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Oncology

Evaluation of serum mid-infrared spectroscopy as new prognostic marker for first-line bevacizumab-based chemotherapy in metastatic colorectal cancer[☆]



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ABSTRACT

Background and aims: Bevacizumab-based chemotherapy is a recommended first-line treatment for metastatic colorectal cancer (mCRC). Robust biomarkers with clinical practice applicability have not been identified for patients with this treatment. We aimed to evaluate the prognostic yield of serum mid-infrared spectroscopy (MIRS) on patients receiving first-line bevacizumab-based chemotherapy for mCRC. **Methods:** We conducted an ancillary analysis from a multicentre prospective study (NCT00489697). All baseline serums were screened by attenuated total reflection method. Principal component analysis and unsupervised k-mean partitioning methods were performed blinded to all patients' data. Endpoints were progression-free survival (PFS) and overall survival (OS).

Results: From the 108 included patients, MIRS discriminated two prognostic groups. First group patients had significantly lower body mass index ($p = 0.026$) and albumin levels ($p < 0.001$), and higher levels of angiogenic markers, lactate dehydrogenase and carcinoembryonic antigen ($p < 0.001$). In univariate analysis, their OS and PFS were shorter with respective medians: 17.6 vs 27.9 months ($p = 0.02$) and 8.7 vs 11.3 months ($p = 0.03$). In multivariate analysis, PFS was significantly shorter ($HR = 1.74, p = 0.025$) with a similar trend for OS ($HR = 1.69, p = 0.061$).

Conclusion: By metabolomic fingerprinting, MIRS proves to be a promising prognostic tool for patients receiving first-line bevacizumab-based chemotherapy for mCRC.

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1. Introduction

Colorectal cancer (CRC) is the third most frequent cancer worldwide and the second most frequent cause of death by cancer in 2018 [1]. Its metastatic evolution (mCRC) is a major shift in tu-

tumour development and survival rates [2]. Sustained neovascularisation promotes tumoral development and stimulates metastatic evolution by specific cell dissemination and pre-metastatic nests establishment [3]. Vascular endothelial growth factor (VEGF) is the principal trigger of the pro-angiogenic switch [4]. Anti-angiogenic treatments are part of the therapeutic arsenal for mCRC. Bevacizumab is a humanised monoclonal antibody binding with all VEGF-A isoforms limiting interaction with VEGF receptors [5]. Its association with a 5-fluorouracil based bi-chemotherapy in first-line mCRC treatment allows prolonged progression-free survival (PFS) and overall survival (OS) and such combination is the standard of care [6]. Yet, some patients do not benefit from bevacizumab, either due to primary inefficiency or by secondary tumoral resistance mechanisms [7,8]. For the last decade, substantial efforts have been conducted in order to identify predictive and prognostic biomarkers for bevacizumab. Despite encouraging results, studies with contradictory conclusions and the lack of randomized prospective trials limit the validation in clinical practice of these biomarkers [9,10]. The multiplication of potential candidates highlights the complexity of tumoral angiogenic mechanisms and their inter- and intra-individual heterogeneity. Combining several biomarkers seems a more appropriate approach [11,12]. Taking into account the risk of therapeutic failure associated with the cost of monoclonal antibodies, the identification of a method detecting bevacizumab non-responders has become a clinical necessity with an economical requirement [13,14].

Mid-infrared spectroscopy (MIRS) renders the metabolic fingerprint of a sample by detecting prominent biomolecules absorbing infrared radiation [15]. MIRS on serum allows a rapid non-invasive profiling of an individual at low cost and high sensitivity from small samples [16]. It could therefore have a substantial value in diagnostic, prognostic and predictive medicine especially in oncology [15]. This makes it a promising yet barely explored method for mCRC treatment optimisation [15,17].

Our study aimed to evaluate the prognostic potential of MIRS on baseline serum of patients treated by chemotherapy associated with bevacizumab in first line treatment for mCRC.

1.1. Materials and method

1.1.1. Study population

This ancillary study was part of a multicentric prospective study financed by the French National Cancer Institute (INCa) and registered on ClinicalTrials.gov (study NCT00489697; INCA06-FI/STIC-AVASTIN) [18]. It was designed according to the Helsinki Declaration and approved by the French Ethical Committee of Tours University on November 11, 2006. Patients were recruited from January 2007 to January 2011. They all gave their written consent to participate. Eligible and exclusion criteria were described in the original study protocol. Patients needed to have at least one measurable liver metastasis. Every 2 weeks, patients received intravenously 5 mg/kg of bevacizumab associated with a fluoropyrimidine-based chemotherapy. Tumour assessment was done according RECIST v1.1 on CT scans every 12 weeks until progression. End of follow-up was in December 2012.

