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► **To cite this version:**

Morgane Caulet, Thierry Lecomte, Olivier Bouché, Jérôme Rollin, Valérie Gouilleux-Gruart, et al.. Bevacizumab Pharmacokinetics Influence Overall and Progression-Free Survival in Metastatic Colorectal Cancer Patients. *Clinical Pharmacokinetics*, 2016, 55 (11), pp.1381 - 1394. 10.1007/s40262-016-0406-3 . hal-01769811

HAL Id: hal-01769811

<https://hal.science/hal-01769811>

Submitted on 15 Jan 2024

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Bevacizumab Pharmacokinetics Influence Overall and Progression-Free Survival in Metastatic Colorectal Cancer Patients

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Abstract

Objective Clinical response to bevacizumab varies between patients treated for metastatic colorectal cancer (mCRC). The aim of this study was to quantify individual factors affecting bevacizumab pharmacokinetic variability and assess the relationship between bevacizumab concentrations and clinical outcomes.

Methods Bevacizumab pharmacokinetics were assessed in 130 mCRC patients using a two-compartment pharmacokinetic population model. Overall and progression-free survival (PFS) were analyzed using Cox models.

Results The bevacizumab volume of distribution increased with height ($p = 10^{-10}$) and was higher in patients with a 3/3 variable number tandem repeat of the *FCGRT* (Fc

fragment of IgG receptor and transporter) gene ($p = 0.039$). The elimination rate constant increased with baseline carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) concentrations, and was higher in patients with extra-hepatic metastases ($p = 0.00029, 0.011, \text{ and } 0.014$). A bevacizumab trough concentration ≤ 15.5 mg/L was associated with both shorter overall survival and PFS (hazard ratio [95 % CI] 1.90 [1.20–2.99] and 1.76 [1.20–2.58], respectively).

Conclusion High tumour burden is associated with low bevacizumab concentrations, and high bevacizumab concentration are associated with both decreased overall and progression-free survivals.

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Key Points

The pharmacokinetic variability of bevacizumab has been described previously in metastatic colorectal cancer. In the present study, we found that bevacizumab pharmacokinetics were influenced by antigenic burden: bevacizumab concentrations decreased with increasing baseline circulating vascular endothelial growth factor (VEGF) concentrations, increasing carcinoembryonic antigen concentrations, and with the presence of extra-hepatic metastases.

A dose–response relationship of bevacizumab has been previously conducted. In the present study, we found that patients with lower bevacizumab serum concentrations present lower overall and progression-free survival.

Patients with large VEGF concentrations and tumor burden may therefore be underexposed to bevacizumab. These patients may benefit from increased bevacizumab doses.

1 Introduction

Bevacizumab is a humanized immunoglobulin (Ig) G1 monoclonal antibody targeting vascular endothelial growth factor (VEGF). It inhibits neovascularization, a mechanism that is critical for the development and progression of metastases in malignancies [1]. Bevacizumab is approved in several cancers including metastatic colorectal cancer (mCRC) [2, 3].

Despite an overall benefit from bevacizumab of patients treated for mCRC, clinical outcomes are highly variable. Part of this variability could be due to differences in exposure to bevacizumab. Metastatic renal cell carcinoma patients treated with bevacizumab 10 mg/kg had longer time to progression than those treated with 3 mg/kg [4]. An observational cohort study of mCRC patients reported an increase in survival correlating with the total cumulative number of bevacizumab doses after first disease progression [5]. This suggests that a concentration–response relationship may exist in patients treated with bevacizumab: pharmacokinetic variability may influence the variability in clinical response, as for cetuximab [6]. The use of bevacizumab concentrations as a biomarker predicting its efficacy would represent a major advance in therapeutics. However, until now, the influence of bevacizumab concentration on clinical response has not been reported.

Bevacizumab pharmacokinetics have been investigated in two studies [7, 8], both of which observed that part of its variability was associated with several factors, including sex, body weight, and concomitantly administered chemotherapies. Indeed, volumes of distribution and clearance are increased for increasing body weight and in males. However, the influence of the amount of target antigen on bevacizumab pharmacokinetic variability was not investigated, while several therapeutic antibodies, including rituximab [9, 10] and trastuzumab [11], were shown to have increased clearance with a corresponding target amount and/or tumor burden.

In addition, bevacizumab pharmacokinetics may be influenced by genetic polymorphisms. The receptor FcRn, coded by *FCGRT* (Fc fragment of IgG receptor and transporter), is involved in IgG recycling and is therefore responsible for the long half-life of IgG (around 3 weeks); it is also involved in IgG transcytosis [12]. A variable number of tandem repeat (VNTR) in the promoter region of *FCGRT* was shown to influence FcRn expression, individuals homozygous for three repetitions (VNTR 3/3) presenting more *FCGRT* transcripts than other individuals [13]. The VNTR 3/3 genotype was associated with a better clinical efficacy of intravenous IgG (IVIg) in common variable immunodeficiency patients [14], and a higher tissue distribution of cetuximab [15].

The aim of the present study was to (1) quantify the influence of individual sources of variability, including VEGF concentrations and tumor burden and (2) assess the influence of bevacizumab concentrations on clinical outcomes in mCRC patients.

2 Methods

2.1 Study Design

This ancillary study was part of a French multicenter (Tours, Angers, Bordeaux Saint-André, Bordeaux Haut-Levêque, Reims, Paris Pitié-Salpêtrière, Besançon, Poitiers, Rennes, and Nantes Saint-Herblain) non-comparative, prospective, open-label, observational study (NCT00489697) [37]. The main study was designed to evaluate the usefulness of hepatic contrast-enhanced ultrasound to predict response to bevacizumab-based chemotherapy in patients with mCRC. Patients received four cycles of bevacizumab 5 mg/kg intravenously every 2 weeks in combination with chemotherapy. Tumor assessments were done according to RECIST (Response Evaluation Criteria In Solid Tumors) using spiral computer tomography (CT). Patients were enrolled between January 2007 and December 2010. The end of follow-up was December 2012.

This study was designed in accordance with legal requirements and the Declaration of Helsinki and was approved by the ethics committee of Tours University Hospital, Tours, France. All patients gave written informed consent to participate in this study, including constitutional genetic analyses. Eligible patients (18–80 years old) had histologically confirmed mCRC with at least one hepatic metastasis detected by ultrasonography, a life expectancy of more than 2 months, a World Health Organization (WHO) performance status of two or less and were treated with first-line treatment by a bevacizumab-based chemotherapy.

2.1.1 Bevacizumab Concentrations

Blood samples were collected to measure bevacizumab serum concentrations before and 24 h after the first infusion, before the second and third infusions, and 2 months after the beginning of the treatment. Bevacizumab serum concentrations were measured by a validated enzyme-linked immunosorbent assay (ELISA) technique [16]. The limit of detection was 0.033 mg/L, the lower limit of quantification was 0.61 mg/L, and the upper limit of quantification was 75 mg/L. Quality controls were 3.1, 30.9, and 61.9 mg/L, respectively. Corresponding intra- and inter-assay precision and bias were <20 %. All bevacizumab concentrations were measured at CNRS (Centre National de la Recherche Scientifique) UMR (unité mixte de recherche) 7292, Tours, France.

