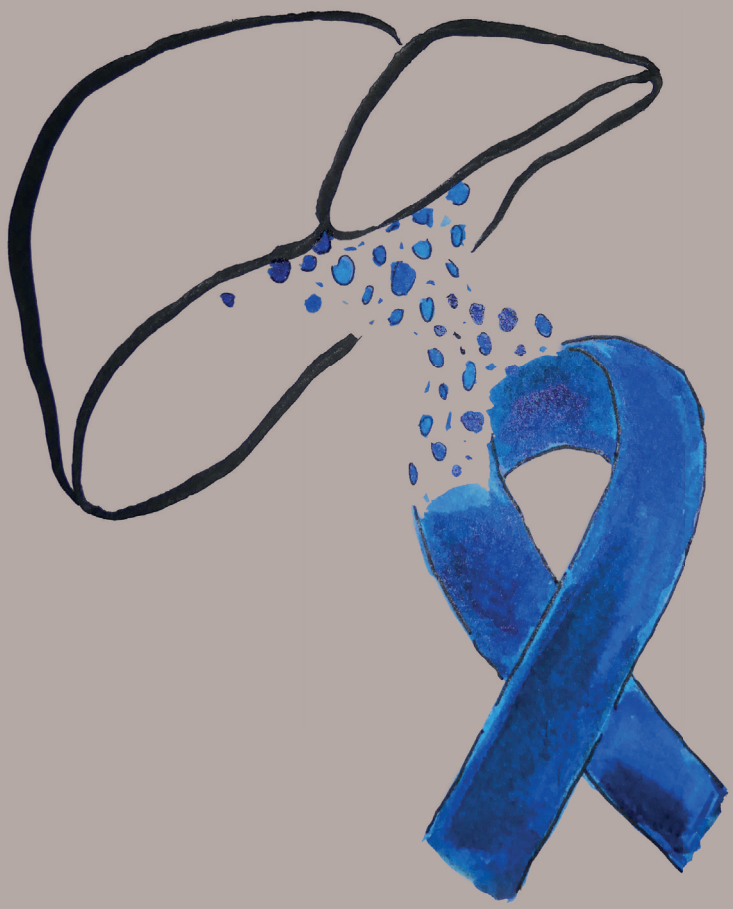
Histopathological Growth Patterns of Colorectal Liver Metastases

A Clinical Evaluation



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This thesis was realised at the department of Surgical Oncology of the Erasmus MC Cancer Institute, Rotterdam, the Netherlands.

Financial support for the production of this thesis was obtained from:
- The Erasmus University Rotterdam
- The Department of Surgery
- Chipsoft
- Blaak & Partners
- Erbe Nederland B.V.
- Raadsheeren B.V.
- Servier Nederland Farma
- ABN AMRO Bank N.V.
- By ease | Leids Congres Bureau
- Coloplast

Research included in this thesis has been financially supported by:
- KWF Kankerbestrijding
- Unrestricted grants from Las Moras and Burando

ISBN: 978-94-6423-336-0
Cover artwork: Marjanne Simons – Vodegel || www.mooiestreken.com
Cover design and layout: Mercedes Benjaminse || www.proefschriftmaken.nl
Printing: Proefschriftmaken || www.proefschriftmaken.nl

Histopathological Growth Patterns of Colorectal Liver Metastases

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Histopathologische groeipatronen van colorectale levermetastasen
Een klinische evaluatie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. F.A. van der Duijn Schouten

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 1 september 2021 om 13.00 uur

door

Pieter Maarten Harry Nierop

geboren te Rotterdam.

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

Promotor:	prof. dr. C. Verhoef
Overige leden:	prof. dr. S. Sleijfer prof. dr. I.D. Nagtegaal prof. dr. C.H.C. Dejong
Copromotor:	dr. D.J. Grünhagen

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PART I

Introduction, aim and outline of this thesis

Introduction

Colorectal cancer

In 2018 over 1.8 million patients were diagnosed with colorectal cancer (CRC) and in the same year 881,000 CRC related deaths occurred worldwide. These numbers entail that CRC ranks third in terms of incidence and second in terms of fatality. [1] The global CRC burden is expected to surpass 2.2 million new cases and 1.1 million CRC related deaths by 2030. [2]

The etiological basis for the development of CRC lies in several aspects of a patients environment and genetics. [3] Although the evidence is not always unambiguous, alcohol consumption, smoking, obesity, diabetes, sedentary lifestyle and an unhealthy diet in general are examples of (partly) lifestyle dependent factors that have been associated with CRC. [3] Dietary patterns, obesity and lifestyle are interlinked factors that are thought to be the underlying reason for the rise in incidence of CRC whereas the mortality declines, as seen in more developed countries, most likely reflect improvements in cancer screening, treatment and management. [1, 4] Despite the fact that most of these factors often relate to social economic status, lower social economic status itself has been independently associated with a higher risk of CRC. [5] Apart from increasing age, one of the least controversial factors associated with CRC remains the inherited risk, but germline cancer susceptibility gene mutations are carried by approximately 10% of patients with CRC. [6] This indicates that most CRC cases are sporadic rather than hereditary.

Colorectal liver metastases

Roughly one in five patients presents with distant metastatic disease at diagnosis of primary CRC (i.e. synchronous metastases). [7-11] In another 20% of patients distant metastases are diagnosed at a later moment in time (i.e. metachronous metastases). [10, 12-14] Development of colorectal liver metastases (CRLM) is common in CRC patients. Cumulatively, approximately 30% of patients are confronted with CRLM at some point in the course of their disease. [11, 14-16] The risk of developing metachronous metastases increases with the stage of the primary tumour. The five years cumulative metastasis rate varies from approximately 3%-5% for patients with stage I tumours, whereas this increases up to 30%-50% for patients with stage III tumours (i.e. tumours with lymph node involvement). [14, 15] Prior to the nineties of the previous century the presence of CRLM was often used as an argument to preclude patients from surgery. [17] The past three decades hepatic resection, if feasible, has been adopted as the standard of care for patients with CRLM. This transition took place despite the absence of prospective randomised controlled trials comparing resection to other treatment modalities (e.g. chemotherapy), but its motivation was and is strengthened by countless retrospective series and very large population based studies. [18] Through time several investigators have advocated a true comparison of liver resection versus systemic therapy for CRLM in the form of a randomised controlled trial. However, already in the early nineties it was concluded that, even if ethical considerations of denying surgery to a patient with resectable CRLM would be disregarded, large quantities of patients had to be included in order to truly evaluate chemotherapy versus resection in patient with

CRLM. [17, 19] With the strongly improved systemic treatment of metastatic CRC, at present even a greater number of patients would be necessary. [17] In addition, several studies have been performed that retrospectively evaluated resection versus systemic therapy in comparable patient cohorts and found results in favour of resection. [20, 21] Based on now outdated series, it was thought that approximately 15%-20% of patients presenting with synchronous CRLM were eligible for metastasectomy. [11, 16] Similarly, metachronous CRLM were deemed treatable with curative intent by means of a partial hepatectomy in approximately 10%-20% of cases. [14, 15] Of note, the boundaries of CRLM resectability are rapidly changing and these data regarding the proportion of patients eligible for CRLM resection are outdated. This was underlined by the most recent national numbers regarding local treatment of CRLM analysed by our group. At present roughly one third of patients with synchronous liver-only metastatic CRC receive local treatment of CRLM. In case of metachronous liver-only metastases more than half receives local treatment of CRLM. (Submitted data Meyer et al.) Patients with CRLM not eligible for surgery have poor prognosis. If patients receive no other treatment than best supportive care, survival is measured in months rather than years. [22] During the nineteen eighties the addition of leucovorin-modulated fluorouracil (5-FU) to the palliative treatment of metastatic CRC led to a median survival of one year. [23] The past four decades chemotherapeutic regimens for the treatment of metastatic CRC have advanced greatly. With the introduction of triplet therapy consisting of oxaliplatin, irinotecan and bevacizumab in the treatment of these patients median survival over 30 months can be reached nowadays. Nevertheless, despite these great advances in systemic therapy options, local treatment remains the only potentially curative treatment for patients with CRLM. Hepatic resections can be performed safely with postoperative mortality of approximately 2% in specialised centres, but varies from roughly 1% to 6% depending on, among other things, the extent of the liver resection. [24] Five-year survival rates of selected patients after resection of CRLM vary from 40%-60%. [18, 25, 26] However, approximately one third of the five-year survivors still succumbs to a cancer-related death. [26] The ten-year survival rate of patients after CRLM resection is around 20%-25% and cancer-related deaths after a decade are extremely rare. [25-27] As can be deduced from these numbers, relapse after CRLM resection unfortunately is common. Relapse rates up to and over 70% generally are reported. [21, 25-33] These high disease relapse rates and the progress made in systemic therapy encouraged numerous researchers to evaluate the possibilities of combining systemic therapy with local therapy in order to improve disease free and overall survival. Several randomised clinical trials assessing this subject have been designed. [34-40] Unfortunately, execution of these trials proved difficult as the majority was forced to close prematurely due to slow patient accrual. [34, 36-38] One randomised trial comparing surgery alone to surgery combined with perioperative systemic chemotherapy in patients with resectable CRLM to have completed its accrual is the European Organisation for Research and Treatment of Cancer (EORTC) intergroup study 40983. [35, 40] The short term results of the EORTC 40983 trial showed a progression free survival benefit in favour of perioperative chemotherapy compared to surgery alone in eligible and resected patients. [35] Several years later, albeit underpowered for such analysis, the mature trial results demonstrated no overall survival benefit whatsoever. [40]

These results displaying a progression free survival benefit in the trial population led to the adaption of perioperative systemic chemotherapy as standard of care in most countries in the treatment of resectable CRLM. However, based on the long-term results demonstrating no overall survival benefit, perioperative chemotherapy is not standard of care according to the Dutch national guidelines. In the Netherlands preoperative chemotherapy is considered an option as a way of downstaging in case of borderline resectable CRLM. Postoperative chemotherapy is generally not recommended.

Prognosis prediction and histopathological growth patterns

Reported five-year survival rates after surgical removal of CRLM greatly vary, with most studies showing a five-year survival rate in selected patients of approximately 50%. [18, 25, 26] There are reports displaying five-year survival rates ranging between 16% and 74%. [25] Accurate prediction of survival after resection of CRLM is important to individualise treatment and inform patients adequately. In order to predict survival after surgery for CRLM several risk scores have been developed. To date, predominantly clinicopathological factors (e.g. lymph node status of the primary tumour, size and number of CRLM) are used as prognostic factors in clinical risk models. [27, 29, 32, 41-44] While these clinical risk models have been validated extensively, it has been shown that a significant proportion of clinically low-risk patients experiences rapid recurrence and cancer-related death and, vice versa, high-risk features were present in long-term survivors. [26] This emphasises the need for new prognostic biomarkers that adequately reflect tumour biology, in patients with CRLM. [45] To date, none of the scientifically established clinical risk scores impacts clinical management of patients with resectable CRLM.

As liver resection for CRLM became accepted as standard of care towards the end of the millennium, higher quantities of CRLM tissue became available as more resections were performed. It became noted by several unrelated groups that different patterns of tumour growth could be distinguished when reviewing CRLM under the microscope. [46, 47] Different nomenclature was used in various parts of the world, but essentially describe the same types of growth patterns (GP). Nagashima and colleagues first described several different types of GPs: the invasive GP (subdivided in infiltrative and expansive), the marginal fibrosis GP and the lymphocytic infiltration GP. As the name implies, in the infiltrative GP liver plates are directly infiltrated by tumour cells. The expansive GP indicates that the tumour expands within the liver without the presence separating tissue, but does not infiltrate. In the marginal fibrosis GP the metastasis is separated from the liver parenchyma by fibrosis. The lymphocytic infiltration GP was given if copious amounts of lymphocytes and other inflammatory cells were seen around the metastasis. The infiltrative patterns was associated with worse prognosis after CRLM resection. [46] Shortly thereafter, the currently most often utilised terms were first described consisting of the desmoplastic (d) histopathological growth pattern (HGP), the replacement (r) HGP and the pushing (p) HGP. [47] HGPs describe the manner of growth of a CRLM at transition border from metastasis to liver parenchyma. In dHGP metastases are separated from the liver parenchyma by a fibrotic capsule consisting of desmoplastic stroma and a dense lymphocytic infiltrate is

practically always present. The architecture of the liver parenchyma is not “preserved” and these metastases are dependent on neoangiogenesis for their blood supply. No direct contact between hepatocytes and tumour cells is observed. The rHGP owes its name to the fact that tumour cells “replace” hepatocytes while conserving the reticulin network of the parenchyma and thereby preserving the architecture of the liver. The rHGP is characterised by minimal neoangiogenesis, instead blood supply is acquired by means of vessel co-option. This means that the existing liver sinusoidal blood vessels falls victim to hostile takeover by the metastasis that thereby bypasses the need for newly formed vasculature. Intimate direct cell-cell contact is seen between hepatocytes and cancer cells. The pHGP describes a pattern of growth in which the liver cell plates are pushed aside, but infiltrative growth and desmoplastic stroma are absent. HGPs have been proposed as a potentially strong predictor of tumour biology and prognosis in patients with CRLM. [47]

The current thesis describes studies aimed at evaluating the clinical value and applicability of histopathological growth patterns displayed by colorectal liver metastases.

Outline of this thesis

Previous studies evaluating the prognostic impact of HGPs were limited in several ways. [48-54] Firstly, these studies did not adequately differentiate between patients that were and were not preoperatively treated with systemic chemotherapy. Preoperative chemotherapy may influence the type of HGP observed, which could have biased the outcomes. Secondly, these studies were limited by short follow-up and small sample size. Therefore, a large study on HGPs, stratified for preoperative treatment and corrected for other known risk factors, was needed. The aim of **Chapter 1** was to determine the prognostic impact of dHGP in a large cohort of chemonaive patients with long-term follow-up after resection for CRLM. Secondary objectives were to determine the prevalence of dHGP after treatment with preoperative chemotherapy and to assess the prognostic impact of dHGP amongst preoperatively treated patients. Knowledge on diagnostic accuracy and reproducibility of HGPs is a prerequisite for evaluation of the clinical applicability of HGPs. To that end the reliability and replicability of HGPs and learnability HGP assessment were evaluated in **Chapter 2**. A much debated subject in the prognostication for patients undergoing resection of CRLM is the resection margin. More specifically, the question whether cancer biology is the true risk factor for positive margins rather than surgical technique. **Chapter 3** aimed to investigate whether the non-dHGP is associated with a higher risk of positive resection margins after resection of CRLM as HGPs have been proposed as a surrogate for tumour biology. Unfortunately, the majority of patients has disease recurrence after surgical treatment of CRLM. **Chapter 4** investigated the impact of HGP type on the pattern and treatment of recurrences after first resection of CRLM. In patients presenting with synchronous liver metastases of locally advanced rectal cancer multiple treatment sequences are possible. One of the possibilities is the liver-first approach. [55] Regardless of treatment sequence, approximately one third of patients does complete the entire treatment sequence with curative intent. [56] **Chapter 5** focussed on the prediction of the non-completion of the liver-first approach and in particular whether HGPs might be utilised to this end. Preoperative systemic chemotherapy might influence the HGP. This potential influence was assessed within three separate patient cohorts including a subset of the EORTC 40983 trial [35, 40] in **Chapter 6**. The prognostic value HGPs was evaluated by means of a post-hoc analysis of the two prospective randomised controlled trials, the EPOC and the New EPOC trial [35, 39, 40, 57] in **Chapter 7**. In the **Chapters 8**, **9** and **10** a general discussion, a summary of this thesis and future perspectives for the (surgical) management of patients with CRLM in the context of HGPs are provided. Lastly, in **Chapter 11** the appendices are presented which entail scientific output, contributing authors, acknowledgements and an about the author section.

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PART II

Histopathological growth patterns of colorectal liver metastases

Chapter 1

Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases

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Angiogenesis. 2019 May;22(2):355-368

Abstract

Background:
In patients with resectable colorectal liver metastases (CRLM), distinct histopathological growth patterns (HGPs) develop at the interface between the tumour and surrounding tissue. The desmoplastic (d) HGP is characterised by angiogenesis and a peripheral fibrotic rim whereas non-angiogenic HGPs co-opt endogenous sinusoidal hepatic vasculature. Evidence from previous studies has suggested that patients with dHGP in their CRLM have improved prognosis as compared to patients with non-desmoplastic HGPs. However, these studies were relatively small and applied arbitrary cut-off values for the determination of the predominant HGP. We have now investigated the prognostic effect of dHGP in a large cohort of patients with CRLM resected either with or without neoadjuvant chemotherapy.

Methods:
All consecutive patients undergoing a first partial hepatectomy for CRLM between 2000 and 2015 at a tertiary referral centre were considered for inclusion. HGPs were assessed in archival H&E stained slides according to recently published international consensus guidelines. The dHGP was defined as desmoplastic growth being present in 100% of the interface between the tumour and surrounding liver.

Results:
In total, HGPs in CRLMs from 732 patients were assessed. In the chemo-naive patient cohort (n=367), the dHGP was present in 19% (n=68) and the non-dHGP was present in 81% (n=299) of patients. This dHGP subgroup was independently associated with good overall survival (OS) (HR: 0.39, p<0.001) and progression free survival (PFS) (HR: 0.54, p=0.001). All patients with any CRLM with a non-dHGP had significantly reduced OS compared to those patients with 100% dHGP, regardless of the proportion of non-dHGP (all p-values ≤0.001).

In the neoadjuvantly treated patient cohort (n=365), more patients were found to express dHGP (n=109, 30%) (adjusted odds ratio: 2.71, p<0.001). On univariable analysis dHGP was associated with better OS (HR: 0.66, p=0.009) and PFS (HR: 0.67, p=0.002). However, after correction for confounding by means of multivariable analysis no significant association of dHGP with OS (HR: 0.92, p=0.623) or PFS (HR: 0.76, p=0.065) was seen.

Conclusions:
The current study demonstrates that the angiogenic dHGP in CRLM resected from chemo-naive patients acts as a strong, positive prognostic marker, unmatched by any other prognosticator. This observation warrants the evaluation of the clinical utility of HGPs in prospective clinical trials.