1.1.2. Data extraction

Clinical data and bevacizumab-related adverse events (BRAE) were collected for each patient. Biological data were obtained when possible and included: *KRAS* (codons 12, 13, 61, and 146) and *BRAF* (Val600Glu) mutational status, carcinoembryonic antigen (CEA), albumin, lactate dehydrogenase (LDH), vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) concentrations. Blood samples were collected in the eight days preceding bevacizumab first infusion. Cut-off values of CEA, albumin, LDH and Ang-2 were respectively fixed at 100 µg/L, 35 g/L, 300 U/L

and 5 µg/L as in previous studies [19–22]. Median VEGF level (200 µg/mL) was fixed as cut-off value.

1.1.3. Mid-infrared spectroscopy

1.1.3.1. Spectral acquisition. All spectral measures were recorded using a LUMOS microspectroscope with a RockSolid™ interferometer (Bruker®). Attenuated total reflection (ATR) technique was carried out on a germanium single reflexion crystal. Absorption wavenumbers were circumscribed within 4000–400 cm⁻¹. Spectral resolution was set to 4 cm⁻¹ with a zero-filing factor of 2 yielding a discrete spectral point spacing of 2 cm⁻¹. For each measure, a 5 µl serum sample was dropped on a single-used microscope slide and at least 5 spectral acquisitions were completed in different zones. Serums were preliminarily dried at 30 °C for 15 min to decrease the water contribution to the signal (dried droplet method).

1.1.3.2. Spectral treatment. Spectral data were analysed within the 3800–800 cm⁻¹ frequency range. Analyses were blinded to patient data. There was no spectral domain eliminated during analysis. Second derivatives were calculated, then smoothed using a Savitzky-Golay moving filter algorithm (width = 11). Spectral data were then processed by vector normalisation. Quality assessment and data homogeneity by outlier identification was investigated by principal component analysis (PCA). Unsupervised k-mean partitioning method was performed to separate individuals in N groups according to the highest inter-/intra-group variance ratio (silhouette function). Visual comparison and mathematical verification for each group spectral means were done to identify discriminative spectral variables. A correlation analysis of these discriminative spectral variables was performed using PCA data. It was graphically represented in a correlation circle.

1.1.4. Statistical analysis

Continuous variables were described by median and interquartile range. Categorical variables were described by frequency. Means were compared using parametrical Student *t*-test in case of normal distribution and non-parametrical Mann-Whitney test otherwise. Continuous variables were tested for normal distribution with the Shapiro-Wilk test. Categorical variables were compared by the Chi-squared (χ^2) test or Fisher's exact test according to sample size.

Primary endpoints were overall survival (OS) and progression-free survival (PFS). They were defined as the time from the first bevacizumab infusion to death from any cause and to disease progression according to RECIST v1.1 respectively. Time was censored at the last known follow-up visit if a patient had no event. Actuarial survival curves were calculated by Kaplan-Meier method and compared by log-rank test. Hazard ratios (HR) and their 95 % confidence intervals (CI) were estimated by univariate and multivariate Cox proportional hazards regression models. All variables related to OS and/or PFS in univariate analysis with a *p*-value <0.2 were integrated in the multivariate analysis by ascending selection. Best objective response rate (bORR) was defined as the percentage of patients who experienced partial or complete response as their best response according to RECIST v1.1 during their follow-up.

For all analyses, *p*-values were two-sided and considered significant when < 0.05. Statistical analysis was performed using R® software version 3.6.1 (R foundation for statistical computing, Vienna, Austria).

2. Results

2.1. Population

Out of the 137 patients included in STIC-Avastin study, 108 were analysed in our ancillary study. Principal reasons for exclusion are

