



HAL
open science

**Metastatic colorectal carcinoma with signet-ring cells:
Clinical, histological and molecular description from an
Association des Gastro-Entérologues Oncologues
(AGEO) French multicenter retrospective cohort**

Marion Allart, Florence Leroy, Stephano Kim, David Sefrioui, Mihane Nayeri,
Aziz Zaanan, Benoit Rousseau, Meher Ben Abdelghani, Christelle de la
Fouchardière, Wulfran Cacheux, et al.

► **To cite this version:**

Marion Allart, Florence Leroy, Stephano Kim, David Sefrioui, Mihane Nayeri, et al.. Metastatic colorectal carcinoma with signet-ring cells: Clinical, histological and molecular description from an Association des Gastro-Entérologues Oncologues (AGEO) French multicenter retrospective cohort. Digestive and Liver Disease, 2022, 54 (3), pp.391-399. 10.1016/j.dld.2021.06.031 . hal-04522449

HAL Id: hal-04522449

<https://hal.science/hal-04522449>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Original article in *Digestive and Liver Disease*

2

3 **Metastatic colorectal carcinoma with signet-ring cells: clinical, histological**
4 **and molecular description from an AGEO French multicenter retrospective**
5 **cohort**

6 M. Allart^{1*} ; F. Leroy² ; S. Kim³ ; D. Sefrioui⁴ ; M. Nayeri⁵ ; A. Zaanani⁶ ; B. Rousseau⁷ ; M.
7 Ben Abdelghani⁸ ; C. de la Fouchardière⁹ ; W. Cacheux¹⁰ ; R. Legros¹¹ ; S. Louafi¹² ; D.
8 Tougeron¹³ ; O. Bouché¹⁴ ; N. Fares¹⁵ ; G. Roquin¹⁶ ; A.L. Bignon¹⁷ ; M. Maillet¹⁸ ; A.
9 Pozet^{19*} and V. Hautefeuille^{1*} For the AGEO investigators[#]

10 *(1) Department of Gastroenterology and Digestive Oncology, Amiens University Hospital –*
11 *Amiens, (2) Department of Cancer Medicine, Gustave Roussy Institute – Villejuif, (3)*
12 *Department of Medical Oncology, Jean Minjot University Hospital - Besançon, (4)*
13 *Department of Hepato-Gastroenterology, Rouen University Hospital – Rouen, (5) Department*
14 *of Digestive and Oncological Surgery, Lille University, Claude Huriez University Hospital –*
15 *Lille, (6) Department of Gastroenterology and Digestive Oncology, European Georges*
16 *Pompidou Hospital, APHP, Univ. Paris– Paris, (7) Department of Medical Oncology, Henri*
17 *Mondor University Hospital – Créteil, Memorial Sloan Kettering Cancer Center – New York*
18 *- USA, (8) Department of Medical Oncology, Paul Strauss Center – Strasbourg, (9)*
19 *Department of Medical Oncology, Leon Berard Center – Lyon, (10) Department of Medical*
20 *Oncology, Private Hospital Pays de Savoie,– Annemasse, (11) Department of*
21 *Gastroenterology, Limoges University Hospital – Limoges, (12) Department of Medical*
22 *Oncology, Oncology Federation of Essonne – Corbeil-Essonnes, (13) Department of*
23 *Gastroenterology, Poitiers University Hospital – Poitiers, (14) Department of*
24 *Gastroenterology and Digestive Oncology, Reims University Hospital – Reims, (15)*

25 *Department of Hepato-Gastroenterology, Toulouse University Hospital – Toulouse, (16)*
26 *Department of Gastroenterology and Digestive Oncology, Angers University Hospital –*
27 *Angers, (17) Department of Hepato-Gastroenterology and Nutrition, Caen University*
28 *Hospital – Caen, (18) Department of Gastroenterology, Saint Louis Hospital, APHP – Paris,*
29 *(19) Methodology and Quality of Life in Oncology Unit, INSERM UMR 1098, Besançon*
30 *University Hospital – Besançon.*

31 * Equally contributed, # Please see Supplementary Appendix 1 for a list of other
32 Investigators/Collaborators.

33 ***Corresponding author:***

34 Dr Vincent Hautefeuille

35 Department of Gastroenterology and Digestive Oncology, Amiens University Hospital,
36 80054, Amiens, France

37 Phone number: (33) 3 22 08 88 54

38 Fax number: (33) 3 22 08 97 35

39 E-mail address: hautefeuille.vincent@chu-amiens.fr

40 ORCID id: 0000-0003-2735-6448

41

42 ***Electronic word count:***

43 Abstract: 198 words

44 Manuscript (excluding title page, figure legends and abstract): 3299 words

45 Number of manuscript pages: 12

46 3 figures and 3 tables

47 Number of references: 49

48

49 *Article type:* Article, Clinical study

50 **Abstract:**

51 **Background:** Metastatic signet-ring cell colorectal carcinoma is rare. We analyzed its
52 clinicopathological and molecular features, prognostic factors and chemosensitivity.

53 **Methods:** Retrospective study from 2003-2017 in 31 French centers, divided into three
54 groups: curative care (G1), chemotherapy alone (G2), and best supportive care (G3).

55 **Results:** Tumors were most frequently in the proximal colon (46%), T4 (71%), and poorly
56 differentiated (86%). The predominant metastatic site was peritoneum (69%). Microsatellite
57 instability and *BRAF* mutation were found in 19% and 9% (mainly right-sided) of patients and
58 *RAS* mutations in 23%. Median overall survival (mOS) of the patients (n=204) was 10.1
59 months (95%CI: 7.9;12.8), 45.1 for G1 (n=38), 10.9 for G2 (n=112), and 1.8 months for G3
60 (n=54). No difference in mOS was found when comparing tumor locations, percentage of
61 signet-ring cell contingent and microsatellite status. In G1, relapse-free survival was 14
62 months (95%CI: 6.5-20.9). In G2, median progression-free survival (PFS) was 4.7 months
63 (95%CI: 3.6;5.9]) with first-line treatment. Median PFS was higher with biological agents
64 than without (5.0 vs 3.9 months, p=0.016).

65 **Conclusions:** mSRCC has a poor prognosis with specific location and molecular alterations
66 resulting in low chemosensitivity. Routine microsatellite analysis should be performed
67 because of frequent MSI-high tumors in this population.

68 **Keywords:** colorectal cancer, metastatic, signet-ring cell, molecular

69

70 **1. Introduction**

71 Signet-ring cell colorectal carcinoma (SRCC) is a rare entity and published data are
72 scarce, especially for metastatic disease. SRCC represents 1% of colorectal carcinomas
73 (CRCs) [1,2] and is characterized by the presence of signet-ring cells (more than 50%)
74 containing intracytoplasmic mucin, which displaces the nucleus to the cell periphery. They
75 are disseminated in an abundant fibrotic stroma and can give the appearance of a
76 macroscopically rigid structure called linitis. The epidemiological characteristics of SRCC are
77 well described in the literature, particularly at stage III and IV [3,4], but few molecular data
78 are available and are limited to the metastatic setting. The few reported series included a small
79 number of patients (range of 10-40). The largest study of the molecular characteristics of
80 SRCC is that of Korphaisarn et al., with 93 patients [5], including 28 with a signet-ring cell
81 component of less than 50%. While outcomes for early stage SRCC have been well
82 documented, the benefit derived from chemotherapy and/or biological agents remains to be
83 determined for patients with advanced disease.

