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Emeric Boisteau, Alexandra Lespagnol, Marie de Tayrac, Sébastien Corre, Anthony Perrot, et al.. MiR-31-3p do not predict anti-EGFR efficacy in first-line therapy of RAS wild-type metastatic right-sided colon cancer. Clinics and Research in Hepatology and Gastroenterology, 2022, 46 (5), 11 p. 10.1016/j.clinre.2022.101888 . hal-03595901

HAL Id: hal-03595901
https://hal.science/hal-03595901

Submitted on 1 Apr 2022

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Original article

MiR-31-3p do not predict anti-EGFR efficacy in first-line therapy of *RAS* wild-type metastatic right-sided colon cancer

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Highlights

- MiR-31-3p expression is predictive of anti-EGFR efficacy in mCRC.
- Primary tumor side is prognostic and predictive of anti-EGFR efficacy in mCRC
- In right-sided RAS-wt mCRC, miR-31-3p expression do not predict anti-EGFR efficacy and bevacizumab is the targeted therapy of choice whatever miR-31-3p expression level.

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Abstract:

Background: Low miR-31-3p expression was identified as predictive of anti-EGFR efficacy in *RAS*-wt mCRC. Primary tumor side was also proposed as a predictive factor of anti-EGFR benefit. This retrospective multicentric study evaluated the predictive role of miR-31-3p in right-sided *RAS*-wt mCRC patients treated with first-line CT+anti-EGFR or CT+bevacizumab (Beva).

Methods: Seventy-two right-sided *RAS*-wt mCRC patients treated in first-line with CT+anti-EGFR (n=43) or Beva (n=29) were included. Overall survival (OS), progression-free survival (PFS) and response rate (RR) were analyzed and stratified according to tumor miR-31-3p expression level and targeted therapy (TT).

Results: *BRAF* V600E mutation was more frequent in high vs low miR-31-3p expressers (60.6% vs 15.4%, $P < 0.001$). PFS was significantly longer with CT+Beva than with CT+anti-EGFR (13 vs 7 months; $P = 0.024$). Among low miR-31-3p expressers, PFS, OS and RR were not significantly different between the two groups, while in high miR-31-3p expressers, only PFS was longer in the CT+Beva group (11 vs 6 months; $P = 0.03$). In patients treated with CT+anti-EGFR, low miR-31-3p expressers had a significantly longer OS (20 vs 13 months; $P = 0.02$) than high miR-31-3p expressers. ORR was not significantly different between the two groups of treatment, in both low and high miR-31-3p expressers. MiR-31-3p expression status was statistically correlated between primary tumors and corresponding metastases.

Conclusion: In this study, miR-31-3p couldn't identify a subgroup of patients with right-sided *RAS*-wt mCRC who might benefit from anti-EGFR and suggest that Beva is the TT of choice in first-line treatment of these patients.

Keywords: colorectal cancer, metastasis, biomarker, miR-31-3p, anti-EGFR mAb, bevacizumab

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer, with an estimated worldwide incidence of over 1.8 million in 2018 [1]. During the last fifteen years, progress has been made in the treatment of metastatic CRC (mCRC) with the development of targeted therapies, including the anti-EGFR (epidermal growth factor receptor) monoclonal antibodies (mAbs) cetuximab (Cetux) and panitumumab (Pani) and the anti-VEGF (vascular endothelial growth factor) mAb bevacizumab (Beva) [2–4]. *RAS* mutation is a predictive marker of anti-EGFR resistance in mCRC, and therefore the use of this therapeutic class is restricted to patients with a *RAS* wild-type (wt) tumor [5–7]. However, only 40 to 70% of *RAS* wt patients achieve an objective response to anti-EGFR therapy with or without chemotherapy (CT) [5-6, 8]. The efficacy of first-line CT plus anti-EGFR or Beva in *RAS* wt patients remains to be defined given the discrepant results of randomized studies [8-9]. Therefore, it is important to find new predictive biomarkers to better select patients who will truly benefit from anti-EGFR mAbs, avoid unnecessary and potentially deleterious exposure to their toxicity and help clinicians choose the best targeted therapy in first-line of *RAS* wt mCRC.

MicroRNAs control gene expression and are deregulated in many types of cancers [10]. MiR-31 promotes cell migration and invasion in CRC cells [11] and is frequently overexpressed in CRC [12]. Overexpression of miR-31-3p, a mature sequence of miR-31, was shown to be correlated with advanced disease and poor response to anti-EGFR mAbs in several retrospective studies [13–15] and in a post-hoc analysis of the FIRE-3 trial [16]. The latter study observed that Cetux was superior to Beva in terms of overall survival (OS), progression-free survival (PFS)

and objective response rate (ORR) only for patients with low miR-31-3p expression [16]. Moreover, primary tumor side has been reported as a prognostic and predictive factor of anti-EGFR efficacy in *RAS* wt mCRC [17–19]. In the FIRE-3 post-hoc analysis, left-sided *RAS* wt mCRC patients benefited more from anti-EGFR mAbs than Beva regardless of miR-31-3p expression level, while those with a right-sided primary tumor and high miR-31-3p expression seemed to benefit more from CT+Beva [16].

