Histopathological Growth Patterns and Survival After Resection of Colorectal Liver Metastasis: an External Validation Study

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Abbreviations

CEA: carcinoembryonic antigen
CI: confidence interval
CRC: colorectal cancer
CRLM: colorectal liver metastases
DFS: disease-free survival
H&E: hematoxylin & eosin
HAIP: hepatic arterial infusion pump
HGP: histopathological growth pattern
HR: hazard ratio
IQR: interquartile range
MSI: microsatellite instability
OS: overall survival
Abstract

**Background:** After resection of colorectal cancer liver metastases (CRLM) two main histopathological growth patterns can be observed; a desmoplastic and a non-desmoplastic subtype. The desmoplastic subtype has been associated with superior survival. These findings require external validation.

**Methods:** An international multicenter retrospective cohort study was conducted in patients treated surgically for CRLM at three tertiary hospitals in the US and the Netherlands. Determination of histopathological growth patterns was performed on hematoxylin & eosin stained sections of resected CRLM according to international guidelines. Patients displaying a desmoplastic histopathological phenotype (only desmoplastic growth observed) were compared to patients with a non-desmoplastic phenotype (any non-desmoplastic growth observed). Cut-off analyses on the extent of non-desmoplastic growth were performed. Overall (OS) and disease-free (DFS) survival were estimated using Kaplan-Meier and multivariable Cox analysis. All statistical tests were 2-sided.

**Results:** In total 780 patients were eligible. A desmoplastic phenotype was observed in 19.1% and was associated with microsatellite instability (14.6% versus 3.6%, p=0.01). Desmoplastic patients had superior 5-year OS (73.4% [95% CI = 64.1-84.0] versus 44.2% [95% CI = 38.9-50.2], p<0.001) and DFS (32.0% [95% CI = 22.9-44.7] versus 14.7% [95% CI = 11.7-18.6], p<0.001) compared to their non-desmoplastic counterparts. A desmoplastic phenotype was associated with an adjusted hazard ratio for death of 0.36 (95% CI = 0.23-0.58), and 0.50 (95% CI = 0.37-0.66) for cancer recurrence. Prognosis was independent of *KRAS* and *BRAF* status. The cut-off analyses found no prognostic relationship between either OS or DFS and the extent of non-desmoplastic growth observed (all p>0.1).

**Conclusions:** This external validation study confirms the remarkably good prognosis after surgery for CRLM in patients with a desmoplastic phenotype. The extent of non-desmoplastic growth does not impact prognosis.
Introduction

During the course of their disease, up to 30% of patients with colorectal cancer (CRC) present with or develop liver metastases.\(^1\) Surgical removal or ablation of colorectal cancer liver metastases (CRLM) remains the only potentially curative treatment in these patients, resulting in a 5 years overall survival (OS) of 40%-60\(^\%\).\(^2\)

At pathological examination of CRLM two clinically relevant histopathological subtypes can be observed, namely a desmoplastic histopathological growth pattern (HGP) and a non-desmoplastic HGP. Considerable biological differences between both pathological subtypes have been demonstrated.\(^3\) The desmoplastic HGP has been associated with increased angiogenic capacity and increased infiltration of cytotoxic T cells, while non-desmoplastic HGP tumors mostly establish vascularization by means of co-option of pre-existing hepatic sinusoidal vessels. In addition, a reduced infiltration of immune cells and increased cancer motility is observed in these tumors.\(^4\)-\(^6\)

Over the years the HGP subtypes have gained interest and a potential impact on prognosis and the effectiveness of chemotherapy has been demonstrated.\(^7\),\(^8\) The largest patient cohort to date was published by our group, showing substantial differences in 5 years OS outcomes between patients expressing a desmoplastic HGP (78\%) and patients expressing any non-desmoplastic HGP (37\%).\(^7\)

HGP\(s\) can easily be assessed on hematoxylin & eosin (H&E) stained tissue sections, and evaluation of HGP\(s\) results in low inter- and intra-observer variability.\(^9\) Importantly, centers should be able to assess HGP\(s\) with minimal additional costs. In view of their potential clinical implications, HGP\(s\) could be an interesting biomarker to further incorporate into the clinical practice of patients with CRLM.

Prior to the implementation of HGP\(s\) in the clinic, external validation is required. This study therefore aims to evaluate the prognostic impact of HGP\(s\) after resection of CRLM in an international
multicenter external validation cohort. Secondly, we sought to validate the optimal cut-off for HGP classification.
Methods

Patient selection and data

Patients who underwent complete surgical treatment for CRLM at either the Erasmus MC Cancer Institute (Rotterdam, the Netherlands), Memorial Sloan Kettering Cancer Center (New York, NY, USA), or Radboud University Medical Center (Nijmegen, the Netherlands) from 2000 till 2019 were potentially eligible for inclusion. Complete surgical treatment was defined as resection (with or without ablation) of all known CRLM and extrahepatic metastases if present. Patients had to have had their primary colorectal malignancy resected as well. Patients receiving adjuvant therapies (systemic chemotherapy and/or hepatic arterial infusion pump (HAIP) chemotherapy) were excluded for two reasons. Firstly, the current study entails an external validation of a previously described cohort which only included patients who did not receive adjuvant therapy.\(^7\) In this external validation study a comparable but independent cohort of patients was selected. Secondly, a recent paper suggested modification of the effect of postoperative systemic chemotherapy by HGP, resulting in a survival benefit for the adjuvantly treated non-desmoplastic patients only.\(^8\) Exclusion of these patients ensures unbiased evaluation of the prognostic effect unaltered by postoperative therapies.