84 SRCC of all tumor stages affects younger persons (25% before 60 years) [2–4], is
85 more frequent in the proximal colon (50-75%) [1,3–6], is diagnosed at a more advanced stage
86 than typical adenocarcinomas (29-40% vs 20% at stage IV) [1,3,4], has more frequent lymph
87 node and peritoneal invasion at diagnosis (67% vs 25% and 50-90% vs 10-20%, respectively)
88 [6–9], and is generally poorly differentiated [1,2]. SRCC is more aggressive and has a lower
89 survival rate than adenocarcinomas, with a median overall survival (mOS) of 16.4 months vs
90 47.2 months for typical metastatic CRC adenocarcinomas in the Korphaisarn et al. study
91 [1,4,5,10]. Moreover, a minor signet-ring cell component (< 50%) is sufficient to confer a
92 poor prognosis, with an mOS of 19.3 months for mCRC with a signet-ring cell component <
93 50% [5,11,12].

94 *RAS* mutations seem to be less frequent in SRCC of all stages [11,13–15], as well as in
95 metastatic disease, with only 11% of *KRAS* mutations [5]. However, the *BRAF V600E*
96 mutation seems to be more frequent in SRCC, whatever the stage [11,13,14]. Scarce data in
97 advanced settings are available for *BRAF* mutations: the cohort of Korphaisarn et al. revealed
98 *BRAF* mutations in 7.7%, 25%, and 8.6% of adenocarcinomas, adenocarcinomas with a
99 signet-ring cell component and SRCC, respectively. Microsatellite instability (MSI-H, or
100 deficient mismatch repair (dMMR) status) seems to be 3 times more frequent in SRCC than in
101 classic adenocarcinomas (31% vs 11%), and it seems to be similar in a metastatic setting
102 (14% vs 5%) [1,13,14]. To our knowledge, no study has focused on an exclusively metastatic
103 population, thus precluding definitive conclusions about the best chemotherapy regimen.
104 Moreover, no study has confirmed the prognostic value of dMMR status in SRCC [1,11,16–
105 18].

106 There are no specific guidelines for treatment of SRCC or CRC with a signet-ring cell
107 component [19]. The benefit of surgery for the primary tumor and the metastases is not clear
108 [8,20–23]. No data on chemosensitivity are available, in particular on whether there is a
109 benefit in using anti-vascular endothelial growth factor (anti-VEGF) or anti-epidermal growth
110 factor receptor (anti-EGFr). The OS of mSRCC patients who have undergone curative surgery
111 is lower than that classic adenocarcinomas (5-year survival rate of 10% vs 25%, or even
112 higher in recent prospective studies [24]) and the 6-month postoperative relapse rate seems
113 higher (25% vs 13%) [20,25].

114 In this observational multicenter retrospective study, we aimed to confirm the clinical,
115 biological and molecular characteristics of mSRCC (CRC with a signet-ring cell component
116 >50% and <50%) and to analyze prognosis and assess chemosensitivity.

117 **2. Patients and methods**

118 *2.1 Study population*

119 This retrospective multicenter cohort included all consecutive patients diagnosed after
120 2003 with histologically proven CRC with signet-ring cells (various signet-ring cell
121 components, i.e. higher or lower than 50%) and with radiological evidence of metastases
122 (synchronous or metachronous). Data were collected in 31 French centers between November
123 2016 and July 2017, through the AGEO (Association des Gastro-Entérologues Oncologues)
124 network, anticancer centers and university hospitals. A personal or family history of gastric
125 signet-ring cell carcinoma and a personal history of inflammatory bowel disease were also
126 collected.

127 Patients with another type of cancer without curative treatment, with an unknown
128 primary tumor location, or with a high-grade neuroendocrine component were excluded.

129 Location of the primary tumor, TNM classification, grade and percentage of signet-
130 ring cells were analyzed. When available, *RAS* (mainly *KRAS*)/*BRAF* mutations and MMR
131 status (MSI and/or MMR immunohistochemistry) were collected. The method for *RAS*
132 mutation analysis was not specified.

133 The cohort was divided into three groups: Group 1 (G1) included patients with
134 curative treatment of the metastatic disease (chemotherapy, radiotherapy and surgery), Group
135 2 (G2) included patients treated with chemotherapy alone. Group 3 (G3) included patients
136 with best supportive care alone. The OS for the entire cohort and for each group was
137 calculated. For Group 1, recurrence-free survival (RFS) after curative treatment of the
138 metastases was analyzed. The chemotherapy regimen, response rate for each treatment line
139 and progression-free survival (PFS) for G2 were analyzed.

140 The study was conducted in accordance with the Declaration of Helsinki and French
141 regulatory requirements (*Commission Nationale Informatique et Libertés*). All patients alive

142 at the time of the study received appropriate verbal information and gave their consent for
143 anonymous data collection. In accordance with French national laws and clinical research
144 guidelines, this retrospective observational study did not require formal ethics committee
145 approval.

146 *2.2 Objectives and statistical analysis*

147 The primary objective was to characterize clinical, biological and molecular features
148 of mSRCC. Secondary objectives were to analyze OS, PFS and chemosensitivity. Continuous
149 variables were expressed as the median with interquartile range. Categorical variables were
150 expressed as percentage and total population. mOS was calculated from the date of diagnosis
151 of metastatic disease and the date of death from any cause. Disease-free survival (DFS) was
152 calculated from the date of the end of local cancer management and the date of metastatic
153 progression. RFS in G1 was calculated from the date of the end of metastatic curative-intent
154 care and the date of metastatic recurrence. PFS in G2 was calculated at each line of
155 chemotherapy from the date of the beginning of chemotherapy and the date of progression.
156 Response rate (overall response rate and disease control rate), progression rate and
157 chemosensitivity were assessed on a CT scan or MRI according to the RECIST 1.1 criteria.

158 Median follow-up was computed using the reversed Kaplan-Meier non-parametric
159 method. OS, DFS and PFS were assessed using the Kaplan-Meier non-parametric method and
160 are expressed as the median with 95% confidence interval (95% CI). Comparisons were
161 performed using the log-rank test. For all tests, $p < 0.05$ was considered to be statistically
162 significant.

163 Univariate and multivariate analyses were performed using the Cox model to assess
164 factors associated with OS. A factor with a p -value less than 0.2 in univariate analysis was

165 integrated in the multivariate analysis. Adjusted hazard ratios (HR) and 95% confidence
166 interval were used for estimation.