2.1.2 Other Laboratory Analyses

Blood samples were collected 1–8 days before the first bevacizumab infusion. Baseline VEGF plasma concentrations were measured with a commercial ELISA kit (R&D Systems, Minneapolis, MN, USA). The methodology used for the determination of baseline serum carcinoembryonic antigen (CEA) levels was at the discretion of each site. VNTR polymorphism in the promoter region of *FCGRT* consists in one to five repeats of a 37 bp motif (VNTR); it was measured as previously described [14]. Concentrations of VEGF and *FCGRT* genotyping were assessed at CNRS UMR 7292.

2.2 Pharmacokinetic Analysis

2.2.1 Software

Pharmacokinetic data were analyzed by a population approach using the non-linear mixed-effects program MONOLIX 4.2.2 software (Lixoft, Antony, France), which combines the stochastic approximation expectation-maximization (SAEM) algorithm and a Markov Chain Monte-

Carlo procedure for likelihood maximization. To ensure the best possible convergence, 600 K1 and 200 K2 (where K1 and K2 refer to the SAEM procedure of MONOLIX, called ‘iteration kernels’) and simulated annealing were used to impose the convergence of the SAEM algorithm toward the global maximum of the likelihood. The Fisher information matrix was computed using stochastic approximation. The objective function, which is the -2Ln likelihood (-2LL), was computed using importance sampling. Each run was made three times to ensure that the estimated parameters and likelihood remained stable. The random seed was changed between each of the three runs.

2.2.2 Structural Model

Bevacizumab concentrations were described using compartmental pharmacokinetic models. One and two mammillary models with first-order distribution and elimination rate constants were tested. Structural models were compared using Akaike’s information criterion (AIC), defined as: $\text{AIC} = \text{OFV} + 2 \cdot p$, where OFV is the value of the objective function and p is the number of model parameters to be estimated. The OFV was -2LL . The model with the lowest AIC was selected.

2.2.3 Inter-Individual Model

The inter-individual variability of the pharmacokinetic parameters was described using an exponential model: for each parameter, variance was removed if it could not be properly estimated.

2.2.4 Error Model

Additive, proportional, and mixed additive–proportional models were tested. For example, the combined additive–proportional model was implemented as follows:

$$Y_{O,ij} = Y_{P,ij} \cdot (1 + \varepsilon_{\text{prop},ij}) + \varepsilon_{\text{add},ij}$$

where $Y_{O,ij}$ and $Y_{P,ij}$ are observed and predicted j th measurements for the i th patient, respectively, and $\varepsilon_{\text{prop},ij}$ and $\varepsilon_{\text{add},ij}$ are proportional and additive errors, with a mean of 0 and variances σ_{prop}^2 and σ_{add}^2 , respectively.

2.2.5 Model Comparison and Evaluation

Inter-individual and residual models were compared using OFV. From pairs of nested models, the one with the lowest OFV was selected. This was assessed by a likelihood ratio test (LRT) in which the difference in -2LL between two models is assumed to follow a Chi-squared (χ^2) distribution.

Model validation was based on plots of population-predicted and individual-predicted versus observed

bevacizumab concentrations and bevacizumab concentrations versus time, and on graphic inspection of population-weighted residual (PWRES) and individual-weighted residual (IWRES) distributions, prediction-corrected visual predictive checks (pcVPC) [17] and normalized prediction distribution errors (NPDEs) [18].

2.3 Survival Analysis

The aim of the survival analysis was to assess the influence of bevacizumab exposure on overall survival (OS) and progression-free survival (PFS). The primary endpoint was OS, defined as the time from the first infusion of bevacizumab to death from any cause. The secondary endpoint was PFS, defined as the time from the first infusion of bevacizumab to documented disease progression or death from any cause. If a patient had no event (progression and/or death), time was censored at the time of the last known follow-up visit. The time-to-event distribution was first described using the Kaplan-Meier estimator. The hazard ratios (HRs) were estimated by univariate and multivariate Cox proportional hazards regression models. Survival analyses were performed using R version 2.14.1 (R foundation for statistical computing, Vienna, Austria).

2.4 Covariate Analysis

2.4.1 Covariates

Individual factors with a potential influence on bevacizumab pharmacokinetics or patient survival were tested as covariates in the models. The tested binary covariates were sex, presence of extra-hepatic (EH) metastases (reference: none vs. at least one), and VNTR polymorphism (reference: genotype 3/3 vs. 2/3 and 3/4). The tested continuous covariates were height, body weight, body surface area (BSA), and baseline serum concentrations of CEA and VEGF. Chemotherapy was not tested as a covariate because the proportions of different chemotherapies were very heterogeneous (Table 1).

The influence of a binary covariate was implemented as follows:

$$\ln(\theta_{TV}) = \ln(\theta_{CAT=0}) + \beta_{CAT=1}$$

where θ is structural parameter, θ_{TV} is the typical value of θ , $\theta_{CAT=0}$ is the value of θ for the reference category, and $\beta_{CAT=1}$ is the parameter that provides the value of θ_{TV} for the other category. Continuous covariates were coded as power functions as follows:

$$\theta_{TV} = \theta_{pop} \cdot (\text{COV}/\text{med}(\text{COV}))^{\beta_{cov}}$$

Table 1 Baseline patient characteristics ($n = 130$)

Characteristic	Median	Interquartile range
Continuous variables		
Age, years	64	58–72
Weight, kg	68	58–77
Height, cm ($n = 129$)	169	163–174
BSA, cm^2 ($n = 129$)	1.75	1.62–1.93
CEA, $\mu\text{g/L}$ ($n = 119$)	59	12–271
VEGF, ng/L ($n = 125$)	169.6	62.4–335.8
C_{trough} , mg/L ($n = 120$)	15.5	11.4–23.0
Time to progression, months	10	7–15
Delay of follow-up, months	24	14–34
	<i>n</i>	<i>%</i>
Categorical variables		
Sex		
Male	79	61
Female	51	39
WHO PS ($n = 124$)		
0	67	54
1	53	43
2	4	3
Primary tumor site		
Colon	93	72
Rectal	37	28
Resection of the primary tumor		
Yes	48	37
No	82	63
Number of sites of extra-hepatic metastases ($n = 128$)		
0	75	59
1	28	22
2	8	6
3	6	5
4	7	5
5	4	3
FCGRT VNTR ($n = 128$)		
2/3	29	22
3/3	97	75
3/4	2	2
Combined chemotherapy		
FOLFIRI	94	73
FOLFOX	25	19
LV5FU2	8	6
Others	3	2

BSA body surface area, CEA carcinoembryonic antigen, C_{trough} serum trough concentration of bevacizumab before the second infusion, FOLFIRI oxaliplatin, fluorouracil, and leucovorin, FOLFOX irinotecan, fluorouracil, and leucovorin, LV5FU2 leucovorin and fluorouracil, VEGF vascular endothelial growth factor, VNTR variable number tandem of repeat, WHO PS World Health Organization performance status

except for VEGF, which was coded as follows:

$$\ln(\theta_{TV}) = \ln(\theta_{pop}) + \beta_{COV} \cdot COV$$

where θ_{pop} is the population value for θ , COV is the continuous covariate value, $\text{med}(\text{COV})$ is the median value of COV, and β_{COV} is the parameter quantifying the relationship between COV and θ_{TV} .

