Dose-finding study and pharmacogenomic analysis of fixed-rate infusion of gemcitabine, irinotecan and bevacizumab in pretreated metastatic colorectal cancer patients

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BACKGROUND: To determine the dose-limiting toxicity (DLT), maximum tolerated dose, recommended dose (RD) and preliminary evidence of activity of escalating doses of irinotecan (CPT-11) fixed-dose-rate infusional gemcitabine (FDR-GMB) and bevacizumab in pretreated metastatic colorectal cancer (mCRC) patients. Pharmacogenomic analysis was performed to investigate the association between VEGF single-nucleotide polymorphisms and clinical outcome.

PATIENTS AND METHODS: A total of 89 mCRC patients were recruited in a two-step study design: 28 were included in the dose-finding study and 59 in the pharmacogenomic analysis. The FDR-GMB of 1000 mg m⁻², bevacizumab 5 mg kg⁻¹ and CPT-11 doses ranging from 100 to 160 mg m⁻² were explored. The VEGF protein serum levels were quantified by EIA. Allelic discrimination was performed to genotype polymorphisms in the VEGF gene.

RESULTS: CPT-11 RD was 150 mg m⁻². Diarrhoea and neutropenia were the DLT. After a median follow-up of 42 months, the median time to progression (TTP) and overall survival were 5.2 and 19.9 months, respectively. VEGF levels were significantly correlated with these genotypes correlated with a longer median TTP (8.8 vs 4.5 months, P = 0.04).

CONCLUSION: The triplet combination tested in this study is effective and well tolerated. A possible predictive role for VEGF gene polymorphisms and baseline VEGF circulating levels is suggested.

Keywords: gemcitabine; irinotecan; bevacizumab; metastatic colorectal cancer; dose finding; VEGF

To date, most patients with relapsed metastatic colorectal cancer (mCRC) receive second or further lines of systemic therapy (Rothenberg, 2004). Overall response rate in the range of 15–25% and survival times around 12 months have been reported, with varying degrees of improved outcome (Maindou-Goebel et al, 1999; Tournigand et al, 2004). Irinotecan-based regimens are commonly used after failure of first-line oxaliplatin/fluoropyrimidine combinations (Tournigand et al, 2004). Although bevacizumab uniformly enhanced the cytotoxic antitumour effect of chemotherapy in the first-line setting of mCRC (Kabbinavar et al, 2003; Hurwitz et al, 2004), at the time we initiated this study, a limited efficacy had been reported with this agent in pretreated mCRC patients, possibly due to the low expected activity of the chemotherapy component (Emmanoulides et al, 2004). On these basis, and taking into account the modest outcome achieved with second-line FOLFIRI (Tournigand et al, 2004), we hypothesised whether the addition of bevacizumab to an irinotecan-based schedule without 5-fluorouracil may represent a promising strategy.

Gemcitabine (GMB), a difluorinated analogue of deoxycytidine, exerts its antitumour activity through inhibition of ribonucleotide reductase and DNA synthesis (Plunkett et al, 1995). Although prolonged exposure of this agent appeared to have superior activity when compared with bolus administration in murine colon tumours (Kornmann et al, 2000), several phase I/II trials of single-agent GMB had demonstrated minimal activity in mCRC patients (Moore et al, 1992; Mani et al, 1998; Lonardi et al, 2004). However clinical outcomes remarkably improve when GMB is used in combination regimens. In fact, a growing body of evidence suggests that GMB synergistically interacts with some of the most widely used agents in mCRC, including fluoropyrimidines (Correale et al, 2003) and oxaliplatin (Fairve et al, 1999). In addition, in vitro blockade of VEGF-receptor activation has proved to enhance the efficacy of GMB (Solorzano et al, 2001).

A synergistic sequence-dependent interaction of GMB and SN-38 has also been found in preclinical models, as the incorporation of GMB into DNA enhances camptothecin-induced topo-I cleavage complexes (Pourquier et al, 2002). Indeed, in colon cancer-derived cell lines, GMB was shown to induce the
expression of all topoisomerase enzymes and cytotoxicity was more relevant when cells lines were treated with GMB and topoisomerase I poisons within a short period of time (Richter et al., 2006).

This preclinical background has prompted the design of clinical studies with GMB-based combinations, mainly oxaliplatin and fluoropyrimidines, in pretreated mCRC patients (Correale et al., 2004), with interesting tumour growth control rates and a favourable toxicity profile.

