Significant effect of *VEGFA* polymorphisms on the clinical outcome of metastatic colorectal cancer patients treated with FOLFIRI-cetuximab

Jérôme Rollin[‡,1,2], Audrey Payancé[‡,1,2,3], Valérie Gouilleux-Gruart[1,4], Michelle Boisdron-Celle[5], Nicolas Azzopardi[1], Alain Morel[5], Yves Gruel[1,2], Gilles Paintaud[1,6], Erick Gamelin[5], Hervé Watier[1,4] & Thierry Lecomte*[,1,3]

[1]CNRS, UMR 7292, GICC & Université Francois-Rabelais, Tours, France
[2]CHRU de Tours, service d'Hématologie-Hémostase, Tours, France
[3]CHRU de Tours, service d'Hépato-Gastroenterologie, Tours, France
[4]CHRU de Tours, service d'Immunologie, Tours, France
[5]INSERM U892 & service d'Oncopharmacologie et Pharmacogénétique, CRCNA, Angers, France
[6]CHRU de Tours, service de Pharmacologie-Toxicologie, Tours, France

Aim: The efficacy of a cetuximab-based regimen used to treat metastatic colorectal cancer (mCRC) could be influenced by *VEGFA* polymorphisms. **Materials & methods**: We studied the effects of five polymorphisms in the *VEGFA* gene (-2549D/I, -1154G/A, -460T/C, +405G/C and +936C/T) on the outcome of 98 mCRC patients treated with FOLFIRI plus cetuximab. **Results**: Patients homozygous for the -2549D, -1154G and -460T alleles did exhibit higher response rates to treatment and longer progression-free survival compared with others. In addition, the DGTGC and IGCGC haplotypes were significantly associated with a lower risk of disease progression. **Conclusion**: These findings suggest that *VEGFA* genetic variations might influence response/resistance of FOLFIRI plus cetuximab treatment in mCRC patients.

Keywords: biomarkers, cetuximab, colorectal cancer, SNPs, VEGFA

Colorectal cancer (CRC) is one of the most common causes of cancer-related death in the Western world, ranking second in Europe and third in USA [1]. In the past few years, the management of metastatic colorectal cancer (mCRC) has been transformed by the availability of EGFR-targeted monoclonal antibodies, including cetuximab. Cetuximab is a chimeric IgG1 monoclonal antibody that binds EGFR with high specificity, and with a higher affinity than EGFR. The binding of cetuximab to EGFR, which leads to the inhibition of ligand-induced downstream signaling, inhibits proliferation, angiogenesis and metastasis, and promotes apoptosis [2]. The efficacy of cetuximab in the treatment of mCRC is limited to patients with a wild-type CRC at exons 2, 3 and 4 of RAS genes *KRAS* and *NRAS* [3]. However, a subset of patients with wild-type RAS tumors do not benefit from cetuximab treatment, and other biomarkers predictive of the response to cetuximab need to be identified. Few studies have investigated the impact of genetic polymorphisms on the effectiveness of cetuximab-based chemotherapy in CRC. It has been shown that polymorphisms in the *FCGR, EGF, let-KRAS, Cyclin D1* or *COX-2* genes could predict patient outcomes of cetuximab-based chemotherapy [4]. In addition, several preclinical studies have suggested that the VEGF pathway is modulated by cetuximab [5–7]. Interestingly, Vincenzi *et al.* showed that circulating levels of VEGF fell during treatment of advanced CRCs with a combination of cetuximab plus irinotecan, and that this decrease was related to the clinical response and survival of patients [8]. On the other hand, Grimminger *et al.* demonstrated that in advanced rectal cancer high intratumoral VEGF mRNA levels before treatment by cetuximab were associated with a complete pathological response in the wild-type *KRAS* subgroup of patients [9]. VEGF is encoded by the *VEGFA* gene, which is known to be highly polymorphic, with several SNPs in the promoter and in the 5′ and 3′-UTR that influence VEGF expression [10]. The -2549DD, -1154GG, -460CC and +405GG genotypes appear to be associated with a higher VEGF expression [11,12], whereas the 936T allele is correlated to lower VEGF expression [13]. We hypothesized that SNPs of *VEGFA* could influence the expres-

sion of VEGF and therefore efficacy of cetuximab-based regimens in mCRC. We analyzed the effect of these five functional polymorphisms in the *VEGFA* gene (-2549 D/I, -1154 G/A, -460 T/C, +405 G/C and +936C/T) on the outcome in 98 mCRC patients treated with FOLFIRI in addition to cetuximab. We also examined the association between levels of circulating VEGF and the response to treatment as well as progression-free survival (PFS).