167 **3. Results**

168 *3.1 Population, patients and tumor characteristics*

169 A total of 204 patients were included. The median age at metastatic diagnosis was 62
170 years [IQR=52-74] and the patients were mainly men (61%) in the entire cohort. Performance
171 status (PS) was 0-1 in 74% of cases and serum carcinoembryonic antigen (CEA) and
172 carbohydrate antigen 19.9 (CA19.9) rates were higher than the upper limit of normal (ULN)
173 (5 ng/mL and 37 IU/mL, respectively) in 61% and 44% of the cases, respectively. The linitis
174 plastica phenotype was present in endoscopy, rectal echo-endoscopy, or medical imaging (CT
175 scan or MRI) in 37% of the cases. Three patients had a personal or family history of lobular
176 breast cancer or gastric signet-ring cell carcinoma. Seven patients had inflammatory bowel
177 disease (3 cases of ulcerative colitis and 4 cases of Crohn's disease). There was no case of
178 Lynch syndrome.

179 The primary tumor was located in the proximal colon, distal colon and rectum in 46%,
180 25% and 29% of cases, respectively. Tumors were T4 in 71% of cases, with lymph node
181 invasion in 95% and were poorly differentiated in 86% of cases. The percentage of signet-ring
182 cells was available for 87 patients, 36% of whom had a percentage lower than 50%. Fifty-
183 eight patients out of 71 had a mucinous component. Metastatic sites were mainly in the
184 peritoneum (69%), lymph nodes (28%) and liver (23%) (**Table 1**).

185 *3.2 Molecular analysis*

186 The distribution of RAS and BRAF mutations in primary tumor sites is represented in
187 **Figure 1**. RAS and BRAF mutations and MMR status were available for 127, 103 and 108

188 patients, respectively. At the time of the study, full RAS mutation (analysis performed in
189 France after 2014) was not available for all patients and a majority only had a KRAS exon 2
190 analysis. KRAS/RAS mutations were found in 23% of cases and did not differ between right-
191 sided and left-sided tumors. BRAF mutations were found in 9% of cases (almost all were
192 p.V600E and one mutation was p.V600G). dMMR was found in 19% of mSRCC cases,
193 mainly in the proximal colon (27% of tumors) and was less frequent in the distal and rectal
194 sites (16% and 9%, respectively). *BRAF* mutations were found in 19% (3/16) of dMMR
195 mSRCC.

196 3.3 Type of treatment

197 Among the 204 patients in the cohort, 34% had surgery of the primary tumor at a
198 localized stage prior to inclusion in the cohort. Antitumor treatment was performed in 168
199 patients: 38 had curative treatment of the metastases (G1) and 112 received chemotherapy
200 alone (G2). Fifty-four patients had best supportive care alone with no surgery and no
201 chemotherapy (G3). Some patients in group 2 may have been operated on, but none of them
202 had curative surgery (i.e. too many liver and/or peritoneal metastases). Patient characteristics
203 at metastatic diagnosis for each group are described in **Table 1**.

204 In G1, half of the patients had neoadjuvant chemotherapy (median of 6 cycles, 9/19
205 received FOLFOX, 6/19 received chemotherapy and bevacizumab). Among the 30 patients
206 with a peritoneal location, 66% underwent cytoreductive surgery followed by HIPEC
207 (Hyperthermic Intra-Peritoneal Chemotherapy). Seventy-nine percent (n=30/38) had adjuvant
208 chemotherapy.

209 In G2, 100% had first-line chemotherapy. Second-line (2L) chemotherapy was carried
210 out in 52% (n=58/112), third-line (3L) in 20% (n=22/112) and fourth-line (4L) in 5%
211 (n=6/112) of the patients. In first-line therapy (1L), 48% received an oxaliplatin-based

212 treatment, 36% had an irinotecan-based treatment and 5% received triplet treatment. Half of
213 the patients received targeted therapy in combination with chemotherapy (29% antiVEGF and
214 21% antiEGFr) in 1L. The 2L, 3L and 4L treatment regimens are detailed in **Table 2**.

215 *3.4 Survival data*

216 The median follow-up period of the cohort was 6.2 years (95% CI: 2.3-9.7). At the last
217 follow-up date, only 18.7% of the patients were still alive. Four patients were lost to follow-
218 up. DFS assessed in the 53 patients who underwent surgery of the primary tumor before
219 diagnosis of metastases was 8.6 months (95% CI: 4.6-10.8).

220 mOS for the entire population was 10.1 months (95% CI: 7.9-12.8) with a 1- and 3-
221 year survival rate of 44% and 15%, respectively (**Figure 2A**).

222 mOS for G1 was 45.1 months (95% CI: 34.2-67.1), with a 1- and 3-year survival rate
223 of 97% and 71%, respectively. mOS for G2 was 10.9 months (95% CI: 8.6-13.8), with a 1-
224 and 3-year survival rate of 43% and 4%. For G3, mOS was only 1.8 months (95% CI: 0.9-
225 2.8), with a 1-year survival rate of 7.9%. mOS was significantly different between the 3
226 groups ($p < 0.0001$) (**Figure 2B**).

227 No difference in mOS was found in the overall population between locations of the
228 primary tumor (proximal colon, distal colon, or rectum, $p = 0.13$) (**Figure 2C**), for signet-ring
229 cell percentage ($\geq 50\%$ vs $< 50\%$, $p = 0.4$) (**Figure 2D**), linitis plastica phenotype (yes vs no vs
230 unknown) ($p = 0.77$), or MMR status (dMMR vs proficient MMR, $p = 0.82$) (**Figure 2E**).

231 The median RFS in G1 at the end of curative treatment of all metastases was 14.0
232 months (95% CI: 6.5-20.9). Median PFS for G2 was 4.7 months in 1L (95% CI: 3.6-5.9), 2.2
233 months in 2L (95% CI: 1.7-2.7), 2.5 months in 3L (95% CI: 1.2-5.0) and 3.1 months in 4L
234 (95% CI: 0.9-11.1) chemotherapy.

235 3.5 Chemosensitivity in group 2

236 Response and disease control rates in 1L were 13.2% and 50.0%, respectively. In 2L
237 and 3L, they were 11.0% and 39.0% and 0.0% and 52.6%, respectively. The disease control
238 rate in 1L was 44.0% for antiVEGF-based therapy, 63.6% for antiEGFr-based therapy and
239 45.5% for chemotherapy alone (**Table 2**).

240 The median PFS in 1L was 6.2 months (95% CI: 3.0-7.8) with antiEGFr compared
241 with 5.1 months with antiVEGF (95% CI: 3.1-9.0) and 3.9 months (95% CI: 2.6-5.5) with
242 cytotoxic agents alone. Targeted treatment (antiVEGF or antiEGFr) was significantly superior
243 to cytotoxic agents alone, with a median PFS in 1L of 5 months (95% CI: 3.7-7.6) compared
244 with 3.9 months (95% CI: 2.6-5.5) ($p=0.02$) (**Figure 3**). There was no difference between PFS
245 in antiVEGF and antiEGFr treatments ($p=0.86$) in 1L, either in right-sided ($p=0.28$) or left-
246 sided ($p=0.27$) tumors.