Introduction

As hepatic tumours develop, histopathological growth patterns (HGPs) appear at the interface between the tumour border and surrounding liver parenchyma. Previous studies have suggested that HGPs have the potential to predict both tumour biology and prognosis in patients with colorectal liver metastasis (CRLM). Three primary HGPs have been identified in CRLM: desmoplastic (d), replacement (r) and the pushing (p) pattern. [1] Over time the classification of HGPs has evolved and ultimately resulted in international consensus guidelines. Applying these guidelines made the dHGP and rHGP the most common types and the pHGP fairly uncommon. [2]

In addition to the fibrotic reaction (desmoplasia) that surrounds the metastases, one of the predominant features of tumours which exhibit dHGP is angiogenesis. These tumours are characterised by elevated endothelial cell proliferation and regions of increased vessel density called vascular hot spots. The new blood vessels appear leaky and functionally impaired with fibrin deposits in the peri-vascular stroma. [3] This is in contrast to the rHGP, in which angiogenesis does not occur, the proportion of proliferating endothelial cells is very low and there are no obvious effects of VEGFA such as fibrin deposition. [3] In rHGP, vascularisation of the tumours is established by co-opting the existing sinusoidal blood vessels of the liver. [3, 4] As the name implies, cancer cells 'replace' the hepatocytes while the stromal architecture of the liver is maintained.

Tumours with rHGP exhibit features that have been associated with aggressive cancer biology and impaired prognosis, including increased cancer cell motility [4], non-angiogenic growth [4] and reduced infiltration of CD8+ immune cells. [5, 6] Previous studies evaluating the prognostic impact of HGPs suggest that the dHGP is associated with improved prognosis. [2, 4, 5, 7-10] These studies were relatively small and applied arbitrary cut-off values for the determination of the predominant growth pattern. If HGPs are an intrinsic reflection of tumour biology, one could hypothesise that the presence of *any* non-desmoplastic HGP (pHGP and/or rHGP) could be of prognostic value. The current study investigated the association of dHGP with survival in a large cohort of patients undergoing resection of CRLM, and the potential correlation between neoadjuvant chemotherapy and HGPs.

Methods

Patient selection and data

All consecutive patients who underwent laparotomy for surgical treatment of CRLM between January 2000 and March 2015 at the Erasmus MC Cancer Institute were considered for inclusion. The Erasmus MC Cancer Institute is a tertiary referral centre for liver surgery. Patients without complete surgical treatment (i.e. resection with or without ablation of all known CRLM) with curative intent were excluded. In addition, patients treated with ablation only were also excluded. Clinicopathological data on primary tumour, CRLM and recurrent metastatic disease were obtained from a prospectively maintained database. HGP assessment was performed retrospectively on H&E stained tissues sections from archival tissue. The current study was performed according to the REMARK guidelines and approved by the medical ethics committee of the Erasmus University Medical Centre Rotterdam (MEC-2016-046). [11]

Prognosis

The primary objective of this study was to evaluate the association between HGPs and prognosis after first hepatectomy for CRLM. In order to analyse this, HGP data of the first hepatectomy were evaluated (i.e. for the survival analyses recurrent CRLM treated with repeat hepatectomy were not evaluated). Survival was measured as progression free survival (PFS) and overall survival (OS). The PFS was defined as the time in months between resection of CRLM and diagnosis of progression of disease or death, whichever occurred first. Disease progression was diagnosed by radiological or histological assessment. The OS was defined as the time in months between surgery for CRLM and death.

Effect of chemotherapy

The secondary objective was to assess the potential association between chemotherapy and the prevalence of HGPs. In order to do so distribution of HGPs amongst chemo-naive and neoadjuvantly treated patients was compared. Patients who had received any chemotherapy within the six months prior to the liver resection were considered neoadjuvantly treated. Patients with a liver recurrence undergoing repeat resection at the Erasmus MC Cancer Institute were identified and subsequently stratified into four distinct treatment groups: chemonaive at both hepatectomies (-/-), neoadjuvantly treated at the first hepatectomy but chemonaive at the second (+/-), chemonaive at the first hepatectomy but neoadjuvantly treated at the second (-/+) and lastly neoadjuvantly treated at both hepatectomies (+/+). Specifically for this secondary objective the HGPs of these recurrent CRLM were determined as well and the prevalence of HGPs at first and second hepatectomy was compared across these four distinct treatment groups.

Chemotherapy and follow-up

In accordance with the Dutch national guidelines, (neo)adjuvant chemotherapy is not standard of care for patients with CRLM. Only in case of initially marginally resectable, synchronous and/or multiple (≥4) resectable CRLM, is neoadjuvant chemotherapy considered. A proportion of patients received neoadjuvant chemotherapy in the referring hospital prior to admission. None of the patients received adjuvant chemotherapy.

Post-operative surveillance is performed for up to five years after surgery for CRLM, using thoracoabdominal computed tomography (CT) and carcinoembryonic antigen (CEA) level measurements every three to six months for three years and then annually thereafter. After five years, further surveillance was performed by the general practitioner. Patients were censored for PFS at date of last follow-up if without disease progression.

Pathological assessment and description of HGPs

HGPs were determined according to the international consensus guidelines of the Liver Metastasis Research Network [2] blinded for patient outcome. HGPs were assessed per patient in all available haematoxylin and eosin (H&E) stained sections from all resected CRLM. In each slide, the interface between tumour border and normal liver parenchyma was evaluated using light microscopy by at least three trained observers (PV, ES, RC, BG, PN, DH). As some CRLM display a combination of HGPs, the entire tumour-liver interface was evaluated for each tissue section. When multiple HGPs were present at the interface, the HGP was scored as a relative proportion of the interface in which each of dHGP, rHGP and/or pHGP occurred. Every fraction of the tumour-liver interface, accounting for 5% or more of the total interface of a metastasis, was taken into account. Average HGP scores were then calculated for each metastasis (in case of multiple slides per CRLM) and patient (in case of multiple CRLM). Tissue sections were considered not suitable for HGP assessment when less than 20% interface was available, when the quality of the H&E tissue section was insufficient for reliable assessment or when viable tumour tissue was absent. [2] Examples of H&E tissue sections with CRLM displaying dHGP, rHGP and pHGP are shown in figure 1A-F. In the dHGP, the cancer cells of the metastasis are separated from the liver tissue by a rim of desmoplastic tissue. The metastasis does not mimic the liver architecture and there is no direct contact between cancer cells and hepatocytes (figures 1A-B). There is often a dense lymphocytic infiltrate at the interface of the desmoplastic rim and the liver tissue. A ‘ductular reaction’, or proliferation of bile ducts, can sometimes be seen surrounding the desmoplastic metastasis. In the pHGP, the liver cell plates that surround the metastasis are pushed away and compressed (figures 1E-F). There is no desmoplastic rim surrounding the metastasis but also no direct contact between cancer cells and hepatocytes within the liver cell plates. As in the dHGP, the metastasis does not mimic the liver architecture. In the rHGP, cancer cells form cell plates that are in continuity with the liver cell plates (figures 1C–D). This permits the cancer cells to replace the hepatocytes within the liver cell plates and allows these metastases to co-opt the sinusoidal blood vessels at the tumour–liver interface, without inducing sprouting angiogenesis. The liver cell plates can sometimes be pushed away while the cancer cells replace the hepatocytes.

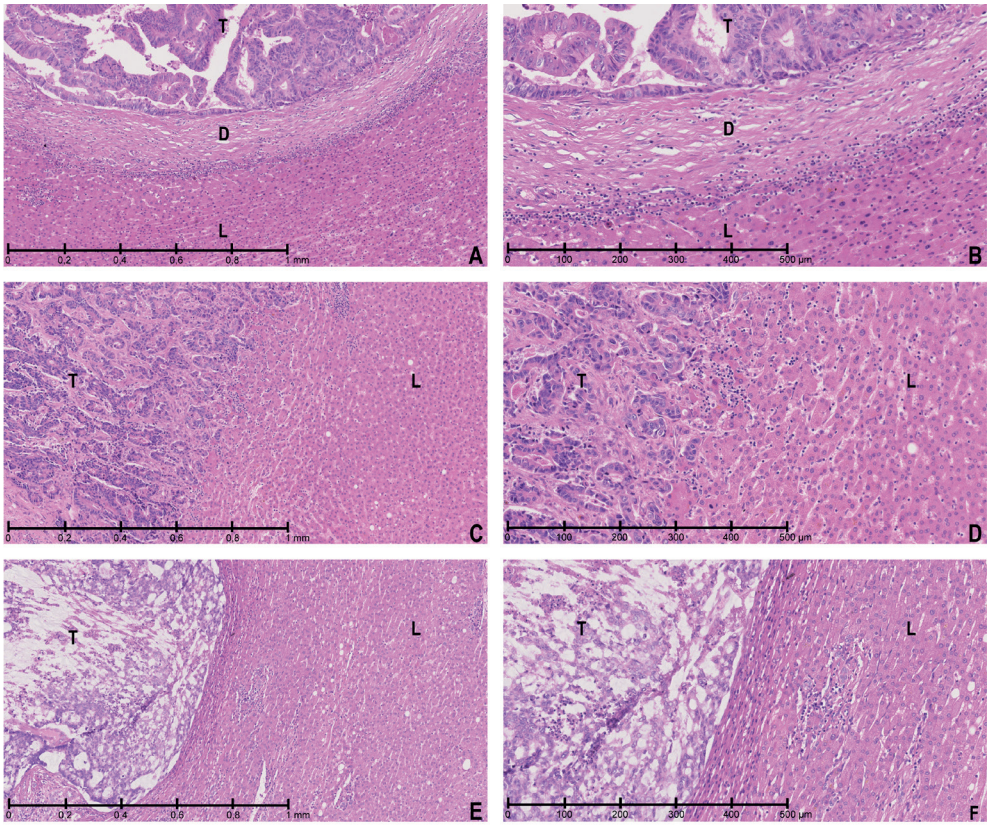


Figure 1A-F. Collated HE tissue sections. 1A-B: dHGP low and high magnification; 1C-D: rHGP low and high magnification; 1E-F: pHGP low and high magnification. T: tumour; D: desmoplastic stroma; L: liver parenchyma.

HGP categorisation

In order to investigate the hypothesis that the presence of *any* non-dHGP determines prognosis, patients were categorised as non-dHGP if any other HGP than dHGP was observed. For supplementary analyses, patients were also categorised according to the 50% cut-off value of the consensus guidelines in which case, patients were categorised as dHGP, rHGP and pHGP when >50% of the interface was scored as such. If none of the three HGP was present at >50% of the interface this was defined as mixed HGP and patients were excluded for further analysis, since no predominant HGP could be determined. In order to compare the angiogenic dHGP to the non-angiogenic rHGP supplementary analyses were also performed for patients with any proportion of rHGP compared to patients with pure (100%) dHGP. To that end, patients without any rHGP in the non-dHGP group were excluded. In this way, the rare pHGP was excluded from the analyses.

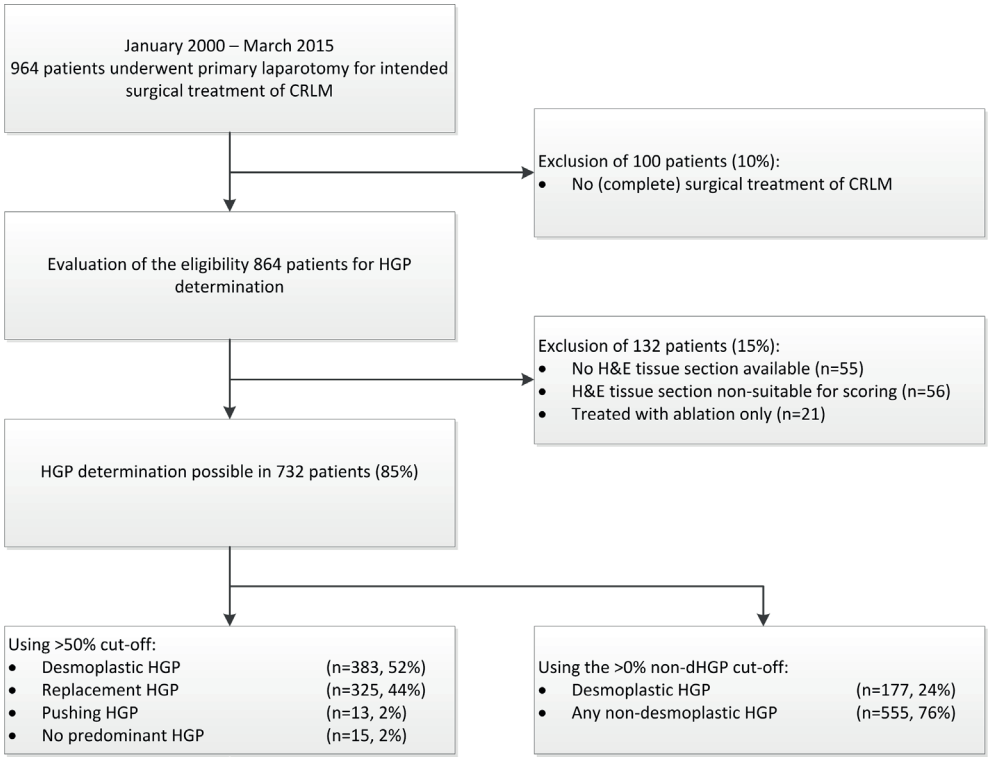
Statistical analysis method

Absolute numbers and percentages were used to present categorical data, while medians (incl. interquartile range (IQR)) were used to display continuous data. The Chi-squared test was used to evaluate differences in proportions. To compare medians between two or three groups the Mann-Whitney U or the Kruskal-Wallis test were used, respectively. Survival was estimated by means of Kaplan-Meier analysis , the curves were computed until 60 months and compared using the logrank test. Uni- and multivariable Cox regression analysis was performed to determine if HGPs remained significantly prognostic when correcting for well-known risk factors. Results of the Cox regression analyses were expressed using hazard ratios (HR) and consequent 95% confidence intervals (CI). In order to test possible statistical interaction between neoadjuvant chemotherapy and the HGP, an interaction term was added to a multivariable Cox regression model analysing the entire study population. Other potential confounders corrected for were age, ASA score, primary tumour location, pathological T-stage, nodal status, disease free interval, number of CRLM, diameter of the largest CRLM, carcinoembryonic antigen level, resection margin and extrahepatic disease. Uni- and multivariable binary logistic regression analysis was performed to determine whether the administration of neoadjuvant chemotherapy was associated with the HGP that was observed. Results of the logistic regression were expressed using odds ratios (OR) and corresponding 95% CI. All analyses were performed for chemo-naive and neoadjuvantly treated patients separately where applicable. Median follow-up time for survivors was estimated using the reversed Kaplan-Meier method. No imputation of missing data was applied. Schoenfeld residuals (for continuous variables) and Kaplan-Meier graphs (for categorical variables) were evaluated, in order to determine whether the proportional hazards assumption was violated. All statistical tests were two-sided and a p-value below 0.05 was considered statistically significant. All analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL) and R version 3.5.1 (http://www.r-project.org).

Results

Patient characteristics

During the study period, 964 consecutive patients underwent laparotomy for intended first surgical treatment of CRLM. One hundred patients (10%) were excluded because no complete surgical treatment was performed. In 132 patients (15%), HGP assessment was not possible due to missing H&E tissue sections (n=55), ablative therapy only (n=21) or H&E tissue sections which were non-suitable for HGP determination (n=56). Ultimately, the HGP could be determined in 732 patients. In 177 patients (24%) dHGP was observed and the other 555 patients (76%) all displayed to some extent a proportion of non-dHGP. A flowchart of the patient inclusion is presented in supplementary figure 1. Median follow-up time for the survivors was 76 months (IQR: 45-116 months), during which time 528 patients (70%) were diagnosed with disease progression and 428 patients (58%) died. Statistical interaction between neoadjuvant chemotherapy and HGP proved significant (p=0.005) on multivariable analysis.



Supplementary figure 1. A flowchart of the patient inclusion.

HGP in chemo-naive patients

Of the 732 patients assessed, 367 (50%) did not receive neoadjuvant chemotherapy. In this subgroup of patients 68 (19%) displayed dHGP only while n=214 (58%) displayed dHGP in combination with non-dHGP, and n=85 (23%) displayed no dHGP. In total, 299 patients (81%) displayed some proportion of non-dHGP (Figure 2A). Baseline characteristics compared for the presence of any non-dHGP are displayed in supplementary table 1.

Table 1. Cox regression analysis for OS and PFS of chemo-naive patients

Overall Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.011 [0.997-1.025]	0.126	1.016 [1.002-1.032]	0.030
ASA > II	1.018 [0.648-1.600]	0.939	0.985 [0.614-1.579]	0.949
Right-sided primary	1.477 [1.053-2.072]	0.024	1.539 [1.074-2.207]	0.019
pT3-4	1.191 [0.852-1.666]	0.306	0.902 [0.626-1.300]	0.579
Node positive primary	1.459 [1.102-1.933]	0.008	1.570 [1.140-2.164]	0.006
Disease free interval (cont.)	0.997 [0.991-1.004]	0.454	0.990 [0.983-0.998]	0.011
Number of CRLM (cont.)	1.145 [1.031-1.273]	0.012	1.095 [0.969-1.237]	0.144
Diameter largest CRLM (cont.)	1.099 [1.041-1.162]	<0.001	1.102 [1.026-1.185]	0.008
Preoperative CEA level (cont.)	1.001 [1.000-1.002]	0.003	1.001 [1.000-1.002]	0.063
R1 resection CRLM	1.321 [0.892-1.956]	0.165	1.116 [0.738-1.685]	0.603
Extra hepatic disease	1.495 [0.896-2.496]	0.124	1.688 [0.930-3.066]	0.085
dHGP	0.314 [0.191-0.515]	<0.001	0.394 [0.233-0.667]	<0.001
Progression Free Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	0.998 [0.987-1.010]	0.769	1.005 [0.993-1.018]	0.387
ASA > II	0.836 [0.554-1.262]	0.394	0.852 [0.555-1.306]	0.462
Right-sided primary	1.179 [0.868-1.602]	0.291	1.232 [0.893-1.698]	0.204
pT3-4	1.175 [0.878-1.573]	0.279	0.873 [0.634-1.203]	0.407
Node positive primary	1.566 [1.224-2.002]	<0.001	1.558 [1.184-2.049]	0.002
Disease free interval (cont.)	0.993 [0.986-1.000]	0.039	0.989 [0.981-0.996]	0.003
Number of CRLM (cont.)	1.215 [1.102-1.340]	<0.001	1.150 [1.029-1.285]	0.013
Diameter largest CRLM (cont.)	1.026 [0.972-1.083]	0.345	1.036 [0.970-1.107]	0.287
Preoperative CEA level (cont.)	1.001 [1.000-1.002]	0.041	1.001 [1.000-1.002]	0.167
R1 resection CRLM	1.620 [1.149-2.285]	0.006	1.376 [0.956-1.982]	0.086
Extra hepatic disease	1.199 [0.760-1.892]	0.434	1.596 [0.953-2.672]	0.076
dHGP	0.452 [0.317-0.645]	<0.001	0.536 [0.366-0.786]	0.001

Abbreviations in alphabetical order: ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; cont.: continuous; CRLM: colorectal liver metastases; dHGP: desmoplastic histopathological growth pattern; R1: irradical resection margin

Patients with dHGP had a five-year OS rate of 78% compared to 37% of patients with any non-dHGP (Figure 3A). After correction for potential confounders, dHGP remained significantly associated with improved OS (adjusted HR: 0.39, p<0.001) compared to non-dHGP (Table 1). Similar results were obtained for PFS. The five-year PFS rate of patients with dHGP was 50% compared to 19% of patients with any non-dHGP. On multivariable analysis dHGP also remained significantly associated with improved PFS (adjusted HR: 0.54, p=0.001) (Table 1 and figure 4A).