Materials & methods

Study design & patients

This ancillary study was a part of a completed multicenter, noncomparative, open-label, Phase II study that included 102 patients (ClinicalTrials.gov identifier: NCT00559741) between October 2005 and January 2008. *KRAS* exon 2 (codons 12 and 13) mutation analysis was retrospectively performed in 61 patients for whom tumor material was available and who gave additional informed consent. There was not enough amount of tumoral DNA available to analyze other RAS mutations. The study design, patient eligibility criteria and treatment regimen have been described previously [14]. Briefly, patients were assigned to receive cetuximab weekly and FOLFIRI every 2 weeks. Dose adjustments of irinotecan and 5FU were decided on the basis of pharmacogenetic and pharmacokinetic parameters, using the internet-based calculator ODPM protocol web access (France). The clinical trial protocol and biomarker studies were approved by the Ethics Committee of Angers University Hospital (France), and blood samples were collected after informed consent had been obtained in accordance with the Declaration of Helsinki. Patients were treated until they improved or until unacceptable toxicity occurred. Tumor response was evaluated 2 months after starting cetuximab plus FOLFIRI using the Response Evaluation Criteria In Solid Tumors (RECIST) criteria. In this study, a patient with complete response (CR), partial response or stable disease was defined as a 'responder'; in contrast, a patient whose cancer progressed (PD) was defined as a 'nonresponder'. Tumoral response data were not available for 14 patients.

VEGFA polymorphisms

We selected five most common polymorphisms of the *VEGFA* gene, that is, -2549D/I (deletion/insertion of 18pb) (rs35569394), -1154G/A (rs1570360), -460C/T (rs833061), +405G/C (rs2010963) and +936C/T (rs3025039) that have been previously involved in regulation of VEGFA expression within tissue or plasma [10]. Therefore, the -2549D/I, -1154G/A and -460C/T are localized in promoter region and -2549D/I has been described in complete linkage disequilibrium (LD) with the -2578C/A polymorphism [15]. In addition, +405G/C and +936C/T are localized in the 5′and 3′ UTR region, respectively. All these SNPs have been previously studied as predictive or prognostic markers in several solid tumors. Germinal DNA was extracted from the peripheral blood of 98 out of 102 patients. SNPs were genotyped by previously described PCR-RFLP methods [16].

Serum VEGF measurement

Serum VEGF concentration was measured 2 h after the first infusion of cetuximab (D0), and before the eighth infusion (after about 60 days; D60) using the Quantikine VEGF immunoassay according to the manufacturer's instructions (R&D system). The detection limit of this assay was 31.2 pg/ml.

Statistical analyze

Wilcoxon's test was used to compare the variation in VEGF concentrations between D0 and D60, and the Mann–Whitney U test to analyze the concentration ratios of VEGF (D60/D0) according to the tumor response. Genotype analysis of each polymorphism was performed using χ^2 or Fisher's exact test. The endpoint assessed in this study was the impact of genotypes and haplotypes of the *VEGFA* gene on PFS, defined as per the protocol as the time from the first day of cetuximab treatment to the date of first disease progression or death due to any cause. The median PFS was estimated by the Kaplan–Meier method. The PFS data were plotted as Kaplan–Meier curves, and the differences between the groups categorized by *VEGFA* gene polymorphisms were compared by the log-rank test. The Hardy–Weinberg equilibrium, LD and haplotypes were analyzed using the THESIAS 3.1 program, which is based on the maximum likelihood method and linked to the SEM algorithm for haplotype–phenotype association [17,18]. This method is used to estimate haplotype frequencies as well as haplotype effects, expressed as odds ratios (ORs) for a binary phenotype (case–control status) by comparison to a reference haplotype. The effects of haplotypes on the survival were expressed as hazard risk ratios (HRRs) and compared with those of a reference haplotype. In all analyses, the most frequent haplotype was used as the reference haplotype. All statistical tests were two-sided, and $p \leq 0.05$ was considered to be statistically significant.

Results

The demographic and clinical data of the population (n = 98) available for genotyping and measurement of circulating VEGF levels are shown in Table 1. Disease control (CR + PD + SD) was obtained in 61.5% of patients,

Table 1. Baseline characteristics of metastatic colorectal cancer patients.