247 3.6 Prognostic factors

248 In the overall population, in univariate analysis, factors associated with poorer OS
249 were PS > 1 (HR=2.61, 95% CI: 1.75-3.90, $p<0.0001$), serum CEA level above the ULN
250 (HR=1.69, 95% CI: 1.11-2.58, $p=0.004$), lymphatic invasion (HR=1.88, 95% CI: 1.08-3.29,
251 $p=0.005$), vascular invasion (HR=1.65, 95% CI: 1.10-2.71, $p=0.008$), major ($\geq 50\%$) signet-
252 ring cell component (HR=1.24, 95% CI: 0.74-2.09, $p=0.003$) and absence of primary tumor
253 surgery (HR=1.78, 95% CI: 1.24-2.54, $p=0.001$). Location of the primary tumor, *RAS*, *BRAF*
254 and MMR status were not predictive of poorer OS.

255 In multivariate analysis, independent factors associated with poorer OS were PS > 1
256 ($p=0.0001$), serum CEA level above the ULN, major ($\geq 50\%$) signet-ring cell component,

257 vascular invasion and metastatic stage. Tumor location and the absence of primary tumor
258 surgery were not statistically associated with poorer OS (**Table 3**).

259 **4. Discussion**

260 To our knowledge, this is the largest study on mSRCC and CRC with a signet-ring cell
261 component to demonstrate that this rare subtype of metastatic CRC does not behave in the
262 same way as typical adenocarcinomas, as it seems to have a distinct natural history. While
263 typical adenocarcinomas are located in the proximal colon (30-40%), distal colon (35-40%)
264 and rectum (20-25%) [4,26], SRCC is more frequently located in the proximal colon (46%)
265 and rectum (29%). In the cohort of Hugen et al. [4], SRCC was also more frequently found in
266 the proximal colon, but less frequently in the rectum.

267 mSRCCs carry tumor factors of aggressiveness, with a vast majority at the T4 stage
268 and with poor differentiation. Surprisingly, the CEA level was low and contrasts with the
269 aggressiveness of mSRCC. Peritoneal carcinomatosis was the most common site of
270 metastases (70% vs 10-20% for adenocarcinomas [6,27]), with fewer liver metastases (23%
271 vs 75% for adenocarcinomas [6]). This specific metastatic spread was also found in the cohort
272 of Korphaisarn et al. [5] and is similar to that of gastric signet-ring cell carcinomas. Indeed,
273 signet-ring cells confer a poor prognosis due to peritoneal dissemination and chemoresistance
274 [28,29]. Three recent studies of patients from the SEER (Surveillance, Epidemiology and End
275 Results) database confirmed these results [30–32].

276 Furthermore, dMMR/MSI status was present in 19% of mSRCCs, which is rather high
277 considering the lower rate of 3.5-5% usually found in classic metastatic adenocarcinomas
278 [5,33–36]. This is higher than the 14% of mSRCCs in the Korphaisarn et al. cohort [5] and
279 suggests that dMMR IHC and MSI testing must be performed in mSRCC in light of the
280 promising results of immune checkpoint inhibitors in dMMR/MSI tumors [37–40]. dMMR

281 status is known to be associated with a poorer prognosis in the metastatic setting [41].
282 Nevertheless, we found no difference in OS when comparing dMMR and pMMR tumors,
283 probably due to the small number of patients studied.

284 It is well established that proximal locations confer a poor prognosis [24,41,42] on
285 typical adenocarcinomas. In our cohort, we found no difference in mOS when comparing
286 proximal and distal mSRCC. Moreover, neither proximal tumor location nor MMR status was
287 predictive of OS in multivariate analysis, suggesting different prognostic factors in mSRCC.
288 However, these groups are small. Mostly *BRAF* (8 out of 10 patients had the p.V600E
289 mutation) mutations were found in 9% of mSRCCs, compared with 7-11% in classic CRC
290 [43,44]. This percentage seems to be low considering the high number of right-sided and
291 dMMR/MSI tumors. While a *BRAF* mutation was found in about 30-40% of dMMR
292 adenocarcinomas [41], we found *BRAF* mutations in only 19% of dMMR mSRCCs. Finally,
293 although most patients had a *KRAS* exon 2 mutation analysis (but not a full *RAS* analysis since
294 a number of patients were included before 2010), *KRAS* mutations were found in only 23% of
295 tumors. This is low compared with the 35-40% of exon 2 *KRAS* mutations [45] and 50-58% of
296 *RAS* mutations in classic CRC [43,46]. Again, these findings are consistent with the 11%
297 reported by Korphaisarn et al. [5]. All of these clinical and molecular features may explain
298 their particular behavior and suggest a specific carcinogenesis in mSRCC.

299 In our cohort, DFS for patients who underwent local surgery of the primary tumor was
300 only 8.6 months, which is indicative of the aggressiveness and high rate of relapse of this
301 subtype of CRC.

302 In the metastatic setting in the entire cohort, mOS was 10.1 months, which is very low
303 and may be explained by the aggressiveness of mSRCCs, but also by the inclusion of all
304 consecutive patients, especially those belonging to group 3. With first-line chemotherapy,

305 mOS and mPFS were only 10.9 months and 4.7 months vs 25-35 months and 10-12 months,
306 respectively, in the studies using bichemotherapy plus biological agent for typical CRC [47–
307 49]. These data are quite similar to those reported by Korphaisarn et al. [5], with an mOS of
308 approximately 16 months, attesting to the limited chemosensitivity of mSRCC when using
309 classic CRC cytotoxic drug regimens. When possible, “aggressive” chemotherapy with triplet
310 therapy plus biologics would be an interesting choice.

311 mSRCC shows resistance to systemic treatment, with low response rates after first-line
312 palliative chemotherapy (response and control rates of 13.2% and 50%, respectively). Typical
313 CRCs have higher objective response rates (60 to 70% with bichemotherapy + antiEGFr
314 [24,49] or 50 to 55% with bichemotherapy + antiVEGF [24,47]).

315 Nevertheless, we observed a benefit with use of a biotherapy-based treatment
316 (antiVEGF or antiEGFr) in 1L compared with the use of cytotoxic agents alone. However,
317 this PFS was not adjusted for major confounding factors. The gain of 1.1 months is weak and
318 would require the assessment of other therapeutic options such as trichemotherapy + targeted
319 therapy, or chemotherapy + immunotherapy (anti-PD1/PDL1 or anti-CTLA4), especially in
320 the dMMR SRCC subgroup. The small number of patients in each group of chemotherapy did
321 not allow us to draw definitive conclusions on the most effective chemotherapeutic regimen.
322 Finally, we did not find any difference in PFS when comparing anti-VEGF and anti-EGFr in
323 either right-sided or left-sided tumors.

324 There was no difference in the OS analysis when comparing the mOS of patients with
325 a high ($\geq 50\%$) vs a low rate ($< 50\%$) of signet-ring cells (LogRank, $p=0.04$), even though this
326 rate was a prognostic factor in the multivariate OS analysis. This was mainly due to missing
327 data for this factor and the number of patients that could be included in the multivariate
328 analysis.

