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ORIGINAL PAPER

Pathological response after neoadjuvant bevacizumab- or cetuximab-based chemotherapy in resected colorectal cancer liver metastases

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Abstract Neoadjuvant chemotherapy (NACT) prior to liver resection is advantageous for patients with colorectal cancer liver metastases (CLM). Bevacizumab- or cetuximab-based NACT may affect patient outcome and curative resection rate, but comparative studies on differential tumour regression grade (TRG) associated with distinct antibodies-associated regimens are lacking. Ninety-three consecutive patients received NACT plus bevacizumab ($n = 46$) or cetuximab ($n = 47$) followed by CLM resection. Pathological response was determined in each resected metastasis as TRG rated from 1 (complete) to 5 (no response). Except for KRAS mutations prevailing in bevacizumab versus cetuximab (57 vs. 21 %, $p = 0.001$), patients characteristics were well balanced. Median follow-up was 31 months (IQR 17–48). Bevacizumab induced significantly better pathological response rates (TRG1–3: 78 vs. 34 %, $p < 0.001$) as well as complete responses (TRG1: 13 vs. 0 %, $p = 0.012$) with respect to cetuximab. Three-year progression-free survival (PFS) and overall survival (OS) were not significantly different in the two cohorts. At multivariable analysis, significant association with pathological response was found for number of resected metastases ($p = 0.015$) and bevacizumab allocation ($p < 0.001$), while KRAS mutation showed only a trend. Significant association with poorer PFS and OS was found for low grades of pathological response ($p = 0.009$ and $p < 0.001$, respectively), R2 resection or presence of extrahepatic disease (both $p < 0.001$) and presence of KRAS mutation ($p = 0.007$ and $p < 0.001$, respectively). Bevacizumab-based regimens, although influenced by the number of metastases and KRAS status, improve significantly pathological response if compared to cetuximab-based NACT. Possible differential impact among regimens on patient outcome has still to be elucidated.

Keywords Liver metastases · Colorectal cancer · Bevacizumab · Cetuximab · Pathological response · Neoadjuvant chemotherapy

Introduction

Treatment of colorectal cancer liver metastases (CLM) is a major therapeutic challenge. When resection is achievable—being the definition of resectability variable according to surgeons experience and centre volume—combined approaches incorporating chemotherapy offer the best outcomes [1]. Although liver metastases are

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considered resectable if a R0 procedure is achievable with sufficient liver remnant after hepatectomy, in most instances timing for surgery and duration of chemotherapy are arbitrarily determined, due to the lack of as prospective trials comparing different strategies and regimens.

Several studies demonstrated the efficacy of neoadjuvant chemotherapy (NACT) in increasing R0 resection of borderline CLM or sparing unnecessary surgery to patients with progressive disease (PD) [2, 3]. In resectable, high-risk CLM—namely liver metastases with positive hilum and/or primary tumour lymph nodes, synchronicity, carcinoembryonic antigen (CEA) ≥200 ng/mL, size ≥5 cm and number >4—NACT can improve long-term outcome [4]. Some studies suggested a correlation between response rate to chemotherapy and resection rate [5]. The addition of cetuximab to standard chemotherapy improves response rate if the KRAS is wild type [6–8], and overall survival (OS) is significantly prolonged when inoperable CLM are converted to R0 resection [9, 10].

Patient- and tumour-related prognostic factors of CLM undergoing liver resection are well known, although their relative weight in real-life decision-making is very heterogeneous and centre specific [11–14]. Among them, pathological response following NACT is an objective parameter and several investigators proposed it as a surrogate marker for improved outcome, possibly due to the eradication of micrometastatic disease [15, 16]. It has repeatedly demonstrated that patients who achieve a pathological complete response (pCR) have an excellent 5-year survival [16, 17]. The measurement of tumour regression grade (TRG) according to objective criteria may be the best method for assessing complete or major pathological response (TRG1–2) versus minimal or no response (TRG4–5) [16].

Type of chemotherapy influences TRG, as oxaliplatin-containing combinations are associated with the highest rates of pathological response, if compared to fluoropyrimidines alone or irinotecan-based doublets. In retrospective series, the addition of bevacizumab to NACT significantly increased pathological response among patients undergoing resection of CLM [16–19]. Notably, the lack of prospective trials comparing bevacizumab- versus cetuximab-based NACT has fuelled heterogeneities in chemotherapy combinations and that has affected endpoints assessment and potential patients' advantages related to different tumour responses.