Patient characteristics	Patients (n)	%
Sex:		
– Male	55	56.1
– Female	43	43.9
WHO baseline performance status:		
– 0	60	61.2
– 1	31	31.6
– 2	7	7.2
Primary tumor site:		
– Colon	58	59.2
– Rectum	26	26.5
– Hinge rectosigmoid	13	13.3
– Other	1	1
Tumor KRAS status:		
– Mutated	26	26.5
– Wild-type	35	35.7
– Nonassessable	37	37.8
Line before FOLFIRI CETUX:		
– 0	14	14.3
– 1	47	47.9
– 2 or 3	37	37.8
RECIST (M2):		
– CR	1	1.0
– PR	24	24.5
– SD	35	35.7
– PD	24	24.5
– ND	14	14.3
	Median	**Range**
Age (years)	62	38–79
Bodyweight (kg)	70.5	34–113
Progression-free survival (months)	6.5	0.2–19.3

CR: Complete response; ND: Not determined; PD: Progressive disease; PR: Partial response; RECIST (M2): Response Evaluation Criteria in Solid Tumors after 2 months of treatment; SD: Stable disease.

who were therefore included in the 'responder' group; conversely, disease progression was observed 2 months after initiation of the cetuximab regimen in 24.5% of patients. The median time to progression was about 6.5 months in the 98 patients treated with FOLFIRI-cetuximab. In addition, no difference in PFS was found in relation to the *KRAS* exon 2 mutation detected in 42.6% of analyzed tumors (median = 6.62 months in mutated *KRAS* vs 6.65 months in nonmutated *KRAS*).

Genotype frequencies of the *VEGFA* gene are summarized in Table 2. The allelic frequencies did not deviate significantly from those expected according to the Hardy–Weinberg equilibrium hypothesis. Genotype analysis demonstrated that the proportion of -2549D homozygous patients (absence of a 18bp insertion) was significantly higher in the 'responder' than in the 'non-responder' group (30 vs 4%, respectively; p = 0.02; OR = 10; 95% CI: 1.2–100). Similar results were observed with the -460TT genotype, which was also associated with a significantly higher rate of response to treatment (30.5 vs 4%, respectively; p = 0.02; OR = 10; 95% CI: 1.2–100). In addition, -1154G homozygous patients

Table 2. Genotypes and minor allele frequency of *VEGFA* single nucleotide polymorphisms according to Response Evaluation Criteria in Solid Tumors criteria.

SNPs of VEGFA	Genotypes	All (n = 98)	'Responders' (n = 60)	'Nonresponders' (n = 24)	OR (95% CI)	p-value
-2549 D/I (rs35569394)						
Genotypes, n (%)	DD	20 (20.5)	18 (30)	1 (4)	10 (1.2–100)	p = 0.02
	II + ID	78 (79.5)	42 (70)	23 (96)		
MAF	I	0.46	0.45	0.71	3 (1.4–6.2)	p = 0.004
-460 T/C (rs833061)						
Genotypes, n (%)	TT	20 (20.5)	18 (30.5)	1 (4)	10 (1.2–100)	p = 0.02
	TC + CC	77 (79.5)	41 (69.5)	23 (96)		
MAF	C	0.46	0.44	0.71	3.1 (1.5–6.6)	p = 0.003
-1154 G/A (rs1570360)						
Genotypes, n (%)	GG	38 (38.5)	29 (48.5)	4 (16.5)	5 (1.4–16.5)	p = 0.015
	GA + AA	60 (61.5)	31 (51.5)	20 (84.5)		
MAF	A	0.37	0.30	0.52	2.4 (1.2–4.8)	p = 0.025
+405 G/C (rs2010963)						
Genotypes, n (%)	GG	50 (51)	29 (48.5)	13 (54)		NS
	GC + CC	48 (49)	31 (51.5)	11 (46)		
MAF	C	0.28	0.32	0.23		NS
+936 C/T (rs3025039)						
Genotypes, n (%)	CC	65 (66.5)	37 (61.5)	18 (75)		NS
	CT + TT	33 (33.5)	23 (38.5)	6 (25)		
MAF	T	0.17	0.20	0.125		NS

CR: Complete response; MAF: Minor allel frequency; 'Nonresponders' = PD; NS: Not significant; OR: Odds ratio; PD: Progressive disease; 'Responders' = CR + PD + SD; SD: Stable disease.

were more in numbers in the group responding to cetuximab than in the 'nonresponder' group (48.5 vs 16.5%, respectively; p = 0.015; OR = 5; 95% CI: [1.4–16.5]). Neither of the two other tested polymorphisms (i.e., +405G/C and +936C/T) was associated with tumor response.