The VEGF gene expression is upregulated in colorectal cancer and can be predictive of invasiveness, metastases, recurrence and prognosis (George et al., 2001). Numerous single-nucleotide polymorphisms (SNPs) in the promoter, 5'- and 3'-untranslated regions have been described (Brogan et al., 1999), although their predictive value regarding bevacizumab efficacy in mCRC remains to be determined.

On the basis of these considerations, we initiated this pilot study with a double aim; first, to determine the dose-limiting toxicity (DLT), the recommended dose (RD) and preliminary evidence of activity of this triplet combination. The second objective was to explore the association of baseline VEGF circulating levels, VEGF gene SNPs and clinical outcome.

PATIENTS AND METHODS

Eligibility

Eligibility criteria included age ≥18 years and a histologically confirmed mCRC progressed after one prior oxaliplatin/fluoropyrimidine-based chemotherapy regimen for metastatic disease. Pre-trial disease progression was radiologically confirmed and independently reviewed. Patients who had only received adjuvant chemotherapy were not included. In addition, an Eastern Cooperative Oncology Group performance status score 0–2, a life expectancy >12 weeks, an adequate hepatic, renal and haematological function, and measurable disease by RECIST criteria was required.

Exclusion criteria included active second malignancy, prior anti-VEGF therapy, brain metastases, uncontrolled severe infection, major organic failure, ischaemic cardiopathy, bleeding or clotting diatheses and requirement for systemic anticoagulation.

Pretreatment baseline evaluation included a complete medical history, physical examination, full blood count, biochemistry including carcinoembryonic antigen, and a CT scan of the chest, abdomen and pelvis. During treatment, a physical examination and blood cell counts were performed biweekly. Treatment was delayed until recovery in case of neutrophils <1500 mm
3, platelets <75 000 mm
3 or diarrhoea or stomatitis grade >1 on the planned day of treatment. If treatment had to be delayed for longer than 2 weeks, or any drug discontinued permanently, patients were excluded from the study. Dose reductions of GMB and irinotecan were allowed based upon treatment tolerability. Bevacizumab was withheld in case of gastrointestinal perforation, grade 3–4 haemorrhage, uncontrollable ischaemic cardioopathy and arterial thrombosis.

Maintenance therapy with single-agent bevacizumab was allowed in those patients achieving disease control after six cycles of therapy upon investigators’ discretion.

A refractory disease was defined in those patients in whom progressive disease to the previous line of therapy was documented as best response. Patients who achieved an objective response (complete (CR)/partial responses (PR)) or disease stabilisation (s.d.) but progressed during or within 3 months thereafter from the end of that therapy were considered to have a resistant disease.

Patients’ characteristics and their outcomes were unknown to investigators performing genetic analyses. The local institutional review board approved the study and all patients provided written informed consent before recruitment.

Study design and treatment

Between January 2005 and October 2008, a total of 89 mCRC patients were enrolled in this two-step study.

Step 1: Dose-finding study

The dose-finding part of the study was designed considering the previous RD for biweekly GMB within a multidrug regimen in mCRC (Correale et al., 2004), the reported lack of a dose–response effect for this agent (Mavroudis et al., 2003) and taking into account that CPT-11 efficacy and toxicity are both dose dependent (Abigerges et al., 1994). Thus, in the dose-finding part of the study, GMB (1000 mg m
–2 at a fixed dose rate of 10 mg m
–2 min
–1) followed by irinotecan (starting dose of 100 mg m
–2, with 10 mg m
–2 increments) and bevacizumab (5 mg kg
–1) were administered on a biweekly basis. Consecutive cohorts of at least three patients were recruited until the maximum tolerated dose (MTD) was defined. If one out of three patients experienced a DLT, a minimum of three additional patients was enrolled at the same dose level. An MTD was defined if two out of three patients experienced a DLT. The RD was the dose level just below the MTD.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). The DLTs included grade 4 neutropenia lasting >7 days, grade 3–4 neurogenic fever, grade 4 thrombocytopenia, grade 3–4 haemorrhage, grade 3–4 non-haematological toxicity, except for alopecia, nausea or vomiting, gastrointestinal perforation and treatment delay of >4 weeks as a result of toxicity.