Patient demographics, clinicopathological disease characteristics and survival data were extracted from the respective center’s prospectively maintained databases. The study adheres to the REMARK guidelines for tumor marker prognostic studies.\(^9\) Institutional ethical review and approval was obtained from the medical ethics committee of the Erasmus University Medical Center Rotterdam (MEC-2018-1743), which granted a waiver for informed consent.

Treatment strategy and postoperative course

The Erasmus MC Cancer Institute, Memorial Sloan Kettering Cancer Center, and the Radboud University Medical Center are tertiary referral centers for liver surgery. All patients with suspected
CRLM were discussed by a multidisciplinary team of surgical oncologists, medical oncologists, radiation oncologists, and radiologists. Presence of limited extrahepatic disease amenable to local treatment did not preclude complete surgical treatment. Noticeable practice differences between centers exist in use of perioperative chemotherapeutic therapies. HAIP chemotherapy is commonly used at the Memorial Sloan Kettering Cancer Center and is administered frequently in selected patients, whereas in the Netherlands HAIP chemotherapy is only administered within the context of randomized controlled clinical trials. Moreover, perioperative systemic chemotherapy is considered standard of care throughout the United States. In the Netherlands, guidelines advocate to only administer preoperative chemotherapy to increase resectability in patients with unresectable disease, or to facilitate a parenchymal sparing approach. Postoperative systemic chemotherapy is not advocated. Practice variation regarding perioperative systemic chemotherapy does however exist in the Netherlands.

Postoperative surveillance in all three centers consists of outpatient visits, serial blood serum carcinoembryonic antigen (CEA) assessments and medical imaging by computed tomography and/or magnetic resonance imaging. Postoperative surveillance is generally scheduled every three to six months for the duration of five years, or longer at the patients’ discretion. In the case of recurrent disease, optimal treatment strategy is again determined by each center’s multidisciplinary team.

Pathological assessment

Pathological assessment of HGP was performed retrospectively on H&E sections by at least two trained observers simultaneously and blinded for patient characteristics and outcome. Dedicated liver pathologists were consulted when necessary. All available H&E tissue sections of all resected CRLM of each individual patient were assessed for HGP phenotype by light microscopy or digital evaluation of digitalized sections.
In accordance with international consensus guidelines, the tumor-liver interface was evaluated for pathological phenotype. The three previously described HGP phenotypes are discussed in depth in these guidelines. In summation, the desmoplastic phenotype is characterized by separation of tumor and liver parenchyma by a band of desmoplastic stroma (Figure 1A). This band of desmoplastic stroma separating cancer cells from the liver parenchyma is absent in the non-desmoplastic phenotypes (Figure 1B). As multiple phenotypes can appear in conjunction, the relative proportion of each phenotype is estimated on each H&E section and expressed as percentage. The final patient-level score is the average of each metastasis with equal weights assigned to discrete metastases and to individual slides within metastases. There is no minimum section requirement for HGP assessment. Sections are considered unsuitable if only a small fraction of the tumor-liver interface (less than 20%) is assessable, if tissue preservation quality is deemed unsuitable (e.g. tear of tissue at the transition zone) or when viable tumor tissue is absent (i.e. complete pathological response). Patients were classified as desmoplastic if all slides of all resected CRLM uniformly displayed a desmoplastic phenotype (i.e. 100% desmoplastic, Figure 1A), and as non-desmoplastic if any non-desmoplastic phenotype was observed in any slide of any resected CRLM (i.e. <100% desmoplastic, Figure 1B). For cut-off analyses patients were classified in subgroups according to the extent of non-desmoplastic phenotypes observed: 100% desmoplastic versus 0.1%-33%, 33.1%-67% and 67.1%-100% non-desmoplastic, respectively.

Outcomes

Overall (OS) and disease-free survival (DFS) were evaluated. OS was defined as time from surgical resection to death. DFS was defined as the time from surgical resection to cancer recurrence or death, whichever came first. Patients were censored if alive with no evidence of disease. Outcomes were additionally evaluated stratified for preoperative chemotherapy status.
Statistical analyses