Therefore, the aim of our study was to evaluate the prognostic and predictive role of miR-31-3p in a non-selected cohort of patients with right-sided *RAS* wt metastatic colon cancer treated with first-line CT + anti-EGFR or Beva using the REporting recommendations for tumor MARKer prognostic studies (REMARK) [20]. As it is still not clear which targeted therapy should be used in first-line for these patients, we assessed whether miR-31-3p could be a predictor of the targeted therapy to use in this setting. Our secondary objectives were to study the concordance of miR-31-3p between paired samples of primary tumors and metastases.

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2. Materials and Methods

2.1. Patients

This retrospective multicentric study included all consecutive patients treated with an anti-EGFR (Pani or Cetux) or Beva in association with a first-line CT for a histologically proven right-sided *RAS* wt mCRC from January 2014 to June 2017 in 12 French centers (6 university hospitals, 4 general hospitals, 1 cancer center and 1 private center). The exclusion criteria were histology other than adenocarcinoma and

double localization (right and left) of CRC. For these patients, the following data were collected from medical files: demographics, tumor stage at the time of diagnosis, primary tumor resection and date of surgery, type and date of adjuvant CT, type and number of metastatic sites, metachronous or synchronous metastases, surgical resection or percutaneous destruction of metastases and their date, *BRAF* and MMR (mismatch repair) status, Eastern Cooperative Oncology Group (ECOG) performance status (PS) before the start of first-line CT, type of targeted therapy and associated CT and their toxicities, best response to treatment (RECIST v1.1), cause of first-line CT discontinuation, date of progression or death and death related cause, number and type of 2nd line or further CT. Tumor response was assessed every 2 months by computed tomography (CT) scan and/or magnetic resonance imaging. As the proportion of tumors with high miR-31-3p expression in our cohort of patients treated with anti-EGFR was lower than expected based on literature data, miR-31-3p expression was also analyzed in an independent cohort of right-sided colon tumors (of all stages) from consecutive patients who underwent surgical resection of their primary tumor at the University Hospital of Rennes from January 2012 to December 2018. From the two independent cohorts of patients, paired samples of both primary tumor and metastasis were available for 31 patients.

The study was reviewed and approved by the Rennes University Hospital Ethics Committee for all participating centers (registration No. 18.37) and obtained the authorization of the National Commission for Data Protection and Liberties (CNIL) (number 2005096v0). According to French regulations, patients were informed of the study and did not express opposition, except one who was excluded from the analysis.

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2.2. MiR-31-3p analyses

Tumor samples were obtained before the first-line treatment from the primary tumor or a metastatic site if primary tumor tissue was not available. All samples were reviewed by a pathologist, and the tumor area was marked for subsequent macrodissection. Only samples with a tumor cell percentage ≥ 20% were selected for DNA extraction [21-22]. For each tumor sample, slides of 10 µm thickness were obtained from the tumor area, and total RNA was extracted using the miRNeasy FFPE extraction kit (Qiagen, ref: 217504) according to the manufacturer's instructions. Total RNA quantity and quality were evaluated using a bioanalyzer. MiR-31-3p expression was quantified by RT-qPCR using a miRpredX 31-3p kit (Integragen, ref: IG-500-001). Expression levels were normalized to a reference miRNA and to a standard sample (provided with Integragen kit) using the ΔΔCt method. A previously reported cutoff value of 1.36 was initially used to define miR-31-3p low and high expresser patients [23]. However, according to the manufacturer's instructions, we considered values between 1.25 and 1.47 as uncertain. If the value lay within this zone, a new miR-31-3p quantification was performed, and if it was still uncertain, the sample was excluded from the analysis. This cutoff was defined to enable the identification of two subgroups of patients with mCRC with differential anti-EGFR treatment effects [21-22].

2.3. Statistical analysis

ORR was defined according to RECIST 1.1 criteria [24]. OS was defined as the time between the day of first administration of the first-line CT and death or date of last news or date of point. PFS was calculated from the first day of first-line CT to the date of first progression or death from any cause. Survival data (OS and PFS) were

estimated by the Kaplan-Meier method and compared by the log-rank test. A univariate Cox regression model was used to evaluate the association of the survival time of patients with each variable of interest, and a multivariate analysis was performed for parameters that were significant in univariate analysis. Differences in ORR according to the treatment arm were tested by Fisher's exact test. The concordance was estimated by calculating Cohen's kappa coefficient (κ) with the Kappa function of the R package vcd. The association between miR-31-3p expression level and *BRAF* V600E mutation status was estimated with the chi-square test. All statistical analyses were carried out with the R statistical environment (http://www.R-project.org/).