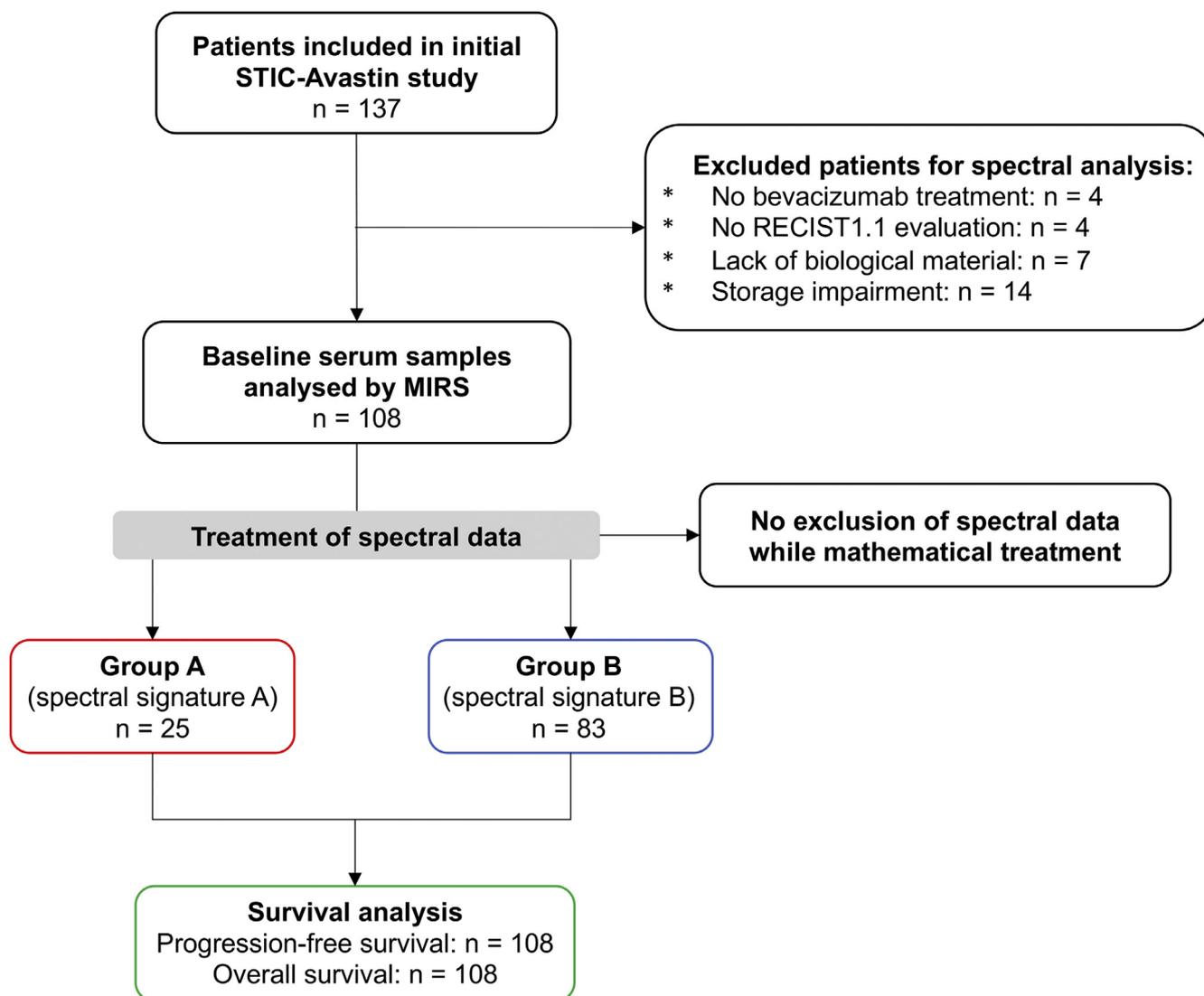


Fig. 1. Flow chart. STIC-Avastin study was a multicentred, non-comparative, prospective, open-labelled study registered on ClinicalTrials.gov (study NCT00489697; INCA06-FT/STIC-AVASTIN). Mid-infrared spectroscopy (MIRS) on baseline serum samples was done on all patients except those not treated with bevacizumab ($n = 4$), not evaluated by RECIST v1.1 criterion ($n = 4$), with insufficient biological material ($n = 7$) or sample storage impairment ($n = 14$). All spectral data were mathematically processed. Two groups of patients were dichotomised, and each defined by a specific spectral signature: group A ($n = 25$) and B ($n = 83$) with respective spectral signature A and B. Survival analysis was carried out on all patients ($n = 108$). Primary endpoints were progression-free survival and overall survival.

mentioned in Fig. 1. Their characteristics are described in Table 1. Median follow-up was 24 months.

2.2. Spectral analysis

Serum MIRS analysis was conducted homogeneously on samples of all 108 patients. All spectra were validated for follow-up treatment. Unsupervised k-mean method separated patients in two groups (A and B) each with a specific spectral signature.

2.2.1. Spectral comparisons of groups A and B

Both group spectral discrimination allowed to identify six significant absorption bands (Fig. 2). These discriminative spectral variables are assigned with methyl or ester group lipids for 2923, 2853 and 1740 cm^{-1} bands; primary amid proteins for 1623 and 1629 cm^{-1} bands; and nucleic acid or saccharide for the 1073 cm^{-1} band (Supplementary Table 1). Taking into account the precision limits of MIRS for millimolar concentrations in serum, it is most likely that the 1073 cm^{-1} band features saccharides rather than less represented circulating nucleic acids.

Correlation analysis of these six variables is represented in Supplementary Figure 1. Two groups of variables are isolated: proteins/sugar (1629, 1633 and 1073 cm^{-1}) and lipids (2923, 2853 and 1740 cm^{-1}). Using the coordinates of these variables according to their principal component, 92.1 % of population variance was characterised. Intra-group variables are strongly correlated whereas both groups are poorly correlated. Comparing spectral means of groups A and B (Fig. 2), lipid variables are more present in patients with signature B. Also, there is an inversion of protein population characterised by a spectral peak shift reflecting global changes in protein's secondary structures suggesting variations in the prominent circulating protein pool. Variations in the sugar spectral domains are less clearly interpretable.