The polymorphisms at positions -2549, -460 and -1154 in the *VEGFA* gene were also associated with PFS. Patients homozygous for the -2549D allele did exhibit a significantly longer PFS than those carrying the I allele (median PFS 9.7 vs 6 months, respectively; p = 0.03; Figure 1A). In addition, the same impact on median PFS was observed when the homozygous -460TT genotype was compared with the other genotypes (p = 0.03, data not shown). Median PFS was also significantly longer in patients homozygous for the -1154G allele than in patients carrying the -1154A allele (9.4 vs 6 months, respectively; p = 0.007; Figure 1C). Interestingly, in the subgroup of patients without the *KRAS* mutation (n = 35), those with the -1154GG genotype exhibited a median PFS of about 10 months, compared with 6 months in those carrying the -1154A allele (p = 0.04; Figure 1D). In contrast, in the subgroup of patients with the *KRAS* mutation, the SNP -1154G/A did not appear to influence survival (median PFS = 7.8 vs 6.5 months for noncarriers and carriers of the -1154A allele, respectively, p = 0.4). No association was found between the other studied *VEGFA* polymorphisms (405C/G and 936C/T) and PFS.

A complete LD was evidenced between SNPs at positions -2549 and -460, whereas the linkage between the three other SNPs were weak (range of D': 0.06–0.47). Haplotype analyses were conducted to evaluate the combined effect of these five *VEGFA* gene polymorphisms on the efficacy of treatment. Ten haplotypes were identified, and the effects of those exhibiting fre-

quencies greater than 1% were evaluated (Table 3). The results obtained showed that carriers of the DGTGC or IGCGC haplotypes had a lower risk of progression than in patients with the IACGC haplotype, used as reference (HRR = 0.48; p = 0.004 and HRR = 0.45; p = 0.003, respectively; Table 3). In the subgroup of patients with the wild-type *KRAS* (n = 35), the DGTGC haplotype was still significantly associated with a lower risk of progression than the IACGC haplotype (HRR = 0.31; 95% CI: 0.1–0.97; p = 0.045).

Finally, serum concentrations of VEGF were also studied in 84 out of 98 patients for whom both D0 and D60 blood samples were available. Median serum VEGF levels at the beginning of treatment with FOLFIRI-cetuximab were similar to that measured after 2 months (median of concentration = 187 vs 195 pg/ml, respectively; p = 0.9, Figure 2A). However, VEGF concentration ratios ($[VEGFA]_{D60}/[VEGFA]_{D0}$) were lower in 'responders' than in 'nonresponders' (median ratio = 0.77 vs 1.15 respectively; p = 0.03, Figure 2B), but without any significant association with the PFS (data not shown). In addition, no relationship was found between any of the gene polymorphisms or haplotypes studied and serum concentrations of VEGFA.

Discussion

Drugs that target the EGFR have had a major impact in CRC therapy. In the present study, the influence of -2549D/I (rs35569394), -1154G/A (rs1570360), -460T/C (rs833061), +405C/G (rs2010963) and +936C/T (rs3025039) polymorphisms of *VEGFA* gene on the response to FOLFIRI-cetuximab therapy was investigated in a cohort of 98 patients with mCCR. Allelic and genotype frequencies observed were similar to those previously described in CRC patients [10,19–20] and in the French general population [16]. We demonstrated that the -2549DD genotype was associated with a higher response rate and longer PFS. Similar results were found for the -460TT genotype, explained by the complete LD between the -2549I/D and -460C/T polymorphisms. In addition, the PFS of -1154GG homozygous patients was ~50% longer than that of