3. Results

3.1. Patients' characteristics and outcome

During the study period, 57 right-sided *RAS* wt mCRC patients were treated with CT+anti-EGFR and 34 with CT+Beva. The flow chart is represented in Figure 1. After applying the exclusion criteria previously described, 72 patients were included in the cohort of patients treated with CT+ targeted therapy (43 patients in the anti-EGFR group and 29 in the Beva group) (Table S1). Among the 26 patients constituting the independent cohort of right-sided colon tumors, tumor analysis was carried out in only 22 of them after 4 exclusions. Finally, of the 31 patients with available paired samples of both primary tumor and metastasis, only 16 patients had both samples with a tumor cell percentage > 20% (5 treated with anti-EGFR, 6 treated with Beva and 5 from the independent cohort). In the cohort of patients treated with first-line CT

+ targeted therapy, miR-31-3p expression analysis was measured in primary tumors and metastases in 93% and 7% of cases, respectively.

The baseline characteristics of patients are reported in Table 1. Patients treated with CT+Beva more often had a primary tumor resection (P = 0.037), ≥ 2 metastatic sites (P = 0.043), or a CT triplet (P = 0.007) than those treated with CT+anti-EGFR. Among the 72 patients, 45.8% were high miR-31-3p expressers (39.5% in the anti-EGFR group and 55.2% in the Beva group). The characteristics of both the low and high miR-31-3p expresser subgroups were comparable, except for the tumor *BRAF* V600E mutation, which was more frequent in high miR-31-3p expressers (60.6% vs 15.4%, P < 0.001) (Table 2). In the independent cohort of 22 primary right-sided colon cancer patients, high miR-31-3p expression was found in 52% of tumor samples.

After a median follow-up of 16.8 months (range 2.1-93), the median PFS and OS were 11 months (95% CI: 8-14 months) and 17 months (95% CI: 14-28 months), respectively. The median PFS was significantly longer in patients treated with Beva than in patients treated with anti-EGFR (13 months vs 7 months; HR = 0.52; 95% CI: 0.29-0.91; P = 0.024) (Figure S1A). We observed a similar tendency in favor of Beva for median OS, but statistical significance was not reached (21 months vs 14 months; HR= 0.57; 95% CI: 0.32-0.99; P = 0.053) (Figure S1B). Tumor response was evaluable for 70 patients. The ORR was not significantly different between the anti-EGFR and Beva groups (42.9% vs 47.6%; P = 0.88).

3.2. Prognostic value of miR-31-3p expression

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In univariate analysis, PS and metastasis resection were significant prognostic factors of PFS, whereas the type of targeted therapy, PS, metastasis resection and the number of metastatic sites were significant prognostic factors of OS (Table S2). As the type of targeted therapy nearly reached significance in univariate analysis for PFS (*P* = 0.053), it was included in multivariate analysis. In multivariate analysis, these factors remained significant for OS and PFS except the ECOG PS (Table S2).

In univariate analysis, PFS and OS of low miR-31-3p expressers were not significantly longer than PFS and OS of high miR-31-3p expressers (PFS:12 months vs 9 months; HR= 1.32; 95% CI: 0.76-2.31; *P* = 0.30; and OS: 28 months vs 13 months; HR= 1.64; 95% CI: 0.93-2.89; *P* = 0.070) (Figures 2A and 2B). Again, for ORR, no significant difference was observed between low and high miR-31-3p expressers (51.3% vs 38.7% respectively; *P* = 0.59).

3.3. Treatment impact on OS, PFS and ORR according to miR-31-3p expression

In the low miR-31-3p expression subgroup (n=39), despite there was a trend for a superiority of CT+Beva compared to CT+anti-EGFR in terms of OS and PFS, the difference was not statistically significant (median PFS 13 months vs 9 months; HR= 0.52; 95% CI: 0.23-1.19; *P* = 0.15; and median OS 44 months vs 20 months; HR= 0.56; 95% CI: 0.25- 1.29; *P* = 0.21) (Figures 3A and 3B). In the high miR-31-3p expresser subgroup (n= 33), the median PFS was significantly longer for patients treated with CT+Beva than for those treated with CT+anti-EGFR (11 months vs 6 months; HR= 0.45; 95% CI: 0.20-0.98; *P* = 0.03). The median OS tended to be longer in the CT+Beva group, but the difference was not statistically significant (20 months vs 13 months; HR= 0.52; 95% CI: 0.24-1.13; *P* = 0.09) (Figures 3C and 3D).

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In both the low and high-expresser subgroups, there was no significant difference in the ORR between patients treated with CT+anti-EGFR or CT+Beva (low expressers: OR= 1.35; 95% CI: 0.29-6.40; *P* = 0.91, high expressers: OR= 1.16; 95% CI: 0.22-6.14; *P* = 1), but the best numeric ORR was observed in the group of low miR-31-3p expressers treated with CT+anti-EGFR (Table 3).