Table 2. Cox regression analysis for OS and PFS of pre-treated patients

Overall Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.021 [1.007-1.036]	0.003	1.034 [1.016-1.051]	<0.001
ASA > II	1.082 [0.675-1.733]	0.744	1.197 [0.728-1.968]	0.479
Right-sided primary	0.877 [0.590-1.304]	0.517	0.954 [0.624-1.459]	0.829
pT3-4	1.476 [0.988-2.204]	0.057	1.402 [0.900-2.183]	0.135
Node positive primary	1.419 [1.050-1.918]	0.023	1.383 [0.994-1.923]	0.054
Disease free interval (cont.)	0.996 [0.985-1.008]	0.541	0.995 [0.982-1.008]	0.452
Number of CRLM (cont.)	1.023 [0.976-1.072]	0.340	1.051 [0.995-1.111]	0.076
Diameter largest CRLM (cont.)	0.997 [0.952-1.045]	0.905	1.026 [0.969-1.086]	0.381
Preoperative CEA level (cont.)	1.000 [1.000-1.000]	0.955	1.000 [1.000-1.000]	0.574
R1 resection CRLM	1.374 [0.989-1.908]	0.058	1.273 [0.867-1.869]	0.218
Extra hepatic disease	1.705 [1.222-2.380]	0.002	1.761 [1.196-2.592]	0.004
dHGP	0.661 [0.484-0.902]	0.009	0.915 [0.643-1.302]	0.623
Progression Free Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.008 [0.996-1.019]	0.188	1.011 [0.998-1.025]	0.106
ASA > II	1.086 [0.731-1.614]	0.682	1.045 [0.682-1.600]	0.840
Right-sided primary	0.936 [0.684-1.282]	0.681	1.053 [0.752-1.474]	0.764
pT3-4	1.420 [1.021-1.974]	0.037	1.440 [1.005-2.065]	0.047
Node positive primary	1.328 [1.032-1.710]	0.028	1.143 [0.869-1.501]	0.339
Disease free interval (cont.)	0.994 [0.985-1.004]	0.234	0.996 [0.986-1.007]	0.462
Number of CRLM (cont.)	1.026 [0.989-1.063]	0.174	1.036 [0.992-1.081]	0.109
Diameter largest CRLM (cont.)	0.993 [0.954-1.034]	0.728	1.000 [0.955-1.048]	0.986
Preoperative CEA level (cont.)	1.000 [1.000-1.000]	0.462	1.000 [1.000-1.000]	0.443
R1 resection CRLM	1.464 [1.101-1.948]	0.009	1.449 [1.043-2.015]	0.027
Extra hepatic disease	1.777 [1.321-2.390]	<0.001	1.912 [1.367-2.674]	<0.001
dHGP	0.671 [0.519-0.867]	0.002	0.762 [0.570-1.017]	0.065

Abbreviations in alphabetical order: ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; cont.: continuous; CRLM: colorectal liver metastases; dHGP: desmoplastic histopathological growth pattern; R1: irradical resection margin

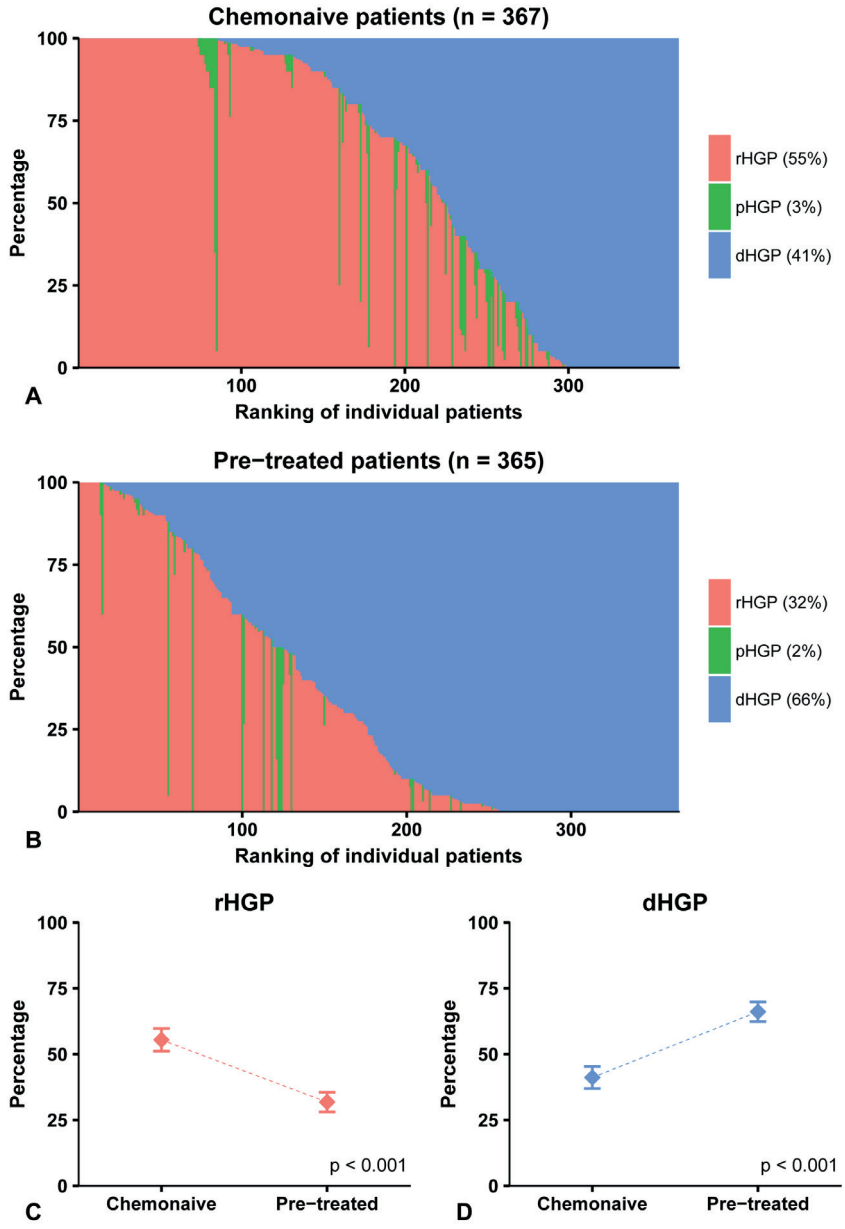


Figure 2A-D. 2A: Distribution of HGPs. Ranking based on percentage dHGP. 2A: distribution of HGPs in the chemo-naive cohort. 3B: distribution of HGPs in the pre-treated cohort. 2C-D: total proportion rHGP (C) and dHGP (D) in chemo-naive patients compared to pre-treated patients. (percentages do not always add up to 100% due to rounding)

When the OS for different percentages of non-dHGP was evaluated (Figure 3B), there were no differences in OS between patients who displayed any non-dHGP, regardless of the percentage of non-dHGP (all p-values >0.2). Kaplan-Meier analysis showed that all patients with any non-dHGP had significantly impaired survival compared to patients who had (100%) dHGP (all p-values ≤0.001). This finding was confirmed on multivariable analysis (Table 3).

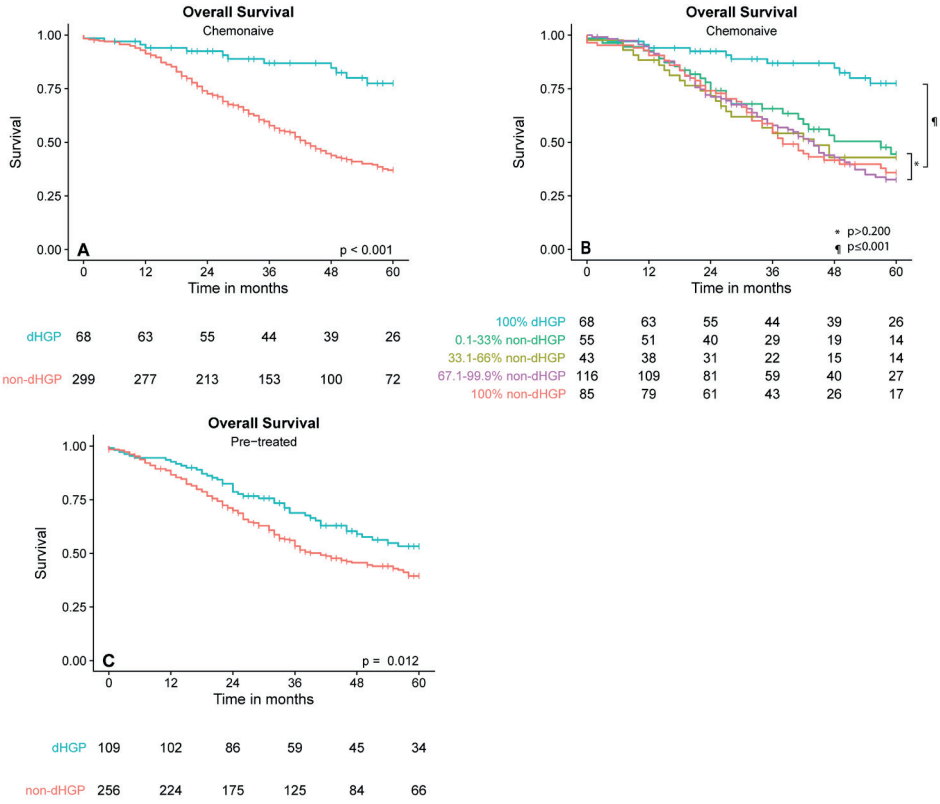


Figure 3A-C. 3A OS chemo-naive patients; 3B Cut-off analysis with OS for percentage dHGP amongst chemo-naive patients; 3C pre-treated patients

HGP in neoadjuvantly treated patients

In total, 365 patients (50%) received chemotherapy within six months prior to liver resection. The distribution of HGPs amongst neoadjuvantly treated patients is displayed in figure 2B. Baseline characteristics of neoadjuvantly treated patients compared for the presence of any non-dHGP are displayed in supplementary table 2. Patients who were treated neoadjuvantly with chemotherapy had a more severe disease burden (Supplementary table 3). The chemotherapeutic regimen was oxaliplatin-based in 309 patients (85%) and irinotecan based in 34 (9%). Fifteen patients received a 5-Fluorouracil derivative only (4%). Six patients (2%) received a combination of oxaliplatin and irinotecan and in one patient the type of chemotherapy was unknown. In 119 patients (33%) bevacizumab was added to the chemotherapy regimen.

Table 3. Cox regression cut-off analysis in the chemo-naive cohort

Overall Survival	Univariable		Multivariable	
Variables	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.011 [0.997-1.025]	0.126	1.017 [1.002-1.032]	0.031
ASA > II	1.018 [0.648-1.600]	0.939	0.968 [0.600-1.564]	0.896
Right-sided primary	1.477 [1.053-2.072]	0.024	1.563 [1.088-2.247]	0.016
pT3-4	1.191 [0.852-1.666]	0.306	0.890 [0.617-1.285]	0.535
Node positive primary	1.459 [1.102-1.933]	0.008	1.583 [1.142-2.194]	0.006
Disease free interval (cont.)	0.997 [0.991-1.004]	0.454	0.990 [0.982-0.998]	0.010
Number of CRLM (cont.)	1.145 [1.031-1.273]	0.012	1.104 [0.974-1.252]	0.122
Diameter largest CRLM (cont.)	1.099 [1.041-1.162]	<0.001	1.105 [1.026-1.189]	0.008
Preoperative CEA level (cont.)	1.001 [1.000-1.002]	0.003	1.001 [1.000-1.002]	0.103
R1 resection CRLM	1.321 [0.892-1.956]	0.165	1.103 [0.727-1.671]	0.645
Extra hepatic disease	1.495 [0.896-2.496]	0.124	1.627 [0.886-2.987]	0.116
100% dHGP	Ref		Ref	
0.1%-33% non-dHGP	2.851 [1.582-5.137]	<0.001	2.350 [1.248-4.425]	0.008
33.1%-67% non-dHGP	2.840 [1.547-5.215]	<0.001	2.458 [1.303-4.639]	0.005
67.1%-99.9% non-dHGP	3.255 [1.924-5.505]	<0.001	2.443 [1.393-4.284]	0.002
100% non-dHGP	3.535 [2.055-6.084]	<0.001	2.858 [1.605-5.088]	<0.001

Of the neoadjuvantly treated patients, 109 (30%) had dHGP and 256 (70%) displayed a proportion of non-dHGP (Figure 2B). dHGP was observed in a greater number of samples from neoadjuvantly treated than chemo-naive patients (30% vs 19%, p<0.001). The total proportion of any dHGP in neoadjuvantly treated patients was 66%, while this was 41% in chemo-naive patients. A similar difference was observed for the total proportion of any rHGP (both p<0.001, figures 2C-D). The association between neoadjuvant chemotherapy and the presence of dHGP remained significant (adjusted OR: 2.71, p<0.001) after correction for several clinicopathological characteristics (Supplementary table 4).

The addition of bevacizumab to the chemotherapeutic regimen was not associated with a significant increase of the proportion of dHGP (35% vs 27%, p=0.120). A subsequently performed multivariable logistic regression model failed to demonstrate a significant association between dHGP and the administration of bevacizumab (adjusted OR: 1.60, p=0.077) (Supplementary table 5).

Five-year OS in neoadjuvantly treated patients with dHGP was 53%, while a five-year OS of 40% was seen in patients with non-dHGP (Figure 3C; p=0.012). When correcting for confounders no significant association of dHGP was observed for OS (adjusted HR: 0.98, p=0.623) (Table 2). Again, similar results were obtained for the PFS. Neoadjuvantly treated patients with dHGP had a five-year PFS rate of 26% compared to 14% in patients with non-dHGP (Figure 4B; p=0.004). On multivariable analysis, only a trend towards a significant association of dHGP with PFS was seen (adjusted HR: 0.76, p=0.065) (Table 2).

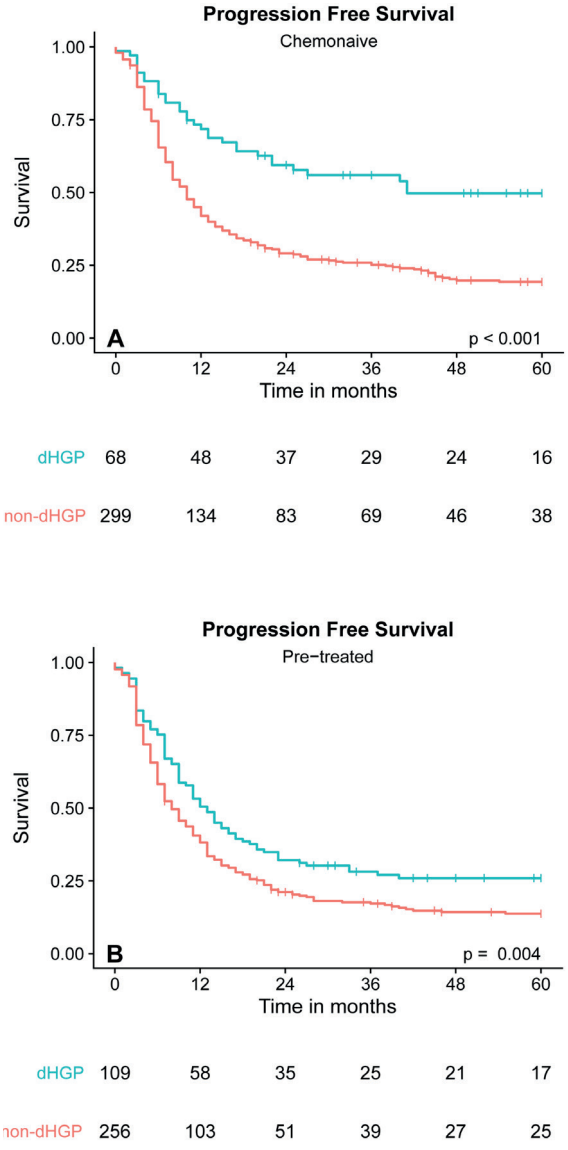


Figure 4A-B PFS. 4A: PFS of chemo-naive patients. 4B: PFS of pre-treated patients.

Additional Kaplan-Meier analyses showed no overall survival difference when adding bevacizumab to the chemotherapeutic regimen in the total group (p=0.754), in the non-dHGP (p=0.854) or in the dHGP subgroup (p=0.411). Similar results were found for PFS in the total group (p=0.806), the non-dHGP (p=0.829) or the dHGP subgroup (p=0.806). Subsequent multivariable analysis in the total neoadjuvantly treated group with bevacizumab entered as potential confounder showed no significant association of bevacizumab with OS (adjusted HR: 1.06, p=0.702; supplementary table 6.) or PFS (adjusted HR: 1.09, p=0.540; supplementary table 7.).

Consensus cut-off

Supplementary analyses performed using the consensus guidelines >50% predominant HGP cut-off confirmed results: superior survival for dHGP, higher proportion of dHGP after neoadjuvant chemotherapy and loss of prognostic impact of dHGP in neoadjuvantly treated patients. These data are presented in supplementary tables 8-12 and supplementary figure 2A-B.

dHGP versus any rHGP

In order to make a direct comparison of angiogenic dHGP versus non-angiogenic rHGP growth, we have performed separate, supplementary analyses which excluded the few cases with angiogenic pHGP. Patients with any proportion of rHGP were compared to patients with pure (100%) dHGP, excluding patients without any rHGP from the non-dHGP group. In total, 26 patients, of which 13 were chemo-naive, without rHGP were observed in the non-dHGP group and excluded for these analyses. Again, all analyses had similar results: superior OS (HR: 0.40, p<0.001) and PFS (HR: 0.55, p=0.002) for chemo-naive patients with dHGP and a reduced prognostic impact of HGPs after neoadjuvant chemotherapy (OS – HR: 0.88, p=0.505; PFS – HR: 0.73, p=0.032).