329 Finally, there is a faint glimmer of hope, as the 38 patients from group 1 who
330 underwent curative metastatic cancer management had an encouraging mOS of 45.1 months
331 and a 3-year survival rate of 71%. However, these results remain below the almost 65 months
332 of OS achieved with R0 resection after first-line treatment including bichemotherapy +
333 targeted therapy reported in the CALGB80405 study [24].

334 This study has some limitations. Firstly, due to its retrospective design some data were
335 missing, in particular the percentage of signet-ring cells. Although SRCC is defined strictly by
336 a greater than 50% presence of signet-ring cells, the mOS was very low regardless of the
337 signet-ring cell percentage (including missing data and low rate of signet-ring cells (<50%)).
338 Secondly, full RAS analysis was not performed before 2014 and some other KRAS or NRAS
339 mutations, although rare, may have been missed. Thirdly, the long period of inclusion leads to
340 heterogeneous conditions of patient care.

341 **5. Conclusion**

342 mSRCC spreads locoregionally, leading to high peritoneal invasion and low liver
343 involvement with specific clinical features and molecular alterations that differ from typical
344 colorectal adenocarcinoma. This results in a poorer prognosis with a median OS of less than
345 one year. Microsatellite analysis should be routinely performed in this subgroup of colorectal
346 cancer given the high rate of dMMR/MSI tumors and the promising results of immune
347 checkpoint inhibitors in dMMR/MSI tumors.

348

349

350

351

352 **Additional information**

353 The authors would like to thank all participating authors for their contributions, Valerie Fong
354 and David Marsh for English review.

355 This study was presented at the ESMO Congress in 2018 in Munich (Poster session, abstract
356 #557P, doi:10.1093/annonc/mdy281.103).

357 MA and VH designed the study. AP performed the statistical analysis. SK, DS, AZ, BR,
358 CDLF, DT, OB, ALB, GP, AP and VH reviewed the article. Data collection was performed
359 by all authors (including those listed in appendix 1).

360 This study was performed in accordance with the ethical principles of the Declaration of
361 Helsinki and French regulatory requirements.

362 Data are the property of VH and the AGEO and are not available.

363 The authors declare no conflict of interest.

364 The authors received no specific funding for this work.

365

366

367

368

369

370

371

372 **References**

- 373 [1] Nitsche U, Zimmermann A, Späth C, et al. Mucinous and signet-ring cell colorectal
374 cancers differ from classical adenocarcinomas in tumor biology and prognosis. *Ann*
375 *Surg.* 2013 Nov;258(5):775–82.
- 376 [2] Chew M-H, Yeo S-AE, Ng Z-P, et al. Critical analysis of mucin and signet ring cell as
377 prognostic factors in an Asian population of 2,764 sporadic colorectal cancers. *Int J*
378 *Colorectal Dis.* 2010 Oct;25(10):1221–9.
- 379 [3] Hyngstrom JR, Hu C-Y, Xing Y, et al. Clinicopathology and outcomes for mucinous and
380 signet ring colorectal adenocarcinoma: analysis from the National Cancer Data Base.
381 *Ann Surg Oncol.* 2012 Sep;19(9):2814–21.
- 382 [4] Hugen N, Verhoeven RH, Lemmens VE, et al. Colorectal signet-ring cell carcinoma:
383 benefit from adjuvant chemotherapy but a poor prognostic factor. *Int J Cancer.* 2015 Jan
384 15;136(2):333–9.
- 385 [5] Korphaisarn K, Morris V, Davis JS, et al. Signet ring cell colorectal cancer: genomic
386 insights into a rare subpopulation of colorectal adenocarcinoma. *Br J Cancer.* 2019 Sep
387 10;121(6):505–10.
- 388 [6] Hugen N, van de Velde CJH, de Wilt JHW, et al. Metastatic pattern in colorectal cancer
389 is strongly influenced by histological subtype. *Ann Oncol.* 2014 Mar;25(3):651–7.
- 390 [7] Chen J-S, Hsieh P-S, Hung S-Y, et al. Clinical significance of signet ring cell rectal
391 carcinoma. *Int J Colorectal Dis.* 2004 Mar;19(2):102–7.

- 392 [8] van Oudheusden TR, Braam HJ, Nienhuijs SW, et al. Poor outcome after cytoreductive
393 surgery and HIPEC for colorectal peritoneal carcinomatosis with signet ring cell
394 histology. *J Surg Oncol*. 2015 Feb;111(2):237–42.
- 395 [9] Lee W-S, Chun H-K, Lee WY, et al. Treatment outcomes in patients with signet ring cell
396 carcinoma of the colorectum. *Am J Surg*. 2007 Sep;194(3):294–8.
- 397 [10] Thota R, Fang X, Subbiah S. Clinicopathological features and survival outcomes of
398 primary signet ring cell and mucinous adenocarcinoma of colon: retrospective analysis
399 of VACCR database. *J Gastrointest Oncol*. 2014 Feb;5(1):18–24.
- 400 [11] Inamura K, Yamauchi M, Nishihara R, et al. Prognostic significance and molecular
401 features of signet-ring cell and mucinous components in colorectal carcinoma. *Ann Surg*
402 *Oncol*. 2015 Apr;22(4):1226–35.
- 403 [12] Tan Y, Fu J, Li X, et al. A minor (<50%) signet-ring cell component associated with
404 poor prognosis in colorectal cancer patients: a 26-year retrospective study in China. *PloS*
405 *One*. 2015;10(3):e0121944.
- 406 [13] Ogino S, Brahmandam M, Cantor M, et al. Distinct molecular features of colorectal
407 carcinoma with signet ring cell component and colorectal carcinoma with mucinous
408 component. *Mod Pathol*. 2006 Jan;19(1):59–68.
- 409 [14] Kakar S, Deng G, Smyrk TC, et al. Loss of heterozygosity, aberrant methylation, BRAF
410 mutation and KRAS mutation in colorectal signet ring cell carcinoma. *Mod Pathol*. 2012
411 Jul;25(7):1040–7.

- 412 [15] Wistuba II, Behrens C, Albores-Saavedra J, et al. Distinct K-ras mutation pattern
413 characterizes signet ring cell colorectal carcinoma. *Clin Cancer Res.* 2003 Sep 1;9(10 Pt
414 1):3615–9.
- 415 [16] Hartman DJ, Nikiforova MN, Chang DT, et al. Signet ring cell colorectal carcinoma: a
416 distinct subset of mucin-poor microsatellite-stable signet ring cell carcinoma associated
417 with dismal prognosis. *Am J Surg Pathol.* 2013 Jul;37(7):969–77.
- 418 [17] Kakar S, Smyrk TC. Signet ring cell carcinoma of the colorectum: correlations between
419 microsatellite instability, clinicopathologic features and survival. *Mod Pathol.* 2005
420 Feb;18(2):244–9.
- 421 [18] Kim IY. Impact of Microsatellite Instability in Signet-Ring Cell and Mucinous
422 Components in Patients With Colorectal Carcinoma. *Ann Coloproctology.* 2016
423 Apr;32(2):45–6.
- 424 [19] Phelip JM, Tougeron D, Léonard D, et al. Metastatic colorectal cancer (mCRC): French
425 intergroup clinical practice guidelines for diagnosis, treatments and follow-up (SNFGE,
426 FFCD, GERCOR, UNICANCER, SFCD, SFED, SFRO, SFR). *Dig Liver Dis.* 2019
427 Oct;51(10):1357–63.
- 428 [20] Shibata J, Kawai K, Nishikawa T, et al. Prognostic Impact of Histologic Type in
429 Curatively Resected Stage IV Colorectal Cancer: A Japanese Multicenter Retrospective
430 Study. *Ann Surg Oncol.* 2015 Dec;22 Suppl 3:S621-629.
- 431 [21] Anthony T, George R, Rodriguez-Bigas M, et al. Primary signet-ring cell carcinoma of
432 the colon and rectum. *Ann Surg Oncol.* 1996 Jul;3(4):344–8.