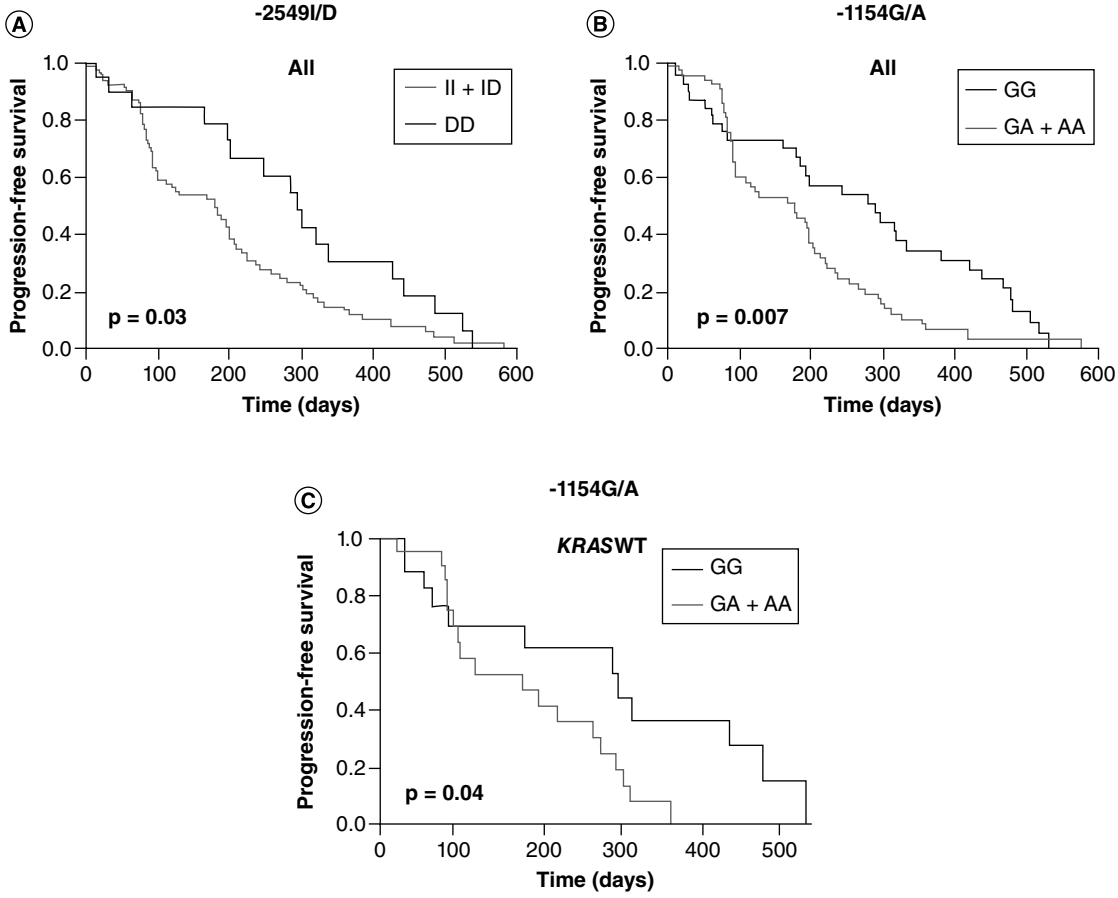

Figure 1. Polymorphisms at positions -2549 and -1154 in the *VEGFA* gene and progression-free survival.
Kaplan–Meier estimates of progression-free survival according to *VEGFA* polymorphism. **(A)** -2549I/D; **(B)** -1154G/A; **(C)** -1154G/A polymorphism in the subgroup of patients without KRAS mutation.

patients carrying the GA and AA genotypes. No difference in the response to treatment or PFS was related to the +405C/G and +936C/T polymorphisms. Only one previous study had evaluated the influence of one *VEGFA* polymorphism on the response to cetuximab in CRC [21]. Our results are in agreement with their findings, as the *VEGFA* +936C/T polymorphism was not related to the response to the cetuximab-based regimen in 39 mCCR patients [21]. However, a recent meta-analysis has demonstrated a significant association between the responsiveness to different chemotherapy in CRC evaluated by RECIST criteria and both VEGF -2578C/A and -460C/T polymorphisms (i.e., CC vs CA [OR = 1.40, 95% CI: 1.00–1.97; p = 0.05] and CC+CT vs TT [OR = 0.71, 95% CI: 0.53–0.96; p = 0.02], respectively). However, no relationship was found between +405C/G and +936C/T polymorphisms and no data were available regarding the -1154G/A polymorphism. Knowing that -2549D/I SNP has been described in absolute LD with the -2578C/A polymorphism, these results were in accordance with our study and suggested that -2549DD and -460TT genotypes increase the response to chemotherapy alone or in association with cetuximab and could lead to better outcome of CRC patients being treated [22].