HGP comparison of multiple hepatectomies

Among the included patients, the HGP of recurrent CRLM could be determined in 66 patients. A similar proportional distribution of HGPs was observed in these patients. After surgery for recurrent CRLM without neoadjuvant chemotherapy dHGP tumours were found in 18% (8/45) of patients, compared to 29% (6/21) in patients who did receive chemotherapy (p=0.318). Four groups (-/-, +/-, -/+, +/+), as described in the methods, were created. Figure 5A-D graphically displays the changes in HGPs found per group. The difference in proportion HGPs between the 1st and 2nd surgery was significant in the +/- group (Figure 5B, p=0.007). The other changes in proportions of HGP between the 1st and 2nd surgery were not significant (all p-values >0.250).

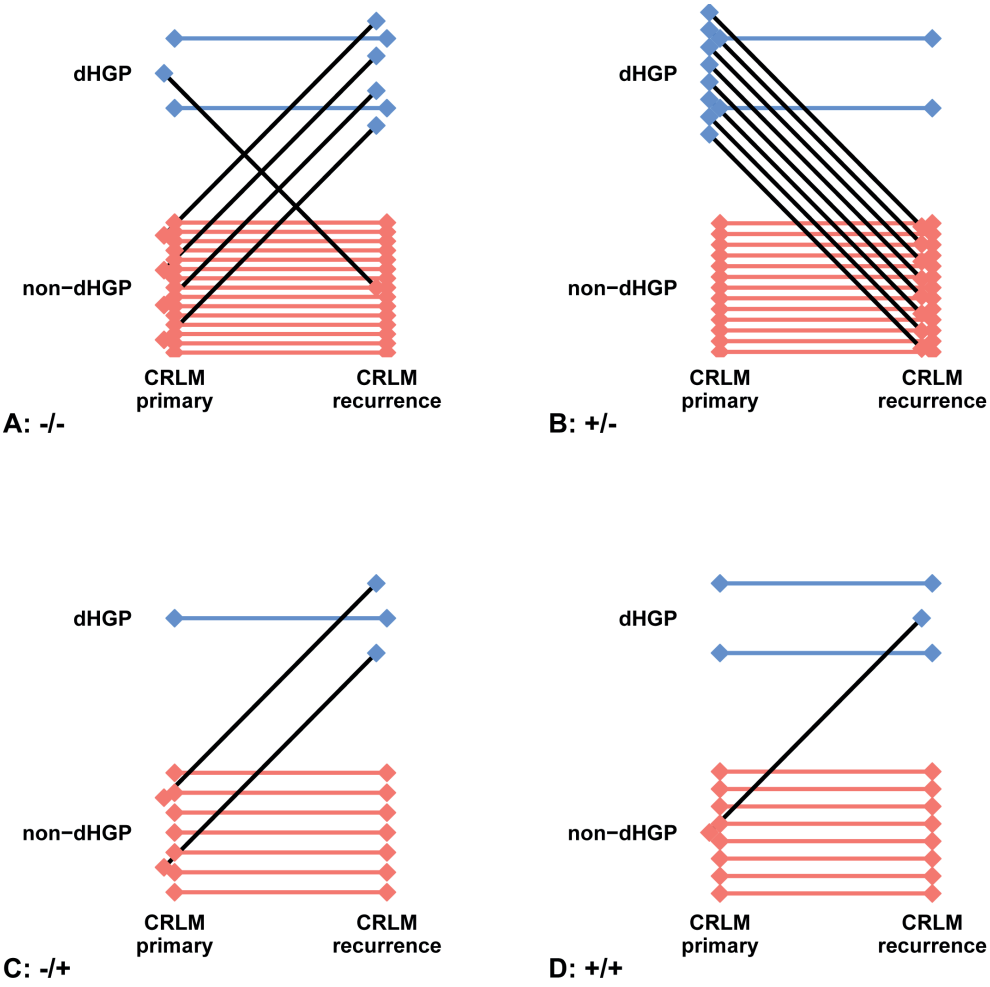


Figure 5A-D. Graphical display of the changes in HGPs between 1st and 2nd surgery for CRLM found per group: 5A: -/-; 5B:+/-; 5C: -/+; 5D: +/+.

Discussion

The current study demonstrates that HGPs have significant prognostic potential for colorectal cancer patients who undergo first resection of CRLM. Our results indicate that in chemo-naive patients the presence of a pure dHGP predicts improved survival with a hazard ratio unmatched by any clinicopathological or biological correlate to date. [12, 13] This is the first study to show that the presence of *any* non-dHGP is sufficient to indicate impaired prognosis. Interestingly, chemotherapy is associated with an increased incidence of CRLM displaying dHGP in the current patient cohort and the prognostic impact of dHGP is reduced in these patients.

Stratifying patient groups for pre-operative treatment status showed that the proportion and prognostic impact of HGPs differs significantly between chemo-naive and neoadjuvantly treated patients. Previous studies examined relatively small and heterogeneous patient groups which hampered adequate multivariable analysis whereas the large number of events in the current study ensured that proper correction for confounders could be performed. [4, 7-10] In addition, preceding studies did not perform cut-off analyses for different proportions of HGPs. The currently performed cut-off analysis showed that an increasing proportion of non-dHGP was not associated with a decrease in prognosis. Therefore, the presence of any non-dHGP, rather than the actual proportion of the tumour-liver interface occupied by non-dHGP, dictates worse survival compared to patients with 100% dHGP. This suggests that an arbitrary cut-off should not be applied to define the non-dHGP growth pattern. This information can be integrated in future consensus guidelines for scoring the HGPs of CRLM.

Neoadjuvant chemotherapy (with or without bevacizumab) has been associated with tumoural fibrosis and necrosis in CRLM. [14, 15] Treatment with bevacizumab has been associated with alterations in the extracellular matrix (ECM) of CRLM [16] and the ECM has been argued to influence the hallmarks of cancer. [17] Given these associations, one could hypothesise that treating CRLM with chemotherapy with or without bevacizumab could induce alterations in the HGP. In the current study it has been possible to determine the prevalence of different HGP types in CRLM relative to chemotherapy status and with the addition of bevacizumab. We observed a higher proportion of 100%dHGP in neoadjuvantly treated patients, but the prognostic impact of this growth pattern was relatively reduced in this patient category. Similar results were found within the subgroup in whom bevacizumab was added to the chemotherapy regimen, but this was not significantly different compared to the group that was treated neoadjuvantly without bevacizumab. Moreover, the previously reported survival benefit of the addition of bevacizumab to chemotherapy in 51 patients with dHGP [4] could not be demonstrated in the current study. At our institution, evident progressive disease during chemotherapy is a contra-indication for surgical treatment of CRLM. As poor pathological and radiological response is associated with rHGP [4], it is possible that progressive patients have CRLM displaying non-dHGP. This could have resulted in a higher relative proportion of dHGP in the neoadjuvantly treated patient cohort. Unfortunately, data on the percentage of patients that were not operated upon because of

disease progression are unavailable in our series. In randomised setting, approximately 7% of patients with resectable CRLM displays progressive disease during chemotherapy. [18] In addition, considerable differences in clinical risk were seen when comparing chemo-naive patients with neoadjuvantly treated patients in this non-randomised cohort. An alternative explanation for both the larger proportion of dHGP and the reduced prognostic impact of HGPs in the neoadjuvantly treated cohort is that a biological response to chemotherapy is a histological conversion to dHGP, the relevance of which we have yet to determine. Of patients considered chemonaive for their recurrent CRLM 18% (8/45) had recurrent CRLM displaying dHGP compared to 29% (6/21) in patients treated neoadjuvantly for their recurrent CRLM. This difference in proportional distribution of recurrent HGPs was not significant. Nevertheless. it was similar to the proportional distribution of HGPs observed after first hepatectomy in which the difference was significant. When taking neoadjuvant treatment status of both resections into account, in the +/- group 35% (8/23) changed from dHGP (1st surgery) to non-dHGP (2nd surgery), while this change was only seen in 5% (1/22) of the -/- group. These data could support the hypothesis of potential conversion of the HGP as a consequence of chemotherapy. An alternative explanation for this observation could be that patients who at first have dHGP CRLM, but develop non-dHGP CRLM at recurrence as the disease might acquire a more aggressive tumour biology. In addition, Frentzas et al. also found a relatively large proportion of rHGP in recurrent CRLM, albeit after combination therapy of chemotherapy and bevacizumab for the recurrent CRLM. [4] The value of these data remains limited, because of its retrospective nature, selected population and low patient numbers. Further study of the HGPs in chemo-naive versus neoadjuvantly treated CRLM is required to investigate this concept and more specifically, data from randomised studies will be needed to further evaluate this hypothesis.

The biological mechanisms that underlie the association of non-dHGP with impaired survival remain largely unknown. The non-dHGP cohort in this study consists almost exclusively of patients with liver metastases that display the vessel co-opting, non-angiogenic rHGP. An important difference between rHGP and dHGP is indeed the mechanism of vascularization. The desmoplastic growth pattern of liver metastases has an elevated fraction of proliferating endothelial cells and blood vessels are organised in vascular hot spots [3, 19], both clear features of angiogenesis. The vascular architecture of the metastasis does not resemble the vascular architecture of the adjacent liver tissue. These findings also apply to the pushing growth pattern. In the replacement growth pattern, on the contrary, a low endothelial cell proliferation fraction and a lack of vascular hot spots are observed. [3, 19] The tumour tissue mimics the liver tissue by growing along and using the sinusoidal blood vessels. The preservation of the normal tissue architecture is indicative of non-angiogenic tumour growth. The co-opted capillary bed from normal liver is highly efficient and liver metastases with a rHGP display minimal hypoxia and vascular leakage as opposed to the desmoplastic liver metastases with their vasculature created in an angiogenic environment in which tortuous, disrupted, leaking and dysfunctional blood vessels result in hypoxia. [3] The association between growth patterns and the means of tumour vascularization (by angiogenesis or by vessel co-option) is not limited to tumour growth in the liver, but has also been described in,

for example, the lungs, the lymph nodes and the skin. [20] The motile and invasive cancer cells present in replacement metastases enables the incorporation of normal surrounding tissue stroma and creates the typical irregular tumour border. Up-regulation of signalling pathways of cell motility has been described in pre-clinical models of CRC liver metastases and primary liver cancer. [4, 21] Similarly, molecular signatures of cancer cell motility and invasion have been identified in angiotropism, a process of perivascular growth that closely resembles vascular co-option during replacement growth. [22, 23] Co-localisation of cancer cells and endothelial cells during vascular co-option also results in angiocrine signalling. Soluble ligands of the notch-pathway produced by endothelial cells induce stemness in adjacent cancer cells which is associated with both cancer cell motility and with resistance to chemotherapy. [24] Again, similar observations have been reported for angiotropic tumours. [23] Beyond the intrinsic changes in the tumour and stroma observed in replacement metastases, an effective immune response in patients with dHGP also might contribute to the difference in survival outcomes between these two HGPs [5, 25]. Brunner et al. demonstrated that capsule formation in dHGP strongly correlates with high levels of peri-tumour infiltration of CD4+, CD45RO+ and CD8+ cells. [5] Taken together, these findings corroborate the less favourable prognosis of patients with liver metastases that have the ability to perform non-desmoplastic growth.

For a more direct comparison of angiogenic dHGP and non-angiogenic rHGP growth, we have excluded the few cases with angiogenic pHGP in separate analyses. Non-angiogenic replacement HGP has been associated with aggressive tumour growth in which normal sinusoidal liver capillaries are co-opted by the metastasis. The pHGP can be difficult to distinguish from the rHGP when during replacement growth the liver cell plates are also pushed aside. This HGP assessment problem has been extensively addressed in the international consensus for scoring the histopathological growth patterns of liver metastases. [2] This, however, is an additional reason to selectively study the impact on survival of pure (100%) dHGP. It will be necessary to assemble a large cohort of patients with pHGP to accurately study the impact of this growth pattern on outcome.

The results of the current study should be interpreted in the light of its limitations. The HGP data were collected retrospectively, in 55 potentially eligible patients tissue sections were missing and there were 56 patients with unsuitable H&E tissue sections. It was also not possible to examine CRLM from patients with progressive disease during chemotherapy as this as a contra-indication for surgical treatment at our institution. This study was also limited by the unavailability of RAS and BRAF mutational status. Both mutations have been suggested as prognostic biomarkers for survival after liver resection for CRLM [13, 26, 27] In addition, Brudvik et al. proposed an enhanced clinical risk score, including the RAS mutational status. The authors demonstrated improved performance of the prognostic model. [28] In an attempt to overcome this shortcoming, the current study was corrected for right-sidedness of the primary tumour, which is associated with KRAS [29, 30] and BRAF [29-31] mutational status.

In conclusion, the current study demonstrates in the largest patient cohort to date with multivariable analyses that HGPs, distinguishing angiogenic from non-angiogenic growth, have considerable prognostic impact in patients who are treated surgically for CRLM. The presence of *any* non-desmoplastic, non-angiogenic HGP displaying vessel co-opting growth, rather than the actual proportion of non-dHGP, determines prognosis suggesting that future studies and guidelines should focus upon this distinction.

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Figure captions

Figure 1A-F. Collated HE tissue sections. 1A-B: dHGP low and high magnification; 1C-D: rHGP low and high magnification; 1E-F: pHGP low and high magnification. T: tumour; D: desmoplastic stroma; L: liver parenchyma.

Figure 2A-D. 2A: Distribution of HGPs. Ranking based on percentage dHGP. 2A: distribution of HGPs in the chemo-naive cohort. 3B: distribution of HGPs in the pre-treated cohort. 2C-D: total proportion rHGP (C) and dHGP (D) in chemo-naive patients compared to pre-treated patients. (percentages do not always add up to 100% due to rounding)

Figure 3A-C. 3A OS chemo-naive patients; 3B Cut-off analysis with OS for percentage dHGP amongst chemo-naive patients; 3C pre-treated patients

Figure 4A-B PFS. 4A: PFS of chemo-naive patients. 4B: PFS of pre-treated patients

Figure 5A-D. Graphical display of the changes in HGPs between 1st and 2nd surgery for CRLM found per group: 5A: -/-; 5B:+/-; 5C: -/+; 5D: +/+.

Table 1. Cox regression analysis for OS and PFS of chemo-naive patients

Table 2. Cox regression analysis for OS and PFS of pre-treated patients

Table 3. Overall Survival Cox regression cut-off analysis in the chemo-naive cohort

Supplementary tables and figures

Supplementary table 1. Baseline characteristics compared for the presence of any non-dHGP

Supplementary table 2. Baseline characteristics pre-treated patients dHGP vs non-dHGP

Supplementary table 3. Baseline characteristics chemo-naive versus pre-treated patients

Supplementary table 4. Uni- and multivariable logistic regression analysis for association with dHGP

Supplementary table 5. (Rebuttal table 2.) Uni- and multivariable logistic regression analysis for association with dHGP in the neoadjuvantly treated group

Supplementary table 6. Overall Survival Cox regression analysis all neoadjuvantly treated patients +/- Bevacizumab

Supplementary table 7. Progression Free Survival Cox regression all neoadjuvantly treated patients +/- Bevacizumab

Supplementary table 8. Baseline characteristics chemo-naive patients 50% cut-off

Supplementary table 9. Uni- and multivariable Cox regression analysis OS of chemo-naive patients >50% cut-off

Supplementary table 10. Uni- and multivariable logistic regression analysis for association with dHGP >50% cut-off

Supplementary table 11. Baseline characteristics pre-treated patients >50% cut-off

Supplementary table 12. Uni- and multivariable Cox regression analysis for OS of pre-treated patients >50% cut-off

Supplementary figure 1. A flowchart of the patient inclusion.

Supplementary figure 2A-B. OS using the >50% cut-off. 2A: OS chemo-naive patients. 2B: OS pre-treated patients

Supplementary materials

Supplementary table 1. Baseline characteristics chemo-naive patients dHGP vs non-dHGP

		dHGP	non-dHGP	p-value
		N=68 (19%)	N=299 (81%)	
General characteristics				
Age at resection (median [IQR])		68.0 [59.0, 75.2]	66.0 [59.0, 73.0]	0.180
Gender (%)	Female	25 (37)	109 (36)	0.962
	Male	43 (63)	190 (64)	
ASA classification(%)	ASA Class I-II	57 (85)	265 (91)	0.142
	ASA Class >II	10 (15)	26 (9)	
	Missing (N=9)			
Primary tumour characteristics				
Location (%)	Right-sided	11 (16)	51 (17)	0.468
	Left-sided	32 (47)	115 (38)	
	Rectum	22 (32)	124 (41)	
	Double tumour	3 (4)	9 (3)	
Pathological T-stage (%)	pT 0-2	19 (28)	61 (21)	0.188
	pT 3-4	49 (72)	235 (79)	
	Missing (N=3)			
Pathological N-stage (%)	N0	35 (52)	118 (40)	0.070
	N+	32 (48)	176 (60)	
	Missing (N=6)			
Adjuvant chemotherapy (%)	No	59 (87)	231 (77)	0.082
	Yes	9 (13)	68 (23)	
CRLM characteristics				
Synchronous CRLM (%)	Metachronous	43 (63)	212 (71)	0.215
	Synchronous	25 (37)	87 (29)	
DFI (median [IQR])		10.0 [0.0, 20.0]	13.0 [0.5, 25.5]	0.154
Number of CRLM (median [IQR])		1.0 [1.0, 2.0]	1.0 [1.0, 2.0]	0.195
Largest diameter CRLM (median [IQR])	*Missing (N=1)*	2.0 [1.5, 3.6]	3.2 [2.2, 4.1]	<0.001*
Preoperative CEA (median [IQR])	*Missing (N=14)*	6.0 [3.0, 12.0]	13.6 [4.7, 44.2]	<0.001*
Bilobar (%)	Unilobar	54 (79)	232 (78)	0.744
	Bilobar	14 (21)	67 (22)	
Extrahepatic disease (%)	No	64 (94)	277 (93)	0.669
	Yes	4 (6)	22 (7)	
Resection margin (%)	R0	65 (96)	259 (87)	0.048*
	R1	3 (4)	38 (13)	
	Missing (N=2)			
CRS (%)	Low (0-2)	53 (80)	230 (78)	0.746
	High (3-5)	13 (20)	63 (22)	
	Incomplete (N=8)			
Major resection (≥3 complete segments) (%)	No major resection	59 (87)	226 (76)	0.046*
	Major resection	9 (13)	73 (24)	
Major complications (i.e. Clavien-Dindo ≥3)	No	61 (90)	277 (93)	0.363
	Yes	7 (10)	21 (7)	
	Missing (N=1)			
Postoperative death (%)	No	67 (99)	293 (98)	0.770
	Yes	1 (1)	6 (2)	