The influence of each of these covariates was tested (1) on pharmacokinetic parameters (central volume of distribution [V_C] and first-order elimination rate constant [k_{10}]) and (2) on survival models.

2.4.1.1 Pharmacokinetic Endpoints The association of bevacizumab pharmacokinetics with both OS and PFS was tested using each sampling time as the pharmacokinetic endpoints. Each pharmacokinetic endpoint was dichotomized according to the median value, and the group with values less than or equal to the median was considered as the reference.

2.4.2 Management of Missing Values

Only variables with less than 10 % missing values were analyzed. Missing values of continuous variables were replaced by the corresponding median value of the total population. The missing categorical variables were substituted by the most represented class. Missing values of bevacizumab concentrations were replaced by concentrations estimated by the pharmacokinetic model at the corresponding time. Variables with more than 10 % missing values were baseline lactate dehydrogenase and serum albumin concentrations and leukocyte, platelet, and lymphocyte counts, and were therefore not assessed.

2.4.3 Covariate Model Building

Selection of competing models was based on the LRT for pharmacokinetics and on Wald's tests for survival analysis. The influence of patient characteristics (covariates) was assessed in two steps:

1. *Univariate step* The influence of each factor on pharmacokinetic parameters associated with inter-individual variability was tested. Covariates were separately included into the base model. Covariates showing a significant influence ($p < 0.1$ and $p < 0.05$ for pharmacokinetic and Cox models, respectively) were kept for the multivariate step.
2. *Multivariate step* A forward and backward stepwise analysis was performed: the covariates of the full model were added and then removed one by one. Covariates whose removal resulted in a statistically significant LRT or Wald's test (LRT $p < 0.05$ and

Wald's test $p < 0.05$ for pharmacokinetic and Cox models, respectively) were retained in the model.

Structural parameters and pharmacokinetic endpoints (bevacizumab concentrations and area under the plasma concentration–time curve [AUC]) were plotted against covariate values.

3 Results

3.1 Patients

This study included 137 patients with mCRC. Pharmacokinetics were not assessable because no sample was collected for seven patients. Seventy-nine patients (61 %) were male, with a median age of 64 years; 93 patients (72 %) had a colon cancer, primary cancers were not resected in 82 patients (63 %), and 75 (55 %) had liver-only metastases (Table 1). The chemotherapy associated with bevacizumab was FOLFIRI (oxaliplatin, fluorouracil, and leucovorin) in 94 patients (73 %). Eight patients did not receive four injections of bevacizumab and therefore were not eligible for survival analysis. The survival analysis was made in the remaining 122 patients (Fig. 1). Median follow-up was 24 months.

3.2 Pharmacokinetic Analysis

A total of 486 bevacizumab serum concentrations (mean of four samples per patient) were measured in the 130 evaluable patients (Fig. 1). Bevacizumab concentrations were best described with a two-compartment model. The first modelling attempts revealed that better model performances were obtained by estimating V_C , k_{10} , and the first-order central to peripheral (k_{12}) and peripheral to central (k_{21}) distribution rate constants as structural parameters rather than estimating central (V_C) and peripheral (V_P) volumes of distribution, and systemic (CL) and intercompartmental (Q) clearances, considered as secondary parameters. The inter-individual variances of k_{12} and k_{21} were not estimable (shrinkage >70 %) and were therefore fixed to 0. The 'post hoc' distribution of CL was obtained using individual CL calculated using post hoc estimates of V_C and k_{10} . The best error model was mixed additive–proportional. Plots of model-predicted versus observed concentrations showed satisfactory prediction of bevacizumab concentrations (Fig. 2). The PWRES, IWRES, NPDE, and pcVPC plots showed that there was no obvious model misspecification (Fig. 2). The pharmacokinetic parameters were estimated with good precision (Table 2). The influence of covariates was tested on V_C and k_{10} . Estimates (inter-individual standard deviation) of the

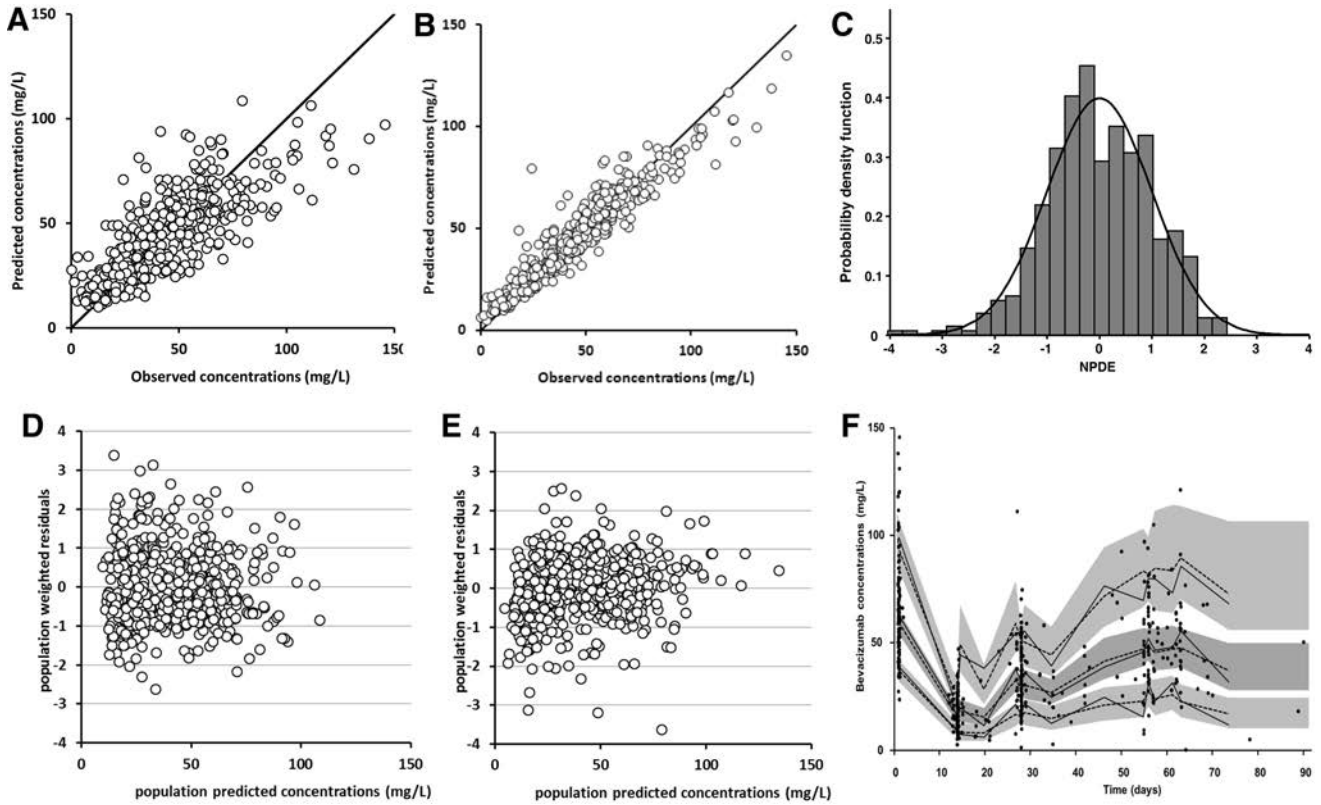


Fig. 1 Diagnostic plots of the pharmacokinetic model: **a** observed vs. population model-predicted bevacizumab concentrations; **b** observed vs. individual model-predicted bevacizumab concentrations; **c** normalized prediction distribution errors vs. gaussian law; **d** population weighted residuals vs. population-predicted bevacizumab concentrations; **e** individual weighted residuals vs. individual-predicted