Our study confirmed that the promoter and the 5′UTR of the *VEGFA* gene harbor a high degree of LD and haplotype analyses of this region may provide further information compared with the study of individual SNPs. In this regard, DGTGC and IGCGC haplotype carriers had a lower risk of death, about 50% lower than patients carrying the IACGC haplotype suggesting that the -1154G allele has a strong influence on the response to FOLFIRI plus cetuximab therapy. Several studies have shown that the homozygous -1154GG genotype is associated with higher cell expression of *VEGFA* than other genotypes [23,24]. Stevens *et al.* using MCF-7 cells (a breast cancer cell line) demonstrated that both -460C/T and +405C/G SNPs could influence the promoter effect of *VEGFA,* but this effect is strongly dependent on other proximal polymorphisms, including the -1154G/A SNP [24]. In addition, higher intratumoral mRNA levels of VEGF were found in CRC in patients carrying the DGG haplotype (i.e., -2549D/I, -1154G/A, 405C/G) [19]. In the present study, haplotypes harboring the DG-G- allele were associated with better outcomes. However, no association was observed between VEGF serum concentrations and the *VEGFA* polymorphisms or haplotypes studied. However, it is important to outline that serum concentrations of VEGF are likely not reflecting the expression of *VEGFA* in the primary tumor. Interestingly, study in advanced rectal cancer showed that high intratumoral VEGFA mRNA levels before treatment by cetuximab were associated with a pathological response in the wild-type *KRAS* subgroup of patients [9]. The authors thus suggested that upregulation of *VEGFA* expression could be due to an indirect effect induced by high *EGFR* signaling and high EGFR activity could therefore be associated to a strong cetuximab efficiency. In addition, we also speculated that SNPs of *VEGFA* associate to high VEGF tumors expression which could lead to the greater drug delivery but additional studies are required to investigate this hypothesis. Inversely, Vallböhmer *et al.* studied a small cohort of mCCRs (n = 31) treated with cetuximab without any other chemotherapy and showed that a high intratumoral mRNA level of *VEGFA* was associated with reduced tumor response probably due to increase in neoangiogenesis of the tumor although no association was found with PFS [25].

Circulating serum concentrations of VEGF were measured in 84 patients when cetuximab therapy was initiated and after 2 months of treatment, and VEGF concentration ratios (D60/D0) were lower in the 'responder' group than in the 'nonresponder' group. These results partly confirm the findings of a previous study performed in a cohort of 45 patients with mCCR treated with cetuximab and for which a

Table 3. Association between *VEGFA* haplotypes and progression-free survival.

***VEGFA* haplotypes (-2549; -1154; -460; +405; +936)**	**Frequency**	**HRR (95% CI)**	**p-value**
IACGC	0.28	–[†]	
DGTCC	0.21	0.81 (0.50–1.20)	NS
DGTGC	0.17	0.48 (0.29–0.78)	0.004
IGCGC	0.15	0.45 (0.26–0.77)	0.003
IACGT	0.073	0.82 (0.50–1.60)	NS
DGTCT	0.072	0.50 (0.25–1.10)	NS
IGCGT	0.03	0.80 (0.30–2.00)	NS

[†]Reference haplotype.
HRR: Hazard risk ratio; NS: Not significant.

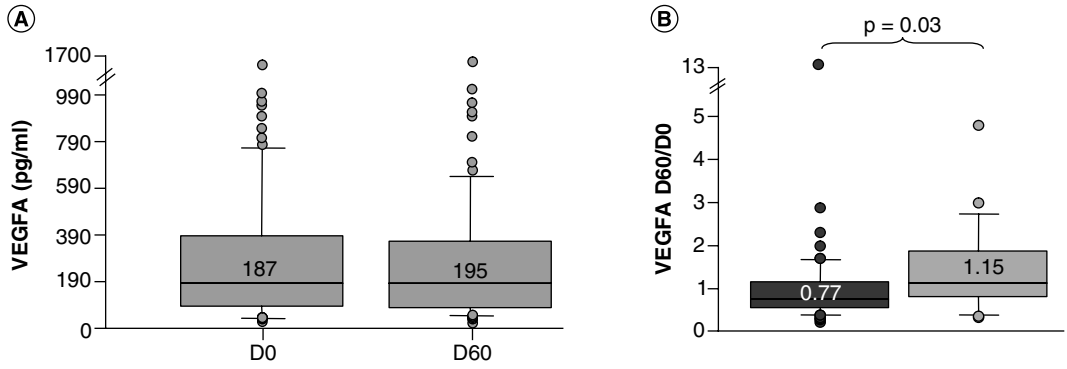

Figure 2. Serum VEGFA concentration of metastatic colorectal cancer patients treated with FOLFIRI-cetuximab.
(A) VEGFA serum concentration on day 0 and after 2 months of treatment were similar. **(B)** The VEGFA concentration ratios calculated for each patient ($[VEGFA]_{D60}/[VEGFA]_{D0}$) were lower in 'responders' (black) than in 'nonresponders' (gray).
D: Day(s).

decrease in VEGFA concentration was associated with the tumor response and PFS [8]. However, we did not find any relation between PFS and changes in serum concentration of VEGFA.