Supplementary table 2. Baseline characteristics pre-treated patients dHGP vs non-dHGP

		dHGP	non-dHGP	p-value
		N=109 (30%)	N=256 (70%)	
General characteristics				
Age at resection (median [IQR])		63.0 [55.0, 70.0]	63.0 [56.8, 68.2]	0.858
Gender (%)	Female	40 (37)	89 (35)	0.724
	Male	69 (63)	167 (65)	
ASA classification(%)	ASA Class I-II	100 (92)	234 (92)	0.995
	ASA Class >II	9 (8)	21 (8)	
	Missing (N=1)			
Primary tumour characteristics				
Location (%)	Right-sided	20 (18)	38 (15)	0.657
	Left-sided	45 (41)	115 (45)	
	Rectum	42 (39)	101 (39)	
	Double tumour	2 (2)	2 (1)	
Pathological T-stage (%)	pT 0-2	20 (19)	36 (16)	0.483
	pT 3-4	86 (81)	192 (84)	
	Missing (N=31)			
Pathological N-stage (%)	N0	44 (42)	77 (34)	0.152
	N+	61 (58)	151 (66)	
	Missing (N=32)			
Adjuvant chemotherapy (%)	No	105 (96)	228 (90)	0.056
	Yes	4 (4)	24 (10)	
	Missing (N=4)			
CRLM characteristics				
Synchronous CRLM (%)	Metachronous	22 (20)	60 (23)	0.495
	Synchronous	87 (80)	196 (77)	
DFI (median [IQR])		0.0 [0.0, 2.0]	0.0 [0.0, 2.2]	0.822
Number of CRLM (median [IQR])		3.0 [1.0, 4.0]	3.0 [2.0, 5.0]	0.018
Largest diameter CRLM (median [IQR])	*Missing (N=1)*	2.9 [2.1, 4.7]	3.4 [2.4, 5.3]	0.047
Preoperative CEA (median [IQR])	*Missing (N=18)*	12.2 [3.6, 51.2]	21.0 [7.0, 93.0]	0.008
Bilobar (%)	Unilobar	55 (50)	94 (37)	0.015
	Bilobar	54 (50)	162 (63)	
Extrahepatic disease (%)	No	94 (86)	213 (83)	0.468
	Yes	15 (14)	43 (17)	
Resection margin (%)	R0	97 (90)	200 (78)	0.009
	R1	11 (10)	56 (22)	
	Missing (N=1)			
CRS (%)	Low (0-2)	48 (48)	89 (37)	0.073
	High (3-5)	53 (52)	151 (63)	
	Incomplete (N=24)			
Major resection (≥3 complete segments) (%)	No major resection	67 (61)	129 (50)	0.052
	Major resection	42 (39)	127 (50)	
Major complications (i.e. Clavien-Dindo ≥3)	No	99 (91)	228 (89)	0.614
	Yes	10 (9)	28 (11)	
Postoperative death (%)	No	109 (100)	249 (97)	0.081
	Yes	0 (0)	7 (3)	

Supplementary table 3. Baseline characteristics chemo-naive versus pre-treated patients

		Total N=732	Chemo-naive N=367 (50%)	Pre-treated N=365 (50%)	P- value
Gender	Male	469 (64%)	233 (64%)	236 (65%)	0.742
	Female	263 (36%)	134 (37%)	129 (35%)	
Age	Median (IQR)	64 (58-71)	66 (59-73)	63 (56-69)	<0.001*
ASA	ASA I-II	656 (91%)	322 (90%)	334 (92%)	0.398
	ASA > II	66 (9%)	36 (10 %)	30 (8%)	
	Missing	*10 patients*			
Primary tumour characteristics					
Location	Right-sided	120 (16%)	62 (17%)	58 (16 %)	0.194
	Left-sided	307 (42%)	147 (40%)	160 (44%)	
	Rectum	289 (40%)	146 (40%)	143 (39%)	
	Double tumour	16 (2%)	12 (3%)	4 (1%)	
pTumour stage	pT0-2	136 (20%)	80 (22%)	56 (17%)	0.082
	pT3-4	562 (81%)	284 (78.0%)	278 (83.2%)	
	Missing	*34 patients*			
Nodal status	N0	274 (40%)	153 (42%)	121 (36%)	0.104
	N+	420 (61%)	208 (58%)	212 (64%)	
	Missing	*38 patients*			
Adjuvant chemotherapy	No	623 (86%)	290 (79%)	333 (92%)	<0.001*
	Yes	105 (14%)	77 (21%)	28 (8%)	
	Missing	*4 patients*			
CRLM characteristics					
Synchronous CRLM	No	337 (46%)	255 (70%)	82 (23%)	<0.001*
	Yes	395 (54%)	112 (31%)	283 (78%)	
Disease-free interval	Median (IQR)	1 (0-17)	13 (0-25)	0 (0-2)	<0.001*
Number of CRLM	Median (IQR)	2 (1-4)	1 (1-2)	3 (2-5)	<0.001*
Size of largest CRLM	Median (IQR)	3.1 (2.1-4.7)	3.0 (3.0-4.0)	3.2 (2.3-5.2)	0.002*
	Missing	*2 patients*			
Preoperative CEA	Median (IQR)	14.7 (4.8-51.8)	11.0 (4.2-29.8)	19.7 (5.3-74.0)	<0.001*
	Missing	*32 patients*			
Fong CRS	Low	420 (60%)	283 (79%)	137 (40%)	<0.001*
	High	280 (40%)	76 (21%)	204 (60%)	
	Incomplete CRS	*32 patients*			

		Total N=732	Chemo-naive N=367 (50%)	Pre-treated N=365 (50%)	P- value
Bilobar metastases	No	435 (59%)	286 (78%)	149 (41%)	<0.001*
	Yes	297 (41%)	81 (22%)	216 (59%)	
Resection margin	R0	621 (85%)	324 (89%)	297 (82%)	0.006*
	R1	108 (15%)	41 (11%)	67 (18%)	
	Missing	*3 patients*			
HGP type	Desmoplastic	177 (24%)	68 (19%)	109 (30%)	<0.001*
	Replacement	86 (12%)	73 (20%)	13 (4%)	
	Mixed	469 (64%)	226 (62%)	243 (67%)	
Extra Hepatic Disease	No	648 (89%)	341 (93%)	307 (84%)	<0.001*
	Yes	84 (12%)	26 (7%)	58 (16%)	
Major liver resection	<3 complete segments	481 (66%)	285 (78%)	196 (54%)	<0.001*
	≥3 complete segments	251 (34%)	82 (22%)	169 (46%)	
Major complications	No	665 (91%)	338 (92%)	327 (90%)	0.193
	Yes	66 (9%)	28 (8%)	38 (10%)	
	Missing	*1 patient*			
Postoperative death	No	718 (98%)	360 (98%)	358 (98%)	0.992
	Yes	14 (2%)	7 (2%)	7 (2%)	

Percentages do not always add up to 100% due to rounding. Abbreviations in alphabetical order: ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastases; CRS: clinical risk score; HGP: histopathological growth pattern; IQR: interquartile range; R1: irradical resection margin

Supplementary table 4. Uni- and multivariable logistic regression analysis for association with dHGP

	Univariable		Multivariable	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Right-sided primary	1.112 [0.710-1.742]	0.644	1.264 [0.789-2.026]	0.330
pT3-4	0.786 [0.517-1.196]	0.261	0.849 [0.534-1.351]	0.491
Node positive primary	0.702 [0.495-0.995]	0.047*	0.611 [0.415-0.901]	0.013*
Disease free interval (cont.)	0.989 [0.978-1.000]	0.049*	0.992 [0.980-1.005]	0.227
Number of CRLM (cont.)	0.977 [0.909-1.050]	0.530	0.872 [0.790-0.962]	0.006*
Diameter largest CRLM (cont.)	0.904 [0.832-0.982]	0.017*	0.898 [0.822-0.981]	0.017*
Preoperative CEA level (cont.)	1.000 [0.999-1.000]	0.800	1.000 [0.999-1.001]	0.932
Preoperative chemotherapy	1.872 [1.325-2.646]	<0.001*	2.709 [1.746-4.203]	<0.001*

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; cont.: continuous CRLM: colorectal liver metastases; dHGP: desmoplastic histopathological growth pattern

Supplementary table 5. Uni- and multivariable logistic regression analysis for association with dHGP in the neoadjuvantly treated group

	Univariable		Multivariable	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Right-sided primary	1.289 [0.711-2.337]	0.403	1.421 [0.757-2.670]	0.274
pT3-4	0.806 [0.441-1.473]	0.484	0.805 [0.417-1.552]	0.517
Node positive primary	0.707 [0.440-1.137]	0.152	0.655 [0.388-1.107]	0.114
Disease free interval (cont.)	1.005 [0.988-1.024]	0.556	1.001 [0.980-1.023]	0.926
Number of CRLM (cont.)	0.906 [0.829-0.991]	0.032	0.891 [0.803-0.989]	0.030
Diameter largest CRLM (cont.)	0.933 [0.850-1.024]	0.145	0.936 [0.847-1.035]	0.200
Preoperative CEA level (cont.)	1.000 [0.999-1.000]	0.728	1.000 [0.999-1.001]	0.912
Bevacizumab	1.449 [0.906-2.317]	0.121	1.595 [0.951-2.675]	0.077

Supplementary table 6. Cox regression all neoadjuvantly treated patients +/- Bevacizumab

Overall Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.021 [1.007-1.036]	0.003	1.034 [1.016-1.052]	<0.001
ASA > II	1.082 [0.675-1.733]	0.744	1.195 [0.726-1.967]	0.484
Right-sided primary	0.877 [0.590-1.304]	0.517	0.952 [0.623-1.456]	0.821
pT3-4	1.476 [0.988-2.204]	0.057	1.398 [0.896-2.182]	0.140
Node positive primary	1.419 [1.050-1.918]	0.023	1.382 [0.990-1.928]	0.057
Disease free interval (cont.)	0.996 [0.985-1.008]	0.541	0.996 [0.983-1.009]	0.532
Number of CRLM (cont.)	1.023 [0.976-1.072]	0.340	1.052 [0.995-1.112]	0.074
Diameter largest CRLM (cont.)	0.997 [0.952-1.045]	0.905	1.025 [0.968-1.086]	0.394
Preoperative CEA level (cont.)	1.000 [1.000-1.000]	0.955	1.000 [1.000-1.000]	0.558
R1 resection CRLM	1.374 [0.989-1.908]	0.058	1.274 [0.868-1.872]	0.216
Extra hepatic disease	1.705 [1.222-2.380]	0.002	1.725 [1.164-2.558]	0.007
dHGP	0.661 [0.484-0.902]	0.009	0.906 [0.635-1.293]	0.587
Bevacizumab	1.001 [0.758-1.324]	0.992	1.063 [0.777-1.456]	0.702

Supplementary table 7. Cox regression all neoadjuvantly treated patients +/- Bevacizumab

Progression Free Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.008 [0.996-1.019]	0.188	1.012 [0.998-1.026]	0.091
ASA > II	1.086 [0.731-1.614]	0.682	1.048 [0.683-1.608]	0.830
Right-sided primary	0.936 [0.684-1.282]	0.681	1.046 [0.747-1.466]	0.791
pT3-4	1.420 [1.021-1.974]	0.037	1.442 [1.004-2.070]	0.047
Node positive primary	1.328 [1.032-1.710]	0.028	1.143 [0.867-1.507]	0.343
Disease free interval (cont.)	0.994 [0.985-1.004]	0.234	0.997 [0.986-1.008]	0.578
Number of CRLM (cont.)	1.026 [0.989-1.063]	0.174	1.036 [0.993-1.082]	0.103
Diameter largest CRLM (cont.)	0.993 [0.954-1.034]	0.728	1.000 [0.954-1.048]	0.989
Preoperative CEA level (cont.)	1.000 [1.000-1.000]	0.462	1.000 [1.000-1.000]	0.489
R1 resection CRLM	1.464 [1.101-1.948]	0.009	1.456 [1.046-2.026]	0.026
Extra hepatic disease	1.777 [1.321-2.390]	<0.001	1.872 [1.336-2.625]	<0.001
dHGP	0.671 [0.519-0.867]	0.002	0.752 [0.562-1.007]	0.055
Bevacizumab	0.986 [0.776-1.253]	0.908	1.087 [0.833-1.419]	0.540

Supplementary table 8. Baseline characteristics chemo-naive patients 50% cut-off

		>50% dHGP	>50% rHGP	>50% pHGP	p-value
		142 (39%)	**213 (58%)**	**8 (2%)**	
General characteristics					
Age at resection (median [IQR])		68.0 [58.2, 76.0]	65.0 [60.0, 72.0]	68.0 [55.5, 73.2]	0.320
Gender (%)	Female	46 (32)	80 (38)	4 (50)	0.426
	Male	96 (68)	133 (62)	4 (50)	
ASA classification(%)	ASA Class I-II	122 (87)	188 (91)	8 (100)	0.290
	ASA Class >II	18 (13)	18 (9)	0 (0)	
	Missing (N=9)				
Primary tumour characteristics					
Location (%)	Right-sided	23 (16)	37 (17)	1 (12)	0.700
	Left-sided	62 (44)	82 (38)	2 (25)	
	Rectum	53 (37)	87 (41)	4 (50)	
	Double tumour	4 (3)	7 (3)	1 (12)	
Pathological T-stage (%)	pT 0-2	36 (25)	40 (19)	3 (38)	0.210
	pT 3-4	106 (75)	170 (81)	5 (62)	
	Missing (N=3)				
Pathological N-stage (%)	N0	75 (53)	75 (36)	2 (25)	0.004*
	N+	66 (47)	133 (64)	6 (75)	
	Missing (N=6)				
Adjuvant chemotherapy (%)	No	120 (85)	161 (76)	7 (88)	0.107
	Yes	22 (15)	52 (24)	1 (12)	
CRLM characteristics					
Synchronous CRLM (%)	Metachronous	95 (67)	152 (71)	4 (50)	0.333
	Synchronous	47 (33)	61 (29)	4 (50)	
DFI (median [IQR])		13.0 [0.0, 26.0]	13.0 [0.0, 24.0]	2.0 [0.0, 6.5]	0.044
Number of CRLM (median [IQR])		1.0 [1.0, 2.0]	1.0 [1.0, 2.0]	1.0 [1.0, 1.0]	0.271
Largest diameter CRLM (median [IQR])	Missing (N=1)	2.8 [1.9, 4.0]	3.0 [2.0, 4.0]	4.0 [2.9, 4.9]	0.066
Preoperative CEA (median [IQR])	Missing (N=14)	7.8 [3.4, 17.5]	13.7 [5.0, 51.5]	20.6 [2.9, 23.3]	0.002*
Bilobar (%)	Unilobar	110 (77)	167 (78)	7 (88)	0.796

		>50% dHGP	>50% rHGP	>50% pHGP	p-value
		142 (39%)	**213 (58%)**	**8 (2%)**	
	Bilobar	32 (23)	46 (22)	1 (12)	
Extrahepatic disease (%)	No	136 (96)	194 (91)	8 (100)	0.171
	Yes	6 (4)	19 (9)	0 (0)	
Resection margin (%)	R0	133 (94)	181 (86)	8 (100)	0.040*
	R1	9 (6)	30 (14)	0 (0)	
	Missing (N=2)				
CRS (%)	Low (0-2)	112 (81)	162 (78)	6 (75)	0.692
	High (3-5)	26 (19)	47 (22)	2 (25)	
	Incomplete (N=8)				
Major resection (≥3 complete segments) (%)	No major resection	118 (83)	158 (74)	7 (88)	0.112
	Major resection	24 (17)	55 (26)	1 (12)	
Major complications (i.e. Clavien-Dindo ≥3)	No	127 (89)	199 (94)	8 (100)	0.220
	Yes	15 (11)	13 (6)	0 (0)	
	Missing (N=1)				
Postoperative death (%)	No	140 (99)	208 (98)	8 (100)	0.757
	Yes	2 (1)	5 (2)	0 (0)	

Supplementary table 9. Uni- and multivariable Cox regression analysis OS of chemo-naive patients >50% cut-off

Overall Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.012 [0.998-1.026]	0.100	1.016 [1.001-1.032]	0.038
ASA > II	1.021 [0.650-1.606]	0.927	1.047 [0.650-1.685]	0.850
Right-sided primary	1.472 [1.046-2.073]	0.027	1.540 [1.066-2.224]	0.021
pT3-4	1.142 [0.816-1.599]	0.438	0.868 [0.604-1.248]	0.446
Node positive primary	1.421 [1.072-1.883]	0.015	1.473 [1.068-2.030]	0.018
Disease free interval (cont.)	0.998 [0.991-1.005]	0.535	0.991 [0.984-0.999]	0.022
Number of CRLM (cont.)	1.138 [1.022-1.266]	0.018	1.164 [1.025-1.322]	0.019
Diameter largest CRLM (cont.)	1.096 [1.037-1.159]	0.001	1.124 [1.044-1.209]	0.002
Preoperative CEA level (cont.)	1.001 [1.001-1.002]	0.002	1.001 [1.000-1.002]	0.155
R1 resection CRLM	1.278 [0.852-1.915]	0.236	1.063 [0.689-1.640]	0.781
Extra hepatic disease	1.490 [0.879-2.526]	0.139	1.719 [0.925-3.196]	0.087
dHGP	Ref		Ref	
rHGP	2.154 [1.581-2.935]	<0.001	1.917 [1.367-2.688]	<0.001
pHGP	5.073 [2.113-12.177]	<0.001	4.398 [1.829-10.577]	<0.001

Supplementary table 10. Uni- and multivariable logistic regression analysis for association with dHGP >50% cut-off

	Univariable		**Multivariable**	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Right-sided primary	1.030 [0.691-1.533]	0.886	1.046 [0.676-1.617]	0.841
pT3-4	0.709 [0.480-1.047]	0.084	0.750 [0.482-1.166]	0.201
Node positive primary	0.564 [0.411-0.773]	<0.001*	0.499 [0.348-0.715]	<0.001*
Disease free interval (cont.)	0.988 [0.979-0.997]	0.006*	0.995 [0.985-1.005]	0.326
Number of CRLM (cont.)	1.093 [1.023-1.168]	0.008*	0.965 [0.891-1.046]	0.390
Diameter largest CRLM (cont.)	1.058 [0.991-1.129]	0.089	1.055 [0.977-1.139]	0.175
Preoperative CEA level (cont.)	1.000 [0.999-1.000]	0.726	0.999 [0.999-1.000]	0.056
Preoperative chemotherapy	3.228 [2.370-4.395]	<0.001*	4.052 [2.708-6.063]	<0.001*