In the cohort of patients treated with CT+anti-EGFR, median PFS of low miR-31-3p expressers was not significantly different compared to high miR-31-3p expressers (9 months and 6 months, respectively, HR= 1.69; 95% CI: 0.83-3.42; *P* = 0.10). However, median OS was significantly longer in low miR-31-3p expressers compared to high miR-31-3p expressers (20 months and 13 months, respectively, HR= 2.04; 95% CI: 0.98-4.24; *P* = 0.02) (Figures S2A and S2B). Despite there was a trend for a better ORR in patients with low miR-31-3p expression, it was not significantly different from that of patients with high miR-31-3p expression (53.8% vs 37.5%; OR= 1.48; 95% CI: 0.36- 6.37; *P* = 0.75) (Table 3).

In the cohort of patients treated with CT+Beva, there was no statistically significant difference in PFS, OS or ORR between patients with low and high miR-31-3p expression (Figure S3A and S3B).

3.4. Correlation of miR-31-3p expression status between paired samples of primary tumors and metastases

MiR-31-3p expression status (low vs high) was statistically correlated between the primary tumors and corresponding metastases in 15 of the 16 patients for whom paired samples were available for analysis (Kappa concordance test = 0.871). In the remaining one patient, miR-31-3p expression was low in the primary tumor but high in the corresponding skin metastasis (Table S3).

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Six patients received CT between the sampling of the primary tumor and the metastasis, including 5 with an associated targeted therapy (4 anti-EGFR and 1 Beva). No change in miR-31-3p expression status between paired samples was observed in these patients.

4. Discussion

Our study confirms the poor prognosis of patients with right-sided *RAS* wt mCRC, with a median PFS of 11 months and a median OS of 17 months. Previous pooled or meta-analyses have clearly demonstrated lower survival in patients with right-sided mCRC compared to those with left-sided tumors and have reported median PFS ranging from 7 to 12.6 months and median OS between 11 months and 23 months [17–19,25]. Moreover, we found a longer OS and PFS in patients treated with CT+Beva compared to those treated with CT+anti-EGFR, with the difference in OS reaching statistical significance, and no difference between the two targeted therapies in terms of ORR, which is also consistent with the literature [17,19,25]. In these meta-analyses, anti-EGFR mAbs appear to be more effective than Beva as first-line treatments for left-sided mCRC. In right-sided mCRC, however, anti-EGFR mAbs were inferior to Beva, which was associated with a significantly increased PFS and a numerically increased OS. This has led the US National Comprehensive Cancer Network to recommend that "only patients whose primary tumors originated on the left side of the colon should be offered Cetuximab or Panitumumab in the first-line treatment of metastatic disease" [26].

Three previous studies, including right and left mCRC, have shown an independent prognostic value of miR-31-3p expression [13,16,27]. In our study, although the survival of low expresser patients was numerically longer than that of

high expressers in terms of PFS and OS, the difference was not significant, probably because of a lack of power and because our study was focused only on right-sided mCRC. However, in our right-sided mCRC patients, we confirmed the previously identified correlation between high miR-31-3p expression and *BRAF* V600E mutation in the overall mCRC population [16,27]. The mechanisms underlying this correlation remain to be elucidated.

Previous reports have demonstrated that the miR-31-3p expression level within the *RAS* wt population was predictive of anti-EGFR efficacy first in pretreated patients and then in first-line treatment of mCRC, with better outcomes and response rates in patients with low miR-31-3p expression [13–16, 27, 28]. However, given the lower efficacy of anti-EGFR in right-sided mCRC reported in several studies [17, 19], the predictive value of miR-31-3p on the efficacy of cetuximab or panitumumab in these right-sided tumors remains to be determined and may inform whether the low expresser subgroup could still benefit from these targeted therapies. However, our results failed to demonstrate that miR-31-3p expression could identify a subgroup of patients with right-sided *RAS* wt mCRC who might benefit from anti-EGFR. Even if OS was significantly longer in low vs high miR-31-3p expressers who received CT+anti-EGFR, survival was never higher with an anti-EGFR than with Beva in low expressers and even tended to be better with Beva, regardless of miR-31-3p expression, with a statistically longer median PFS in high expressers. These results are consistent with those previously reported by Laurent-Puig *et al*. from the FIRE-3 trial, who found that OS was improved in low expresser patients treated with FOLFIRI+Cetux compared to those treated with FOLFIRI+Beva, except for patients with right-sided tumors [16]. Moreover, FOLFIRI+Beva was associated with a longer but not significantly different PFS compared to FOLFIRI+Cetux in the overall

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population comprising left- and right-sided mCRC patients [16]. In this trial, patients were selected and received FOLFIRI in combination with Cetux or Beva. Our study led to the same conclusions in “real life” in unselected patients who received either Cetux or Pani as anti-EGFR therapy and various associated backbone chemotherapies (oxaliplatin and/or irinotecan-based regimens), reflecting all first-line therapeutic practices. Taken together, these data suggest that an anti-EGFR should not be recommended in first-line treatment of right-sided *RAS* wt mCRC patients.