Interestingly, despite the limited number of patients with wt *KRAS* exon 2 tumors in our cohort (n = 35), a significantly longer PFS was still observed in homozygous carriers of the -1154G allele and of the DGTGC haplotype. Mutations of *KRAS* exon 2 are well-established negative predictive factors for clinical response to anti-EGFR monoclonal antibodies [26]. However, the tumor *KRAS* exon 2 status did not appear to exert a significant influence on PFS in our patients. Patients were recruited from 2004, and tumor tissue was available in 61 cases only, and this negative result could thus be attributable to the insufficient power of our study regarding this issue. Individual dosage adaptation of conventional chemotherapy scheduled in the clinical study also possibly contributed to the lack of effect of *KRAS* exon 2 mutation on the response to cetuximab. Another study limitation is that the extended RAS panel was not analyzed. Recent data demonstrate that other RAS mutations impact response to cetuximab, in particular, mutations in *KRAS* exons 3 and 4 and *NRAS* exons 2, 3 and 4 [3]. In our study, the extended RAS panel was not analyzed because tumoral DNA was no longer available and its potential interference with the *VEGFA* SNP in the present study remained unknown.

Conclusion & future perspective

In summary, our findings show that germline polymorphisms of the *VEGFA* gene may predict the clinical outcome in mCRC patients receiving cetuximab-based chemotherapy. Our assessment of the *VEGFA* SNP gene as a predictor of the efficacy of FOLFIRI plus cetuximab treatment in patients with mCRC suggests that three *VEGFA* polymorphisms and two *VEGFA* haplotypes are strongly associated with PFS. These findings indicate that alternative treatment approaches should be developed for patients with these genetic variants. However, our findings obtained with a small number of patients might be confirmed and validated in larger prospective and randomized controlled clinical trials.

Acknowledgements

The authors would like to thank the Investigators who included patients in the study: O Capitain, J-P Metges, A Adenis, J-L Raoul, Y Heng Lam, R Faroux, C Masliah and V Berger. The authors also thank M-N Marsson for her help in the genotyping of VEGFA. Genotyping was carried out within the CePiBAc platform.

Financial & competing interests disclosure

CePiBAc is funded by the EU and the region Centre. This work received funding in the form of Research Program grants from the Cancéropôle Grand-Ouest, from the French National Society of Gastroenterology, and from la Ligue contre le Cancer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Drugs that target EGFR, including cetuximab, have had a major impact in metastatic colorectal cancer (mCRC) therapy.
- Despite the overall success of FOLFIRI-cetuximab treatment, clinical efficacy is variable and some patients are refractory to these therapies.
- We analyzed the effect of five functional polymorphisms in the *VEGFA* gene (-2549 D/I, -1154 G/A, -460 T/C, +405 G/C and +936C/T] on the outcome in 98 mCRC patients treated with FOLFIRI in addition to cetuximab.

Results

- The *VEGFA* genotypes -2549DD, -1154GG and -460TT did exhibit higher response rates to treatment and longer progression-free survival.
- The DGTGC and IGCGC haplotypes were significantly associated with a lower risk of disease progression.
- The -1154GG genotype and the DGTGC haplotype were associated with a better progression-free survival in a subgroup of patients whose tumors wild-type for *KRAS*.

Conclusion

- *VEGFA* genetic variations, and particularly the -1154G/A polymorphism, could help to predict the clinical outcome in mCRC patients receiving FOLFIRI plus cetuximab treatment.
- Alternative therapeutic approaches for patients affected by a mCRC should be developed according to *VEGFA* genetic variants.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127, 2893–2917 (2010).

2 Vincenzi B, Schiavon G, Silletta M, Santini D, Tonini G. The biological properties of cetuximab. *Crit. Rev. Oncol. Hematol.* 68(2), 93–106 (2008).

3 Van Cutsem E, Lenz HJ, Köhne CH *et al.* Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J. Clin. Oncol.* 33, 692–700 (2015).

4 Custodio A, Feliu J. Prognostic and predictive biomarkers for epidermal growth factor receptor-targeted therapy in colorectal cancer: Beyond KRAS mutations. *Crit. Rev. Oncol. Hematol.* 85, 45–81 (2013).

5 Perrotte P, Matsumoto T, Inoue K *et al.* Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin. Cancer Res.* 5, 257–265 (1999).