bevacizumab concentrations; **f** prediction-corrected visual predictive check (observed concentrations [black circles], theoretical [dashed bold lines], empirical [continuous thin lines] percentiles [from bottom to top: 10, 50, and 90 % percentiles] and prediction intervals (shaded areas are, from bottom to top: 10, 50, and 90 % prediction intervals, respectively). NPDE normalized prediction distribution error

base model were $V_C = 4.6 \text{ L}$ (0.34), $k_{10} = 0.050 \text{ day}^{-1}$ (0.39), $k_{12} = 0.15 \text{ day}^{-1}$ (-), and $k_{21} = 0.09 \text{ day}^{-1}$ (-). Secondary parameters were $CL = 0.19 \text{ L/h}$ (0.60), $V_P = 7.7 \text{ L}$ (-), and $Q = 0.69 \text{ L/h}$ (-).

During the univariate step, sex, VNTR polymorphism, height, body weight, BSA, and baseline CEA concentrations were found to significantly influence V_C . However, height, body weight, and BSA were correlated and therefore could not be kept together in the final model. Since height led to a larger reduction of objective function value (OFV) than body weight and BSA, height was kept in the model. In addition, sex, height, body weight, BSA, baseline CEA and VEGF concentrations, and presence of EH metastases significantly influenced k_{10} .

In the final multivariate model, V_C was found to increase with height and to be higher in VNTR 3/3 patients ($p = 10^{-10}$ and 0.039, respectively), whereas k_{10} was found to increase with baseline CEA and VEGF concentrations and to be higher in patients with EH metastases

($p = 0.00029$, 0.014, and 0.011, respectively; Table 2; Fig. 3). The final model was written as follows:

$$V_{C,TV} = V_{C,pop} \cdot \left(\frac{HT}{\text{med}(HT)} \right)^{\beta_1} \cdot e^{\beta_2 \cdot \text{VNTR}}$$

$$k_{10,TV} = k_{10,pop} \cdot e^{\beta_3 \cdot \text{EH}} \cdot e^{\beta_4 \cdot \text{VEGF}} \cdot \left(\frac{\text{CEA}}{\text{med}(\text{CEA})} \right)^{\beta_5}$$

where $V_{C,TV}$ is the typical value of V_C , $V_{C,pop}$ is the population value of V_C , HT is height, β_1 is the coefficient of association of HT and V_C , $\text{med}(HT)$ is the median value of HT, VNTR is variable number tandem repetition (reference: 3/3 vs. 2/3 and 3/4), β_2 is the coefficient giving the V_C value for genotypes 2/3 and 3/4, $k_{10,TV}$ is the typical value of k_{10} , $k_{10,pop}$ is the population value of k_{10} , EH is the number of extra-hepatic metastases (reference: none vs. at least one), β_3 is the coefficient giving the k_{10} value for at least one EH metastasis, VEGF is the baseline VEGF concentration, β_4 is the coefficient of association of k_{10} and

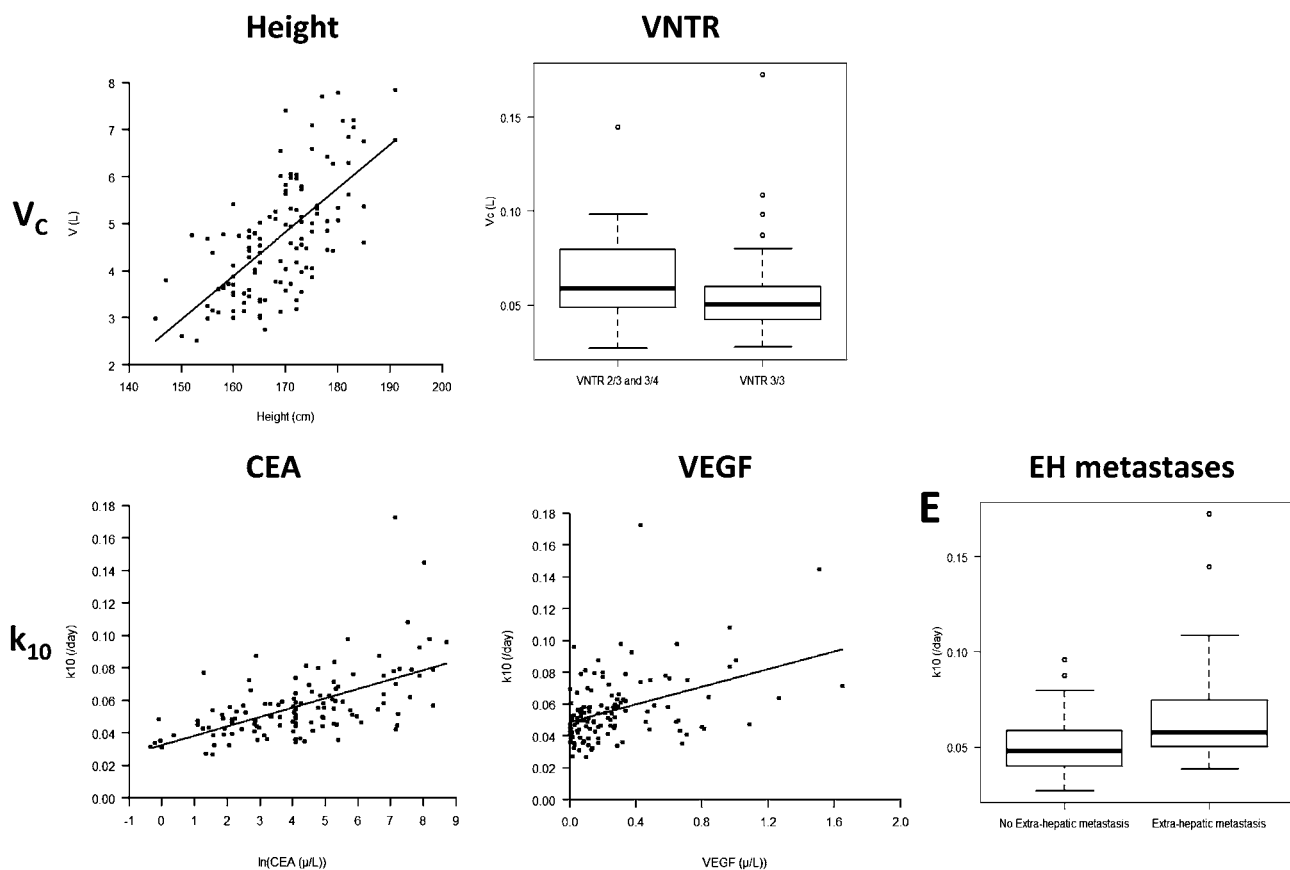


Fig. 2 Individual pharmacokinetic parameter estimates vs. covariates: central volume of distribution (V_C) vs. height (**a**) and vs. variable number tandem repeat (VNTR) genotype (**b**); elimination rate constant (k_{10}) vs. logarithm of carcinoembryonic antigen (CEA)

value (**c**), circulating vascular endothelial growth factor (VEGF) concentration (**d**) and presence or absence of extra-hepatic (EH) metastases (**e**). Horizontal lines of boxplots represent, from bottom to top, 5th, 25th, 50th, 75th, and 95th percentiles of V_C and k_{10}