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; cont.: continuous CRLM: colorectal liver metastases; dHGP: desmoplastic histopathological growth pattern

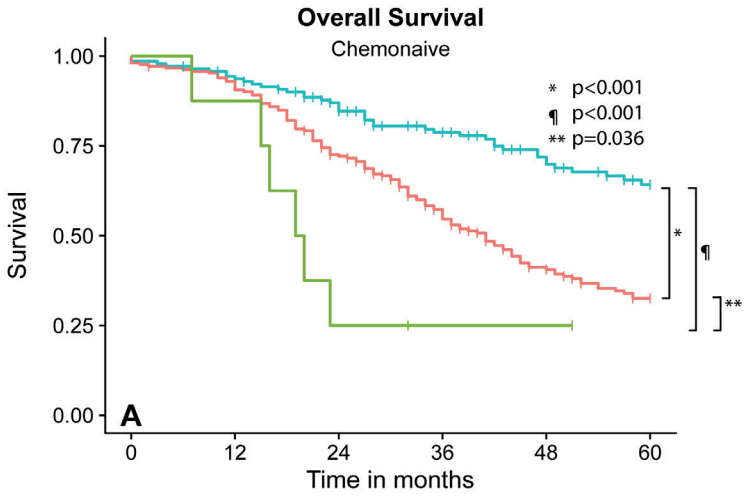
Supplementary table 11. Baseline characteristics pre-treated patients >50% cut-off

		>50% dHGP	>50% rHGP	>50% pHGP	p-value
		N=241 (67%)	N=112 (31%)	N=5 (1%)	
General characteristics					
Age at resection (median [IQR])		63.0 [56.0, 70.0]	64.0 [57.8, 69.0]	62.0 [60.0, 64.0]	0.422
Gender (%)	Female	84 (35)	42 (38)	1 (20)	0.682
	Male	157 (65)	70 (62)	4 (80)	
ASA classification(%)	ASA Class I-II	219 (91)	103 (92)	5 (100)	0.773
	ASA Class >II	21 (9)	9 (8)	0 (0)	
	Missing (N=1)				
Primary tumour characteristics					
Location (%)	Right-sided	41 (17)	16 (14)	0 (0)	0.637
	Left-sided	100 (41)	54 (48)	4 (80)	
	Rectum	97 (40)	41 (37)	1 (20)	
	Double tumour	3 (1)	1 (1)	0 (0)	
Pathological T-stage (%)	pT 0-2	44 (20)	11 (11)	1 (20)	0.174
	pT 3-4	181 (80)	88 (89)	4 (80)	
	Missing (N=31)				
Pathological N-stage (%)	N0	93 (41)	24 (24)	2 (40)	0.015
	N+	132 (59)	74 (76)	3 (60)	
	Missing (N=32)				
Adjuvant chemotherapy (%)	No	224 (94)	100 (90)	4 (80)	0.282
	Yes	15 (6)	11 (10)	1 (20)	
	Missing (N=4)				
CRLM characteristics					
Synchronous CRLM (%)	Metachronous	58 (24)	22 (20)	1 (20)	0.646
	Synchronous	183 (76)	90 (80)	4 (80)	
DFI (median [IQR])		0.0 [0.0, 3.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.608
Number of CRLM (median [IQR])		3.0 [2.0, 5.0]	3.0 [2.0, 5.2]	6.0 [2.0, 10.0]	0.322
Largest diameter CRLM (median [IQR])	*Missing (N=1)*	3.3 [2.3, 5.1]	3.1 [2.0, 5.2]	5.1 [2.5, 5.4]	0.619
Preoperative CEA (median [IQR])	*Missing (N=18)*	19.7 [5.2, 65.2]	21.0 [6.8, 112.5]	3.0 [2.4, 21.0]	0.170

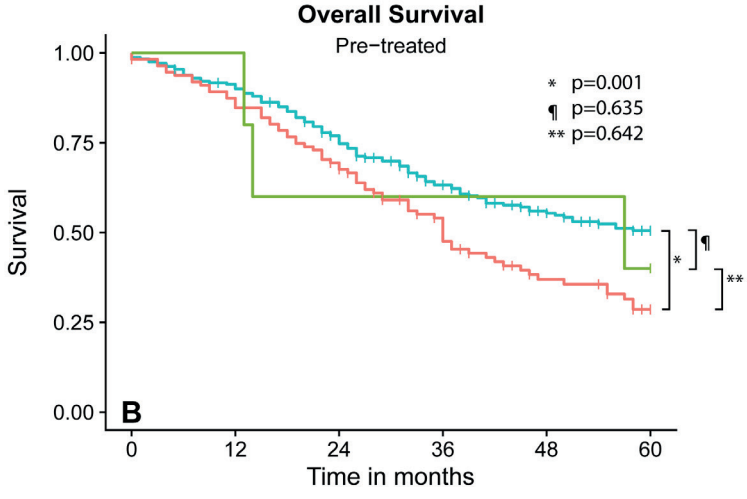
		>50% dHGP	>50% rHGP	>50% pHGP	p-value
		N=241 (67%)	N=112 (31%)	N=5 (1%)	
Bilobar (%)	Unilobar	101 (42)	44 (39)	2 (40)	0.896
	Bilobar	140 (58)	68 (61)	3 (60)	
Extrahepatic disease (%)	No	203 (84)	95 (85)	5 (100)	0.625
	Yes	38 (16)	17 (15)	0 (0)	
Resection margin (%)	R0	199 (83)	87 (78)	5 (100)	0.281
	R1	41 (17)	25 (22)	0 (0)	
	Missing (N=1)				
CRS (%)	Low (0-2)	102 (45)	32 (31)	1 (20)	0.047
	High (3-5)	126 (55)	70 (69)	4 (80)	
	Incomplete (N=24)				
Major resection (≥3 complete segments) (%)	No major resection	139 (58)	52 (46)	2 (40)	0.117
	Major resection	102 (42)	60 (54)	3 (60)	
Major complications (i.e. Clavien-Dindo ≥3)	No	218 (90)	97 (87)	5 (100)	0.407
	Yes	23 (10)	15 (13)	0 (0)	
Postoperative death (%)	No	238 (99)	108 (96)	5 (100)	0.323
	Yes	3 (1)	4 (4)	0 (0)	

Supplementary table 12. Uni- and multivariable Cox regression analysis for OS of pre-treated patients >50% cut-off

Overall Survival	**Univariable**		**Multivariable**	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	1.021 [1.007-1.036]	0.004	1.032 [1.015-1.050]	<0.001
ASA > II	1.089 [0.680-1.746]	0.722	1.218 [0.741-2.001]	0.436
Right-sided primary	0.919 [0.618-1.369]	0.679	0.988 [0.646-1.511]	0.954
pT3-4	1.476 [0.988-2.206]	0.057	1.341 [0.860-2.092]	0.196
Node positive primary	1.466 [1.081-1.989]	0.014	1.411 [1.010-1.972]	0.044
Disease free interval (cont.)	0.997 [0.986-1.009]	0.640	0.995 [0.983-1.008]	0.448
Number of CRLM (cont.)	1.024 [0.977-1.073]	0.324	1.058 [1.000-1.121]	0.051
Diameter largest CRLM (cont.)	0.994 [0.949-1.043]	0.817	1.031 [0.973-1.093]	0.299
Preoperative CEA level (cont.)	1.000 [1.000-1.000]	0.938	1.000 [1.000-1.000]	0.504
R1 resection CRLM	1.364 [0.979-1.902]	0.067	1.246 [0.851-1.825]	0.258
Extra hepatic disease	1.746 [1.243-2.454]	0.001	1.815 [1.221-2.698]	0.003
dHGP	Ref		Ref	
rHGP	1.570 [1.183-2.084]	0.002	1.282 [0.922-1.784]	0.140
pHGP	1.020 [0.324-3.209]	0.973	0.829 [0.245-2.801]	0.763



dHGP	142	132	112	88	70	51
rHGP	213	197	150	106	67	46
pHGP	8	7	2	1	1	0



dHGP	241	218	176	129	96	78
rHGP	112	97	76	50	28	18
pHGP	5	5	3	3	3	2

Supplementary figure 2A-B. OS using the >50% cut-off. 2A: OS chemo-naive patients. 2B: OS pre-treated patients

Chapter 2

Histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy

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Clinical and Experimental Metastasis. 2019 Aug;36(4):311-319

Abstract

Introduction:
Colorectal liver metastases (CRLM) exhibit distinct histopathological growth patterns (HGPs) that are indicative of prognosis following surgical treatment. This study aims to assess the reliability and replicability of this histological biomarker.

Methods
Within and between metastasis HGP concordance was analysed in patients who underwent surgery for CRLM. An independent cohort was used for external validation. Within metastasis concordance was assessed in CRLM with ≥2 tissue blocks. Similarly, concordance amongst multiple metastases was determined in patients with ≥2 resected CRLM. Diagnostic accuracy (expressed in area under the curve [AUC]) was compared by number of blocks and number of metastases scored. Interobserver agreement (Cohen's *k*) compared to the gold standard was determined for a pathologist and a PhD candidate without experience in HGP assessment after one and two training sessions.

Results:
Both the within (95%, n=825) and the between metastasis (90%, n=363) HGP concordance was high. These results could be replicated in the external validation cohort with a within and between metastasis concordance of 97% and 94%, respectively. Diagnostic accuracy improved when scoring 2 vs. 1 blocks(s) or CRLM (AUC=95.9 vs. 97.7 [p=0.039] and AUC=96.5 vs. 93.3 [p=0.026], respectively), but not when scoring 3 vs. 2 blocks or CRLM (both p>0.2). After two training sessions the interobserver agreement for both the pathologist and the PhD candidate were excellent (*k*=0.953 and *k*=0.951, respectively).

Conclusions:
The histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy, making them a reliable and replicable histological biomarker.

Introduction

Colorectal cancer (CRC) is one of the most prevalent solid malignancies in the world with approximately one third of patients developing hepatic metastases[1-5]. Even though surgical treatment is seen as the only potentially curative treatment option, reported 5-year survival rates vary widely (from 20% to 70%). [6-13]

Recently, a new potential histological biomarker has been described. [14, 15] Colorectal liver metastases (CRLM) grow in three distinct histopathological growth patterns (HGP), the desmoplastic, the replacement and the pushing type, each with unique morphological and biological features (figure 1 a-f). These distinct features have previously been described in detail. [16-18] In short: HGP assessment is performed by assessing the proportion (expressed as percentage) of each distinct HGP observed at the tumour-liver interface on H&E stained tissue sections. [14] Previous studies suggest that a high relative proportion of the replacement type is prognostic for an impaired overall survival. [19-22] The largest and most recent study analysed a cohort of 732 patients and found that it is the presence rather than the relative proportion of any non-desmoplastic type HGP (i.e. pushing and/or replacement type) that dictates poor prognosis. [15] In terms of clinical relevance, HGPs can therefore be classified into two categories: either pure desmoplastic (dHGP) or any observed non-desmoplastic type HGP (non-dHGP). [15]

While interesting from a biological point of view, this new classification raises methodological concerns. For if classification is based on either 100% dHGP or <100% dHGP, assessment could be more susceptible to sampling and reading error. In order to validate HGPs as a histological biomarker, knowledge on HGP concordance within a single and amongst multiple metastases within the same patient is essential, especially considering the growing evidence of (non-)genetic intra-tumoural heterogeneity in CRC. [23] Knowledge on diagnostic accuracy and learnability of HGP assessment is also necessitated to determine the reliability and replicability of this histological biomarker. This study therefore analyses within and between metastasis HGP concordance within the same cohort as described by Galjart et al.[15], as well as an external validation cohort. [24] In addition, diagnostic accuracy is determined for scoring a single or multiple Formalin-Fixed Paraffin-Embedded (FFPE) tissue blocks per CRLM and for scoring a single or multiple CRLM per patient. Lastly, the learning curve associated with HGP assessment is determined in two observers (pathologist and PhD candidate) without prior experience in HGP assessment.

Methods

The current study was approved by the medical ethics committee of the Erasmus University Medical Center (MEC-2018-1743). The need for informed consent was waived by the ethics committee due to the retrospective and non-invasive nature of the study. Drafting of the manuscript was performed in accordance with the REMARK guidelines. [25]

Patient selection
The patient selection for the current study was performed in the same cohort as described by Galjart et al.[15]. Patients undergoing resection of CRLM at the Erasmus MC Cancer Institute, the Netherlands, between January 2000 and March 2015 were eligible for inclusion.

Routine pathological assessment
During macroscopic pathological assessment of the surgical specimens of resected CRLM, representative sections (e.g. tumour, tumour with relation to the surgical margin(s), capsule, background liver, non-tumorous liver in distance) were considered for preparation of FFPE tissue blocks. A 5µm section per block was cut and stained with Haematoxylin and Eosin (H&E) for pathological interpretation. If needed, deeper levels of the block were cut and stained with H&E.

Assessment of HGPs
H&E stained slides retrieved from the archive of the Pathology Department of the Erasmus MC were retrospectively reviewed by light microscopy (figure 1 a-f). Scoring of the HGPs was performed in accordance with international consensus guidelines. [14] For each block subjected to review the relative presence (in percentage %) at the tumour-liver interface of the distinct HGP's (pushing, desmoplastic and replacement type) was estimated. The metastasis HGP was defined as the pooled estimate (average with equal weights per block) of all blocks of a single CRLM. Concordantly, the patient HGP was defined as the pooled estimate (average with equal weights per CRLM) of all resected CRLM within a single patient. Given recent findings [15], block, metastasis and patient HGP were classified as dHGP if only the desmoplastic type was observed (i.e. 100% dHGP), and as non-dHGP if any percentage of pushing and/or replacement type was observed (i.e. <100% dHGP). Due to this on/off classification, if non-dHGP is observed on a single block, corresponding metastasis and patient HGP is classified as non-dHGP, regardless of the HGP of other blocks within the same metastasis or other CRLM within the same patient.

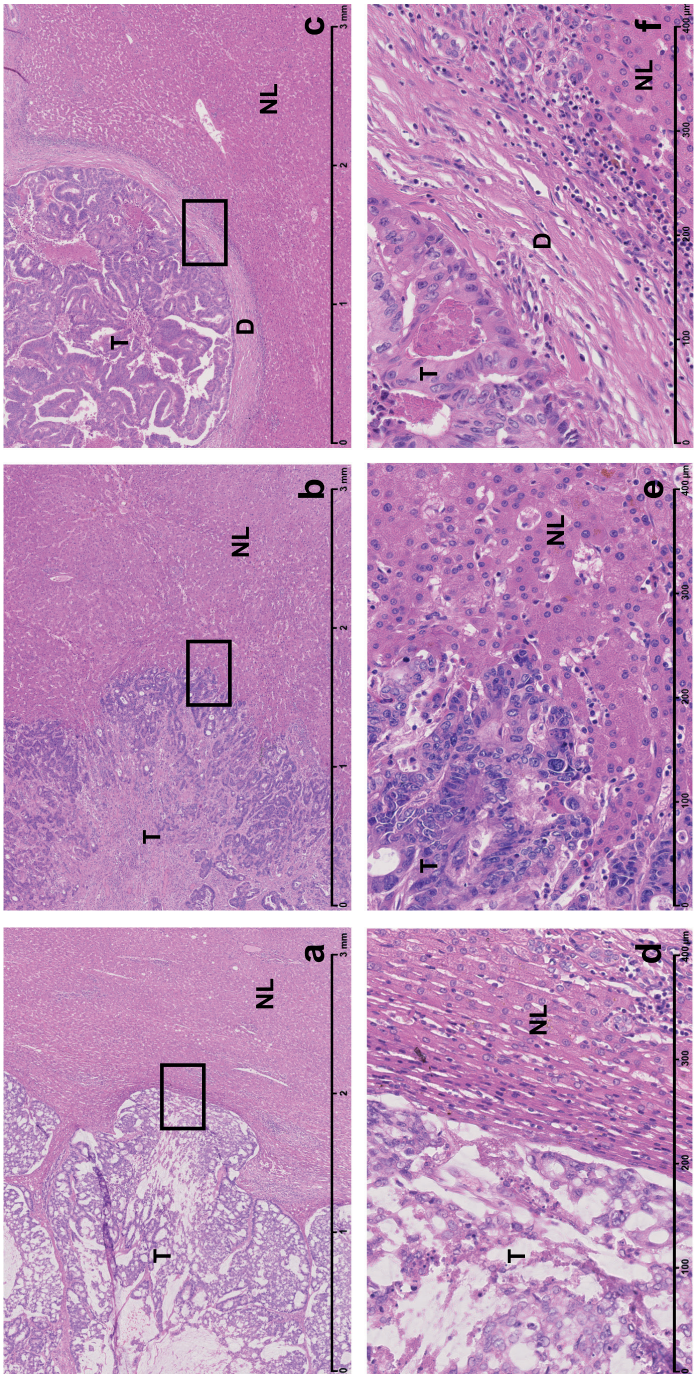


Figure 1. Three distinct types of histopathological growth patterns (HGPs) can be identified on H&E stained tissue blocks. (a, b & c) 2.5x magnification. (d, e & f) 20x magnification. (a&d) Pushing type HGP. (b&e) Replacement type HGP. (c&f) Desmoplastic type HGP. T: tumour, NL: normal liver & D: desmoplastic stroma.

For the within metastasis analysis, concordance (yes/no) of block HGP to metastasis HGP was recorded for all resected CRLM with ≥2 tissue blocks. Within metastasis concordance was defined as the proportion of concordant tissue blocks. Since a lesion represents a three dimensional structure, consecutive slides from a single block (i.e. deeper levels) do not adequately represent its three dimensional nature. As such, consecutive slides from a single block were excluded from the within metastasis analysis. For the between metastasis analysis, concordance (yes/no) of metastasis HGP to patient HGP was determined in all patients with ≥2 CRLM resected in a single time-frame (e.g. no recurrent CRLM). Between metastasis concordance was defined as within patient proportion of concordant CRLM. Patient information and data on primary CRC and CRLM were extracted from a prospectively maintained database. Regarding systemic treatment status, patients were considered chemo-naive if they did not receive any form of chemotherapy within the six months prior to resection. Multivariable logistic regression analysis was performed for within metastasis discordance (yes/no) with primary tumour characteristics, known clinical risk factors, systemic treatment status and number of blocks scored entered into the model. Significant predictor(s) found for within metastasis discordance were used as stratification factor(s) for between metastasis analysis. Identical models were fitted within each stratum (if applicable) to predict discordance (yes/no) amongst multiple metastases. Mean within metastasis concordance was compared across number of blocks scored. Similarly, mean between metastasis concordance was compared within strata (if applicable) and by number of CRLM resected.

