tie2 and similar findings have been reported in glioblastoma,33 colorectal cancer and in solid tumours treated with a cediananb–gefitinib combination.29

Patients with increasing levels of VEGFA at any time had a worse outcome for both PFS and OS, in patients who received cediranib, this detrimental outcome was attenuated. This, again, is consistent with the known mechanism of action of cediananb.16 This suggests that the changes in circulating VEGFA correlates with the potential benefit from treatment with cediranib.

Using multivariable models for biomarkers predictive of outcome, rising circulating levels of Ang2 were shown to be
Fig. 3  Circulating tumour cells. Association between CTC count at baseline and Cycle 3 and PFS (a) and OS (b): patients who had CTCs enumerated using Cell Search both at baseline (BL) and at the start of Cycle 3 of treatment, were divided into two categories; Group 1 had no CTCs at BL and C3 (n=35) and Group 2 had at least 1 CTC at either BL or C3, or both time-points (n=22). The range of CTCs observed in this patient set was 0-44, with a median of 0 and a mean of 2 CTCs. *As both of these curves overlap, this p-value may not be reliable. c Shows change in CTC count (as absolute numbers at C3), shaded by best response. The hypothesis would be that the patients who had the biggest decrease in CTCs, would have better outcomes (which is not the case). d Using the data collected from the n=56 patients who had CTCs enumerated at both baseline and C3, this shows that combining baseline and C3 CTC counts appears less discriminatory than considering baseline CTC counts alone.
Ang2 is a growth factor ligand of the Tie family of protein receptor tyrosine kinases. Ang2 promotes the dissociation of pericytes and loosens the cellular junctions, which results in unstable blood vessels. Increasing levels of circulating Ang2 in this setting would appear to be indicative of effective tumour destabilisation.

Conversely, rising levels of CK18 were associated with a shorter OS. Cancers of epithelial origin are known to contain relatively large intracellular pools of soluble and insoluble cytokeratins. However, during necrotic and apoptotic cell death, CK18 and other cytokeratins are released into the blood in either their intact or their caspase-cleaved forms, where they remain relatively stable in the circulation of patients with cancer. CK18 is proposed as a surrogate measure of disease burden/drug-induced cell death, and it would appear that rising levels in this patient population is indicative of impending disease progression. Similarly, rising levels of VEGFR2 (in all patients) were associated with a shorter OS. As a target of cediranib, it is not unexpected to observe a fall in levels of circulating VEGFR2, but it is interesting to note that an increase in the levels in all patients is associated with disease progression and suggests a potential mechanism of resistance to chemotherapy.

Whilst we reported that CellSearch-identified CTCs were prognostic at baseline in ABC-03, the data presented here does not support their role as predictive biomarkers for cediranib. A
limitation of these data is that only a subgroup (43 patients for the CTC subgroup) had complete data. Moreover, the CellSearch platform captures only EpCam-expressing CTCs; in many epithelial cancer types, this represents only a subset of CTCs not measuring, for example, the CTCs undergoing epithelial-to-mesenchymal transition. Further studies would be required using marker-independent CTC platforms, which accommodate phenotypic heterogeneity coupled with molecular analysis of DNA profiles of the isolated CTC candidates, to allow detailed evaluation of their utility in the clinical setting. Future studies would also benefit from the collection of the genomic profiling data, which may provide methods for treatment selection.

This translational component to the clinical study was set out to evaluate the biomarkers usefulness, as suggested by others.38 These data provide additional information about a panel of circulating biomarkers, which may predict the benefit from the combination of chemotherapy and cediranib.

These data suggest that the treatment with cediranib may attenuate the increased risk of progression and death associated with high circulating levels of VEGFA. It is not known whether this is true for other VEGFA inhibitors such as bevacizumab.

The strength of this study is the prospective evaluation of the sequential biomarker analysis in a randomised cohort of patients against a control, as described in the Cancer Research UK biomarker roadmap (www.cruk.org.uk). We have been able to differentiate between chemotherapy- and cediranib-related effects, and have demonstrated that week 9 (cycle 3) is a suitable time point for the biomarker estimation.

The primary limitation of this study was the necessary “self-selection” of patients for whom the data were available, as only patients who were deemed to have derived clinical benefit (by the absence of disease progression on treatment) contributed longitudinal biomarker data. In addition, this biomarker substudy serves as an exploratory dataset that was not powered a priori to identify the robust subgroups and not adjusted for multiple testing; the findings would need to be validated in an independent dataset, according to the REMARK guidelines.39

CONCLUSION
Unravelling the complexity of circulating biomarkers is best achieved though prospective randomised trials such as ABC-03. These data propose that the detrimental outcome observed on PFS and OS associated with circulating VEGFA levels in patients with advanced biliary tract cancer treated with cisplatin and gemcitabine may be attenuated by cediranib. This is in keeping with its known mechanism of action. The role of VEGF inhibition requires further evaluation to identify and validate biomarker-defined potentially responsive subgroups. Surrogate measures of tumour burden (rising CK18) and potential treatment resistance (rising VEGFR2) were associated with worse outcomes and warrant validation.

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AUTHOR CONTRIBUTIONS
ABC-03 was developed through and supported by the Hepatobiliary Subgroup of the UK National Cancer Research Institute Upper GI Clinical Studies Group and was led by the trial management group composed of J.W.V., J.A.B., H.W., S.B., M.D., A.L. and A.C.B. A.L. was responsible for statistical analysis. The translational aspects of the study were led by C.D., J.W.V. and A.C.B. M.D. and S.B. were responsible for the conduct of the trial, ensuring all required approvals were in place, and for collection and verification of the integrity of the data. Study results were interpreted by the trial management group; the trial management group also drafted the manuscript and collated the responses from all co-authors.

ADDITIONAL INFORMATION
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Consent for publication: All authors gave final approval of the version to be published.

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