VEGF, CEA is the baseline CEA concentration, and β_5 is the coefficient of association of k_{10} and CEA. Associations of pharmacokinetic parameters (V_C , k_{10} , and CL) with covariates are given in Fig. 3. Of note, contrary to other covariates, including CEA concentrations and presence of EH metastases, baseline VEGF concentrations had no visible effect on the serum trough concentration of bevacizumab before the second infusion (C_{trough}). Variations of pharmacokinetic parameters without each covariate (reference vs. other category) were as follows:

- $V_C = 4.7$ and 4.2 L for VNTR 3/3 vs. other genotypes, respectively;
- $k_{10} = 0.045$ and 0.055 day^{-1} for absence vs. presence of EH metastases, respectively;
- $CL = 0.22$ and 0.32 L/day for absence or presence of EH metastases, respectively.

Variations of pharmacokinetic parameters with each continuous covariate (first quartile, median, and third quartile covariate values; Table 1) were as follows:

- With height, $V_C = 3.7$, 4.2 , and 4.6 L, respectively;
- with baseline VEGF concentrations, $k_{10} = 0.046$, 0.048 , and 0.050 day^{-1} , respectively, and $CL = 0.26$, 0.27 , and 0.28 L/day , respectively;
- with baseline CEA concentrations, $k_{10} = 0.040$, 0.045 , and 0.050 day^{-1} , respectively, and $CL = 0.23$, 0.26 , and 0.29 L/day , respectively.

The pharmacokinetic model was used to compute bevacizumab concentrations 14 days after the first infusion of bevacizumab (C_{trough}). For a ‘reference’ patient, with VNTR polymorphism 2/3 or 3/4, a low baseline CEA concentration ($10 \mu\text{g/L}$), and no antigenic mass (no EH metastases and undetectable baseline VEGF concentrations), the predicted C_{trough} value was 21.2 mg/L . This concentration was lower for VNTR 3/3 (18.9 mg/L), and an additional decrease occurred if VNTR 3/3 patients had a CEA concentration of $100 \mu\text{g/L}$ (18.5 mg/L), if EH metastases were present (16.9 mg/L), and if the baseline VEGF concentration was 1000 ng/L (14.2 mg/L).

Table 2 Pharmacokinetic parameter estimates

Parameter (unit)	Estimate	RSE (%)	<i>p</i> value ^a
V_C (L)	4.2	6	
Height (cm) on V_C	3.2	14	10^{-10}
VNTR 3/3 on V_C	0.12	51	0.039
k_{10} (/day)	0.045	10	
CEA ($\mu\text{g/L}$) on k_{10}	0.072	28	0.00029
EHM on k_{10}	0.2	41	0.011
VEGF ($\mu\text{g/L}$) on k_{10}	0.3	40	0.014
k_{12} (/day)	0.16	17	
k_{21} (/day)	0.1	16	
CL (L/day)	0.19		
V_P (L)	7.7		
Q (L/day)	0.69		
ω_{V_C}	0.23	9	
$\omega_{k_{10}}$	0.32	12	
ω_{CL}	0.60		
σ_{add} (mg/L)	2.8	17	
σ_{prop} (%)	0.19	7	

Estimates (inter-individual standard deviation) of the base model were $V_C = 4.6$ L (0.34), $k_{10} = 0.050$ day⁻¹ (0.39), (-), $k_{12} = 0.15$ day⁻¹ (-), and $k_{21} = 0.09$ day⁻¹ (-). Secondary parameters (CL, V_P , Q , and ω_{CL}) were calculated from primary parameters and reported in the table

CEA serum carcinoembryonic antigen concentration, CL clearance, EHM number of extra-hepatic metastases, k_{10} first-order elimination rate constant, k_{12} first-order central to peripheral transfer rate constant, k_{21} first-order peripheral to central transfer rate constant, Q intercompartmental clearance, RSE relative standard error, V_C central volume of distribution, VEGF serum vascular endothelial growth factor concentration, VNTR variable number of tandem repeat polymorphism, V_P peripheral volume of distribution, ω inter-individual standard deviation, σ_{add} additive error, σ_{prop} proportional error

^a Obtained with a likelihood ratio test

3.3 Survival Analysis

3.3.1 Overall Survival

Median OS [95 % confidence interval (CI)] was 24.4 months [20.6–31.3]. The univariate analysis showed that among the different sampling times, the C_{trough} was the most strongly related to OS, and identified the presence of EH metastases, high baseline VEGF and CEA concentrations, and low C_{trough} values as risk factors of death (Table 3). The multivariate analysis showed that only a high baseline CEA concentration and $C_{\text{trough}} \leq 15.5$ mg/L were independent risk factors of death (HR = 1.15 [1.04–1.27] and 1.90 [1.20–3.00], respectively; Table 3). Median OS was 17.3 months for patients with a $C_{\text{trough}} \leq 15.5$ mg/L and 33.9 months for patients with a $C_{\text{trough}} > 15.5$ mg/L (Fig. 4).

3.3.2 Progression-Free Survival

Median PFS [95 % CI] was 10.6 months [9.6–12.1]. The univariate analysis identified the presence of EH metastases, high baseline VEGF and CEA concentrations, and low C_{trough} values as risk factors of progression (Table 4). The multivariate analysis showed that only baseline VEGF concentration and $C_{\text{trough}} \leq 15.5$ mg/L were independent risk factors for progression (HR = 2.25 [1.26–4.00] and 1.76 [1.20–2.58], respectively; Table 4). Median PFS was 8.7 months for patients with a $C_{\text{trough}} \leq 15.5$ mg/L and 13.2 months for patients with a $C_{\text{trough}} > 15.5$ mg/L (Fig. 4).

4 Discussion

This is the first study showing a relationship between bevacizumab serum concentrations and both PFS and OS. In addition, even if bevacizumab pharmacokinetics in mCRC have been described using population compartmental modeling before [7, 8, 16], our study is the first to report the influence of both plasma VEGF concentrations (i.e., the circulating target antigen of bevacizumab) and tumor burden, as assessed by baseline CEA concentration, on bevacizumab pharmacokinetic variability.