Categorical data are reported as absolute count with corresponding percentage. Non-parametric continuous data are reported as median with corresponding interquartile range (IQR). Differences in proportions were evaluated by means of the Chi-squared test. Medians were compared by the Kruskall-Wallis test. Survival curves were estimated according to Kaplan-Meier analysis and compared by means of the log-rank test. Five-year survival estimates with corresponding 95% confidence intervals (CIs) are reported. Median follow-up for survivors was determined using the reverse Kaplan-Meier method. Uni- and multivariable Cox proportional hazards regression survival analyses were performed and reported as hazard ratios (HRs) with corresponding 95% CIs. All known clinicopathological risk factors were added to the regression models. With regards to missing data, full case analyses were performed. The proportional hazards assumption was visually assessed by plotting Schoenfeld residuals and Kaplan-Meier curves. Since data on KRAS and BRAF mutational status was only available for less than half of the patients, separate Cox regression models were computed with additional correction for these genetic risk factors. Cox regression models with interaction terms were created to evaluate effect modification of HGP by preoperative chemotherapy. All log-rank tests and Cox regression analyses were performed with center as stratification factor. The statistical significance level was set at an $\alpha$ of .05. All statistical tests were 2-sided and were performed using the R Project for Statistical Computing version 4.0.3 (https://www.r-project.org/) with the packages ggplot2 (v3.3.2), rms (6.0-1), survival (v3.2-7), survminer (v0.4.8) and tableone (v0.12.0).

Results

Between 2000 and 2019 a total of 2708 consecutive patients underwent resection of CRLM at either the Erasmus MC Cancer Institute (n=1044), Memorial Sloan Kettering Cancer Center (n=1352) or Radboud University Medical Center (n=312) and had resection specimens suitable for pathological
HGP assessment. Of these, 732 patients treated at the Erasmus MC Cancer Institute are described in our previous paper, 582 received perioperative HAIP chemotherapy, 446 were treated with postoperative systemic chemotherapy, and 168 did not undergo complete surgical treatment, resulting in a total of 780 patients included in the current external validation study. Baseline characteristics stratified by center are reported in Supplementary Table 1. A total of 213 patients were treated at the Erasmus MC Cancer Institute, 338 at the Memorial Sloan Kettering Cancer Center, and 229 at the Radboud University Medical Center. Of the 213 newly described patients treated at the Erasmus MC Cancer Institute, 163 (76.5%) underwent surgery outside (i.e. after March 2015) the inclusion period of the previous study, 10 (4.7%) were additionally identified through data requests at the IT department, and for the remaining 40 (18.7%) H&E resection specimens were previously missing but have since been recovered. Primary tumor and CRLM clinicopathological characteristics were comparable between centers, with the exception of the number of CRLM, presence of extrahepatic disease, and the disease-free interval between resection of primary tumor and detection of liver metastasis, all being more favorable in patients treated at the Radboud University Medical Center (Supplementary Table 1).

A desmoplastic histopathological phenotype was observed in 149 (19.1%) patients and was equally distributed across centers (Table 1). About half (n=373, 47.8%, Table 1) of all patients were treated with preoperative systemic chemotherapy, although this did differ between treatment centers (Supplementary Table 1). A desmoplastic phenotype was more often found in the pre-treated subpopulation: 22.7% (n=85 of 373) versus 15.7% (n=64 of 407) (p=0.01). Patients with a non-desmoplastic phenotype had slightly larger CRLM (median = 3.0 cm versus 2.2 cm, p<0.001), a longer disease-free interval (median = 2 versus 0 months, p=0.03), higher preoperative serum CEA levels (median = 11.2 versus 5.3 μg/L, p<0.001), and more often had extrahepatic disease (11.9% versus 6.0%, p=0.04) (Table 1). Data on KRAS, BRAF and microsatellite stability status was available for 42.3%, 37.1%, and 23.1% of patients. The mutation rate of KRAS (50.0% versus 43.0%, p=0.33) and BRAF (4.0% versus 3.3%, p=0.82) did not differ between patients with a desmoplastic and a non-desmoplastic
phenotype, respectively. Microsatellite instability (MSI) was however more often seen in the desmoplastic phenotype (14.6% versus 3.6%, p=0.01).

Overall and disease-free survival

The median follow-up for survivors was 42 months (IQR = 21-66 months). During follow-up 501 (64.2%) patients experienced recurrence and 294 (37.7%) died. Patients with a desmoplastic phenotype had statistically significantly longer OS compared to their non-desmoplastic counterparts, with 5-year OS estimates of 73.4% (95%CI = 64.1%-84.0%) for desmoplastic versus 44.2% (95%CI = 38.9%-50.2%) for non-desmoplastic (Figure 2A, p<0.001). Similar differences were observed for DFS, with 5-year estimates of 32.0% (95%CI = 22.9%-44.7%) for desmoplastic versus 14.7% (95%CI = 11.7%-18.6%) for non-desmoplastic (Figure 2B, p<0.001). The overall recurrence rate was statistically significantly lower for the patients with a desmoplastic HGP (45.6% versus 68.6%, p<0.001). In the full case multivariable analysis of 625 (80.1%) patients, a desmoplastic phenotype resulted in an adjusted HR (95%CI) of 0.36 (0.23-0.58) for OS and 0.50 (0.37-0.66) for DFS (Table 2). Considering KRAS and BRAF mutation status, 227 (29.1%) full cases were available for multivariable analysis and a desmoplastic phenotype remained independently (adjusted HR [95%CI]) associated with both OS (0.43 [0.20-0.92]) and DFS (0.42 [0.25-0.70]) (Table 3).