- 433 [22] Song W, Wu S, He Y, et al. Clinicopathologic features and survival of patients with
434 colorectal mucinous, signet-ring cell or non-mucinous adenocarcinoma: experience at an
435 institution in southern China. *Chin Med J (Engl)*. 2009 Jul 5;122(13):1486–91.
- 436 [23] Lee H-S, Soh JS, Lee S, et al. Clinical Features and Prognosis of Resectable Primary
437 Colorectal Signet-Ring Cell Carcinoma. *Intest Res*. 2015 Oct;13(4):332–8.
- 438 [24] Venook AP, Niedzwiecki D, Lenz H-J, et al. Effect of First-Line Chemotherapy
439 Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS
440 Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial.
441 *JAMA*. 2017 20;317(23):2392–401.
- 442 [25] Fu J, Wu L, Jiang M, et al. Signet ring cell carcinoma of resectable metastatic colorectal
443 cancer has rare surgical value. *J Surg Oncol*. 2016 Dec;114(8):1004–8.
- 444 [26] Qiu M, Hu J, Yang D, et al. Pattern of distant metastases in colorectal cancer: a SEER
445 based study. *Oncotarget*. 2015 Nov 17;6(36):38658–66.
- 446 [27] Franko J, Shi Q, Meyers JP, et al. Prognosis of patients with peritoneal metastatic
447 colorectal cancer given systemic therapy: an analysis of individual patient data from
448 prospective randomised trials from the Analysis and Research in Cancers of the
449 Digestive System (ARCAD) database. *Lancet Oncol*. 2016 Dec;17(12):1709–19.
- 450 [28] Jung K, Park MI, Kim SE, et al. Borrmann Type 4 Advanced Gastric Cancer: Focus on
451 the Development of Scirrhus Gastric Cancer. *Clin Endosc*. 2016 Jul;49(4):336–45.
- 452 [29] Voron T, Messenger M, Duhamel A, et al. Is signet-ring cell carcinoma a specific entity
453 among gastric cancers? *Gastric Cancer* 2016 Oct;19(4):1027–40.

- 454 [30] Yang L-L, Wang M, He P. Clinicopathological characteristics and survival in colorectal
455 signet ring cell carcinoma: a population-based study. *Sci Rep.* 2020 Jun 26;10(1):10460.
- 456 [31] Diao J-D, Ma L-X, Wu C-J, et al. Construction and validation a nomogram to predict
457 overall survival for colorectal signet ring cell carcinoma. *Sci Rep.* 2021 Feb
458 9;11(1):3382.
- 459 [32] Shi T, Huang M, Han D, et al. Chemotherapy is associated with increased survival from
460 colorectal signet ring cell carcinoma with distant metastasis: A Surveillance,
461 Epidemiology, and End Results database analysis. *Cancer Med.* 2019;8(4):1930–40.
- 462 [33] Venderbosch S, Nagtegaal ID, Maughan TS, et al. Mismatch repair status and BRAF
463 mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO,
464 CAIRO2, COIN, and FOCUS studies. *Clin Cancer Res.* 2014 Oct 15;20(20):5322–30.
- 465 [34] Smith CG, Fisher D, Claes B, et al. Somatic profiling of the epidermal growth factor
466 receptor pathway in tumors from patients with advanced colorectal cancer treated with
467 chemotherapy ± cetuximab. *Clin Cancer Res.* 2013 Aug 1;19(15):4104–13.
- 468 [35] Koopman M, Kortman G a. M, Mekenkamp L, et al. Deficient mismatch repair system
469 in patients with sporadic advanced colorectal cancer. *Br J Cancer.* 2009 Jan
470 27;100(2):266–73.
- 471 [36] Evrard C, Tachon G, Randrian V, et al. Microsatellite Instability: Diagnosis,
472 Heterogeneity, Discordance, and Clinical Impact in Colorectal Cancer. *Cancers.* 2019
473 Oct 15;11(10).
- 474 [37] Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic
475 DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer

476 (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.*
477 2017;18(9):1182–91.

478 [38] Morse MA, Hochster H, Benson A. Perspectives on Treatment of Metastatic Colorectal
479 Cancer with Immune Checkpoint Inhibitor Therapy. *The Oncologist.* 2020 Jan;25(1):33–
480 45.

481 [39] Morse MA, Overman MJ, Hartman L, et al. Safety of Nivolumab plus Low-Dose
482 Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-
483 Deficient Metastatic Colorectal Cancer. *The Oncologist.* 2019;24(11):1453–61.

484 [40] André T, Shiu K-K, Kim TW, et al. Pembrolizumab in Microsatellite-Instability–High
485 Advanced Colorectal Cancer. *N Engl J Med.* 2020 Dec 3;383(23):2207–18.

486 [41] Tougeron D, Cohen R, Sueur B, et al. A large retrospective multicenter study evaluating
487 prognosis and chemosensitivity of metastatic colorectal cancer with microsatellite
488 instability. *ESMO 2017, Madrid, Abstr#533P.*

489 [42] Lenz HJ et al. Independent radiological evaluation of objective response, early tumor
490 shrinkage, and depth of response in FIRE-3 in the final RAS evaluable population.
491 *ESMO 2014, Madrid, LBA11.*

492 [43] Folprecht G, Beer P, Salazar R, et al. Frequency of potentially actionable genetic
493 alterations in EORTC SPECTAcolor. *ESMO 2016 Cph Abstr458O.*

494 [44] Bokemeyer C, Van Cutsem E, Rougier P, et al. Addition of cetuximab to chemotherapy
495 as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis
496 of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer.* 2012
497 Jul;48(10):1466–75.