To address the issue, we conducted a study on prospectively evaluated pathological response after preoperative bevacizumab- or cetuximab-based chemotherapy in patients with resected CLM. The study aimed at assessing whether pathological response to NACT correlated with the type of used monoclonal antibody (bevacizumab or cetuximab) and patient outcome.

Patients and methods

Patient population

At Fondazione IRCCS Istituto Nazionale dei Tumori of Milan, NACT associated with a monoclonal antibody (bevacizumab or cetuximab) was indicated after multidisciplinary discussion in 93 consecutive patients with borderline or high-risk resectable CLM collected from October 2006 to December 2012. KRAS testing was not part of routine assessment before 2009, and 19 patients with unknown KRAS status were treated in that period with cetuximab. After 2009, cetuximab was restricted to patients with KRAS wild type. Patients who received prior chemotherapy for advanced disease were excluded. Clinical characteristics including age, gender, type and length of chemotherapy, radiological and pathological response, and type of liver surgery were recorded. Primary tumour location and stage, number and size of CLM, KRAS mutation, microscopic margins of resected tumours, status of hilum lymph nodes, presence of extrahepatic disease were analysed.

Mutational analysis of KRAS exons 2–4 was assessed on formalin-fixed, paraffin-embedded primary and matched-pair metastatic tumour tissue, as previously described [20]. KRAS exon 2 status was further confirmed through a specific mutant-enriched technique [21].

All clinical decisions were made at multidisciplinary hepato-oncology board, and patients gave their informed consent to proposed treatment strategy and tissue analysis. Data collection and study design were approved by the institutional review board.

Assessment of response to neoadjuvant chemotherapy

Pre-surgical radiological response to NACT was rated according to RECIST 1.1 criteria [22]. Patients were considered suitable for resection when R0 resection was expected, and there would be enough hepatic parenchyma left to avoid post-resection liver failure. Pathological response to NACT was classified on a 5-point scale according to the degree of treatment-induced fibrosis and necrosis in each removed nodule [16]. Untrimmed three-category analysis was favoured by merging TRG1 and 2 as major pathological response, while TRG3 qualified partial response and TRG4–5 absence of response. Both the 5- and 3-points TRG scoring systems were assessed by two blinded pathologists.

Two different approaches were considered for TRG analyses: (1) *patient-related:* considering the worst TRG value as reference in patients with multiple metastases; (2) *tumour-related*: considering all the TRG values detected in each liver metastasis removed at surgery.

Statistical analysis

The analyses were carried out using the SAS® and R software (http://www.r-project.org/, last access 22 June, 2013). To compare the two treatment groups according to continuous variables, the Mann–Whitney–Wilcoxon test was used, while categorical variables were assessed through exact Chi-square or Fisher's test, when appropriate. A multivariable binary logistic model was applied to perform patient-related TRG analysis, while a generalised estimating equations (GEE) model [23] was fitted to response data in tumour-related analysis. In both cases, a binary response variable was defined combining major (TRG1–2) with partial response (TRG3) versus minor and no response (TRG4–5). Overfitting due to the logistic model high dimensionality in relation to the low number of responses was controlled through the penalised maximum-likelihood estimation method as proposed by Firth [24]. In the GEE model, the intra-patient correlation among different nodule responses was accounted for assuming an exchangeable correlation structure. The model covariates were: patient's age, lymph nodes status of the primary tumour (positive vs. negative), KRAS status (mutated vs. wild type), liver metastases presentation (synchronous vs. metachronous), number of hepatic nodules per patient (in patient-related TRG analysis), liver hilum lymph nodes status (positive vs. negative), treatment type (cetuximab- vs. bevacizumab based), microscopic margin infiltration (R1 vs. R0) and R2 margins/extrahepatic disease (yes vs. no).

Progression-free survival (PFS) was calculated from the date of surgery to the date of progression or death without evidence of disease, whichever occurred first, or censored at the date of last follow-up for alive and progression-free patients. Patient overall survival (OS) was calculated from date of surgery to the date of death due to any cause, or censored at the date of last follow-up for living patients. Kaplan–Meier PFS and OS curves were estimated and compared to the log-rank test. Cox models were fitted in a multivariable setting; the covariates were the same as in the logistic or GEE model, and patient- or tumour-related TRG was also included. In addition, we modelled the interaction between treatment and TRG for investigating whether the prognostic impact of chemotherapy could be different according to different response. Overfitting control in the Cox models was achieved by applying Ridge penalised maximum-likelihood estimation methods with cross-validation of penalty parameter [25]. In all the models, patient's age, TRG and number of liver metastases per patient were modelled as continuous variables by means of three-knot restricted cubic splines [26], whereas the remaining clinical–biological variables were modelled as categorical by dummy variables. The interaction between treatment and TRG was not statistically significant in any of the models and so were the cubic splines nonlinear terms for continuous variables; thus we only show the results obtained from the models without interaction with linearly modelled continuous variables.