2.2.2. Clinical and biological comparisons of groups A and B

Characteristics of each group are described in Table 1. There were less patients in group A ($n = 25$) than B ($n = 83$). Patients in group A were less overweight ($p = 0.026$), more nutritionally deficient ($p < 0.001$), less fit ($p = 0.054$) and had more frequent surgical tumoral removal ($p = 0.002$). They also had higher base-

Table 1
Description of patients' characteristics.

Characteristics	Global: n = 108 Median (IQ) Number (%)	Group A: n = 25; 23.1 % Median (IQ) Number (%)	Group B: n = 83; 76.9 % Median (IQ) Number (%)	p value
Age (years)	65 (13.8)	63 (12.0)	66 (12.5)	0.19
≤ 70	73 (67.6)	19 (76.0)	54 (65.1)	
> 70	35 (32.4)	6 (24.0)	29 (34.9)	
Gender				0.63
Male	69 (63.9)	17 (68.0)	52 (62.7)	
Female	39 (36.1)	8 (32.0)	31 (37.4)	
BMI (kg/m ²)	24.1 (4.97)	23.2 (4.44)	24.5 (5.44)	0.026*
< 25	66 (61.1)	18 (72.0)	48 (57.8)	
≥ 25	42 (38.9)	7 (28.0)	35 (42.2)	
Performance Status				0.054
0	56 (54.4)	8 (34.8)	48 (60.0)	
1	47 (45.6)	15 (65.2)	32 (40.0)	
Tumour location				0.96
Colon	73 (67.6)	17 (68.0)	56 (67.5)	
Rectum	35 (32.4)	8 (32.0)	27 (32.5)	
Tumour resection				0.002*
Yes	41 (38.0)	16 (64.0)	25 (30.1)	
No	67 (62.0)	9 (36.0)	58 (69.9)	
Number of EH metastatic sites				0.24
0	67 (62.0)	13 (52.0)	54 (65.1)	
1	23 (21.3)	8 (32.0)	15 (18.1)	
2	5 (4.63)	0 (0)	5 (6.02)	
>2	13 (12.0)	4 (16.0)	9 (10.8)	
Chemotherapy protocol				0.54
FOLFIRI	78 (72.2)	20 (80.0)	58 (69.9)	
FOLFOX	21 (19.4)	3 (12.0)	18 (21.7)	
LV5FU2	6 (5.56)	2 (8.00)	4 (4.82)	
Other**	3 (2.78)	0 (0)	3 (3.61)	
KRAS and BRAF mutational status				0.59
KRAS mutated	24 (22.2)	4 (16.0)	20 (24.1)	
KRAS wild-type	24 (22.2)	5 (20.0)	19 (22.9)	
BRAF mutated	2 (1.85)	0 (0)	2 (2.41)	0.70
Missing data	60 (55.6)	16 (64.0)	44 (53.0)	
Ang-2 (µg/L)	4.57 (4.23)	9.23 (5.61)	4.15 (2.40)	<0.001*
< 5	60 (57.7)	3 (12.0)	57 (72.1)	
≥ 5	44 (42.3)	22 (88.0)	22 (27.9)	
VEGF (µg/mL)	192 (265)	336 (600)	132 (227)	<0.001*
< 200	55 (51.9)	8 (32.0)	47 (58.0)	
≥ 200	51 (48.1)	17 (68.0)	34 (42.0)	
Albumin (g/L)	38.0 (6.00)	34.5 (3.35)	39.3 (5.6)	<0.001*
≥ 35	46 (70.8)	7 (36.8)	29 (86.7)	
< 35	19 (29.2)	12 (63.2)	7 (15.6)	
LDH (U/L)	380 (333)	630 (899)	314 (224)	<0.001*
< 300	24 (34.3)	2 (10.5)	22 (43.1)	
≥ 300	46 (65.7)	17 (89.5)	29 (56.7)	
CEA (µg/L)	59 (186)	222 (1266)	55.9 (128)	<0.001*
< 100	66 (61.1)	8 (32.0)	58 (69.9)	
≥ 100	42 (38.9)	17 (68.0)	25 (30.1)	

IQ: interquartile range; EH: extra-hepatic; FOLFIRI: irinotecan, leucovorin, 5-fluorouracil; FOLFOX: oxaliplatin, leucovorin, 5-fluorouracil; LV5FU2: leucovorin, 5-fluorouracil; KRAS: *Kirsten RAt Sarcoma* gene mutations on codons 12, 13, 61 or 146 of respective exons 2, 2, 3 and 4; BRAF: *B-Raf* gene mutation on V600E (p.Val600Glu) of exon 15; Ang-2: angiotensin-2; VEGF: vascular endothelial growth factor; LDH: lactate dehydrogenase; CEA: carcinoembryonic antigen.

FOLFOXIRI (oxaliplatin, irinotecan, leucovorin, fluorouracil) (n = 1),

irinotecan and raltitrexed (n = 1),

and irinotecan monotherapy (n = 1).

* p < 0.05;.

** other chemotherapy protocols included.

line concentrations of Ang-2, VEGF, LDH and CEA (p < 0.001, for all).