When evaluating the optimal cut-off for HGP determination, no statistically significant differences in either OS or DFS were observed between patients with a 0.1%-33%, 33.1%-67% and 67.1%-100% relative presence of non-desmoplastic HGP (all p>0.1). Patients with a desmoplastic phenotype displayed superior survival compared to all other subgroups (all p<0.001, Figure 2C and D). For both OS and DFS similar results were obtained in multivariable analysis (n=625 full cases, all p<0.01, Supplementary Table 2).
Effect of preoperative chemotherapy

No statistically significant interaction between preoperative chemotherapy and HGP was observed (OS $p=0.61$, DFS $p=0.64$). OS and DFS differed statistically significantly between desmoplastic and non-desmoplastic HGP patients in both the chemo-naive and pre-treated subpopulations.

In chemo-naive patients the 5-year OS estimate for a desmoplastic phenotype was 81.5% (95%CI = 68.9%-96.5%) compared to 51.8% (95%CI = 44.4%-60.5%) for a non-desmoplastic phenotype (Figure 3A, $p<0.001$). Again, similar differences were observed for DFS, with 5-year DFS estimates of 36.4% (95%CI = 22.6%-58.6%) for desmoplastic versus 19.9% (95%CI = 15.0%-26.2%) for non-desmoplastic (Figure 3B, $p<0.001$).

For pre-treated patients the 5-year OS for a desmoplastic phenotype was 67.1% (95%CI = 54.6%-82.5%) compared to 37.1% (95%CI = 30.2%-45.6%) for a non-desmoplastic phenotype (Figure 3C, $p<0.001$). Subsequently, the 5-year DFS was 29.0% (95%CI = 18.3%-46.0%) for pre-treated desmoplastic versus 8.6% (95%CI = 5.5%-13.3%) for pre-treated non-desmoplastic (Figure 3D, $p<0.001$).

After correction for potential confounding, a desmoplastic phenotype was associated with superior survival outcomes in both the chemo-naive (n=352 full cases, adjusted HR [95%CI] OS = 0.29 [0.13-0.65]; DFS = 0.53 [0.34-0.82], Supplementary Table 3) and pre-treated subpopulations (n=273 full cases, adjusted HR [95%CI] OS = 0.43 [0.23-0.79]; DFS = 0.43 [0.29-0.64], Supplementary Table 4).

Discussion

In this study, we present the results of an international multicenter external validation study on the prognostic value of HGPs after complete surgical treatment of CRLM. A desmoplastic phenotype was independently associated with superior OS and DFS outcomes in both chemo-naive and pre-treated patients. As the extent of HGP phenotypes observed can vary both within the same
tumor, as well as across multiple tumors in the same patient, external validation of the optimal cut-off for classification was also performed. In line with previous reports this external validation study confirms that it is the presence of any non-desmoplastic phenotype, rather than the relative quantity, that drives prognosis.

The first report of HGPs in CRLM was published in 1991 by Morino et al.\textsuperscript{16}, and since then several reports have followed.\textsuperscript{15,17} Due to heterogeneity in histopathological assessment, cut-offs, and terminology, formal meta-analysis of the available data is not possible, but most studies demonstrate favorable outcomes in patients with a predominant desmoplastic phenotype.\textsuperscript{17} The largest study to date was published by our group and reported a 5-years OS of 78\% in chemo-naive patients with a desmoplastic HGP.\textsuperscript{7} In the present study we observed a 5 year OS of 73.4\% in all patients with a desmoplastic phenotype, and a comparable 5-year OS of 81.5\% within the chemo-naive subpopulation. In line with these results, lower recurrence rates and superior DFS were seen in patients with a desmoplastic phenotype, reflecting the remarkably good cancer-related outcomes in these patients with metastatic CRC. In addition, our study is the first to investigate the prognostic impact of HGPs in light of \textit{KRAS} and \textit{BRAF} mutational status. Although data on these genetic risk factors was only available for approximately 40\% of patients, no association between the histopathological phenotype and mutations in either of these genes was observed, and after correction for these genetic risk factors a desmoplastic phenotype was still independently associated with good overall and cancer-free survival.

In order to standardize assessment of HGPs, international consensus guidelines have been established.\textsuperscript{15} In these guidelines classification of HGP is based on predominance, with an advocated cut-off value of 50\%. Both our previous paper and the current external validation study – which represent the two largest studies to date – demonstrate that predominance of a distinct HGP is irrelevant. Superior survival outcomes were only observed in patients with a uniform desmoplastic phenotype. In the patients with any observed non-desmoplastic growth, the extent of this observation...
does not seem to bear any prognostic consequences. We therefore deem reappraisal of the current guidelines for HGP assessment necessary; classification of HGPs in CRLM should be based on the presence or absence of non-desmoplastic growth.