Step 2: Clinical and pharmaco genomic analysis

Once the irinotecan RD was established in a limited dose-finding assessment, this cohort was further expanded in order to prospectively perform an efficacy analysis and an exploratory angiogenesis-directed pharmaco genomic profiling of the combination. Sites of metastatic disease were radiologically re-evaluated every 8 weeks according to standard RECIST criteria unless clinically otherwise indicated (Therasse et al., 2000). All responses were independently reviewed and had to be confirmed ≥28 days after the initial documentation of response. At the time of maximum response, determined by serial CT scans or positron emission tomography if clinically indicated, patients were evaluated by a multidisciplinary team that included surgeons, medical oncologists, hematologists and interventional radiologists. In this evaluation it was ruled out whether a consolidative approach should be attempted. These approaches consisted of surgical removal of all macroscopic remaining disease, radiofrequency ablation or liver radioembolisation with Yttrium 90 microspheres. Serum samples were obtained by centrifugation at 3000 r.p.m. for 10 min and stored at −80 °C until use. Serum levels of VEGF, normalised by the patients platelet count, were determined using a VEGF ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions.

VEGF gene polymorphisms (Supplementary Table 1) were selected if a reported minor allelic frequency >0.20 in a Caucasian population was recorded in the SNP database (http://www.ncbi.nlm.nih.gov/SNP), and/or if the given polymorphism may alter the function of the gene in a biologically relevant manner.

DNA was extracted from EDTA-anticoagulated peripheral blood using the DNAeasy Mini kit (Qiagen, Valencia, CA, USA). Candidate SNPs were genotyped with Taqman-based real-time PCR using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Primers and probes were obtained from Applied Biosystems as Assays-on-Demand SNP genotyping product (Supplementary Table 1).
Table 1A  Irinotecan doses level and DLTs

| Dose (mg·m⁻²) | N  | Number of patients with DLT |
|--------------|----|-----------------------------|
| 100–140      | 15 | 0                           |
| 150          | 12 | 1                           |
| 160          | 3  | 2                           |

| Type of DLT | Efficacy |
|-------------|----------|
| Grade 3 asthenia | 2 CR, 5 PR, 6 SD, 2 PD |
| Grade 3 febrile neutropenia | Grade 3 asthenia | 1 CR, 4 PR, 6 SD, 1 PD |

Abbreviations: CR = complete response; DLT = dose-limiting toxicity; PD = progressive disease; PR = partial response; SD = stable disease.

Table 1B  Worst-grade toxicity per patient

| Event      | Level 1–5 (n = 15) | Level 6 (n = 12) | Level 7 (n = 3) |
|------------|---------------------|------------------|-----------------|
| Leucopenia | Grade 1–2           | 5                | 5               | 2               |
|            | Grade 3–4           | 1                | 0               | 1               |
| Neutropenia| Grade 1–2           | 2                | 2               | 1               |
|            | Grade 3–4           | 0                | 0               | 1               |
| Anaemia    | Grade 1–2           | 6                | 2               | 2               |
|            | Grade 3–4           | 1                | 0               | 0               |
| Thrombocytopenia | Grade 1–2 | 2                | 2               | 0               |
|            | Grade 3–4           | 0                | 0               | 0               |
| Diarrhoea  | Grade 1–2           | 2                | 4               | 2               |
|            | Grade 3–4           | 0                | 0               | 0               |
| Vomiting   | Grade 1–2           | 4                | 5               | 2               |
|            | Grade 3–4           | 0                | 0               | 0               |
| Asthenia   | Grade 1–2           | 11               | 3               | 1               |
|            | Grade 3–4           | 0                | 1               | 2               |
| Fever      | Yes                 | 1                | 0               | 1               |
|            | No                  | 14               | 11              | 2               |

Statistical analysis

Descriptive statistical methodology was used to design and analyse the dose-finding part of the study. Once the RD was achieved, the primary study end point was response rate; secondary end points included characterisation of time to progression (TTP), overall survival (OS) and treatment safety. Analysis of baseline circulating VEGF levels and VEGF gene SNPs as predictors of TTP were evaluated at an exploratory level. A two-staged Simon accrual design was adopted with a minimum target activity level (CR + PR) of 20% (Tournigand et al, 2004), with the initial stage accruing 24 response-assessable patients. Early discontinuation of the study was planned in the case of less than five responses in the first 24 patients. A minimum planned sample size of 48 evaluable patients was chosen to better estimate efficacy, and a total of 59 patients were recruited. The probability of erroneously concluding that the new treatment is active (P ≥ 0.20) when it is actually ineffective (P ≤ 0.04) is < 0.05 (α). The probability of erroneously concluding that the treatment is ineffective (P < 0.20) when it is actually effective (P ≤ 0.04) is < 0.05 (β).