It should however be noted that the ORR of low miR-31-3p expressers treated with CT+anti-EGFR was the best ORR observed in our study. This could mean that in patients with right-sided *RAS* wt mCRC, miR-31-3p expression might identify patients who could benefit from anti-EGFR therapy when the objective is to obtain the best tumor response, as is the case in patients with potentially resectable or symptomatic metastases. Nevertheless, this hypothesis needs to be confirmed in a study with larger population. Furthermore, it should be kept in mind that an intensification of the chemotherapy backbone with a combination of 5-FU, oxaliplatin and irinotecan (FOLFOXIRI or FOLFIRINOX), with or without a targeted therapy, is also a therapeutic option associated with high response rates and must be considered in patients who can receive it [29–31]. These considerations do not concern patients with a tumor harboring a deficient MMR status (more frequently found in right-sided colon cancer) for whom immunotherapy has been proven to be superior to standard chemotherapy [32].

We found a good concordance of miR-31-3p expression status (low or high) between the primary tumor and corresponding metastases in the 16 patients with paired samples analyzed. Data on this concordance and the impact of CT and/or anti-EGFR therapy on miR-31-3p levels are discordant in the literature [28,33]. Our

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results are concordant with a study performed in chemorefractory mCRC patients treated with Cetux as a single agent, where a good correlation of miR-31-3p expression (evaluated before Cetux administration) was found between primary tumors and metastasis [33]. In this study, the authors also compared miR-31-3p expression levels in sequential metastatic tissue biopsies collected before, during and after cetux administration and found no changes in miR-31-3p scoring. This would mean that sampling from either the primary tumor or a metastasis could be used to determine miR-31-3p expression level.

MiR-31-3p expression level and cutoff value determination has been well described in a previous study and has proven to be a reproducible and simple clinical test [21, 23]. While miR-31-3p levels have been mostly determined by qPCR on tumor material until now, new quantification techniques have recently been developed. A recent study [33] described the use of in situ hybridization for miR-31-3p quantification and revealed that the results of this technique were well correlated with qPCR results. Measurement of circulating tumor DNA is another promising technique for the quantification of biomarkers predictive of response to treatment. This method has been developed for *RAS* and *BRAF* status in mCRC and is still under development for miR-31-3p expression [34,35].

Our study has some limitations. First, its retrospective design, which explains the differences in characteristics between patients treated with CT+anti-EGFR and those treated with CT+Beva, should make us take the results with caution. However, while patients treated with CT+Beva more often had a primary tumor resection and a CT triplet, they also more frequently had ≥ 2 metastatic sites, which may have counterbalanced the former two favorable parameters. Second, the conclusions may have been partially influenced by different care depending on the centers, and the

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distribution between CT + anti-EGFR and CT + Beva in each center was not similar as it depends on various factors, such as resectability of the metastases, the disease-related symptoms, patient comorbidities and/or investigator's choice between the two targeted therapies assumed to be equally effective in the first line setting at the time of the study, where primary tumor side was not taken into account. Third, although this cohort dedicated to *RAS* wt right mCRC is one of the largest to date, the number of patients is limited because right-sided *RAS* wt mCRC represent only 15% to 20% of all mCRC. In addition, we had to exclude all patients who did not receive targeted therapy as first-line therapy and all those for whom the samples could not be analyzed for miR-31-3p expression. The small sample size may have limited the power of our results.

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5. Conclusion

In conclusion, the poor prognosis of *RAS* wt right-sided mCRC observed in our study is concordant with the literature. We found a strong correlation between high miR-31-3p expression and the presence of the *BRAF* V600E mutation. Our results failed to demonstrate that miR-31-3p expression could identify a subgroup of patients with right-sided *RAS* wt mCRC who might benefit from anti-EGFR and suggest that Beva should be the targeted therapy of choice in first-line treatment of these patients, regardless of miR-31-3p expression level.

Acknowledgments: We particularly thank Integragen SA, Evry, France, for its support in generously supplying the miRpredX-31-3p® kits. We thank Pascale

Bellaud and Roselyne Viel from H2P2 (Rennes 1 University) for their valuable collaboration. We thank Dr Alain Bargain (Bargain laboratory), Dr Anne-Sophie Lajarte-Thirouard (Ouest-Pathologie laboratory) and Nantes Hospital University tumorothèque for their collaboration in supplying tumor material of participating centers.

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Ethics approval: The study was approved by the Ethics Committee of Rennes University Hospital for all participating centers (registration No. 18.37). It obtained the authorization of the National Commission for Data Protection and Liberties (CNIL) (number 2005096v0).

Consent to participate: According to French regulations, patients were informed of the study and did not express opposition, except one who was excluded from the analysis.

Consent for publication: All authors have read and agreed to the published version of the manuscript.

Data availability: The datasets generated and analyzed during the current study are not publicly available due to patients confidentiality and protection of data but are available from the corresponding author on reasonable request.