Besides implications for HGP assessment and postoperative prognosis, this observation is also interesting from a cancer biology perspective as it suggests that HGPs can be regarded as a binary biological switch. While this paper does not provide a clear indication for the actual underlying process, in the 23% of patients with available data we did observe a statistically significant association between MSI and a desmoplastic phenotype. Because of their genetic hypermutability MSI tumors express more mutational neoantigens which can become targets for T cells.\textsuperscript{18,19} The more potential immune targets are present, the more likely an effective antitumor response can be elicited.\textsuperscript{19} This is why MSI tumors are thought to form metastases less often and why MSI represents the only indication for systemic immunotherapy in metastatic CRC so far.\textsuperscript{20,21} Since MSI tumors only accounted for 15% of patients with a desmoplastic phenotype in our study, a desmoplastic HGP could reflect more a state of (hepatic) anticancer immunity. This is supported by several other studies which demonstrated that a desmoplastic phenotype was associated with an enrichment of immune cells in the tumor microenvironment, specifically CD8\textsuperscript{+} T cells.\textsuperscript{5,6} One could therefore hypothesize that a non-desmoplastic histopathological phenotype, observed in however small a quantity, may be a reflection of the tumor’s intrinsic or obtained ability to evade the anticancer immune response. Our study is however at serious risk of selection bias regarding availability of MSI status and validation should therefore be pursued, as well as research into the other biological and immunological aspects of these histopathological phenotypes.

Preoperative chemotherapy was administered in approximately half of the patients in this validation cohort. It has been suggested that response to chemotherapy might induce misclassification of HGP type, which could limit the applicability of HGPs in patients receiving preoperative chemotherapy.\textsuperscript{7} In our previous study, no statistically significant impact of HGPs in pre-treated
patients was found in multivariable OS analysis. Although this study also found a diminished adjusted HR for OS in pre-treated patients, a desmoplastic phenotype remained associated with superior survival after correction for confounders. The results of this external validation study are promising to increase the applicability of this biomarker, as administration of preoperative chemotherapy is standard of care in many countries.

Many reports evaluating HGPs are now available, most of which demonstrate relevant prognostic and clinical implications.\textsuperscript{6,7,9,15,17,22-30} In addition, the effect of HGPs on survival (adjusted HR 0.36) is considerable, underlining its importance. We therefore feel that application in clinical practice should be pursued. An important step would be incorporation of the desmoplastic and non-desmoplastic phenotypes in the standard pathological report after resection of CRLM. This can be done on standard H&E slides with excellent intra-observer agreement\textsuperscript{9}, limited resources, and minimal additional time or medical costs required. If included in the standard pathological assessment, this prognostic information becomes readily available for clinicians and could be incorporated in individual counseling of patients. Herein a desmoplastic phenotype could be considered a marker for good prospects regarding survivorship. In addition, efforts should be made to determine whether the effectiveness of postoperative chemotherapy can be predicted by the HGP phenotype. Buisman et al. showed no benefit of postoperative chemotherapy in patients with a desmoplastic HGP, but validation of these results is needed.\textsuperscript{8} Being a postoperative pathology-based biomarker, the impact on preoperative decision making is absent for now. Cheng et al. showed that preoperative assessment of HGPs can however be done on imaging with an area under curve of over 0.9.\textsuperscript{31} When validated and optimized for use in clinical practice, HGPs could also be assessed and used in preoperative medical decision making.

This study presents the largest cohort investigating the prognostic impact of HGPs after resection of CRLM currently available and validates findings from previous studies. Nevertheless, the study has its limitations which are mostly related to its retrospective nature. An important limitation
also remains the limited data on established genetic risk factors, since KRAS and BRAF mutation status were only available for less than half of patients.\textsuperscript{32} Many of the patients in the current study were treated before the introduction of standard molecular testing, and in earlier years mutation status was only determined in patients with disease recurrence for choice of palliative systemic chemotherapy regimens, underscoring the risk of selection bias. Nevertheless, in those patients with data on KRAS and BRAF no association or impact on prognosis was seen. In addition, correction for sidedness of the primary tumor, which can be considered a weak proxy for mutational status\textsuperscript{33-37}, also did not diminish the prognostic value of a desmoplastic phenotype. Similar risk for selection bias exists regarding MSI status, which we found to be associated with a desmoplastic phenotype. While our study therefore does assess HGPS in light of KRAS, BRAF, and MSI status, in-depth genetic association studies on these histopathological phenotypes are needed to limit potential bias, confirm our findings, and also to investigate other CRC driver genes.

In conclusion, this study validates the prognostic impact of a desmoplastic phenotype in a large international multicenter cohort of surgically treated CRLM patients. We were able to confirm that patients with a desmoplastic phenotype have superior survival outcomes when compared to patients with any observed non-desmoplastic phenotype. The extent of non-desmoplastic growth does not impact prognosis. These data show that histopathological growth patterns harbor important prognostic value, warranting implementation in clinical practice.

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**Data Availability**

The data underlying this article cannot be shared publicly due to US and Dutch laws governing patient privacy and personal data. The authors nevertheless encourage further research and collaborations into the histopathological growth patterns. As such the data will be shared on reasonable request to all senior authors of the three participating centers and under the provisions of data transfer agreements.