The results were considered statistically significant whenever a two-sided p value below 0.05 was achieved.

Results

Patients

Among the 93 patients analysed, 46 (49 %) were in bevacizumab and 47 (51 %) in cetuximab group. Demographics and clinical characteristics, separated by treatment arm, are shown in Table 1. The groups were well matched, except for higher frequency of KRAS mutations in bevacizumab versus cetuximab groups (57 vs. 21 %; $p < 0.001$), the prior adjuvant chemotherapy (24 vs. 9 %; $p = 0.04$) and NACT backbone (oxaliplatin based: 48 vs. 85 %; $p < 0.001$). Thirty-two (34 %) patients showed a single liver metastasis, whereas 61 (66 %) had multiple metastases. The median number of resected nodules was 3 (interquartile range [IQR] 1–4) in the whole population, with no difference between bevacizumab versus cetuximab groups [3 met (1–5) vs. 2 (1–4) respectively; $p = 0.358$]. Synchronous metastases affected 76 % of patients. Of 22 (24 %) patients with metachronous liver metastases, one half had node-negative primary tumour (stage I and II) and 15 received adjuvant chemotherapy. Overall, 65 (70 %) patients were lymph node-positive at the time of primary tumour surgery. Among the 87 patients with available baseline CEA, the median level was 11.8 ng/mL (IQR 3.07–35.58) and in six patients (7 %) >200 ng/mL. In all but one patient, KRAS status was determined: 56 (60 %) had wild type and 36 (39 %) mutated tumours. There was a 99 % concordance of KRAS status between primary tumour and liver metastases.

Only nine patients (10 %) underwent simultaneous resections on primary tumour and liver metastases after NACT, as all the remaining patients received NACT after primary tumour resection. Major hepatectomies (≥4 liver segments) were performed in 41 (44 %) patients, minor hepatectomies in 29 (31 %) and multiple wedge—non-anatomic resections—in 23 (25 %). Unilobar involvement was observed in 35 patients (38 %) and bilobar involvement in 58 (62 %). These conditions combined with remnant liver considerations led to two-stage hepatectomies with portal vein embolisation in four patients (4 %) and to the combination with single <2 cm nodule radiofrequency ablation in 10 (11 %). Microscopically negative surgical margins (R0) were obtained in 65 (70 %) patients. Lymph

Table 1 Main patient and disease characteristics according to treatment group

	Overall		Bevacizumab		Cetuximab		*p*
	N	(%)	*N*	(%)	*N*	(%)	
Total	93		46	(49)	47	(51)	–
Patient's age (years)							0.451
Median (IQ range)	58 (50–66)		56 (48–67)		59 (52–66)		
Gender							0.616
Male	67	(72)	33	(72)	34	(72)	
Female	26	(28)	13	(28)	13	(28)	
Status of the primary tumour T stage							0.833
T1–T2	10	(11)	4	(9)	6	(13)	
T3–T4	83	(89)	42	(91)	41	(87)	
Status of the primary tumour nodes							0.438
Positive	65	(70)	33	(72)	32	(68)	
Negative	28	(30)	13	(28)	15	(32)	
KRAS status							**<0.001**
Mutated	36	(39)	26	(57)	10	(21)	
Wild type	56	(60)	19	(41)	37	(79)	
Missing	1	(1)	1	(2)	–	–	
Metastases presentation							0.427
Synchronous	71	(76)	36	(78)	35	(75)	
Metachronous	22	(24)	10	(22)	12	(25)	
Number of nodules							0.358
Median (IQ range)	3 (1–4)		3 (1–5)		2 (1–4)		
Status of hilum nodes							0.774
Positive	7	(7)	3	(6)	4	(8)	
Negative	86	(93)	43	(94)	43	(92)	
Resection margins							0.951
R0	65	(70)	29	(63)	36	(77)	
R1	28	(30)	17	(37)	11	(23)	
R2 margins/extrahepatic disease							0.291
Yes	21	(23)	12	(26)	9	(19)	
No	72	(77)	34	(74)	38	(81)	
Prior adjuvant chemotherapy[a]							**0.040**
Yes	15	(16)	11	(24)	4	(9)	
No	78	(84)	35	(76)	43	(91)	
Chemotherapy backbone of NACT							**<0.001**
Oxaliplatin based	62	(67)	22	(48)	40	(85)	
Irinotecan based	28	(30)	21	(46)	7	(15)	
Single-agent fluoropyrimidine	3	(3)	3	(6)	0	(0)	