The TTP and OS were calculated from the first day of treatment to the date of first observation of progressive disease or death, respectively. Patients who underwent consolidative procedures after being downstaged with the use of the study regimen were censored at that time for TTP analysis. Patients without documented OS events were censored at last contact. Kaplan–Meier estimates are provided for median TTP and OS, and the log-rank or Breslow tests were applied to test the differences in time-to-event across different genotypes. Differences in circulating VEGF levels were evaluated using the Mann–Whitney U-test.

We estimated the false-positive report probability (FPRP) for the observed statistically significant associations using the methods described by Wacholder et al (2004). FPRP is the probability of no true association between a genetic variant and a phenotype given a statistically significant finding. It depends not only on the observed P-value but also on the prior probability that the association between the genetic variant and the phenotype is real and the statistical power of the test. In the current study, we set the odds ratio and HR values of 2–4 as a likely threshold value. The prior probability used was 0.25 for all SNPs. The FPRP value for noteworthiness was set at 0.2, which indicates any finding expanded with six more patients, with no further observation of DLT.

Part 2: Clinical and pharmacogenomic analyses

Clinical analysis  Once the RD and preliminary evidence of efficacy were established, 59 additional patients were included in the second part of the trial. Characteristics of these 59 patients are summarised in Supplementary Tables 2 and 3. Most patients (76.3%) received the study regimen as second line. Among them, 28 (62.2%) had received a triplet regimen up-front, (FOLFOXIRI or FOLFOX-Cetuximab), whereas the remaining 17 patients had received first-line therapy with FOLFOX or XELOX. In all, 31 patients (52.5%) treated with the study regimen as second line were considered to have resistant (16 patients) or refractory disease (15 patients). Twenty-one patients received single-agent bevacizumab as maintenance therapy.

The toxicity profile of the combination was mild and it is listed in Supplementary Table 4. The treatment was generally well tolerated in the outpatient setting. The most common grade 3–4 events were haematological. Eight and ten patients had grade 3 leucopenia and grade 3 neutropenia, respectively. Grade 3–4 non-haematological toxicities were rare and included grade 3 diarrhoea.
and grade 3 asthenia in 5 and 7% of the patients, respectively. Hypertension was the most frequently reported bevacizumab-related toxicity, being of grade 3 in 13.5% of the patients. Other grade 3–4 bevacizumab-specific toxicities included gastrointestinal perforation (3.3%), VTE (1.6%) and bleeding (1.6%).

Eleven patients required hospitalisation during the study therapy due to gastrointestinal perforation managed medically (2), haemoptysis (1), neutropenia (5) and fever (3).

On an intent-to-treat basis, overall response rate was 45.7%, with 25 (42.3%) PR and 2 (3.4%) CR. Overall response rate was 63.6 and 30% in patients with sensitive and resistant/refractory disease, respectively ($P = 0.059$). Disease control rate (DCR; CR, PR and SD lasting $>6$ months) was achieved in 32 patients (54.2%). Sixteen patients (27.2%) achieved a sufficient downsstaging to undergo a consolidative procedure, including liver surgery (seven patients), thoracic surgery (five patients) or liver radioembolisation with Yttrium$^{90}$ microspheres (four patients).

After a median follow-up of 42 months (range: 10–59), the median TTP and OS were 5.2 (95% CI: 3.4–6.8) and 19.9 months (95% CI: 32–77), respectively.

In the univariate analysis, risk index according to Köhne classification (Köhne et al., 2002) and DCR achievement were significantly associated with TTP (Supplementary Table 2). A trend classification (Kohne et al., 1995) was also found for response to the preceding line of therapy significantly associated with TTP (Supplementary Table 2). A trend was also observed for response to the preceding line of therapy significantly associated with TTP (Supplementary Table 2).

Serum VEGF levels, VEGF polymorphisms and clinical outcome Although it remains a controversial issue, standardisation of serum VEGF, normalised by the patients platelet count, has been recommended (George et al., 2000), and thus we use this approach in this study.

Baseline VEGF serum levels were significantly higher in Köhne high-risk patients (mean±s.d., 4.42 pg per $10^9$ platelet ± 2.17 pg per $10^9$ platelet) as compared with the low-risk group (mean±s.d., 2.06 pg per $10^9$ platelet ± 1.32 pg per $10^9$ platelet) ($P = 0.01$).