Population pharmacokinetic modeling was previously used in two studies including patients with diverse solid tumors [7, 8]. However, in these studies, the description of inter-individual variability did not investigate the influence of the target antigen burden, which was reported to influence the pharmacokinetics of several therapeutic antibodies [10, 11]. Even if the inter-individual variability of transfer rate constants between compartments was not evaluable, the inter-individual distribution of the V_C and first-order k_{10} were satisfactorily described, allowing the quantification of the influence of target antigen burden on bevacizumab pharmacokinetics. An increase in V_C is associated with decreased bevacizumab concentrations, and an increase in k_{10} corresponds with a faster elimination of bevacizumab and therefore a shorter elimination half-life.

Since body size (e.g., weight and BSA) was reported to influence the pharmacokinetics of most therapeutic antibodies including bevacizumab [7, 8], the influence of height, body weight, and BSA were tested and were all found to be related to V_C . Surprisingly, the strongest influence was observed for height, a finding that has never been reported previously for a therapeutic antibody. The influence of weight or BSA on V_C is usually considered to be due to a correlation between body weight or BSA and serum volume [19]. However, height may be a better predictor for bevacizumab elimination than weight, because it is highly correlated with ideal (fat-free) body weight,

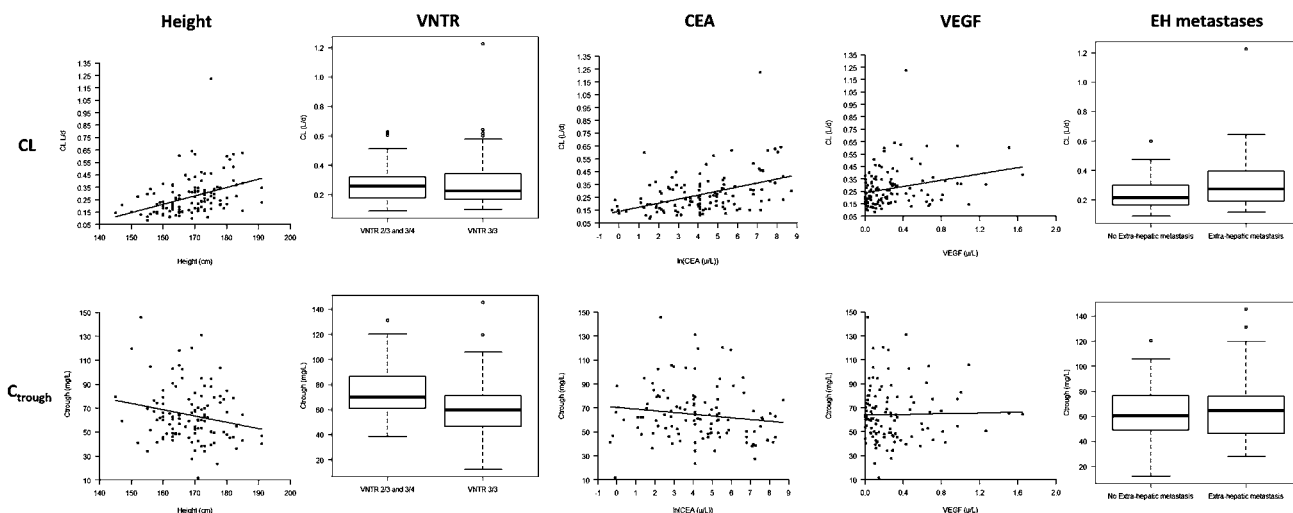


Fig. 3 Individual post hoc clearance (CL , *top*) and observed bevacizumab trough concentrations (C_{trough} , *bottom*) vs. height, variable number tandem repeat (VNTR) genotype, logarithm of carcinoembryonic antigen (CEA) value, circulating vascular

endothelial growth factor (VEGF) concentration, and presence or absence of extra-hepatic (EH) metastases. *Horizontal lines of boxplots* represent, from *bottom* to *top*, 5th, 25th, 50th, 75th, and 95th percentiles of CL and C_{trough}

Table 3 Univariate and multivariate assessment of risk factors for death ($n = 122$)

Covariate	HR	95 % CI	<i>p</i> Value
Univariate analysis			
Age	1.01	0.99–1.03	0.5
Sex (male)	1.45	0.93–2.25	0.097
Extra-hepatic metastases	1.62	1.06–2.46	0.025
VNTR polymorphism (3/3 vs. 2/3 and 3/4)	0.66	0.42–1.05	0.080
VEGF, $\mu\text{g/L}$	2.09	1.10–4.00	0.025
$\log(\text{CEA})$, $\mu\text{g/L}$	1.21	1.1–1.34	0.0001
$C_{\text{trough}} \leq 15.5$ mg/L	2.34	1.53–3.60	10^{-4}
$C_{24 \text{ h}}$	1.15	0.76–1.75	0.501
$C_{28 \text{ d}}$	1.43	0.95–2.21	0.0825
$C_{56 \text{ d}}$	1.64	1.08–2.52	0.0249
Multivariate analysis			
$\log(\text{CEA})$, $\mu\text{g/L}$	1.15	1.04–1.27	0.007
$C_{\text{trough}} \leq 15.5$ mg/L	1.90	1.20–3.00	0.006

$C_{24 \text{ h}}$, $C_{28 \text{ d}}$, $C_{56 \text{ d}}$ bevacizumab serum concentration 24 h, 28 days, and 56 days after the first infusion, respectively, CEA serum carcinoembryonic antigen concentration, CI confidence interval, C_{trough} serum trough concentration of bevacizumab before the second infusion, HR hazard ratio, $VEGF$ serum vascular endothelial growth factor concentration, $VNTR$ variable number of tandem repeat polymorphism

especially in advanced-stage cancer patients who are often underweight. The stronger influence of height than weight or BSA on bevacizumab pharmacokinetic parameters needs to be confirmed in further studies.

In the present study, we found a trend toward a higher V_C for VNTR 3/3 patients than other genotypes and therefore lower bevacizumab concentrations than in other patients. This suggests that the distribution of bevacizumab is affected by the $FCGRT$ VNTR genetic polymorphism,

possibly because of higher transcytosis, as previously reported for cetuximab [15].

We observed an increase in k_{10} with baseline VEGF concentrations. This may be explained by target-mediated drug disposition (TMDD), a mechanism of elimination that has been reported for several monoclonal antibodies [19, 20]. Increased levels of the target antigen result in increased ‘consumption’ of the therapeutic antibody, leading to an increase in its target-mediated elimination,