Bold values indicate statistical significance set at p value <0.05

[a] Adjuvant chemotherapy after primary tumour resection was administered only to patients with metachronous metastases

nodal sampling at the liver hilum was performed in all cases, and in seven out of 93 patients (8 %), microscopic tumour invasion was demonstrated. Overall, 21 (23 %) of patients had macroscopically involved (R2) surgical margins or extrahepatic disease detected at surgery.

The most commonly used NACT was oxaliplatin-based doublets in 22 (48 %) patients in bevacizumab versus 40 (85 %) in cetuximab group. Irinotecan-based regimens were administered with bevacizumab in 21 (46 %) and with cetuximab in seven (15 %). Three patients in the bevacizumab group received single-agent fluoropyrimidines (Table 1). Median treatment duration was 4 versus 3 months in bevacizumab versus cetuximab group. Bevacizumab was always stopped at least 6 weeks prior to surgery.

Tumour-related response

Overall, 76 patients (82 %) achieved pre-surgical partial response (80 %) or complete response (2 %) according to RECIST 1.1, whereas 15 (16 %) had stable disease and two (2 %) had progression. There were no significant differences of radiological responses assessed before surgery between bevacizumab and cetuximab groups (78 vs. 85 %; $p = 0.410$).

When considering the worst TRG as reference in case of multiple metastases (patient-related TRG), the observed pathological response was mostly in the intermediate range as follows: TRG1 in six cases (6 %), TRG2 in seven (8 %), TRG3 in 39 (42 %), TRG4 in 27 (29 %) and TRG5 in 14 (15 %). According to treatment, median TRG was 3 (IQR 2.25–3) in bevacizumab versus 4 (3–4) in cetuximab group ($p < 0.0001$).

The distributions of pathological response rate according to treatment group are detailed in the Supplemental Figure. A higher proportion of pCR was observed in patients treated with bevacizumab as compared to patients receiving cetuximab (13.0 vs. 0 %; $p = 0.012$). The use of bevacizumab increased the rate of major-to-complete pathological response (TRG1–2), with a percentage of 26 % as compared to 2 % among those receiving cetuximab ($p < 0.001$). There was also an increase in the rate of partial-to-complete pathological response (TRG1–3), and the difference was significant (78 vs. 34 %, $p < 0.001$) [28].

Similarly, tumour-related TRG values were significantly better in bevacizumab than in cetuximab groups, with a median TRG of 3 (IQR 2–3) versus 4 (3–4) ($p < 0.0001$).

Multivariable analyses were performed to explore the association between patient- or tumour-related TRG and clinical–biological characteristics. Odds ratio (OR) estimates, namely the chance of not responding (TRG4–5) versus responding (TRG1–3), together with the 95 % confidence interval (CI), are shown in Table 2. In patient-related analysis, only two factors emerged as independently associated with pathological response: the number of liver metastases (OR 1.89, 95 % CI 1.13–3.16; $p = 0.015$) and type of monoclonal antibody, favouring bevacizumab (OR 6.13, 95 % CI 2.29–16.42; $p < 0.001$). The latter emerged as significant also in tumour-related analysis, with bevacizumab predicting pathological response with significantly higher OR with respect to cetuximab (OR 8.37, 95 % CI 3.13–22.33; $p < 0.001$). Notably, at univariable analysis, KRAS mutation was significantly associated with pathological response expressed by patient-related TRG ($p = 0.013$), while at multivariable analysis such trend reached a borderline significance ($p = 0.068$).

Patient-related outcomes

Median follow-up of the whole series was 31 months (IQR 17–48); 27 months (17–41) in the bevacizumab and 31 months (19–48) in the cetuximab group. Overall, 59 (63 %) patients had a documented relapse or PD, and a total of 31 (33 %) patients died. All deaths were due to PD, while one patient was lost to follow-up. The survival curves were truncated at 3 years, namely at a time interval slightly longer than the median follow-up. Three-year PFS and OS were 26.7 % (95 % CI 17.9–39.7 %; median PFS 12 months) and 54.9 % (95 % CI 42.9–70.1 %; median OS 42 months), respectively.