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### Table 1. Baseline characteristics stratified by histopathological phenotype

| Characteristic                                    | Missing, No. (%) | Desmoplastic (n = 149) | Non-desmoplastic (n = 631) | P*  |
|---------------------------------------------------|------------------|------------------------|-----------------------------|-----|
| Treatment center, No. (%)                         |                  |                        |                             |     |
| Erasmus MC                                       | --               | 45 (30.2)              | 168 (26.6)                  | 0.66|
| MSKCC                                             | --               | 63 (42.3)              | 275 (43.6)                  |     |
| Radboud UMC                                       | --               | 41 (27.5)              | 188 (29.8)                  |     |
| Median age at resection CRLM (IQR), y             | --               | 65.0 (52.0, 72.0)      | 65.0 (56.0, 72.0)           | 0.31|
| Sex, No. (%)                                      |                  |                        |                             |     |
| Male                                              | --               | 92 (61.7)              | 374 (59.3)                  | 0.58|
| Female                                            | --               | 57 (38.3)              | 257 (40.7)                  |     |
| ASA classification, No. (%)                       |                  |                        |                             |     |
| ASA I-II                                          | 4 (0.5)          | 87 (59.2)              | 377 (59.9)                  | 0.87|
| ASA >II                                           | --               | 60 (40.8)              | 252 (40.1)                  |     |
| Primary tumor location, No. (%)                   |                  |                        |                             |     |
| Left-sided                                        | 24 (3.1)         | 49 (34.8)              | 254 (41.3)                  | 0.35|
| Right-sided                                       | --               | 41 (29.1)              | 166 (27.0)                  |     |
| Rectal                                            | --               | 51 (36.2)              | 195 (31.7)                  |     |
| T stage, No. (%)                                  |                  |                        |                             |     |
| pT 0-2                                            | 56 (7.2)         | 21 (15.7)              | 76 (12.9)                   | 0.39|
| pT 3-4                                            | --               | 113 (84.3)             | 514 (87.1)                  |     |
| N stage, No. (%)                                  |                  |                        |                             |     |
| N0                                                | 10 (1.3)         | 64 (43.5)              | 220 (35.3)                  | 0.06|
| N+                                                | --               | 83 (56.5)              | 403 (64.7)                  |     |
| Median No. of CRLM (IQR)                          | 2 (0.3)          | 2.0 (1.0, 3.0)         | 2.0 (1.0, 3.0)              | 0.12|
| Median diameter of largest CRLM (IQR), cm         | 3 (0.4)          | 2.2 (1.3, 3.3)         | 3.0 (2.0, 4.6)              | <0.001|
| Median disease-free intervala (IQR), months       | 11 (1.4)         | 0.0 (0.0, 11.8)        | 2.0 (0.0, 16.0)             | 0.03|
| Median Preoperative CEA (IQR), µg/L                | 65 (8.3)         | 5.3 (2.7, 16.4)        | 11.2 (4.2, 32.5)            | <0.001|
| Preoperative systemic chemotherapy, No. (%)       |                  |                        |                             |     |
| No                                                | --               | 64 (43.0)              | 343 (54.4)                  | 0.01|
| Yes                                               | --               | 85 (57.0)              | 288 (45.6)                  |     |
| Resection margin involved, No. (%)                |                  |                        |                             |     |
| No                                                | 1 (0.1)          | 136 (91.3)             | 541 (85.9)                  | 0.08|
| Yes                                               | --               | 13 (8.7)               | 89 (14.1)                   |     |
| Extrahepatic disease, No. (%)                     |                  |                        |                             |     |
| No                                                | --               | 140 (94.0)             | 556 (88.1)                  | 0.04|
| Yes                                               | --               | 9 (6.0)                | 75 (11.9)                   |     |
| KRAS mutational status, No. (%)                   |                  |                        |                             |     |
| Wildtype                                          | 450 (57.7)       | 29 (50.0)              | 155 (57.0)                  | 0.33|
| Mutant | BRAF mutational status, No. (%) |  |  |
|--------|---------------------------------|---|---|
|        | Wildtype                        | 491 (62.9) | 48 (96.0) | 231 (96.7) | 0.82 |
|        | Mutant                          | --          | 2 (4.0)   | 8 (3.3)    |      |
| MSS    | Microsatellite stability status, No. (%) | 600 (76.9) | 35 (85.4) | 134 (96.4) | 0.01 |
| MSI    | --                              | 6 (14.6)    | 5 (3.6)   |            |      |

Note: Categorical variables were compared using the Chi-squared and numerical variables using the Kruskall-Wallis test (two sided). ASA = American Society of Anesthesiologists; CEA = carcinoembryonic antigen; CRLM = colorectal liver metastasis; Erasmus MC = Erasmus MC Cancer Institute; IQR = interquartile range; MSI = microsatellite instable; MSKCC = Memorial Sloan Kettering Cancer Center; MSS = microsatellite stable; Radboud UMC = Radboud University Medical Center.