2.3. Survival analysis and response rate

2.3.1. Progression-free survival

PFS data was available for all 108 patients (Fig. 3A). At the data cut-off point, 100 patients progressed (92.6 %) and the me-

dian PFS time was 10.6 months. Patients from group A had a shorter PFS median of 8.7 months (vs 11.3 months for group B). Kaplan-Meier estimator revealed a significantly shorter PFS time in patients from group A (HR: 1.64, 95 % CI [1.03–2.61], log-rank p = 0.03).

As shown in Table 2B, multivariate Cox regression model identified serum MIRS signature A as independently associated with shorter PFS (HR: 1.74, 95 % CI [1.07–2.82], p = 0.025) when ad-

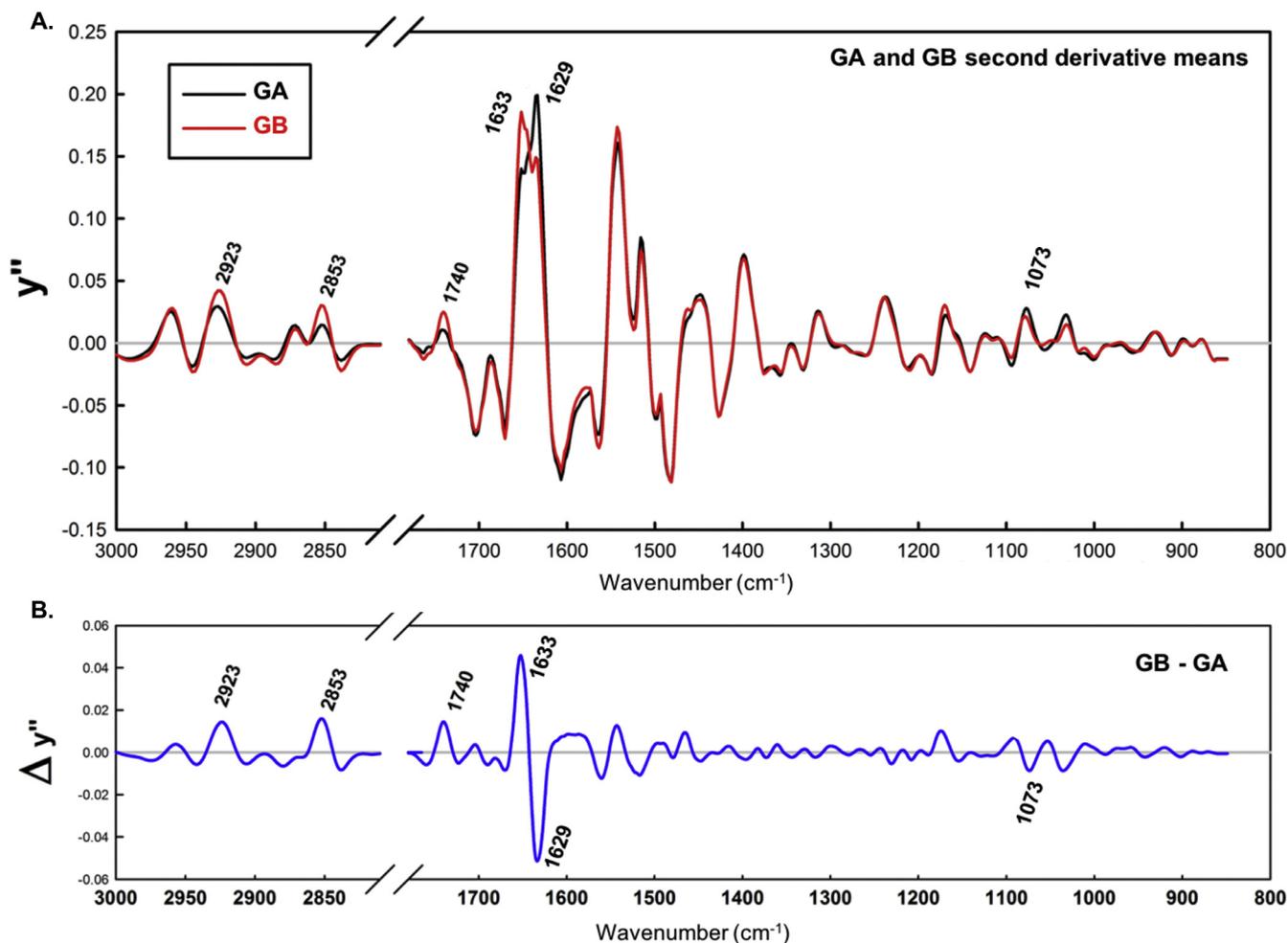


Fig. 2. Mid-infrared spectroscopy (MIRS) signatures. A: mean of spectral signatures in second derivative from patients of group A (GA, black curve) superposed with the respective mean from patients of group B (GB, red curve). Identification of six discriminative spectral bands (2923 cm⁻¹, 2853 cm⁻¹, 1740 cm⁻¹, 1633 cm⁻¹, 1629 cm⁻¹, 1073 cm⁻¹). B: spectral subtraction of second derivative means of patients from group B minus group A. Bands at 2923 cm⁻¹, 2853 cm⁻¹ and 1740 cm⁻¹ are less present in signature A. There is a spectral peak shift in the Amide I protein from 1629 cm⁻¹ (group A) to 1633 cm⁻¹ (group B).