External validation

External validation of mean within and mean between metastasis concordance was performed by retrospective HGP assessment as described previously. The external validation cohort comprised of chemo-naive patients treated surgically for CRLM at the University Hospital of Heidelberg, Germany, between October 2001 and June 2009. [24] H&E stained sections of the validation cohort were provided by the tissue bank of the National Center for Tumor Diseases (NCT). As the external validation cohort consisted of chemo-naive patients, comparisons to the original cohort were performed in (tissues from) chemo-naive patients only.

Diagnostic accuracy

Diagnostic accuracy for scoring a single FFPE block was determined in all CRLM with ≥2 blocks. Of these ≥2 blocks, one individual block was selected at random. The HGP of this randomly selected block was considered the predictor (i.e. test result), while the metastasis HGP – as determined by HGP assessment of all ≥2 blocks of the metastasis in question – was considered the response (i.e. true HGP status). This was done similarly for 2 blocks in all CRLM with ≥3 blocks. Identically, the diagnostic accuracy of scoring a single resected CRLM was determined within patients with ≥2 CRLM resected etc. The area under the curve [AUC] of the corresponding receiver operating characteristic (ROC) curves were compared for 2 vs. 1 block(s) or CRLM scored, and for 3 vs. 2 blocks or CRLM scored, respectively.

Learning curve

A gastro-intestinal pathologist (MD) and a PhD-candidate (DH) without prior pathology experience were recruited for learning curve analysis. Both observers had no prior experience in HGP assessment. The raters received a joint training session by a pathologist with over 10 years of experience in HGP assessment (PV). During this training session, 50 tissue sections were assessed collaboratively. Hereafter, both observers independently scored a test-set of an additional 50 tissue sections. Individual scores of the test-set were reviewed in a joint session with the trainer, followed by a second training session of 50 tissue sections. Subsequently a second test-set of 50 tissue sections was scored independently. After completion scores were again collaboratively reviewed. For both test-sets, interobserver agreement of both observers compared to the gold standard was determined for the dHGP/non-dHGP classification. The scores of the experienced trainer were considered the gold standard.

Statistical analysis

Dichotomous or categorical data are reported as percentage, parametric continuous data are reported as mean (standard deviation [SD]) and non-parametric continuous data are reported as median (inter-quartile range [IQR]). Mean concordances were compared by an independent samples T-test or a one-way analysis of variance (ANOVA), depending on the number of strata. AUC values were compared as described by DeLong[26]. Interobserver agreement was determined using Cohen's kappa. All statistical analyses were performed using R version 3.5.3 (http://www.r-project.org). The R-package 'pROC' was used for comparison of AUC values. A p-value <0.05 was considered statistically significant.

Results

Patient characteristics

In total 785 patients underwent resection of one or more CRLM at the Erasmus MC Cancer Institute in the study period and were consequently scored for HGP. In total 1625 CRLM were resected. Of these, 835 CRLM had two or more H&E stained slides available for review (2135 slides in total) and were considered for within metastasis analysis. Of these, 31 slides of 10 individual CRLM were identified as consecutively cut from single FFPE blocks, and were hence excluded from within metastasis analysis. Resection of two or more CRLM was performed in 382 patients. Nineteen were excluded for between metastasis analysis due to missing data required to link individual tissue samples to individual CRLM. Within the remaining 363 patients a total of 1118 CRLM were resected. Patient characteristics are reported in table 1.

Table 1. Characteristics of patients included for between metastasis concordance analysis

		n=363 (%)
Gender	Female	233 (64)
	Male	130 (36)
Age at resection CRLM - *(median [IQR])*		63.0 [57.0, 70.0]
Primary tumour location	Right-sided	61 (17)
	Left-sided	152 (42)
	Rectal	145 (40)
	Missing	5 (1)
T-stage	pT 0-2	70 (19)
	pT 3-4	265 (73)
	Missing	28 (8)
N-stage	N0	118 (33)
	N+	216 (60)
	Missing	29 (8)
Disease-free interval - months *(median [IQR])*		0.0 [0.0, 9.0]
Diameter of largest CRLM - cm *(median [IQR])*		3.1 [2.0, 4.8]
Preoperative CEA - µg/L *(median [IQR])*		20.0 [5.4, 70.1]
Preoperative CTx status	Chemo-naive	121 (33)
	Pre-treated	242 (67)
Two-staged resection	No	347 (96)
	Yes	16 (4)
Use of RFA or MWA	No	252 (69)
	Yes	111 (31)
Number of CRLM resected	2	175 (48)
	3	87 (24)
	4	58 (16)
	≥5	43 (12)
Histopathological growth pattern	dHGP	72 (20)
	non-dHGP	291 (80)

CRLM: colorectal liver metastasis, IQR: interquartile range, CEA: carcinoembryonic antigen, CTx: chemotherapy, RFA: radiofrequency ablation, MWA: microwave ablation, (non-)dHGP: (non-)desmoplastic type histopathological growth pattern

Within metastasis concordance

Non-dHGP was observed in 72% of reviewed tissue blocks. Results of the multivariable logistic regression model on within metastasis discordance are reported in table 2. Systemic treatment status proved to be a significant predictor for presence of HGP discordance (yes/no) amongst multiple blocks, with an odds ratio (OR) (95%CI) of 2.107 (1.231;3.679) and p=0.007 for pre-treated versus chemo-naive CRLM. Mean within metastasis concordance was 95%. Figure 2a shows the mean within metastasis concordance stratified by number of blocks scored. Mean within metastasis concordance (95%CI) for 2, 3, 4, or ≥5 blocks scored was 96% (95;97), 94% (92;96), 93% (88;98) and 94% (86;100) respectively and was independent of the number of blocks scored (p=0.315).

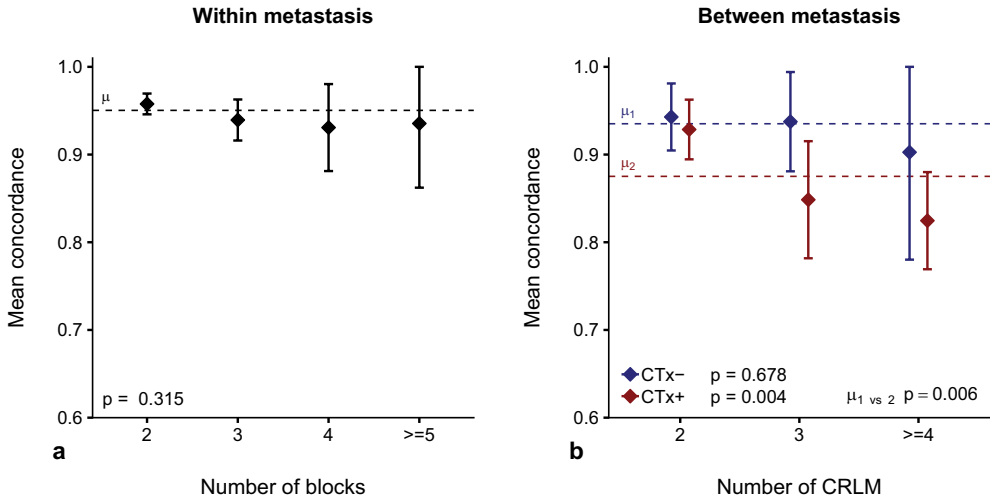


Figure 2. (a) Within metastasis histopathological growth pattern (HGP) concordance within the original cohort stratified by number of blocks scored. Overall mean within metastasis concordance (μ) was 95%. (B) Mean between metastasis HGP concordance within the original cohort stratified by preoperative chemotherapy status and number of colorectal liver metastasis (CRLM) resected. Mean between metastasis concordance in chemo-naive (CTx-) patients was 94% (μ_1). Mean between metastasis concordance in pre-treated (CTx+) patients was 88% (μ_2). Error-bars represent the 95% confidence interval of the estimate.

Between metastasis concordance

Mean between metastasis concordance of all 363 patients was 90%. Since systemic treatment status was a significant predictor for within metastasis discordance, between metastasis analysis was performed in chemo-naive and pre-treated patients separately. Non-dHGP was found in 85% of chemo-naive patients versus 78% in pre-treated patients (p=0.094). Results of the fitted multivariable logistic regression models on presence of HGP discordance (yes/no) amongst multiple resected CRLM are reported in table 2. Within chemo-naive patients, the size of the largest hepatic tumour on preoperative imaging proved a significant predictor for between metastasis discordance with OR (95%CI) 1.461 (1.073;2.145) and p=0.028 for every cm increase in size. The only significant predictor found for between metastasis discordance in pre-treated patients was number of CRLM resected.

Corresponding OR (95%CI) were 3.602 (1.414-9.550) for 3 vs. 2 CRLM resected and 5.887 (2.585;14.356) for ≥4 vs. 2 CRLM resected (p=0.008 and p<0.001). Mean between metastasis concordance (figure 2b) was significantly lower in pre-treated vs. chemo-naive patients (88% vs. 94%, p=0.006). Figure 2b shows the mean between metastasis concordance for chemo-naive and pre-treated patients stratified by number of CRLM resected. In chemo-naive patients, mean between metastasis concordance [95%CI] did not differ amongst 2 (94% [91;98]), 3 (94% [88;99]) or ≥4 (90% [78;100]) CRLM resected (p=0.678). In pre-treated patients mean between metastasis concordance [95%CI] was significantly different amongst 2 (93% [90;96]), 3 (85% [78;92]) and ≥4 (83% [77;88]) CRLM resected (p=0.004).

External validation

The external cohort comprised of 276 patients of whom the HGP could be determined in 251 (91%). In total 168 patients had resection performed of two or more CRLM and could be included for between metastasis analysis. Within metastasis analysis was performed in 270 CRLM with two or more blocks. Baseline characteristics were comparable between the external validation cohort and chemo-naive patients within the original cohort (supplementary table 1). Mean within (96% vs. 97%, p=0.652) and between (94% vs. 94%, p=0.710) metastasis concordance did not differ between the original (chemo-naive patients only) and validation cohort (figure 3).

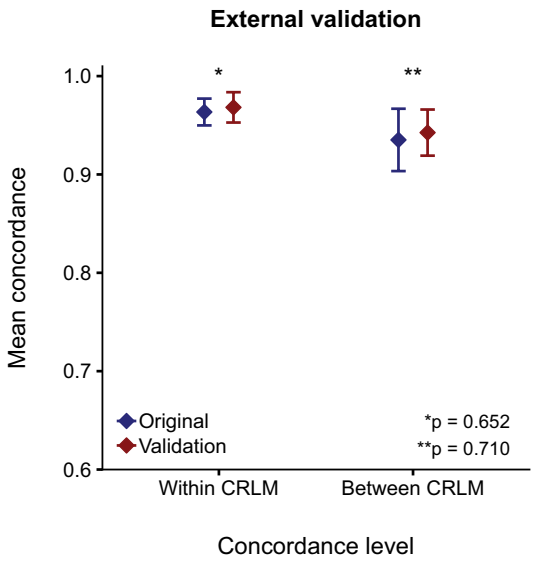


Figure 3. External validation of within and between colorectal liver metastasis (CRLM) concordance of histopathological growth pattern. Comparison was performed between the external validation cohort and (tissue of) chemonaive subjects from the original cohort.

Table 2. Multivariable binary logistic regression models on discordance *(yes/no)* in histopathological growth pattern

		Within metastasis (n = 702)		Between metastasis (n = 308)			
				Chemo-naive (n = 111)		Pre-treated (n = 197)	
Variable		**OR [95% CI]**	**p-value**	**OR [95% CI]**	**p-value**	**OR [95% CI]**	**p-value**
Location of primary	left vs right	1.513 [0.721-3.487]	0.298	0.610 [0.086-5.237]	0.620	0.655 [0.261-1.660]	0.367
	rectal vs right	1.881 [0.881-4.403]	0.120	3.174 [0.673-23.785]	0.186	0.604 [0.221-1.660]	0.324
pT3-4 vs pT0-2		1.259 [0.652-2.551]	0.506	1.163 [0.258-6.011]	0.848	1.193 [0.462-3.265]	0.721
Node-positive primary		0.650 [0.385-1.101]	0.107	0.516 [0.122-2.134]	0.354	0.696 [0.334-1.455]	0.332
Disease-free interval - months [a]		0.998 [0.980-1.014]	0.852	0.995 [0.947-1.034]	0.828	1.017 [0.972-1.062]	0.434
Diameter of largest CRLM - cm [ab]		-	-	1.461 [1.073-2.145]	0.028*	1.139 [0.983-1.321]	0.081
Preoperative CEA - µg/L [a]		0.999 [0.996-1.000]	0.152	0.996 [0.985-1.001]	0.278	1.000 [0.999-1.001]	0.575
Pre-treated vs chemonaive [c]		2.107 [1.231-3.679]	0.007*	-	-	-	-
Number of blocks scored	3 vs 2	1.994 [1.140-3.441]	0.014*	-	-	-	-
	4 vs 2	2.076 [0.788-4.869]	0.111	-	-	-	-
	≥5 vs 2	1.418 [0.322-4.411]	0.589	-	-	-	-
Number of CRLM resected	3 vs 2	-	-	0.868 [0.188-3.443]	0.846	3.602 [1.414-9.550]	0.008*
	≥4 vs 2	-	-	1.617 [0.292-7.394]	0.548	5.887 [2.585-14.356]	<0.001*

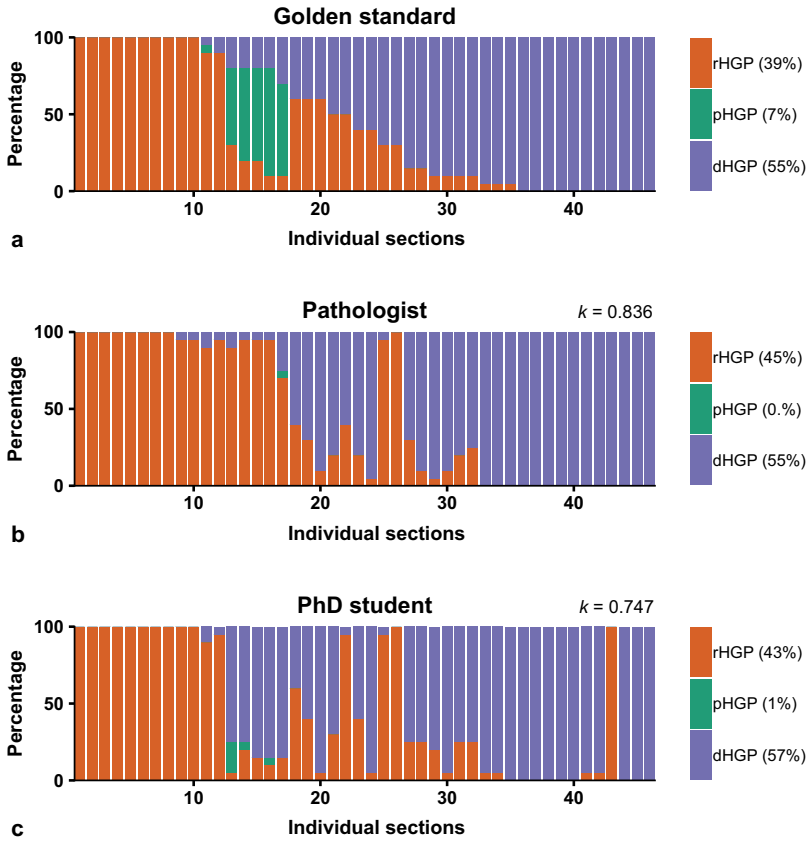
OR: odds ratio, CI: confidence interval, CRLM: colorectal liver metastases and CEA: carcinoembryonic antigen
[a] = continuous data entered into the model
[b] = omitted from within metastasis analysis since it is not representative for individual metastasis
[c] = used as stratification factor for between metastasis analysis
* = $\alpha < 0.05$

Diagnostic accuracy

Supplementary figure 1a displays the AUC for scoring a single (95.9%), two (97.7%) or three blocks (98.8%) per metastasis. A significant increase in diagnostic accuracy was observed for scoring 2 vs. 1 block(s) (p=0.039), but not for scoring 3 vs. 2 blocks (p=0.341). The AUC for scoring a single (93.3%), two (96.5%) or three (98.2%) resected CRLM per patient are reported in supplementary figure 1b. A significant increase in diagnostic accuracy was found for scoring 2 vs. 1 resected CRLM (p=0.026), but not for scoring 3 vs. 2 resected CRLM (p=0.235).

Learning curve

The results of both test-sets as scored by the gold standard, the pathologist and the PhD candidate are graphically displayed in figure 4 a-f. Interobserver agreement was higher in the second test-set for both the pathologist (*k*=0.953 vs. *k*=0.836) and the PhD candidate (*k*=0.951 vs. *k*=0.747). Where in the first test-set a difference in performance was seen between the pathologist and the PhD candidate (*k*=0.836 and *k*=0.747), performance in the second test-set did not differ (*k*=0.953 and *k*=0.951).



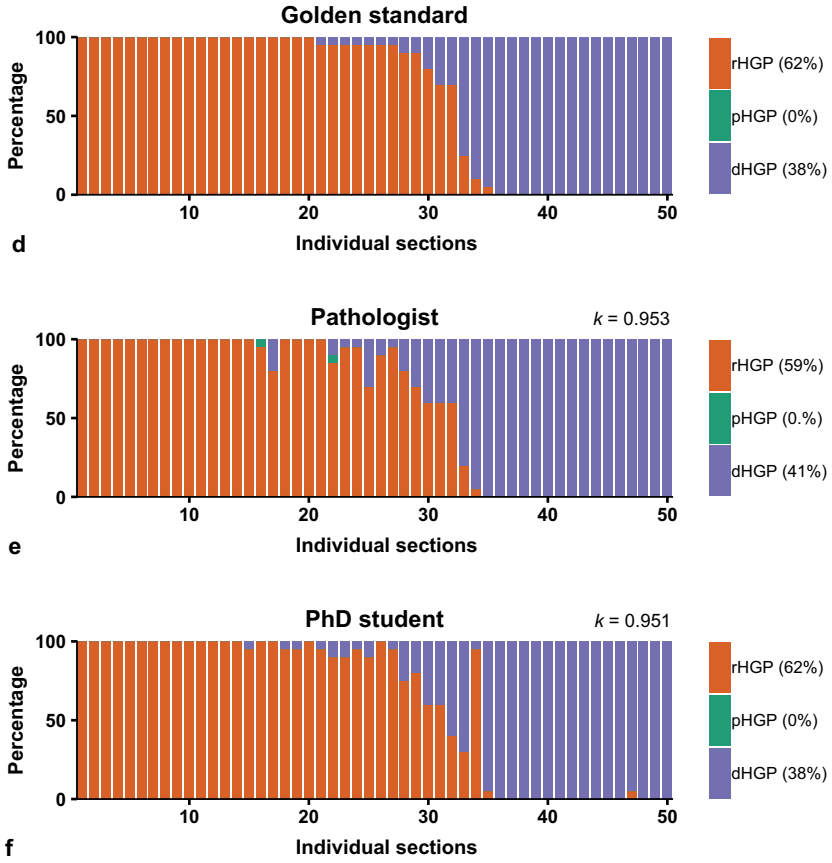


Figure 4. (a&d) Results of the first and second test-set as scored by the experienced trainer (gold standard). (b&e) Results of the first and second test-set as scored by the pathologist. (c&f) Results of the first and second test-set as scored by the PhD candidate. rHGP: replacement type histopathological growth pattern (HGP), pHGP: pushing type HGP & dHGP: desmoplastic type HGP.