• **Demonstrated for the first time in mice model that cetuximab treatment has a significant antitumoral effect mediated in part by inhibition of angiogenesis by decreasing VEGF expression in tumor.**

6 Bruns CJ, Harbison MT, Davis DW *et al.* Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. *Clin. Cancer Res.* 6, 1936–1948 (2000).

7 Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z. The antiepidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. *Oncogene* 24, 4433–4441 (2005).

8 Vincenzi B, Santini D, Russo A *et al.* Circulating VEGF reduction, response and outcome in advanced colorectal cancer patients treated with cetuximab plus irinotecan. *Pharmacogenomics* 8, 319–327 (2007).

•• **These data represent the first evidence that suggests a role of VEGF reduction in the prediction of efficacy of treatment with cetuximab plus weekly irinotecan in heavily pretreated advanced colorectal cancer patients.**

9 Grimminger PP, Danenberg P, Dellas K *et al.* Biomarkers for cetuximab-based neoadjuvantradiochemotherapy in locally advanced rectal cancer. *Clin. Cancer Res.* 17, 3469–3477 (2011).

•• **High intratumoral EGFR and VEGF mRNA expressions were significantly associated with complete response of patient with locally advanced rectal cancer treated by cetuximab-based chemoradiation.**

10 Hansen TF, Jakobsen A. Clinical implications of genetic variations in the VEGF system in relation to colorectal cancer. *Pharmacogenomics* 12, 1681–1693 (2011).

11 Koukourakis MI, Papazoglou D, Giatromanolaki A *et al.* *VEGF* gene sequence variation defines *VEGF* gene expression status and angiogenic activity in non-small cell lung cancer. *Lung Cancer* 46, 293–298 (2004).

12 Yang B, Cross DF, Ollerenshaw M *et al.* Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patients with type 1 diabetes mellitus. *J. Diabetes Complications* 17, 1–6 (2003).

13 Renner W, Kotschan S, Hoffmann C *et al.* A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J. Vasc. Res.* 37, 443–448 (2000).

14 Azzopardi N, Lecomte T, Ternant D *et al.* Cetuximab pharmacokinetics influences progression-free survival of metastatic colorectal cancer patients. *Clin. Cancer Res.* 17, 6329–6337 (2011).

15 Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5′ UTR regions of the human vascular endothelial growth factor gene. *Hum. Immunol.* 60, 1245–1249 (1999).

16 Bruyère F, Hovens CM, Marson M-N *et al.* VEGF polymorphisms are associated with an increasing risk of developing renal cell carcinoma. *J. Urol.* 184, 1273–1278 (2010).

17 Tregouet D-A, Barbaux S, Escolano S *et al.* Specific haplotypes of the P-selectin gene are associated with myocardial infarction. *Hum. Mol. Genet.* 11, 2015–2023 (2002).

18 Tregouet DA, Tiret L. Cox proportional hazards survival regression in haplotype-based association analysis using the stochastic-EM algorithm. *Eur. J. Hum. Genet.* 12, 971–974 (2004).

19 Ungerbäck J, Elander N, Dimberg J, Söderkvist P. Analysis of VEGF polymorphisms, tumor expression of VEGF mRNA and colorectal cancer susceptibility in a Swedish population. *Mol. Med. Rep.* 2, 435–439 (2009).

20 Cacev T, Loncar B, Seiwerth S, Spaventi S, Kapitanovic S. Vascular endothelial growth factor polymorphisms -1154 G/A and -460 C/T are not associated with VEGF mRNA expression and susceptibility to sporadic colon cancer. *DNA Cell. Biol.* 27, 569–574 (2008).

21 Zhang W, Gordon M, Press OA *et al.* Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with Cetuximab. *Pharmacogenet. Genomics* 16, 475–483 (2006).

22 Wang L, Ji S, Cheng Z. Association between polymorphisms in vascular endothelial growth factor gene and response to chemotherapies in colorectal cancer: a meta-analysis. *PLoS ONE* 10, e0126619 (2015).

23 Shahbazi M, Fryer AA, Pravica V *et al.* Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J. Am. Soc. Nephrol.* 13, 260–264 (2002).

24 Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. *Cancer Res.* 63, 812–816 (2003).

25 Vallbohmer D. Molecular determinants of cetuximab efficacy. *J. Clin. Oncol.* 23, 3536–3544 (2005).

- **Suggests that gene-expression levels of EGFR and VEGF in patients with metastatic colorectal cancer may be useful markers of clinical outcome in single-agent cetuximab treatment.**

26 Van Cutsem E, Köhne C-H, Hitre E *et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N. Engl. J. Med.* 360, 1408–1417 (2009).