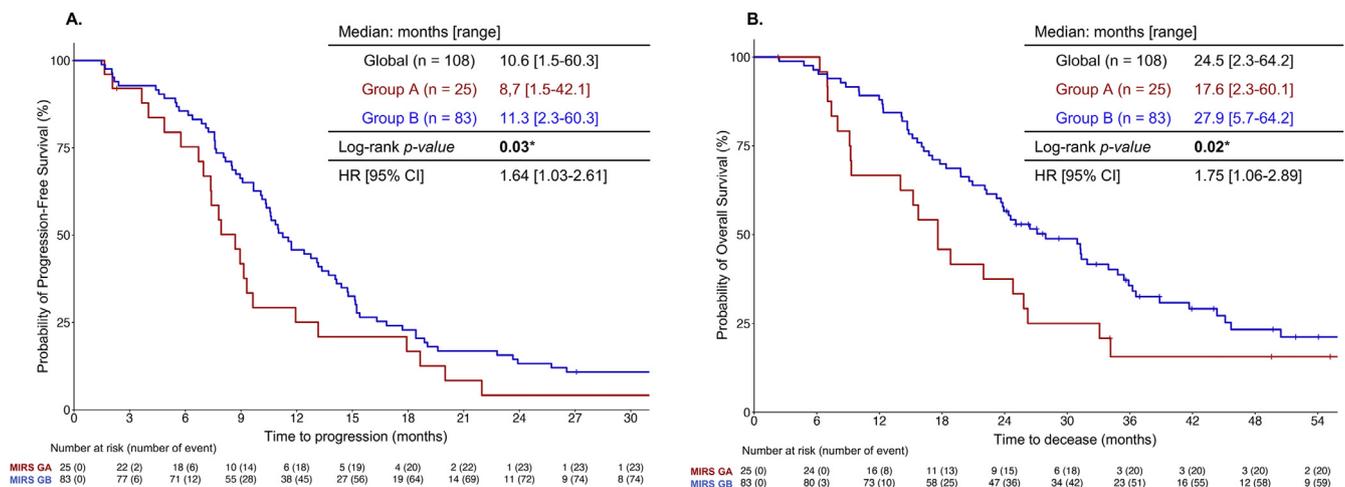


Fig. 3. Survival Kaplan-Meier curves for progression-free survival (A) and overall survival (B) of patients from groups A (red curves) and B (blue curves) defined by two different serum spectral signatures.

Tables 2

Univariate and multivariate analysis by Cox regression model for:

A: Overall survival. **p* < 0.2; ***p* < 0.05

Co-variables	Univariate analysis			Multivariate analysis		
	HR	95 % IC	<i>p</i> value	HR	95 % IC	<i>p</i> value
Age: >70 vs <70 years	1.37	0.86–2.18	0.18*	1.71	1.02–2.87	0.042**
Gender: M vs F	1.46	0.91–2.32	0.11*	1.51	0.93–2.47	0.097
BMI: <25 vs ≥ 25 kg/m ²	1.14	0.73–1.79	0.56			
PS: 1 vs 0	1.10	0.70–1.72	0.68			
Resection: Yes vs No	1.88	1.20–2.95	0.0059**	1.69	1.04–2.75	0.034**
Localisation: Colon vs Rectum	1.24	0.78–1.98	0.37			
EH metastases: Yes vs No	1.53	0.98–2.39	0.059*	1.51	0.92–2.47	0.095
FOLFOX/IRI vs others	1.15	0.53–2.50	0.73			
KRAS: mutation vs wild type	1.39	0.74–2.59	0.31			
BRAF: mutation vs wild type	2.32	0.55–9.84	0.25			
MIRS signatures: A vs B	1.75	1.06–2.89	0.028**	1.69	0.98–2.93	0.061

B: Progression-free survival. **p* < 0.2; ***p* < 0.05.