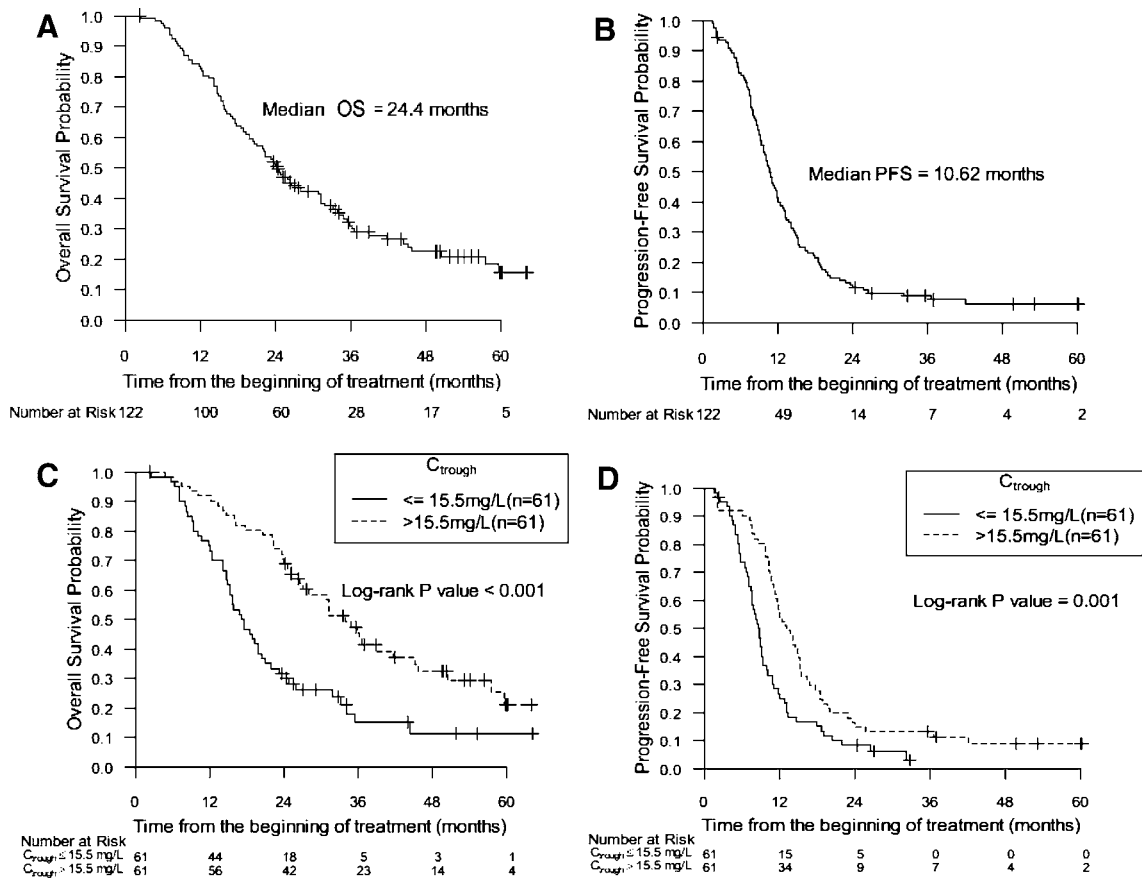


Fig. 4 Kaplan-Meier curves of **a** overall survival and **b** progression-free survival, and **c** overall survival and **d** progression-free survival according to bevacizumab trough concentration (≤ 15.5 vs. >15.5 mg/

L). C_{trough} bevacizumab trough concentration, OS overall survival, PFS progression-free survival

Table 4 Univariate and multivariate assessment of risk factors for progression ($n = 122$)

Covariate	HR	95 % CI	p value
Univariate analysis			
Sex (male)	1.45	0.98–2.14	0.063
Age, years	1.01	0.99–1.02	0.49
Extra-hepatic metastases	1.48	1.02–2.17	0.041
VNTR polymorphism (3/3 vs. 2/3 and 3/4)	0.72	0.47–1.10	0.13
VEGF, $\mu\text{g/L}$	2.51	1.42–4.43	0.0015
log(CEA), $\mu\text{g/L}$	1.1	1.02–1.19	0.017
$C_{trough} \leq 15.5$ mg/L	1.86	1.27–2.72	0.0013
$C_{24\text{ h}}$	0.99	0.68–1.44	0.968
$C_{28\text{ d}}$	1.32	0.90–1.92	0.15
$C_{56\text{ d}}$	2.52	1.02–4.02	0.0551
Multivariate analysis			
VEGF, $\mu\text{g/L}$	2.25	1.26–4.00	0.0058
$C_{trough} \leq 15.5$ mg/L	1.76	1.20–2.58	0.0039

$C_{24\text{ h}}$, $C_{28\text{ d}}$, $C_{56\text{ d}}$ bevacizumab serum concentration 24 h, 28 days, and 56 days after the first infusion, respectively, CEA serum carcinoembryonic antigen concentration, CI confidence interval, C_{trough} serum trough concentration of bevacizumab before the second infusion, HR hazard ratio, $VEGF$ serum vascular endothelial growth factor concentration, $VNTR$ variable number of tandem repeat polymorphism

and therefore in its ‘total’ elimination (k_{10}), which has been previously reported for rituximab [9] and trastuzumab [11]. For bevacizumab, a previous study described bevacizumab pharmacokinetics accounting for VEGF-mediated bevacizumab disposition using a TMDD model [21], but this study accounted only for circulating VEGF concentrations. However, the absence of a visible effect of the circulating VEGF concentration on C_{trough} may indicate the effect of other covariates, including CEA concentrations and the presence of EH.

The circulating VEGF concentration constitutes only a part of the total amount of VEGF that may be targeted by bevacizumab. Indeed, VEGF concentrations were shown to be higher inside the tumor and in its microenvironment than in serum [22], and the relationship between tumor tissue and circulating VEGF levels remains unclear [23]. Therefore, the total amount of VEGF should increase with tumor volume. Interestingly, k_{10} was also influenced by CEA concentrations, a biomarker known to increase with tumor volume [24]. As for baseline circulating VEGF, the influence of CEA/tumor volume on bevacizumab elimination may be explained by antibody ‘consumption’. An increase of tumor volume should lead to an increase in the VEGF amount and therefore in bevacizumab target-mediated elimination. Similarly, the influence of the presence of EH metastases on k_{10} may be explained by an increased amount of tumor VEGF and therefore an increased bevacizumab target-mediated elimination. In conclusion, circulating VEGF concentrations, the baseline CEA concentration, and the number of hepatic metastases may provide information on the total burden of bevacizumab target antigen independent of the circulating VEGF level. Baseline circulating VEGF and CEA concentrations may be interpreted as steady-state concentrations, resulting from an equilibrium between input and output, and therefore provide a global information of circulating target amount. Therefore, the decrease in these concentrations during treatment and the re-increase after the end of treatment should lead to a variable target-mediated elimination of bevacizumab, which cannot be described in this study because only baseline concentrations were available.

Surprisingly, the elimination half-life of bevacizumab was higher in the present study (44.6 days) than has been previously reported (around 21 days [7, 8, 16]). Such a large difference in the elimination half-life has been reported for rituximab, an anti-CD20 monoclonal antibody used in B lymphocyte malignancies. The elimination half-life of rituximab was found to be around 21 days in follicular non-Hodgkin lymphoma [25] and rheumatoid arthritis [26] patients, but was 37 days in diffuse large B cell lymphoma patients [27]. In addition, discrepancies have been reported for trastuzumab, an anti-HER2 monoclonal antibody. In metastatic breast cancer patients, Bruno

et al. [11] reported higher V_p and elimination half-life values than were reported in non-metastatic breast cancer patients [28], with an increased target-mediated clearance in both studies. A possible explanation of this phenomenon may be the retention of monoclonal antibodies by their tumor target antigen. Being retained by VEGF inside and near the tumor, bevacizumab may be slowly released from the tumor into the serum. Retention and slow release of bevacizumab by tumor VEGF could explain the higher elimination half-life associated with lower bevacizumab serum concentrations. These differences may be due not only to the amount of target antigen, but also the turnover, density, and distribution of these targets, as well as differences in bevacizumab–target complex elimination. A TMDD model could not be tested in the present study because circulating VEGF and CEA concentrations were not available across time, and this hypothesis needs further investigation.