Between resection of primary tumor and detection of CRLM.
Table 2. Uni- and multivariable Cox regression analyses for overall and disease-free survival

| Characteristic                              | Overall survival (n=625) | Disease-free survival (n=625) |
|---------------------------------------------|--------------------------|-------------------------------|
|                                             | Univariable HR (95%CI)   | p-value                       | Multivariable HR (95%CI) | p-value |
|                                             |                         |                               |                         |         |
| Age at resection CRLM - years               | 1.01 (1.00-1.02)         | 0.01                          | 1.00 (0.99-1.00)         | 0.34    |
|                                             |                          |                               | 1.00 (0.99-1.01)         | 0.95    |
| ASA classification - >II vs I-II            | 1.26 (0.94-1.71)         | 0.13                          | 1.14 (0.91-1.41)         | 0.25    |
|                                             |                          |                               | 1.22 (0.95-1.57)         | 0.12    |
| Right-sided primary - yes vs no             | 1.46 (1.13-1.88)         | 0.004                         | 1.05 (0.86-1.27)         | 0.65    |
|                                             |                          |                               | 1.03 (0.82-1.29)         | 0.81    |
| T-stage - pT3-4 vs pT0-2                    | 1.36 (0.92-2.00)         | 0.12                          | 1.24 (0.95-1.61)         | 0.11    |
|                                             |                          |                               | 1.09 (0.81-1.46)         | 0.57    |
| N-stage - N+ vs N0                          | 1.18 (0.93-1.51)         | 0.18                          | 1.29 (1.08-1.55)         | 0.005   |
|                                             |                          |                               | 1.24 (1.01-1.53)         | 0.04    |
| Disease-free intervala (cont.) - months     | 1.00 (0.99-1.01)         | <0.001                        | 0.99 (0.99-1.00)         | 0.01    |
|                                             |                          |                               | 0.99 (0.98-1.00)         | 0.01    |
| Number of CRLM (cont.)                     | 1.10 (1.06-1.15)         | <0.001                        | 1.11 (1.08-1.15)         | <0.001  |
|                                             |                          |                               | 1.08 (1.04-1.12)         | <0.001  |
| Diameter of largest CRLM (cont.) - cm       | 1.06 (1.03-1.10)         | <0.001                        | 1.06 (1.03-1.09)         | <0.001  |
|                                             |                          |                               | 1.05 (1.01-1.09)         | 0.009   |
| Preoperative CEA (cont.) - 100 µg/L         | 1.01 (1.00-1.02)         | 0.006                         | 1.01 (1.00-1.02)         | 0.09    |
|                                             |                          |                               | 1.01 (1.00-1.02)         | 0.24    |
| Resection margin involved - yes vs no       | 1.83 (1.36-2.47)         | <0.001                        | 1.84 (1.47-2.31)         | <0.001  |
|                                             |                          |                               | 1.46 (1.11-1.92)         | 0.007   |
| Extrahepatic disease - yes vs no            | 1.63 (1.15-2.29)         | 0.005                         | 1.85 (1.44-2.38)         | <0.001  |
|                                             |                          |                               | 2.21 (1.64-2.98)         | <0.001  |
| Preoperative chemotherapy - yes vs no       | 1.25 (0.96-1.62)         | 0.10                          | 1.45 (1.20-1.74)         | <0.001  |
|                                             |                          |                               | 1.26 (1.01-1.56)         | 0.04    |
| Desmoplastic phenotype - yes vs no          | 0.39 (0.27-0.56)         | <0.001                        | 0.44 (0.35-0.56)         | <0.001  |
|                                             |                          |                               | 0.50 (0.37-0.66)         | <0.001  |

a Between resection of primary tumor and detection of CRLM. ASA = American Society of Anesthesiologists; Cont. = entered as continuous variable; CEA = carcinoembryonic antigen; CRLM = colorectal liver metastasis.
Table 3. Uni- and multivariable Cox regression analyses for overall and disease-free survival including KRAS and BRAF status