VEGF levels were dichotomised into two categories around the median value (platelet-normalised VEGF baseline levels $>0.3$ or $<3$) to better describe its association with survival times. A significant relationship was found between platelet-normalised VEGF baseline levels and TTP ($P = 0.02$; Breslow test) (Figure 1A), with a median TTP of 2.4 (0.83–3.89) months and 8.2 (5.1–11.2) months for patients with high and low VEGF baseline levels, respectively.

A significant association was also found between platelet-normalised VEGF baseline levels and OS ($P = 0.034$; log-rank test) (Figure 1B), with a median OS of 5.2 (0.27–10.1) months and 21.3 (0.3–47.3) months for patients with high and low VEGF baseline levels, respectively.

As VEGF SNPs contribute to a high variability in VEGF circulating plasma concentrations (Watson et al., 2000), we searched for this association in our patients cohort. The VEGF genotype frequencies are shown on Supplementary Table 5. Genotype frequencies of all SNPs followed the Hardy–Weinberg equilibrium. Platelet-normalised serum circulating VEGF baseline levels were significantly lower in VEGF-2578AA and VEGF-460CC carriers ($P = 0.008$) and a trend was also observed for VEGF+405GG genotype (Supplementary Figures 1A–C). These results led us to further investigate the correlation between low-VEGF level-associated SNPs and clinical outcome. Patients

Figure 1 Kaplan–Meier curves for time to progression (TTP) (A) and overall survival (OS) (B) according to circulating vascular endothelial growth factor (VEGF) serum levels and according to the number of VEGF favourable genotypes (C) TTP outcome stratified on basis of the number of favourable clinical and molecular factors (D).
carrying the VEGF-2578AA genotype had a longer TTP than those
with the combined CA and CC genotypes (8.8 vs 5 months; 
P = 0.080; Supplementary Figure 2A). Similar data were found for
the VEGF-460CC genotype (9.6 vs 4.5 months; P = 0.054; Supple-
mentary Figure 2B). There was also a trend for a longer TTP
according to VEGF + 405 polymorphism (P = 0.13; Supplementary
Figure 2C). Patients harbouring at least one of these genotypes
(VEGF-2578 AA, VEGF-460 CC or VEGF + 405 GG) showed a
significantly longer median TTP than patients possessing none of
them (8.8 vs 4.5 months; P = 0.043; Figure 1C).

In an attempt to classify different risk groups in this population,
a predictive-risk score was calculated according to the number of
favourable clinical (Köhne low-risk category and DCR achieve-
ment) and molecular (any favourable VEGF genotype) factors.
This analysis rendered four different risk groups, with a median
TTP ranging from 2.3 months (95% CI: 0.49 – 4.25) to 11.4 months
(95% CI: 8.5 – 14.4) corresponding to the groups with none and
three favourable factors, respectively (Figure 1D).

In the multivariate model, including the VEGF favourable
genotypes and relevant clinical factors according to the univariate
analysis (Köhne risk index and DCR), the presence of any
favourable VEGF genotype and DCR achievement were both
significantly associated with TTP (Table 2). The FPRP was 0.163
for patients carrying any favourable VEGF genotype, indicating
noteworthiness.

DISCUSSION

To our knowledge, this is the first study to report mature results
on the combination of GMB, irinotecan and bevacizumab in
oxaliplatin/fluoropyrimidines pretreated mCRC. Although it is a
heterogeneous group, with most patients receiving the regimen as
second line and some as an even later line, if we take into account
the type of previous systemic therapies and the high percentage of
patients considered to have a truly resistant or refractory disease,
our results seem to be encouraging and compare favourably with
other irinotecan-based regimens in oxaliplatin-pretreated mCRC
(Recchia et al, 2004; Tournigand et al, 2004; Bidard et al, 2009).
Furthermore, they overlap with those achieved when bevacizumab
is combined with a more active partner than 5-FU (Giantonio et
al, 2007; Lievre et al, 2009) and with other reported GMB-based
schedules (Correale et al, 2004, 2005; Ziras et al, 2006; Lopes et
al, 2007; Bitossi et al, 2008; Merl et al, 2009). Although 27% of
the patients underwent a consolidative procedure, this finding
should be viewed with caution, as the trial was not specifically designed
to rule out the resectability rate achieved with this combination.