- 498 [45] Modest DP, Ricard I, Heinemann V, et al. Outcome according to KRAS-, NRAS- and
499 BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized
500 trials in metastatic colorectal cancer by the AIO colorectal cancer study group. *Ann*
501 *Oncol.* 2016 Sep;27(9):1746–53.
- 502 [46] Bachet JB, Bouche O, Taïeb J, et al. RAS mutations concordance in circulating tumor
503 DNA (ctDNA) and tissue in metastatic colorectal cancer (mCRC): RASANC, an AGEO
504 prospective multicenter study. *Ann Oncol.* 2018 May 1;29(5):1211-1219.
- 505 [47] Loupakis F, Cremolini C, Masi G, et al. Initial therapy with FOLFOXIRI and
506 bevacizumab for metastatic colorectal cancer. *N Engl J Med.* 2014 Oct
507 23;371(17):1609–18.
- 508 [48] Van Cutsem E, Lenz H-J, Köhne C-H, et al. Fluorouracil, leucovorin, and irinotecan plus
509 cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol.* 2015 Mar
510 1;33(7):692–700.
- 511 [49] Heinemann V, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus
512 FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal
513 cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2014
514 Sep;15(10):1065–75.
- 515

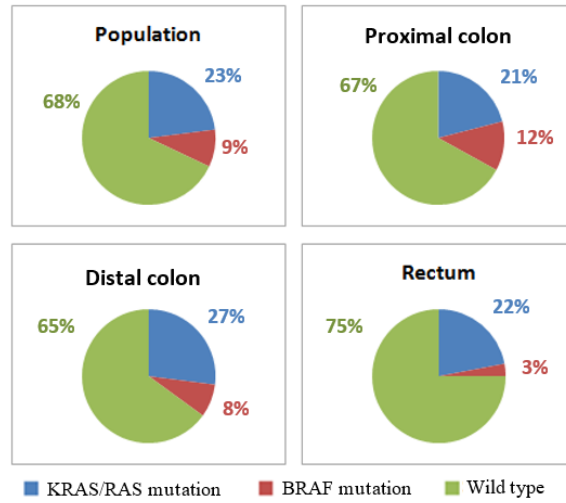


Figure 1: Distribution of RAS and BRAF mutation in primary tumor sites

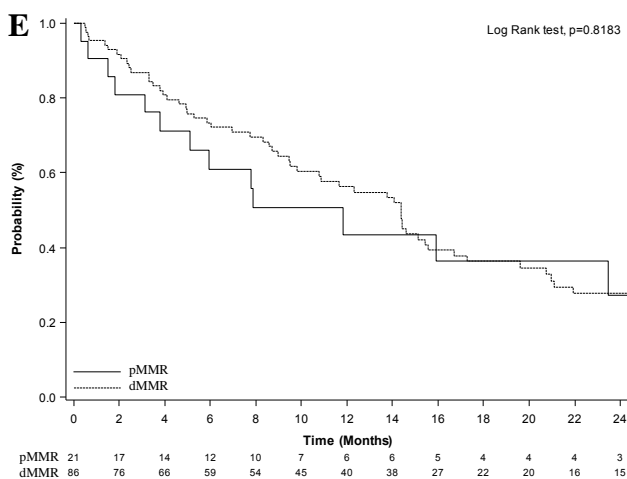
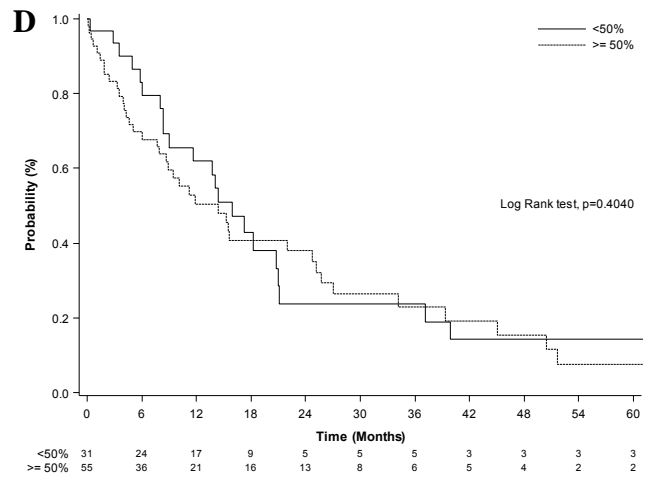
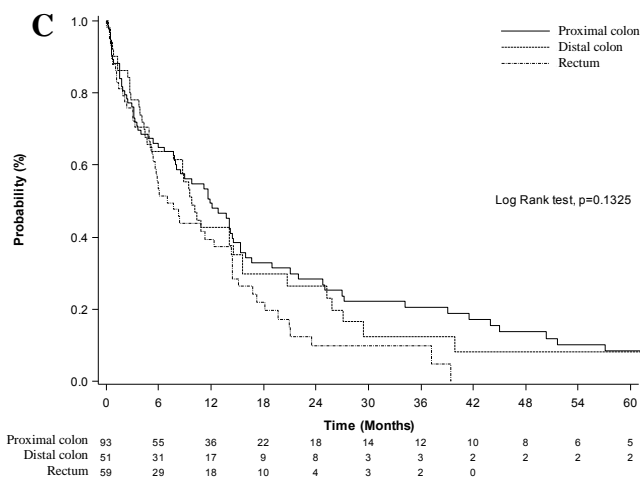
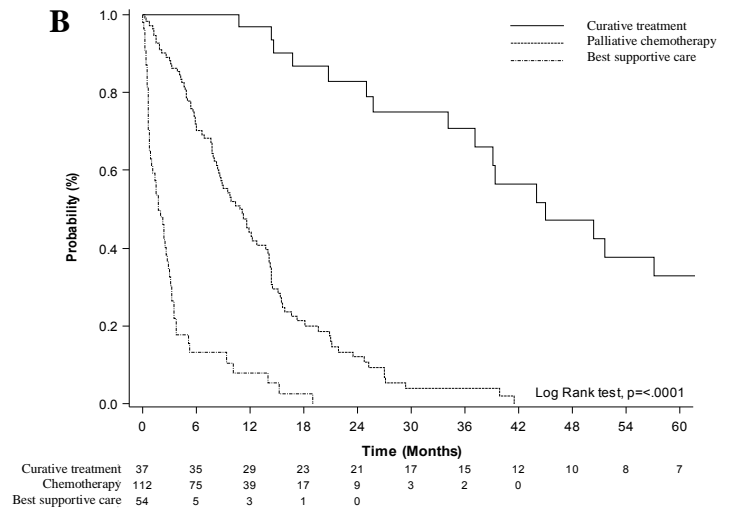
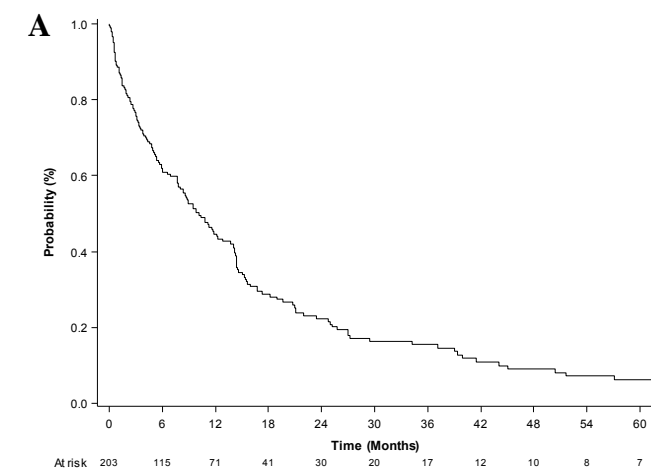


Figure 2: (A) Overall survival of overall cohort and depending on (B) the type of treatment (C) tumor location (D) signet-ring cell's percentage, and (E) microsatellite status.