Co-variables	Univariate analysis			Multivariate analysis		
	HR	95 % IC	<i>p</i> value	HR	95 % IC	<i>p</i> value
Age: >70 vs <70 years	1.45	0.95–2.21	0.082*	1.64	1.04–2.60	0.034**
Gender: M vs F	1.41	0.93–2.13	0.11*	1.21	0.78–1.89	0.39
BMI: <25 vs ≥ 25 kg/m ²	1.05	0.70–1.57	0.81			
PS: 1 vs 0	0.972	0.65–1.46	0.89			
Resection: Yes vs No	1.27	0.84–1.90	0.25			
Localisation: Colon vs Rectum	1.07	0.70–1.64	0.75			
EH metastases: Yes vs No	1.36	0.91–2.04	0.13*	1.33	0.85–2.07	0.21
FOLFOX/IRI vs others	1.21	0.59–2.49	0.61			
KRAS: mutation vs wild type	1.36	0.75–2.47	0.32			
BRAF: mutation vs wild type	1.36	0.33–5.71	0.67			
MIRS signatures: A vs B	1.64	1.03–2.61	0.036**	1.74	1.07–2.82	0.025**

BMI: body mass index; M: male; F: female; PS: performance status; EH: extra-hepatic; FOLFOX: oxaliplatin, leucovorin and 5-fluorouracil; FOLFIRI: irinotecan, leucovorin and 5-fluorouracil; MIRS: mid-infrared spectroscopy; HR: hazard ratio; CI: confidence interval.

Table 3

Best objective response rates during study follow-up. **p* < 0.05.

	Global: n (%)	Group A: n (%)	Group B: n (%)	Comparison A vs B: p-value
Objective response rate	74 (68.5 %)	13 (52.0 %)	61 (73.5 %)	0.043*
Complete Response	23 (21.3 %)	0	23 (27.7 %)	
Partial Response	51 (47.2 %)	13 (52.0 %)	38 (45.8 %)	
Stable Disease	25 (23.2 %)	10 (40.0 %)	15 (18.1 %)	
Progressive Disease	9 (8.33 %)	2 (8.00 %)	7 (8.43 %)	

justed with age, gender and extra-hepatic metastasis. Age >70 years was also an independent factor of shorter PFS.

2.3.2. Overall survival

OS data was available for all 108 patients (Fig. 3B). At the data cut-off point, 81 patients were deceased (75.0 %) and the median OS time was 24.5 months. Patients from group A had a shorter OS median of 17.6 months (vs 27.9 months for group B). Kaplan-Meier estimator revealed a significantly shorter OS time in patients from group A (HR: 1.75, 95 % CI [1.06–2.89], log-rank *p* = 0.02).

As shown in Table 2A, multivariate Cox regression model identified age and surgical resection of the colorectal tumour as independent factors influencing OS. Serum MIRS signature A showed a trend for independent association with shorter OS (HR: 1.69, 95 % CI [0.98–2.93], *p* = 0.061) when adjusted on age, gender, tumour resection and extra-hepatic metastasis.

2.3.3. Objective response rate

Overall response rates were available for all 108 patients. During follow-up, 74 (68.5 %) patients achieved an objective response at best (Table 3). Patients from group A had a significantly lower bORR (*n* = 13, 52.0 %) than in group B (*n* = 61, 73.5 %) with *p* = 0.04. No complete response was observed in group A.

2.4. Bevacizumab safety profile

BRAE were reported in 62 (57 %) patients overall, including 45 (42 %) and 17 (16 %) with grades 1–2 and 3–4 respectively (Supplementary Table 2). Patients from group A had significantly less grade 1–2 BRAE than in group B: 6 (24 %) vs 39 (47 %), *p* = 0.041. This difference was only observed for grade 1–2 hypertension: 2 (8.0 %) vs 23 (28 %), *p* = 0.041.

3. Discussion

Bevacizumab-based chemotherapy is a recommended first-line treatment of mCRC [6]. Yet, some patients do not benefit from this strategy and have a reduced survival [7,8]. With a promising new technique of metabolomic fingerprinting, we have detected patients at significant risk of progression.

Until now, substantial efforts have been made to identify a prognostic biomarker for mCRC patients treated with bevacizumab-based chemotherapy but without changing our clinical practice [9,10]. Some biomarkers of tumoral aggressiveness and angiogenic potential have been well explored including LDH, CEA, VEGF isoforms (-A and -D) and Ang-2 [23–26]. Yet, discordant results categorising patients with heterogeneity in small retrospective studies with poor statistical impact explain the lack of clinical applicability. The complex landscape of all these studies con-

firms that prognostic evaluation of patients with anti-angiogenic treatment cannot be done with a unique biomarker. Indeed, bevacizumab action mechanisms are not all elucidated [27] and angiogenesis is a complex machinery with an adaptative evolution soliciting diverse biological processes [28]. The combination strategy of associating a panel of biomarkers seems more appropriate compared to isolating one controversial entity [11,12,29]. It is from this assumption that MIRS takes its interest.