Considering the entire series, PFS was significantly improved in patients with pathological response, with TRG1–3-related outcomes largely exceeding those observed in case of TRG4–5. As depicted in Fig. 1a, 3-year PFS was 37.3 % (24.6–56.7 %) versus 13.1 % (5.2–32.9 %) and median PFS 16 versus 8 months, respectively ($p = 0.030$). A more striking difference was observed with restriction to major-to-complete pathological response (TRG1–2) versus partial or no response (TRG3–5): 3-year PFS was 57.7 % (34.7–96.0 %) versus 20.6 % (12.2–34.9 %) and median PFS 48 versus 10 months, respectively ($p = 0.027$). However, the small number of events in TRG1–2 subgroup (13 patients and two events) limited the statistical strength of such observation.

Patients with pathological response (TRG1–3) had a nonsignificantly higher OS as compared to those with TRG4–5 [64.2 % (49.4–83.4 %) vs. 44.3 % (27.8–70.6 %)], respectively, with a median OS of 60 versus 32 months ($p = 0.352$; Fig. 1b). As expected, when analysis was restricted to major-to-complete response (TRG1–2) versus partial or no response (TRG3–5), a significant difference in survival emerged ($p = 0.038$): 3-years OS was 90.0 % (73.2–100.0 %) versus 47.8 % (35.0–65.4 %), and median OS was not reached in the TRG1–2 group at data cut-off analysis versus 34 months in the TRG3–5 group.

Progression-free survival was significantly worsened in the presence of mutated versus wild-type KRAS. Three-year PFS was 5.2 % (0.8–32.6 %) versus 41.0 % (29.0, 58.0 %), with a median of 9 versus 15 months, respectively ($p = 0.009$) (Fig. 2a). Similar features were observed with survival analysis at 3 years: OS was 17.3 % (5.3–56.0 %) versus 74.2 % (61.3–89.8 %), with a median of 24 versus 60 months ($p < 0.001$) (Fig. 2b).

The outcome analysis showed no statistically significant difference among the bevacizumab versus cetuximab groups, being the 3-year PFS 28.7 % (17.2–47.9 %) versus 24.6 % (13.1–46.0 %) ($p = 0.966$) and the 3-year OS 61.9 % (46.9, 81.8 %) versus 47.9 % (31.5–72, 8 %) ($p = 0.702$), respectively.

Table 2 Multivariable analyses of association between the pathological response, expressed in terms of patient- or tumour-related TRG, and patient or disease characteristics

	Patient-related TRG			Tumour-related TRG		
	OR	CI	*p*	OR	CI	*p*
Patient's age (years)			0.450			0.801
66 versus 50[a]	1.32	(0.65–2.68)		1.08	(0.60–1.94)	
Status of the primary tumour nodes			0.116			0.234
Positive versus negative	2.41	(0.81–7.19)		2.11	(0.62–7.26)	
KRAS status			0.068			0.245
Mutated versus Wild type	0.38	(0.13–1.08)		0.52	(0.18–1.56)	
Metastases presentation			0.494			0.934
Synchronous versus metachronous	1.47	(0.49–4.48)		1.05	(0.32–3.46)	
Number of nodules			**0.015**	–		–
4 versus 1[a]	1.89	(1.13–3.16)				
Status of hilum nodes			0.964			0.919
Positive versus negative	1.03	(0.24–4.48)		0.95	(0.33–2.70)	
Resection margins			0.542			0.201
R1 versus R0	0.72	(0.24–2.10)		1.91	(0.71–5.14)	
R2 margins/extrahepatic disease			0.069			**0.007**
Yes versus no	2.72	(0.93–7.98)		3.81	(1.44–10.05)	
Treatment			**<0.001**			**<0.001**
Cetuximab versus bevacizumab	6.13	(2.29–16.42)		8.37	(3.13–22.33)	

Bold values indicate statistical significance set at p value <0.05

OR odds ratio, *CI* 95 % confidence interval of OR, *p* *p* value at two-sided Wald test

[a] The two values are, respectively, the third and first quartiles of the variable distribution