Conflicts of interests: EB reports nonfinancial support from Merck Serono, MSD, Sandoz, Amgen, Pfizer. ALespagnol and AP report nonfinancial support from IntegraGen SA. MDT reports nonfinancial support from IntegraGen SA. NRL reports lectures for Astra Zeneca, Pfizer and Bristol Myers Squib. SML declares non financial support from Integragen SA. PA reports board membership for Roche, Amgen, Merck, Pierre Fabre, Servier, and Bayer, with travel funded by Roche, Servier, Amgen, and Pierre Fabre. PGP reports grants, personal fees and nonfinancial support from Servier, nonfinancial support from Novartis, nonfinancial support from Astellas, nonfinancial support from Amgen, nonfinancial support from AbbVie,

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personal fees and nonfinancial support from Ipsen, personal fees and nonfinancial support from Bayer, and personal fees and nonfinancial support from Sanofi outside the submitted work. JB reports personal fees from Roche, Astra-Zeneca, MSD, Servier, Bayer, and Bristol Myers Squib. TL reports personal fees from Sanofi Genzyme, Merck Serono, Amgen and Servier and nonfinancial support from Amgen and Servier. SG declares personal fees from MSD and AstraZeneca and nonfinancial support from Roche SA, MSD, AstraZeneca and Bristol Myers Squib. DG reports grants from Sanofi Genzyme, Servier, Novartis, Roche. KBLP reports personal fees from IPSEN and Novartis and nonfinancial support from IPSEN. FBibeau reports personal fees from Bristol Myers Squib, MSD, Astra-Zeneca, Merck, Diaceutics, Amgen, and Bayer and nonfinancial support from Bristol Myers Squib and MSD. ALièvre reports grants from Bayer, Lilly, and Novartis; personal fees from AAA, Amgen, Bayer, HalioDx, Incyte, Ipsen, Mylan, Novartis, Pierre Fabre, Roche, Sanofi and Servier; and nonfinancial support from Amgen, Bayer, Boerhinger, Ipsen, Pfizer, Pierre Fabre, Roche, Sandoz, Servier and IntegraGen SA. MDG reports board membership with AZ, Amgen, Pfizer, and Roche and nonfinancial support from IntegraGen SA. PC, LD, DC, CB, RCoriat, FBeuvon, LB, FL, RChautard and RD declare no conflicts of interest.

Funding: This research received no external funding. We thank the Rennes University Hospital and the Centre d'Etude des Maladies Digestives de Rennes (CEMDR) for their financial support. Anthony Perrot was financially supported by the Rennes University Hospital.

Report guidelines: This study was performed using the REporting recommendations for tumor MARKer prognostic studies (REMARK).

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Table 1: Baseline characteristics of patients treated with CT+anti-EGFR or CT+bevacizumab

	Anti-EGFR	Bevacizumab	*P* - value	Total
Number of patients	43	29		72
Age (median, years)	62.3	64.9		63.5
Sex, n (%)				
Male	26 (60.5)	16 (55.2)	0.839	42 (58.3)
Female	17 (39.5)	13 (44.8)		30 (41.7)
PS at metastasis diagnostic n (%)				
0-1	36 (83.7)	28 (96.6)	0.392	64 (88.9)
2	5 (11.6)	1 (3.4)		6 (8.3)
Undetermined	2 (4.7)	0 (0.0)		2 (2.8)
Primary tumor resection, n (%)				
Yes	35 (81.4)	29 (100)	0.037	64 (88.9)
No	8 (18.6)	0 (0.0)		8 (11.1)
Synchronous metastasis, n (%)	32 (74.4)	16 (55.2)	0.148	48 (66.7)
Number of metastatic sites, n (%)				
1	25 (58.1)	9 (31.0)	0.043	34 (47.2)
≥ 2	18 (41.9)	20 (69.0)		38 (52.8)
Liver metastasis, n (%)				
Yes	22 (51.2)	20 (69)	0.208	42 (58.3)
No	21 (48.9)	9 (31.0)		30 (41.7)
Peritoneal metastasis, n (%)				
Yes	25 (58.1)	15 (51.7)	0.767	40 (55.6)
No	18 (41.9)	14 (48.3)		32 (44.4)
Metastasis resection, n (%)				
Yes	11 (25.6)	8 (27.6)	1	19 (26.4)
No	32 (74.4)	21 (72.4)		53 (73.6)
BRAF V600E mutation, n (%)				
Yes	13 (30.2)	13 (44.8)	0.319	26 (36.1)
No	30 (69.8)	16 (55.2)		46 (63.9)
MMR status, n (%)				
MSI	3 (7.0)	6 (20.7)	*	9 (12.5)
MSS	17 (39.5)	6 (20.7)		23 (31.9)
N.A	23 (53.5)	17 (58.6)		40 (55.6)
Chemotherapy–targeted therapy				
LV5FU2	0 (0.0)	2 (6.9)	0.007**	2 (2.8)
FOLFOX	24 (55.8)	12 (41.4)		36 (50.0)
FOLFIRI	18 (41.9)	10 (34.5)		28 (38.9)
FOLFIRINOX	0 (0.0)	5 (17.2)		5 (6.9)
No cytotoxic chemotherapy	1 (2.3)	0 (0.0)		1 (1.4)
Cetuximab	22 (51.2)			
Panitumumab	21 (48.8)			