Dose-limiting toxicities for the combination included grade 3
asthenia and neutropenia. At the RD, toxicity profile was mild.
Altogether, 12% of the patients required hospitalisation, but
toxicities were uneventfully managed and no toxic deaths
were reported. The addition of bevacizumab did not significantly
change the side effect profile associated with GMB and CPT-11

Kakolyris et al, 2002; Nishio et al, 2005). The low incidence of
bevacizumab-specific toxicities encountered in this trial and the
previously reported lack of PK/PD interactions between
irinotecan and bevacizumab (Denlinger et al, 2009) may be partly
responsible for these findings. Nevertheless, these results should
be viewed with caution, as recent work has suggested
that UGT1A1-driven irinotecan dose-escalation studies may more
accurately define the precise dosage for this agent (Goetz et al,
2007).

To date, most of the tested biomarkers have failed to
discriminate patients more likely to benefit from bevacizumab-
containing regimens. Assessment of baseline VEGF circulating
levels has yielded conflicting results (Burstein et al, 2008; Kopetz
et al, 2010). We initially decided to use serum, instead of plasma,
to measure VEGF baseline levels. Plasma VEGF levels are close
to the lower limits of detection of the currently available ELISA and,
subsequently, serum assessments may provide a greater sensitivity
(George et al, 2000). Several studies demonstrated that paired
serum and plasma VEGF levels correlated in mCRC, and both of
them increase with advanced disease stage (Banks et al, 1998). In
this study, significantly longer median survival times were found
in patients with low baseline VEGF levels. Interestingly, low VEGF
level-associated SNPs were also correlated with a better clinical
outcome in terms of TTP. Similar results were obtained for both,
the VEGF-2578A > C and the VEGF-460T > C polymorphisms, in
agreement with a previously described strong linkage disequi-
librium between both loci. In vitro work has also linked the VEGF-
2578AA genotype with a decreased VEGF secretion in peripheral
blood mononuclear cells (Shahbazi et al, 2002) and a lower
immunohistochemical VEGF expression in cancer specimens
(Schneider et al, 2008). Our results show that patients harbouring
the VEGF-2578AA genotype achieve an almost two-fold longer
TTP compared with alternative genotypes. Similar findings have
been reported in metastatic breast cancer patients treated with
bevacizumab-based schedules, with VEGF-2578AA carriers show-
ning longer survival times compared with the VEGF-2578CA + CC
genotype (Schneider et al, 2008). The VEGF-2578CC genotype has
also been associated with an inferior median OS compared with
alternative genotypes in mCRC patients treated with irinotecan-
based chemotherapy and bevacizumab in the first-line setting
(Koutras et al, 2010).

There may be several potential limitations in these findings. The
limited sample size, the exploratory nature of the pharmacogen-
omic analysis and the complex biological network involved in
tumour angiogenesis make it mandatory to confirm these data
in larger, prospectively designed clinical trials. Furthermore,
Köhne low- and intermediate-risk patients were more likely to
have low VEGF baseline levels compared with the high-risk group,
and subsequently, a potential confounding interaction between
these variables cannot be definitively ruled out. Indeed, VEGF
levels have been advocated as a prognostic rather than a predictive
factor by other authors (Bernaards et al, 2010).

In conclusion, this study suggests that the combination of GMB,
irinotecan and bevacizumab may be a valid alternative for oxali-
platin/fluoropyrimidines-pretreated mCRC and deserves further
insight into the possible role of VEGF gene SNPs as surrogate
markers of bevacizumab-based therapy efficacy seems warranted.

ACKNOWLEDGEMENTS

We thank Dra A Patiño for critical reading and discussion of the
paper, and Ines Lopez and Marisol Gonzalez for their excellent
technical assistance. This work was supported by a grant of the
Departamento de Salud-Gobierno de Navarra.

Supplementary Information accompanies the paper on British
Journal of Cancer website (http://www.nature.com/bjc)

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British Journal of Cancer (2010) 103(10), 1529 – 1535

Table 2 Adjusted Cox multivariate analysis for TTP

| Factor | Variable | Hazard ratio | 95% CI | P-value |
|--------|----------|--------------|--------|---------|
| Any favourable VEGF genotype | Yes | 1.0 | — | 0.017 |
| | No | 2.3 | 1.1–4.5 | <0.001 |
| DCR | Yes | 1.0 | — | 0.017 |
| | No | 79.4 | 9.9–630.9 | — |

Abbreviations: CI = confidence interval; DCR = disease control rate; TTP = time to progression; VEGF = vascular endothelial growth factor. In the multivariate model, VEGF favourable genotypes and the relevant clinical factors according to the univariate analysis (Köhne risk index and DCR) have been included.

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