Cox multivariable analyses were carried out to assess whether or not the prognostic variables were associated independently with patients outcome. The strongest associations were seen when considering the intra-patient variability of pathological response, with three adverse prognostic factors showing a significant association with both PFS and OS (Table 3): (a) absence of pathological response ($p = 0.009$ and $p < 0.001$, respectively); (b) R2 resection or extrahepatic disease (both $p < 0.001$); (c) presence of KRAS mutation ($p = 0.007$ and $p < 0.001$, respectively). Similar trends were observed when the worst TRG was adjusted for patients with multiple liver lesions (Supplemental Table). Neither age, metastases presentation (synchronous or metachronous), number of resected nodules, status of hilum lymph nodes, microscopic involvement of surgical margins and type of NACT (cetuximab vs. bevacizumab) had an independent association with outcome. Incidentally, lymph nodal status of the primary tumour showed a significant association only with PFS.

Discussion

On top of the clinical benefit related to CLM downstaging, NACT allows tumour macro- and microscopic responses that lead to safer and more curative surgery.

To facilitate the use of NACT, empirical definitions of pathological response have been developed in the past with the goal of using tumour regression grade (TRG) as early predictor of outcome [15–17]. In such respect, the potential use of pathological response of CLM treated with NACT as a predictor of patient outcome (PFS and OS) conflicted with the low rate (about 4 %) of pCR assessed through unrestricted histology parameters [17]. Conversely, the semi-quantitative grading of pathological response defined by Rubbia-Brandt et al. [16] has allowed efficacious capturing of chemotherapy activity and increased reliability of the assessment of intra- and inter-observer estimations. This has exerted a positive influence on post-operative multidisciplinary decision-making.

Our study confirms that tumours responding to NACT with monoclonal antibodies (bevacizumab or cetuximab) are associated with improved outcome after CLM resection. In prospective randomised trials, the addition of cetuximab to chemotherapy improved RECIST response and conversion to surgery [6–10]. In other studies, bevacizumab improved modestly radiological responses, while showing significant increase in PFS and OS [27]. This suggests that the biological activity of antiangiogenic treatments may not be well captured by standard RECIST, being more accurately defined by other means—such as pathological response [27, 28]. It is known that bevacizumab significantly improves

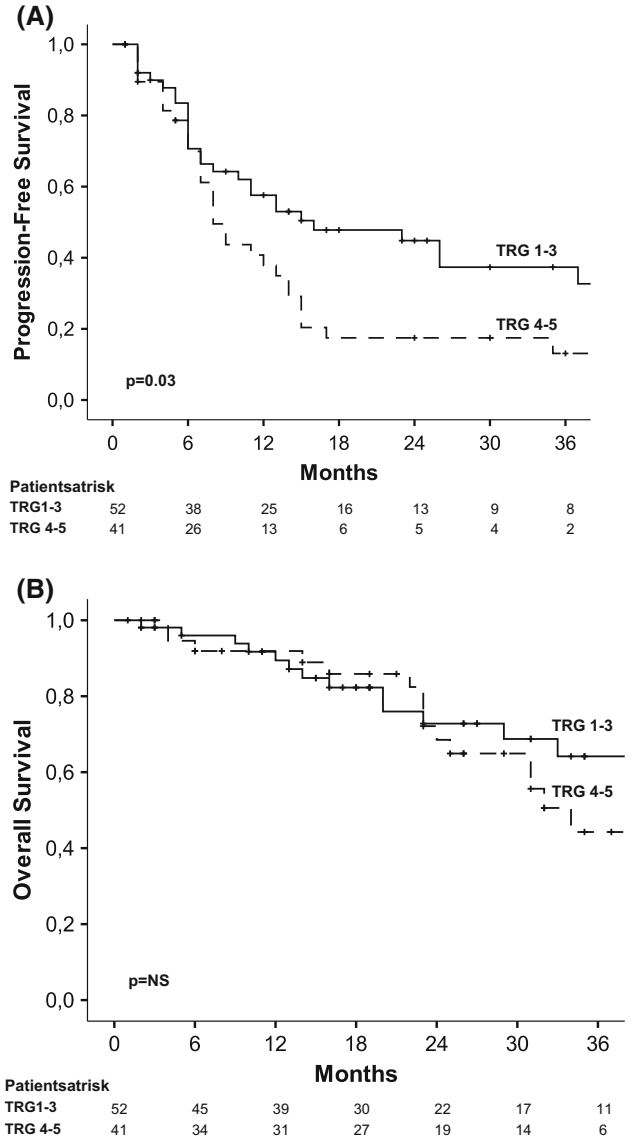

Fig. 1 Progression-free survival (**a**) and overall survival (**b**) in 93 patients undergoing liver resection for metastatic colorectal cancer, according to pathological response assessed as tumour regression grade (TRG1–3 vs. TRG4–5) after neoadjuvant chemotherapy