| Characteristic                                    | Overall survival | Disease-free survival |
|---------------------------------------------------|------------------|-----------------------|
|                                                   | Univariable      | Multivariable (n=227) | Univariable      | Multivariable (n=227) |
|                                                   | HR (95%CI)       | p-value               | HR (95%CI)       | p-value               |
|                                                   |                  |                       |                  |                       |
| Age at resection CRLM - years                     | 1.01 (1.00-1.02) | 0.01                  | 1.02 (1.00-1.04) | 0.05                  | 1.00 (0.99-1.00) | 0.34 | 1.00 (0.99-1.01) | 0.99 |
| ASA classification - >II vs I-II                  | 1.26 (0.94-1.71) | 0.13                  | 0.91 (0.52-1.61) | 0.75                  | 1.14 (0.91-1.41) | 0.25 | 1.02 (0.71-1.48) | 0.91 |
| Right-sided primary - yes vs no                   | 1.46 (1.13-1.88) | 0.004                 | 1.01 (0.59-1.71) | 0.98                  | 1.05 (0.86-1.27) | 0.65 | 0.83 (0.58-1.19) | 0.32 |
| T-stage - pT3-4 vs pT0-2                          | 1.36 (0.92-2.00) | 0.12                  | 1.74 (0.73-4.11) | 0.21                  | 1.24 (0.95-1.61) | 0.11 | 1.48 (0.86-2.56) | 0.16 |
| N-stage - N+ vs N0                                | 1.18 (0.93-1.51) | 0.18                  | 0.98 (0.58-1.66) | 0.95                  | 1.29 (1.08-1.55) | 0.005 | 1.15 (0.80-1.67) | 0.45 |
| Disease-free intervala (cont.) - months           | 1.00 (0.99-1.01) | 0.65                  | 0.97 (0.95-0.99) | 0.003                 | 0.99 (0.99-1.00) | 0.01 | 0.99 (0.97-1.00) | 0.01 |
| Number of CRLM (cont.)                            | 1.10 (1.06-1.15) | <0.001                | 1.03 (0.95-1.11) | 0.46                  | 1.11 (1.08-1.15) | <0.001 | 1.06 (1.00-1.12) | 0.04 |
| Diameter of largest CRLM (cont.) - cm             | 1.06 (1.03-1.10) | <0.001                | 1.02 (0.94-1.11) | 0.56                  | 1.06 (1.03-1.09) | <0.001 | 0.99 (0.93-1.06) | 0.81 |
| Preoperative CEA (cont.) - 100 µg/L                | 1.01 (1.00-1.02) | 0.006                 | 0.95 (0.83-1.10) | 0.53                  | 1.01 (1.00-1.02) | 0.09 | 1.02 (0.91-1.15) | 0.71 |
| Resection margin involved - yes vs no             | 1.83 (1.36-2.47) | <0.001                | 1.87 (1.01-3.47) | 0.05                  | 1.84 (1.47-2.31) | <0.001 | 1.63 (1.07-2.46) | 0.02 |
| Extrahepatic disease - yes vs no                  | 1.63 (1.15-2.29) | 0.005                 | 1.49 (0.81-2.76) | 0.20                  | 1.85 (1.44-2.38) | <0.001 | 2.16 (1.41-3.29) | <0.001 |
| Preoperative chemotherapy - yes vs no             | 1.25 (0.96-1.62) | 0.10                  | 1.44 (0.82-2.51) | 0.20                  | 1.45 (1.20-1.74) | <0.001 | 0.98 (0.68-1.41) | 0.91 |
| KRAS status - mutant vs wildtype                  | 1.55 (1.11-2.18) | 0.01                  | 2.21 (1.33-3.65) | 0.002                 | 1.33 (1.04-1.70) | 0.03 | 1.43 (1.03-1.98) | 0.03 |
| BRAF status - mutant vs wildtype                  | 1.59 (0.58-4.37) | 0.37                  | 3.42 (1.00-11.71) | 0.05                  | 1.08 (0.53-2.23) | 0.83 | 1.03 (0.39-2.72) | 0.95 |
| Desmoplastic phenotype - yes vs no                | 0.39 (0.27-0.56) | <0.001                | 0.43 (0.20-0.92) | 0.03                  | 0.44 (0.35-0.56) | <0.001 | 0.42 (0.25-0.70) | <0.001 |

a Between resection of primary tumor and detection of CRLM. ASA = American Society of Anesthesiologists; Cont. = entered as continuous variable; CEA = carcinoembryonic antigen; CRLM = colorectal liver metastasis
Figure legends

**Figure 1.** Hematoxylin & eosin stained tissue sections of resected CRLM viewed at 5 times magnification are shown with corresponding scale bars in the upper-right. A) Hematoxylin & eosin stained tissue section of a resected colorectal liver metastasis displaying a desmoplastic phenotype. Note the rim of desmoplastic tissue separating the tumor cells (lower-right) from the liver parenchyma (upper-left). B) Hematoxylin & eosin stained tissue section of a resected colorectal liver metastasis displaying a non-desmoplastic phenotype. Note the absence of a desmoplastic rim and the direct contact between the tumor cells (lower-left) and the liver parenchyma (upper-right).

**Figure 2.** Kaplan-Meier overall and disease-free survival estimates are shown. Figures A and B display the overall (A) and disease-free (B) survival estimates of patients with a desmoplastic versus a non-desmoplastic phenotype. Figures C and D display the overall (C) and disease-free (D) survival estimates according to the extent of non-desmoplastic growth observed. The p-values represent the results from the two-sided log-rank tests used to compare the survival estimates.

**Figure 3.** Kaplan-Meier overall and disease-free survival estimates stratified by preoperative chemotherapy are shown. Figures A and B display the overall (A) and disease-free (B) survival estimates for chemo-naive patients with a desmoplastic versus a non-desmoplastic phenotype. Figures C and D display the overall (C) and disease-free (D) survival estimates for pre-treated patients with a desmoplastic versus a non-desmoplastic phenotype. The p-values represent the results from the two-sided log-rank tests used to compare the survival estimates.
Figure 2

(A) Overall survival

Survival vs. time in months for desmoplastic and non-desmoplastic tumors. The p-value is less than 0.001.

(B) Disease-free survival

Survival vs. time in months for desmoplastic and non-desmoplastic tumors. The p-value is less than 0.001.

(C) Overall survival

Survival vs. time in months for different desmoplastic categories. The p-values are less than 0.001.

(D) Disease-free survival

Survival vs. time in months for different desmoplastic categories. The p-values are less than 0.001.
Figure 3

(A) Overall survival

Chemo-naive

Desmoplastic
Non-desmoplastic

\( p < 0.001 \)

(B) Disease-free survival

Chemo-naive

(D) Disease-free survival

Pre-treated

(\( p < 0.001 \)