*: no P-value was calculated because of small effectives

**: Comparison of groups according to mono-, doublet or triplet CT.

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Table 2. Correlation between miR-31-3p expression and *BRAF* V600E mutation

	BRAF V600E status		
miR-31-3p expression level	*BRAF* wt (n= 46)	*BRAF* mutated (n= 26)	$P < 0.001$
Low (n= 39)	33	6	
High (n= 33)	13	20	

Wt= wild-type

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Table 3. Treatment effect on objective response rate according to miR-31-3p expression level

miR-31-3p expression level	**Univariate Odd ratio**	**Response rates (%, number of patients)**	
		CT+Anti-EGFR	**CT+Beva**
Low (n=39)	**1.35 [0.29; 6.40]** ***P* = 0.91**	**53.8% (14/26)**	**46.2% (6/13)**
High (n=31)	**1.16 [0.22; 6.14]** ***P* = 1**	**37.5% (6/16)**	**40% (6/15)**

P = 0.45

CT= chemotherapy

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Figures legends:

Figure 1. Flow chart (CT= chemotherapy, n= number of patients, Beva= bevacizumab, CRC= colorectal cancer, TCP= tumor cell percentage)

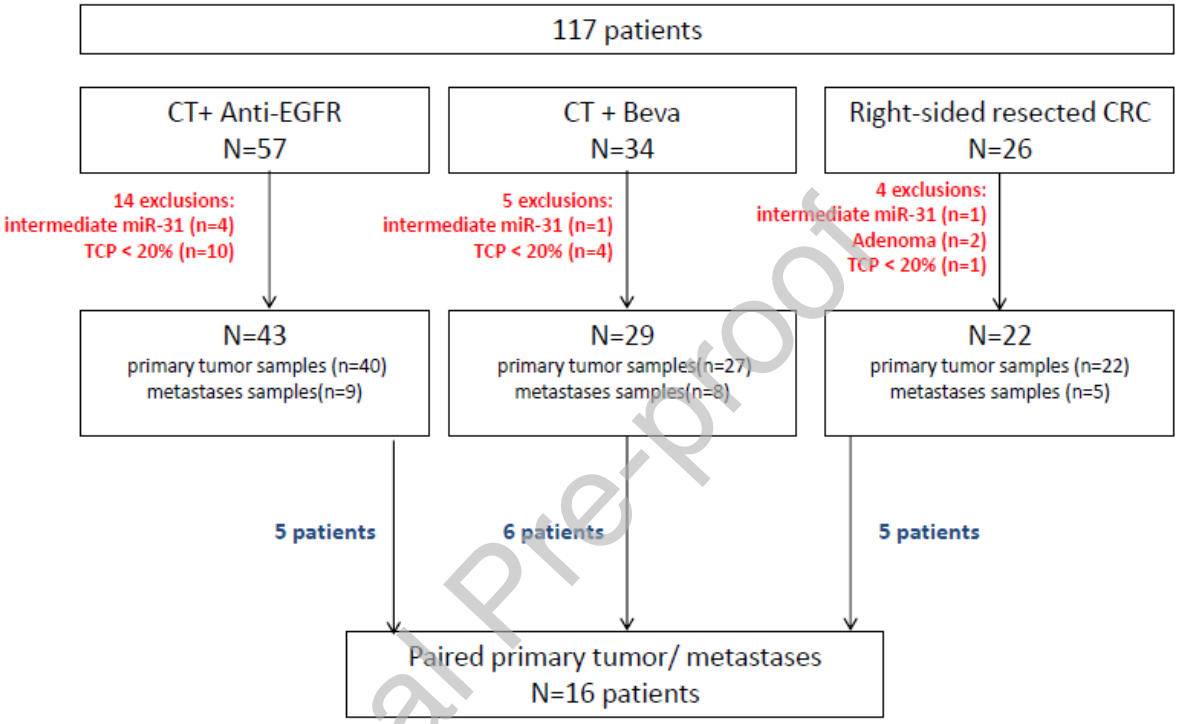

Figure 2. (A) Progression-free survival (PFS) and (B) overall survival (OS) according to miR-31-3p expression in the overall cohort of patients (miR low= low miR-31-3p expression, miR high= high miR-31-3p expression, n= number of patients, mo= months, HR= hazard ratio, CI= confidence interval, pts= patients)