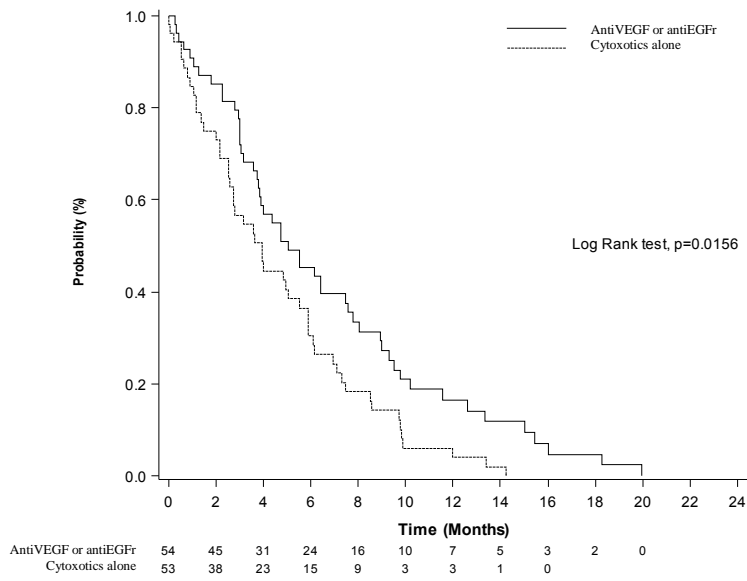


Figure 3: Progression-free survival according to treatment regimen.

Table 1 : Characteristics at metastatic diagnosis.

*median (IQR), performance status (PS), carcinoembryonic antigen (CEA), carbohydrate antigen 19.9 (CA19.9)

Variable	Population (n=204)	Group 1 (n=38)	Group 2 (n=112)	Group 3 (n=54)
Age (years)*	62.3 (52.8-74.6)	56.5 (41.2-63.4)	60.3 (53.7-71)	76.6 (62.9-84)
Gender (n, %)				
Male	124 (60.8)	18 (47.4)	68 (60.7)	38 (70.4)
Female	80 (39.2)	20 (52.6)	44 (39.3)	16 (29.6)
PS (n, %)				
0-1	128 (74.4)	31 (96.9)	86 (81.9)	11 (31.4)
2	26 (15.1)	1 (3.1)	15 (14.3)	10 (28.6)
3-4	18 (10.5)	0	4 (3.8)	14 (40)
missing	32	6	7	19
CEA (ng/mL)*	9.05 (2-35.6)	2 (1.2-7.6)	11.8 (3-50.9)	28.1 (6.4-138.5)
missing	80	15	43	31
CA19.9 (UI/mL)*	28.6 (11-116)	22.2 (15.1-41.2)	28.6 (2.8-129.2)	58.5 (23.2-178.3)
missing	114	19	59	36
Linitis plastic phenotype (n, %)				
Yes	37 (37)	-	-	-
No	63 (63)			
missing	104			
Tumor location (n, %)				
Proximal colon	93 (45.6)	20 (52.6)	45 (40.2)	28 (52)
Distal colon	52 (25.5)	11 (29)	28 (25)	13 (24)

Rectum	59 (28.9)	7 (18.4)	39 (34.8)	13 (24)
Sites of metastases (n, %)				
non regional lymph node	57 (28.2)	8 (21.1)	37 (33)	12 (22.2)
liver	47 (23.3)	6 (15.8)	31 (27.7)	10 (18.5)
lung	27 (13.4)	1 (2.6)	17 (15.2)	9 (16.7)
peritoneum	139 (68.8)	30 (78.9)	72 (64.3)	37 (68.5)
bone	18 (8.9)	0	13 (11.6)	5 (9.3)
brain	3 (1.5)	0	1 (0.9)	2 (3.7)
other	24 (11.9)	4 (10.5)	13 (11.6)	7 (13)
T status (n, %)				
missing	37	2	28	7
T1-T2	1 (0.6)	0	1 (1.2)	0
T3	47 (28.1)	12 (33.3)	20 (23.8)	15 (31.9)
T4	119 (71.3)	24 (66.7)	63 (75)	32 (68.1)
N status (n, %)				
missing	44	3	29	12
N0	8 (5)	4 (11.4)	0	4 (9.5)
N1	35 (21.9)	8 (22.9)	19 (22.9)	8 (19.1)
N2	117 (73.1)	23 (65.7)	64 (77.1)	30 (71.4)
Grade (n, %)				
moderate	21 (14)	8 (25.8)	9 (11.2)	4 (10.3)
poor	129 (86)	23 (74.2)	71 (88.8)	35 (89.7)
missing	54	7	32	15
Signet-ring cells (n, %)				
<50%	31 (35.6)	7 (30.4)	19 (40.4)	5 (29.4)
≥50%	56 (64.4)	16 (69.6)	28 (59.6)	12 (70.6)
missing	117	15	65	37

Table 2: Chemotherapy in patients with no curative treatment (G2). Chemotherapy (CTx), overall response rate (ORR), disease control rate (DCR), progression-free survival (PFS)

Variables	1L	2L	3L	4L
Number of patients (n)	112	58	22	6
CTx (n, %)				
oxaliplatin	54 (48.2)	21 (36.2)	4 (18.2)	0
irinotecan	40 (35.7)	28 (48.3)	8 (36.3)	2 (33.3)
both molecules	5 (4.5)	3 (5.2)	0	1 (16.7)
other	13 (11.6)	6 (10.3)	10 (45.5)	3 (50)
missing				
Biotherapy (n, %)				
none	56 (50)	29 (50)	4 (18.2)	2 (33.3)
antiVEGF	32 (28.6)	18 (31)	7 (31.8)	4 (66.7)
antiEGFr	24 (21.4)	11 (19)	11 (50)	0
ORR (n, %)				
CTx	7/44 (15.9)	-	-	-
CTX + antiVEGF	2/25 (8)	-	-	-
CTx + antiEGFr	3/22 (13.6)	-	-	-
DCR (n, %)				
CTx	20/44 (45.5)	-	-	-
CTx + antiVEGF	11/25 (44)	-	-	-
CTx + antiEGFr	14/22 (63.6)	-	-	-
PFS (months)				
	4.7	2.2	2.5	3.1

Table 3: Univariate and multivariate analysis for overall survival. Performance status (PS), carcinoembryonic antigen (CEA).

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio	p	Hazard ratio	p
	(95%CI)		(95%CI)	
PS				
≤1	1	0.0001	1	0.0001
>1	2.61		2.44	
CEA (ng/ml)				
<5	1	0.004	1	0.016
≥5	1.69		1.78	
Location				
proximal	1	0.13	1	0.16
distal	1.10		1.38	
rectum	1.46		1.41	
Signet-ring cell				
<50%	1	0.003	1.27	0.01
≥50%	1.24			
Vascular invasion				
yes	1	0.008	1	0.01
no	1.65		2.15	
Primary tumor surgery				
yes	1	0.001	1	0.05
no	1.78		1.68	