To assess the relationship between bevacizumab concentration and clinical response, we used OS and PFS as clinical endpoints. Exposure to bevacizumab was quantified using measured bevacizumab concentrations. A bevacizumab $C_{\text{trough}} > 15.5$ mg/L was associated with almost doubled OS (33.9 vs. 15.5 months; $p = 0.006$) and PFS (13.2 vs. 8.7 months; $p = 0.0039$). Of note, a cut-off value may be calculated using the Hothorn-Zeileis method [29] and in our study is 16 mg/L, a value very close to the median value. Increased drug exposure was shown to be associated with better clinical response and/or survival for other monoclonal antibodies, such as rituximab [30], cetuximab [6, 31], and trastuzumab [32], but the influence of the bevacizumab dose on clinical response in mCRC patients was only evoked in previous studies. A small phase II study of bevacizumab combined with fluorouracil and leucovorin suggested that a bevacizumab dose of 10 mg/kg was more effective than 5 mg/kg [33]. The efficacy of two different bevacizumab doses in 387 patients with mCRC has been evaluated in a phase III study [34]. This study observed only a trend for improved PFS in the high-dose arm of the subgroup of patients with baseline CEA >20 ng/mL and metastasis size ≥ 5 cm [34]. More recently, a study in mCRC patients showed that the number of bevacizumab doses was related to post-progression survival [5]. Because a higher number of doses should be associated with increased exposure to bevacizumab, this study suggests that bevacizumab exposure is associated with better survival, but serum concentrations of the monoclonal antibody were not measured.

Theoretically, the AUC is the ‘gold standard’ to describe the concentration–effect relationship. In this study, the AUC from time zero to infinity (AUC_{∞}) was significantly associated (see the figure in the Electronic Supplementary Material) with both OS (HR = 2.43 [95 % CI 1.47–2.76];

$p = 0.0001$) and PFS (HR = 1.96 [95 % CI 1.28–3.03]; $p = 0.0017$). However, an accurate estimation of AUC necessitates rich pharmacokinetic data, which is difficult to obtain in clinical routine.

Our results suggest that increasing the exposure to bevacizumab may increase its efficacy in mCRC patients. One response to this might be to increase bevacizumab dose in patients for whom the C_{trough} is below 16 mg/L. In addition, our results may be used to design a therapeutic drug monitoring tool that allows estimation of the optimal dose for each patient individually. However, the relevance of C_{trough} threshold needs to be shown in a randomized clinical trial; in addition, this threshold will be suitable in a population similar to that in the present study (mCRC patients with hepatic metastases), but may be different in patient groups with a different prognosis. Baseline concentrations of CEA and VEGF were significantly associated with decreased OS and PFS, respectively, a finding confirming that these biomarkers are prognostic factors of disease progression independent of anti-cancer treatments [35, 36]. Of note, the influence of these biomarkers on bevacizumab pharmacokinetics and on survival are independent: if the association between decreased exposure to bevacizumab (and therefore of decreased clinical response) and increased CEA and VEGF concentrations may be explained by bevacizumab ‘consumption,’ the influence of these biomarkers on survival independent of bevacizumab concentrations should reflect the influence of the disease itself on its sensitivity to bevacizumab.

5 Conclusion

Our study is the first to show (1) an influence of both serum VEGF and tumor burden on bevacizumab pharmacokinetics, which may be explained by TMDD, and (2) a relationship between serum concentrations of bevacizumab and clinical efficacy, as patients with a bevacizumab concentration above 15 mg/L 14 days after the first administration of bevacizumab have longer survival.

6 Clinical Implications

With the current dosage regimen of bevacizumab, patients with high VEGF concentrations and tumor burden may be underexposed to bevacizumab and as a result may be at risk of shorter survival than other patients. Therefore, the dosing regimen of bevacizumab may be optimized: patients with low bevacizumab exposure, as assessed by measuring its serum concentration before the second infusion, may benefit from increased bevacizumab doses. This knowledge

may lead to the design of a dosing optimization regimen for each patient individually.

Compliance with Ethical Standards

Acknowledgments This study was funded by the “Institut National du Cancer” (INCa), the “Société Nationale Française de Gastro-Entérologie” (SNFGE), Roche Laboratories, and “Cancéropôle Grand-Ouest.” Measurement of bevacizumab serum concentrations was carried out within the CePiBac (Centre Pilote de suivi Biologique des traitements par Anticorps) platform. CePiBac is supported by the French Higher Education and Research ministry under the program “Investissements d’avenir” Grant Agreement: LabEx MAbImprove ANR-10-LABX-53-01.

Disclosure Morgane Caulet was funded by Roche for the present study. Olivier Bouché reports receiving personal fees from Roche, Merck Serono, and Lilly. Christophe Borg reports grants from Roche and grants and personal fees from Sanofi. Jean-Yves Douillard has participated in steering committees on behalf of Amgen and Bayer, participated in advisory boards, symposia and acted as a consultant for Amgen, Merck Serono, and Roche, participated in an advisory board for Boehringer Ingelheim and Sanofi, and received research funding from Merck Serono. Gilles Paintaud reports grants from Novartis, Roche Pharma, Genzyme, MSD, Servier, and Pfizer. Thierry Lecomte, Jérôme Rollin, Valérie Gouilleux-Gruart, Nicolas Azzopardi, Julie Léger, Sylvain Manfredi, Denis Smith, Olivier Capitain, Aurélie Ferru, Driffa Moussata, Eric Terrebbonne, and David Ternant have no conflict of interest to disclose.

Author Contributions Morgane Caulet analyzed and interpreted the data, and wrote the manuscript. Thierry Lecomte designed the study and was the principal investigator of the study. Olivier Bouché acquired data and reviewed the manuscript. Jérôme Rollin acquired data and reviewed the manuscript. Valérie Gouilleux-Gruart acquired data and reviewed the manuscript. Nicolas Azzopardi participated in data interpretation and reviewed the manuscript. Julie Léger participated in data interpretation and reviewed the manuscript. Christophe Borg acquired data and reviewed the manuscript. Jean-Yves Douillard acquired data and reviewed the manuscript. Sylvain Manfredi acquired data and reviewed the manuscript. Denis Smith acquired data and reviewed the manuscript. Olivier Capitain acquired data and reviewed the manuscript. Aurélie Ferru acquired data and reviewed the manuscript. Driffa Moussata acquired data and reviewed the manuscript. Eric Terebonne acquired data and reviewed the manuscript. Gilles Paintaud designed the study and reviewed the manuscript. David Ternant developed the methodology of data analysis and reviewed the manuscript. As the corresponding author, he confirms that all authors had full access to all data in the study and takes final responsibility for the content of the article.

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