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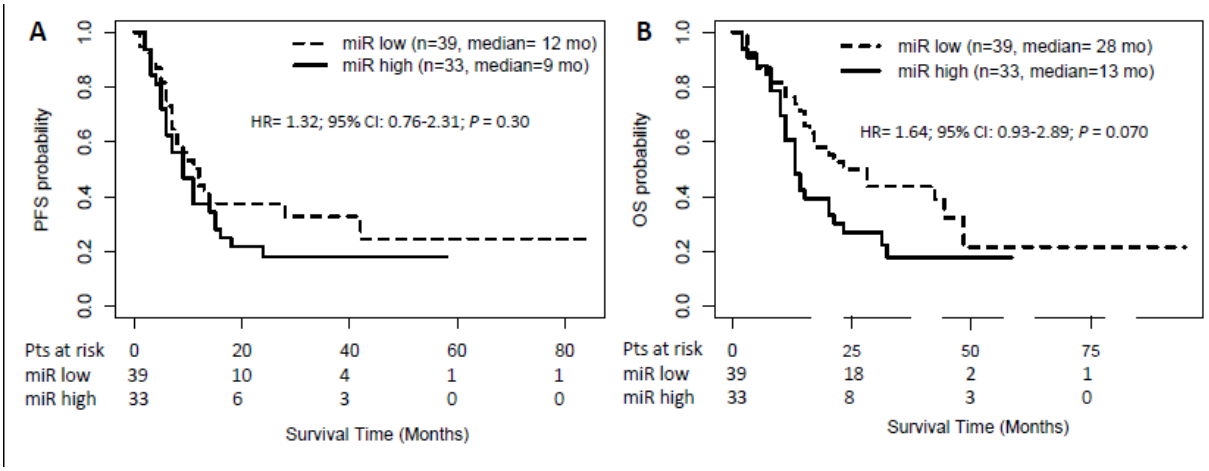
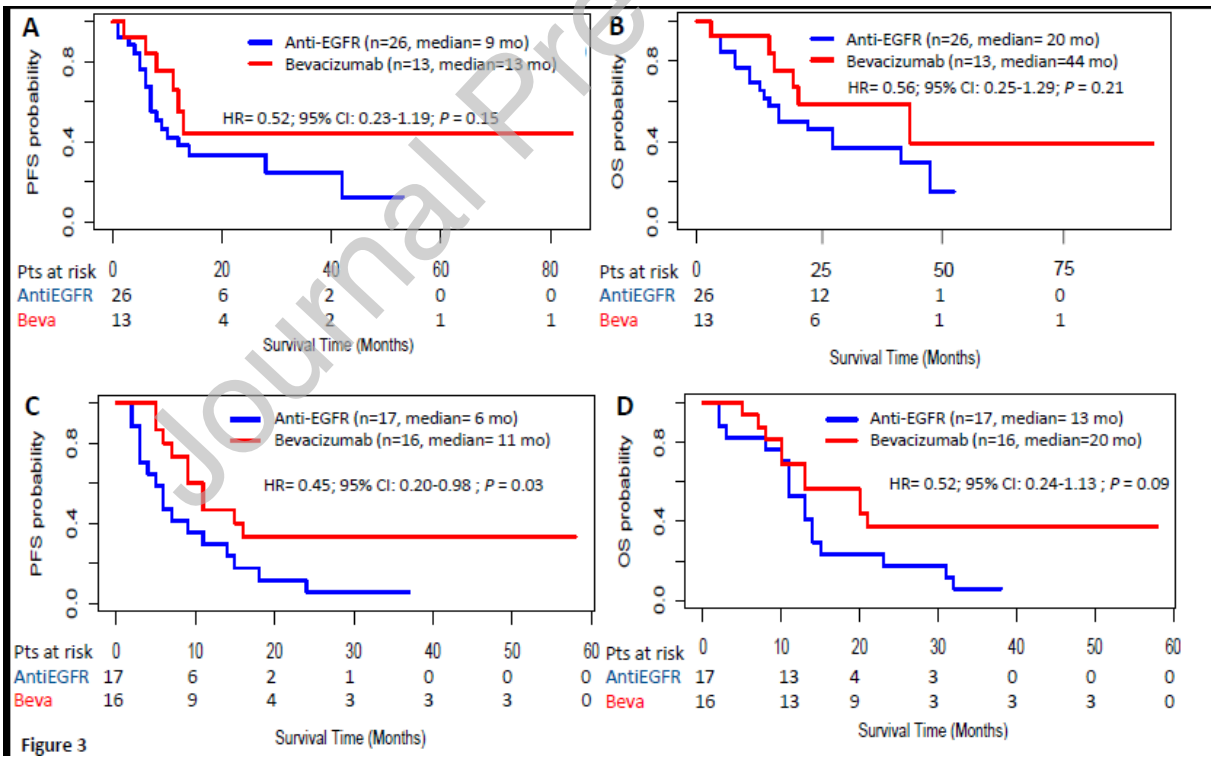


Figure 3. (A) Progression-free survival (PFS) and (B) overall survival (OS) of low miR-31-3p expressers according to targeted therapy; (C) PFS and (D) OS in high miR-31-3p expressers according to targeted therapy (n= number of patients, mo= months, HR= hazard ratio, CI= confidence interval, pts= patients, Beva= Bevacizumab)



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