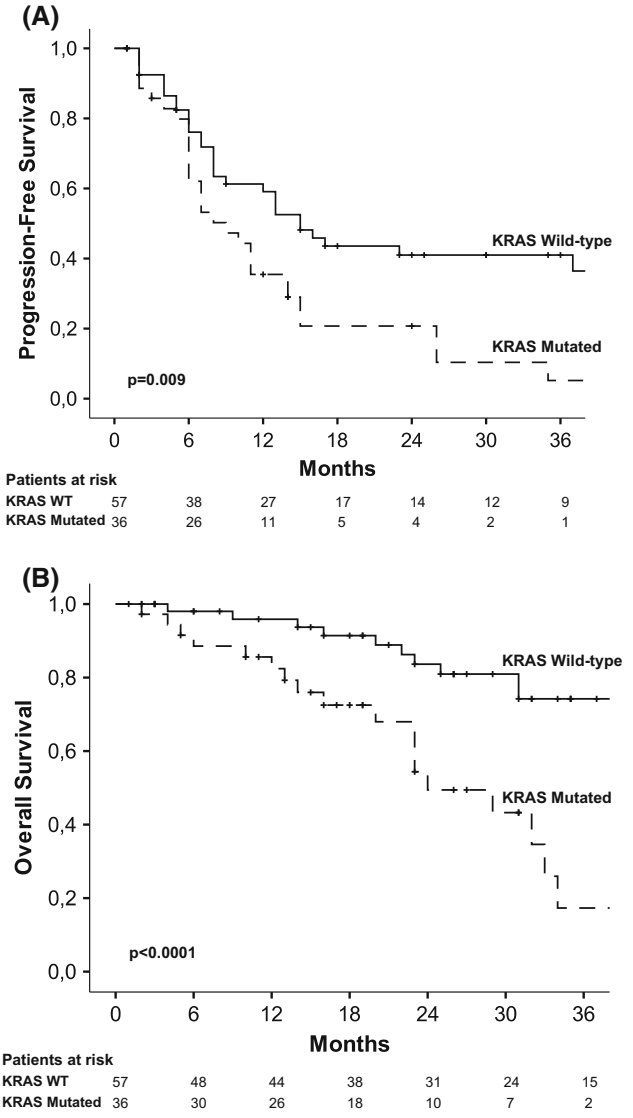

Fig. 2 Progression-free survival (**a**) and overall survival (**b**) in 93 patients undergoing liver resection for metastatic colorectal cancer, according to KRAS tumour status (KRAS wild type vs. mutant)

pathological response, tumour thickness at the tumour–parenchyma interface and necrosis as compared to chemotherapy alone [16, 18, 19, 29, 30], although confirmatory prospective studies are currently ongoing [31]. The effect of cetuximab on pathological response of resected CLM has not been elucidated yet.

However in a non-randomised setting, we provided for the first time a comparison between two cohorts of bevacizumab- versus cetuximab-based NACT, with the aim of assessing possible differences in terms of pathological response in resected CLM. Our data showed that bevacizumab induced a significantly higher pathological response rate at multivariable analysis, being an independent factor along with the number of resected liver metastases.

Despite such differential influence on pathological response, no significant differences on patient outcomes at 3 years were observed among bevacizumab versus cetuximab groups. Notably, the observed patient outcomes (PFS: 26.7 % and OS: 55 %) were quite remarkable in the light of a population undergoing surgery within a detrimental setting of adverse prognostic factors such as 66 % of multiple (median: 3) bilateral metastases, 40 % of KRAS mutations and 76 % synchronous tumours.