Mid-infrared spectroscopy provides the metabolic fingerprint of an analysed sample [15,16]. Various neoplastic conditions have been explored by MIRS [15,30]. Most of these studies analysed tissular samples. Concerning CRC, MIRS is shown to be an interesting method for diagnosis, chemotherapy monitoring and evaluation of post-surgical relapse risk [31–33]. Yet, tissular analysis needs an invasive and restrictive endoscopic or surgical sampling. Compared to tissue analysis, biofluids require very few material and processing [16]. MIRS analysis of serum is a simple, rapid, reproducible, non-destructive, non-invasive metabolomic method at accessible cost and with a high sensitivity from few material quantity [15,17,34]. It therefore appears to be an ideal medical bedside tool with vast applicability. It has been evaluated for diagnosis and classifying different neoplasia including digestive cancers [35,36]. Results are encouraging and prospective trials are emerging [37]. Concerning CRC, we have shown in a recent pre-clinical study that MIRS on serum predicted tumour volume of xenografts in nude mice [38]. Also, major shifts in protein and lipid spectral signatures were correlated with anti-tumoral efficacy of curcumin and inhibitors of specific mitochondrial exchanger mediating calcic extrusion [38]. Only two clinical studies from blood samples, including one on serum, have been published [39,40]. The method proves to be feasible and shows a 81 % sensibility for early diagnostic but with a limited specificity of 71 % [39]. Until now, no study has explored the prognostic potential of serum MIRS analysis in oncology. It is therefore a promising and insufficiently investigated method.

Our study is the first to explore with serum MIRS the survival of patients treated by first-line bevacizumab-based chemotherapy for mCRC. We blindly dichotomised from baseline two groups of patients with significantly different survival patterns and baseline levels of tumour aggressiveness. They also had opposed PS and nutritional characteristics. The aim being to identify patients with poor survival rates in order to optimise mCRC treatment, serum MIRS seems to be an interesting tool by distinguishing patients at significantly high risk of progression. Interestingly, more frequent grade 1–2 BRAE were reported in patients of better prognostic and tumoral response. Bevacizumab-induced hypertension has been correlated with survival in a recent meta-analysis [41]. This might suggest a more bevacizumab-specific predictive potential for MIRS. Yet, our study was not designed to evaluate the predictive yield of serum MIRS. The absence of a control group of patients without bevacizumab prevents us from affirming a predictive impact of MIRS. The feasibility of such design can be ethically arguable since survival is improved when bevacizumab is added to a chemotherapy backbone. The control group would need to be exposed to EGFR inhibitors-based chemotherapy which is a 1st line therapeutic option in mCRC but their characteristics would differ, particularly considering their tumoral molecular landscape. Even so, the encouraging results of our study should incite such a comparison in order to evaluate the full potential of serum MIRS in mCRC.

Spectral discrimination was established from 6 spectral variables. Since it is a pilot study, no comparison with previous results is possible. The asset of MIRS on serum is taking the global metabolomic into account and not only tissular tumoral activity. We have shown that patients with better survival have a richer lipid composition and an inversion of protein population. Variations on the 1100–1000 cm^{-1} spectral domain are less explicit. It

was not possible to link these spectral bands with more precise biochemical elements. Yet, these spectral variables were correlated in a system composed of two groups, one of lipids and one of proteins/sugar. It is fundamental to emphasise that it is the network which structures the spectrum and its fluctuation which holds the prognostic information. The goal of this study is less to specifically identify biomarkers, than to pinpoint a prognostic metabolic fingerprint.

In spite of promising results, development of MIRS in clinical practice is limited by numerous obstacles. A major hurdle is the lack of reproducibility due to absence of standardisation procedures for sampling, storage and conditioning [30]. These are crucial steps since spectral analysis depends on the biological state of elements. In our study, sampling was centre dependent, storage was centralised and standardised, and conditioning was identically reproduced for each patient. Still, 14 patients out of the initial 137 were excluded due to storage impairment. Another obstacle is the lack of statistical power [30,34]. Most of the studies evaluating serum MIRS are retrospective on small cohorts. Our study does not avoid this drawback. We also lacked clinical and biological data on patients, notably tumour laterality and molecular markers such as microsatellite instability. Our promising results need to be confirmed with a validation cohort taking into account these previous drawbacks.

Rapid non-invasive metabolomic fingerprinting is possible by MIRS on serum. This method identified patients at significantly high risk of progression in spite of first-line bevacizumab-based chemotherapy for mCRC. Taking into account this innovating work, MIRS on serum is a promising tool which applicability in clinical practice must be mentored. Large prospective trials are needed to evaluate in a standardised manner how MIRS can optimise mCRC treatment.

Declaration of competing interest

Authors have declared no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence their work regarding this study.

Author contribution

Romain Chautard: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Morgane Caulet:** Data curation, Resources, Writing – review & editing. **Olivier Bouché:** Resources, Writing – review & editing. **Christophe Borg:** Resources, Writing – review & editing. **Sylvain Manfredi:** Resources, Writing – review & editing. **Olivier Capitain:** Resources, Writing – review & editing. **Jean-Philippe Spano:** Resources, Writing – review & editing. **William Raoul:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Maxime Guéguinou:** Writing – review & editing. **Olivier Herault:** Writing – review & editing. **Aurélie Ferru:** Resources, Writing – review & editing. **Cédric Pobel:** Conceptualization, Formal analysis, Software, Writing – review & editing. **Olivier Sire:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – review & editing. **Thierry Lecomte:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Supplementary materials

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