Correlation of pathological response to long-term outcome was beyond the scope of our study, and the possible benefits of

Table 3 Multivariable Cox model analyses of PFS and OS (tumour-related TRG)

	PFS			OS		
	HR	CI	*p*	HR	CI	*p*
Patient's age (years)			0.730			0.260
66 versus 50[a]	1.05	(0.81–1.36)		0.82	(0.58–1.16)	
Status of the primary tumour nodes			**0.038**			0.370
Positive versus negative	1.51	(1.02–2.24)		1.22	(0.79–1.89)	
KRAS status			**0.007**			**<0.001**
Mutated versus wild type	1.45	(1.11–1.89)		1.73	(1.25–2.39)	
Metastases presentation			0.074			0.870
Synchronous versus metachronous	0.72	(0.50–1.03)		1.04	(0.66–1.65)	
Status of hilum nodes			0.085			0.980
Positive versus negative	1.52	(0.94–2.46)		0.99	(0.61–1.62)	
Resection margins			0.290			0.590
R1 versus R0	0.84	(0.61–1.16)		1.10	(0.77–1.59)	
R2 margins/extrahepatic disease			**<0.001**			**<0.001**
Yes versus No	2.54	(1.92–3.37)		3.39	(2.47–4.67)	
Treatment			0.070			0.340
Cetuximab versus bevacizumab	0.74	(0.53–1.03)		1.20	(0.83–1.72)	
Tumour-related TRG			**0.009**			**<0.001**
5 versus 1[b]	2.16	(1.21–3.83)		4.55	(2.08–9.94)	

Bold values indicate statistical significance set at p value <0.05

PFS progression-free survival, *OS* overall survival, *HR* hazard ratio, *CI* 95 % confidence interval of HR, *p* *p* value at two-sided Wald test

[a] The two values are, respectively, the third and first quartiles of the variable distribution

[b] The two values are, respectively, the maximum and the minimum TRG values

bevacizumab remain to be elucidated through adequately powered prospective studies. Since our series confirms that pathological response is associated with patient outcome, we speculate that the beneficial effect of bevacizumab is sustainable, despite the retrospective nature of our collection and the heterogeneity of the chemotherapy regimens used.

A further set of interesting data emerged with respect to the role of KRAS mutations as potential biomarker in the specific setting of NACT preceding CLM resection.

The multivariate analysis on a consistent population undergoing NACT showed the independent role of KRAS mutations in predicting adverse patient outcome after CLM resection, as well as TRG (Table 3), being a statistical trend detectable also for KRAS mutations as predictors of pathological response. This is a signal that warrants further validation in larger data sets since reliable biomarkers predictive of CLM pathological response are still lacking, with KRAS mutations mostly used in clinical practice only for excluding treatment with cetuximab or panitumumab in patients with advanced disease [32].

The prognostic role of KRAS remains controversial, as multiple studies did not show any negative impact on survival for individuals harbouring KRAS mutations [33]. A strong argument in favour of the negative prognostic influence of KRAS mutations is more than a decade old [34] and limited to stage III tumours carrying the specific mutation of glycine to valine in codon 12. More recently, KRAS mutations were found to predict adverse clinical outcomes in selected patients treated with bevacizumab-based NACT prior to liver resection [35]. Similar conclusion was reached in other retrospective studies on both resectable and unresectable CLM [36], being KRAS-mutated tumours also associated with increased risk of lung metastases after curative resection [37]. The presented results originated from two cohorts of patients undergoing CLM resection after NACT are in line with such observations and indicate that KRAS status, in combination with TRG, may function as reliable prognostic marker also in the specific setting of resected CLM—a subgroup of patients with potentials for cure that still lacks specific biological drivers for clinical decision-making.

As in other contests of clinical oncology [38], clinical biomarkers indicating adverse prognosis—as KRAS mutations—are likely to predict good pathological response to specific treatments—as for the bevacizumab-based NACT in CLM—but are also associated to outcome deterioration if compared to patients with KRAS wild type. We showed that prognosis of patients with KRAS mutations who experienced a pathological response after bevacizumab-based regimens may be equalised to that observed in wild-type

tumours. In line with that, patients survival showed to be independently related to KRAS status and TRG after NACT, being those variables strictly intertwined with the chemotherapy regimen and considering that absence of pathological response in mutated tumours led almost exclusively to dismal prognosis.

In conclusion, we hypothesise that bevacizumab-based NACT may significantly improve the pathological response of resected CLM if compared to cetuximab-based regimens [39]. The limited follow-up at 3 years impeded to draw any conclusion on possible differential impact on patient outcome.

KRAS mutation is a negative prognostic factor that, in case of pathologic response to therapy, may lead to non-inferior outcomes with respect to those observed in wild-type tumours. Further studies specifically designed for these patients subgroups with resectable CLM are needed, for better individualisation of treatment choices on the basis of reliable biomarkers.

Conflict of interest None.

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