Discussion

The current study found within metastasis concordance to be high (95%) when classifying the HGP as dHGP or non-dHGP. Furthermore, mean within metastasis concordance was independent of number of FFPE blocks scored. Overall between metastasis concordance was also high (90%), but differed for chemo-naive versus pre-treated patients (94% vs 88%). In chemo-naive patients, mean between metastasis concordance was independent of number of CRLM resected and the only predictor found in multivariable analysis for discordance was size of largest hepatic tumour on preoperative imaging. For pre-treated patients, the number of CRLM resected proved predictive for between metastasis discordance. This finding was supported by a significant difference in mean concordance for 2, 3 or ≥4 resected CRLM within pre-treated patients.

External validation in a large cohort of chemo-naive patients found similarly high numbers of mean within (97%) and between (94%) metastasis concordance. Unfortunately, the external validation cohort comprised of chemo-naive patients only, as such external validation within pre-treated CRLM and patients could not be performed.

The current study suggests that systemic chemotherapy treatment prior to hepatic resection might somewhat affect the reliability of HGP assessment. In the same patient cohort as currently described, Galjart et al. reported a significant increase in dHGP within pre-treated patients [15]. It is as of yet unclear if this difference is due to chemotherapy directly changing HGP morphology, or due to selection bias in that patients with dHGP have improved prognosis and are thus more likely to complete their pre-operative chemotherapy and subsequent liver resection. Although inconclusive, the current study did find a higher heterogeneity amongst the HGP of slides and CRLM of pre-treated patients. This could be the result of chemotherapy having a direct effect on HGP morphology.

Two studies have previously reported on HGP concordance so far. Van Dam et al analysed within metastasis agreement of ≥4 sections in a small sample of 50 CRLM [14] and Eefsen et al. reported on between metastases agreement in a small group of 24 patients with multiple resected CRLM. [17] As both studies applied different cut-off values to determine the HGP (50% and 75% respectively), interpretation of its results in light of the current study is difficult. Considering recent developments, it seems logical that future HGP classification will be based on the dHGP/non-dHGP cut-off.

When determining the diagnostic accuracy of HGP assessment, the current study found high AUC values for scoring a single, two or three blocks (all >95%) or CRLM (all >92%). The currently obtained results show that scoring two instead of one FFPE block(s) per CRLM increased diagnostic accuracy significantly. This increase was not significant when scoring three versus two blocks. As such, scoring two blocks per CRLM seems preferable and little accuracy is gained by further increasing the number of blocks assessed. This could significantly decrease workload, especially considering when non-dHGP is observed in a

single block, the other available blocks of the same or different CRLM do not necessarily have to be assessed, for non-dHGP has readily been determined. Similar results were seen when looking at the diagnostic accuracy for scoring two versus one and three versus two CRLM resected in patients with multiple metastases. These findings suggest that CRLM treated by other modalities (e.g. ablative techniques) can accurately be diagnosed by CRLM resected within the same timeframe, especially in the case of two or more resected metastases.

Analysis of the learning curve showed that after a single training session by an experienced trainer good to excellent (k >0.7) interobserver agreement for dHGP/non-dHGP was reached by two unexperienced observers. As expected, an observer with prior experience in liver pathology had a superior initial performance. After two training sessions however, the interobserver agreement was near perfect (k >0.9) for both raters. Although only two unexperienced raters were included, these results suggest that HGP classification into dHGP or non-dHGP can be taught with relative ease and that interobserver agreement is high. In comparison, Chetty et al. reported on the interobserver agreement of tumour regression grade (TRG), a histopathological assessment within the field of colorectal cancer. [27] The overall agreement (expressed in k) was determined for three separate scoring systems: the Mandard [28], Dworak [29] and the modified rectal cancer regression grading system (m-RCRG). [30] Seventeen experienced rectal cancer pathologists were asked to score 10 slides of 10 separate cases of rectal cancer treated with long-course preoperative chemoradiation. Reported overall agreement for the Mandard, Dworak and m-RCRG were k=0.28, k=0.35 and k=0.38, respectively. [27] Furthermore, these results are also promising for automated HGP determination using digital image slides and 'pathomics', as it has shown great promise in other histological phenotypes. [31] Especially considering the new on/off phenomenon as described by Galjart et al. [15], automated determination on digital sections is something worth investigating and the authors feel could be feasible.

Common biomarkers used in clinical practice for the treatment of colorectal cancer include K-RAS and B-RAF mutational status. Richman et al. reported on within tumour heterogeneity of K-RAS and B-RAF in 69 primary CRC cases. [32] Intra-tumoural heterogeneity was found in 5/69 (7.2%) for K-RAS and 2/69 (2.9%) for B-RAF status. [32] When comparing multiple tumour sites, a recent meta-analysis by Bhullar et al. reported on the concordance of, amongst others, K-RAS and B-RAF between the primary tumour and its corresponding metastases. [33] Median biomarker concordance [range] for K-RAS and B-RAF were 93.7% [67-100] and 99.4% [80-100], respectively. [33]

It appears that little within and between metastasis heterogeneity exists in the HGP of CRLM when classified as dHGP and non-dHGP. In addition, the observed heterogeneity seems comparable to that observed for biomarkers currently used in clinical practice. Furthermore, the diagnostic accuracy and learnability of HGP assessment by light microscopy seems high. These findings suggest that the HGPs of CRLM are a reliable and replicable histological biomarker.

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Figure captions

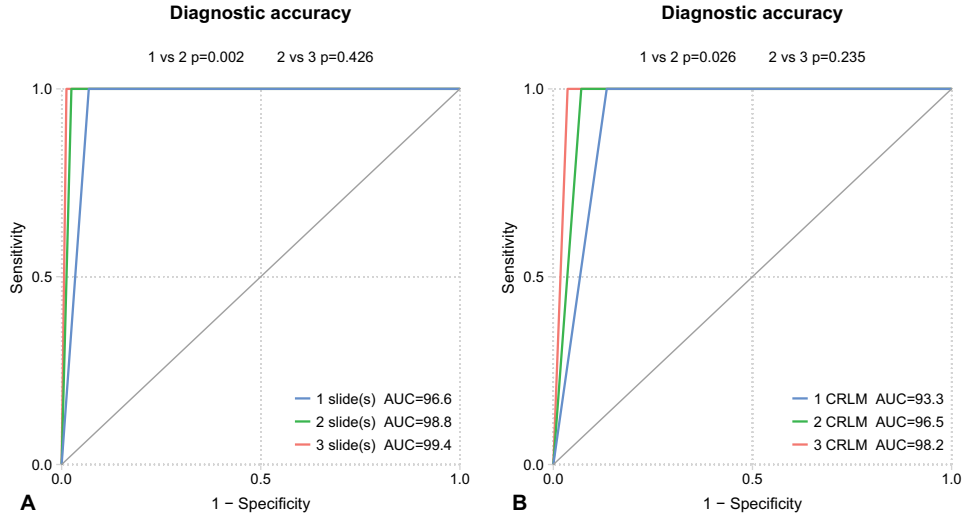
Figure 1. Three distinct types of histopathological growth patterns (HGPs) can be identified on H&E stained tissue blocks. (a, b & c) 2.5x magnification. (d, e & f) 20x magnification. (a&d) Pushing type HGP. (b&e) Replacement type HGP. (c&f) Desmoplastic type HGP. T: tumour, NL: normal liver & D: desmoplastic stroma.

Figure 2. (a) Within metastasis histopathological growth pattern (HGP) concordance within the original cohort stratified by number of blocks scored. Overall mean within metastasis concordance (μ) was 95%. (B) Mean between metastasis HGP concordance within the original cohort stratified by preoperative chemotherapy status and number of colorectal liver metastasis (CRLM) resected. Mean between metastasis concordance in chemo-naive (CTx-) patients was 94% (μ_1). Mean between metastasis concordance in pre-treated (CTx+) patients was 88% (μ_2). Error-bars represent the 95% confidence interval of the estimate.

Figure 3. External validation of within and between colorectal liver metastasis (CRLM) concordance of histopathological growth pattern. Comparison was performed between the external validation cohort and (tissue of) chemonaive subjects from the original cohort.

Figure 4. (a&d) Results of the first and second test-set as scored by the experienced trainer (gold standard). (b&e) Results of the first and second test-set as scored by the pathologist. (c&f) Results of the first and second test-set as scored by the PhD candidate. rHGP: replacement type histopathological growth pattern (HGP), pHGP: pushing type HGP & dHGP: desmoplastic type HGP.

Supplementary materials



Supplementary figure 1. (a) Receiver operating characteristics (ROC) curves for determining metastasis histopathological growth pattern (HGP) by scoring 1, 2 or 3 block(s) respectively. (b) ROC curves for determining patient HGP by scoring 1, 2 or 3 resected CRLM respectively. *AUC: area under the curve.*

Supplementary table 1. Comparison of baseline characteristics of chemo-naive patients in the original and external validation cohort included for between metastasis concordance analysis

		Cohort		
		Original	**Validation**	
		n=121 (%a)	**n=168 (%a)**	**p-value**
Gender	Female	75 (62)	107 (64)	0.717
	Male	46 (38)	60 (36)	
	Missing	0 (0)	1 (1)	
Age at resection CRLM - *(median [IQR])*		63.0 [60.0. 73.0]	63.0 [56.0. 69.0]	0.033
Primary tumour location	Colon	69 (58)	88 (53)	0.437
	Rectum	50 (42)	77 (47)	
	Missing	2 (2)	3 (2)	
T-stage	pT 0-2	29 (24)	26 (16)	0.072
	pT 3-4	91 (76)	140 (84)	
	Missing	1 (1)	2 (1)	
N-stage	N0	43 (36)	56 (34)	0.713
	N+	77 (64)	110 (66)	
	Missing	1 (1)	2 (1)	
Disease-free interval	>1 year	49 (40)	67 (40)	0.982
	≤1 year	72 (60)	99 (60)	
	Missing	0 (0)	2 (1)	
Diameter of largest CRLM	≤5 cm	105 (88)	84 (50)	<0.001
	>5 cm	15 (12)	83 (50)	
	Missing	1 (1)	1 (1)	
Preoperative CEA	≤200 µg/L	108 (92)	151 (90)	0.580
	>200 µg/L	9 (8)	16 (10)	
	Missing	4 (3)	1 (1)	
Histopathological growth pattern	dHGP	18 (15)	22 (13)	0.665
	non-dHGP	103 (85)	146 (87)	

CRLM: colorectal liver metastasis. IQR: interquartile range. CEA: carcinoembryonic antigen and (non-)dHGP: (non-)desmoplastic type histopathological growth pattern
a Percentages are expressed as proportions across each stratum (i.e. excluding missing). Percentages for missing are expressed as proportion of missing values within each stratum.

Chapter 3

Histopathological growth patterns and positive margins after resection of colorectal liver metastases

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HPB (Oxford). 2019 Nov 14. pii: S1365-182X(19)30755-5

Abstract

Background:
Histopathological growth patterns (HGPs) of colorectal liver metastases (CRLM) may be an expression of biological tumour behaviour impacting the risk of positive resection margins. The current study aimed to investigate whether non-desmoplastic growth pattern (non-dHGP) is associated with a higher risk of positive resection margins after resection of CRLM.

Methods:
All patients treated surgically for CRLM between January 2000 and March 2015 at the Erasmus MC Cancer Institute and between January 2000 and December 2012 at the Memorial Sloan Kettering Cancer Center were considered for inclusion. Positive resection margin (R1) was defined as tumour cells at the resection margin.

Results:
Of all patients (n=1302) included for analysis, 13% (n=170) had positive resection margins. Factors independently associated with positive resection margins were the non-dHGP (odds ratio (OR): 1.79, 95% confidence interval (CI): 1.11-2.87, p=0.016) and a greater number of CRLM (OR: 1.15, 95% CI: 1.08-1.23 p<0.001). Both positive resection margins (HR: 1.41, 95% CI: 1.13-1.76, p=0.002) and non-dHGP (HR: 1.57, 95% CI: 1.26-1.95, p<0.001) were independently associated with worse overall survival.

Conclusion:
Patients with non-dHGP are at higher risk of positive resection margins. Despite this association, both positive resection margins and non-dHGP are independent prognostic indicators of worse overall survival.

Introduction

Development of colorectal liver metastases (CRLM) is common in patients with colorectal cancer (CRC). Over one third of patients are confronted with CRLM at some point in the course of their disease.[1-3] Long term survival and even cure can be achieved by surgical resection in a proportion of selected patients.[4] The ability to accurately predict survival of patients currently remains elusive.[5, 6] One prognostic factor that has been the subject of discussion for many years is the hepatic resection margin. It has been postulated that positive resection margins may be more a reflection of underlying tumour biology rather than surgical technique.[7-9]

CRLM grow in three distinct histopathological growth patterns (HGPs); a desmoplastic (dHGP), a pushing and a replacement type.[10] Recently published international consensus guidelines validated HGPs as a prognostic marker in patients undergoing resection of CRLM and provide a uniform and replicable scoring method.[11] Patients with any observed replacement and/or pushing HGP (taken together as non-dHGP) have worse survival compared to patients with dHGP.[12] We hypothesised that patients with non-dHGP are at higher risk of positive resection margins. The aim of this multicentre cohort study was to investigate a possible association between HGPs and margin status after resection of CRLM, while adequately correcting for potential confounders with sufficient statistical power.

Methods

The current study was approved by the institutional review board of the Erasmus University Medical Center (MEC-2018-1743).

Patients

All consecutive patients who underwent resection of CRLM between January 2000 and March 2015 at the Erasmus MC Cancer Institute (Rotterdam, the Netherlands) and between January 2000 and December 2012 at the Memorial Sloan Kettering Cancer Center (New York, USA) were considered for inclusion. Both institutions are tertiary referral centres for liver surgery. Patients without complete resection of CRLM were excluded. Since resection is a prerequisite for HGP and margin assessment, patients treated solely with ablative therapies were not included in the analyses. When HGP assessment was not possible (e.g. missing or unsuitable tissue slides), the resection margin could not be determined (e.g. extensive thermal damage) or when margin status was not recorded, patients were excluded as well.

Study design, variables and outcomes

Prospectively maintained databases were used to extract patient demographics, clinicopathological data, treatment details and survival data. One of the variables that was extracted was the Clinical Risk Score (CRS) and its determinants.[13] Hepatic arterial infusion pump (HAIP) chemotherapy combined with systemic chemotherapy was administered to a proportion of the MSKCC patients pre- and or postoperatively as described previously.[14] Positive resection margin (R1) was defined as tumour cells at the resection margin. Overall survival (OS) was defined as the time in months from the date of CRLM resection until the date of death. When alive, patients were censored at date of last follow-up. As preoperative chemotherapy might influence the risk of positive resection margins, a distinction was made between preoperative systemic chemotherapy without HAIP and preoperative HAIP chemotherapy when investigating possible predictors of R1 resection margins. For survival analyses, a distinction was made between any perioperative systemic chemotherapy without HAIP (i.e. pre- and/or postoperative systemic chemotherapy without HAIP) and any perioperative HAIP chemotherapy (i.e. pre- and/or postoperative HAIP chemotherapy) to correct for the possible prognostic effect of perioperative chemotherapy.

Pathological assessment of HGPs and resection margins

Since the first description of HGPs[10], multiple studies have shown their prognostic value.[15-19] Recently, international consensus guidelines for HGP assessment defined a uniform and replicable scoring system.[11] In order to retrospectively determine the HGPs of all patients at both centres, a per patient re-evaluation was performed of all available haematoxylin and eosin (H&E) stained sections of all resected CRLM from all patients by means of light microscopy. At the Erasmus MC Cancer Institute the HGP assessment for the current study was performed by a dedicated HGP pathologist (PV) and researchers (PN,

DH, ES, BG) trained by the dedicated HGP pathologist according to these guidelines. At the Memorial Sloan Kettering Cancer Center the HGPs were subsequently scored in a similar manner according to the same guidelines by one of the trained researchers (ES) and for difficult cases the dedicated HGP pathologist (PV) was consulted. The currently applied methodology has recently been validated in a previous study regarding the diagnostic accuracy of HGP determination.[20] Interobserver agreement (expressed in Cohen's k) compared to the gold standard was determined for a researcher without experience in HGP assessment. After two training sessions the interobserver agreement for the researcher compared to the gold standard was excellent (k=0.951). The scoring of HGPs was performed with the observers blinded to all patient characteristics and outcome. The entire interface between the tumour border and normal liver parenchyma was evaluated. Every fraction of the tumour-liver interface, accounting for 5% or more of the total interface, was taken into account. In the consensus guidelines a cut-off value of 50% is used for determining the predominant HGP, but new insights with regard to this cut-off value have emerged. The presence of any non-dHGP, rather than the percentage, dictates prognosis.[12] Tumours displaying only dHGP were therefore classified as dHGP and tumours displaying any HGP other than dHGP were categorised as non-dHGP. Examples of dHGP (figure 1A) and non-dHGP (figure 1B) are displayed in figure 1. Tissue sections were considered unsuitable for HGP assessment when less than 20% of the tumour/liver interface was available, when the quality of the H&E tissue section was insufficient or when viable tumour tissue was absent.[11]

Histopathological assessment of the resection margin was executed by the pathologists of both respective institutions. In the case of multiple CRLM, the closest resection margin is reported as the final resection margin.

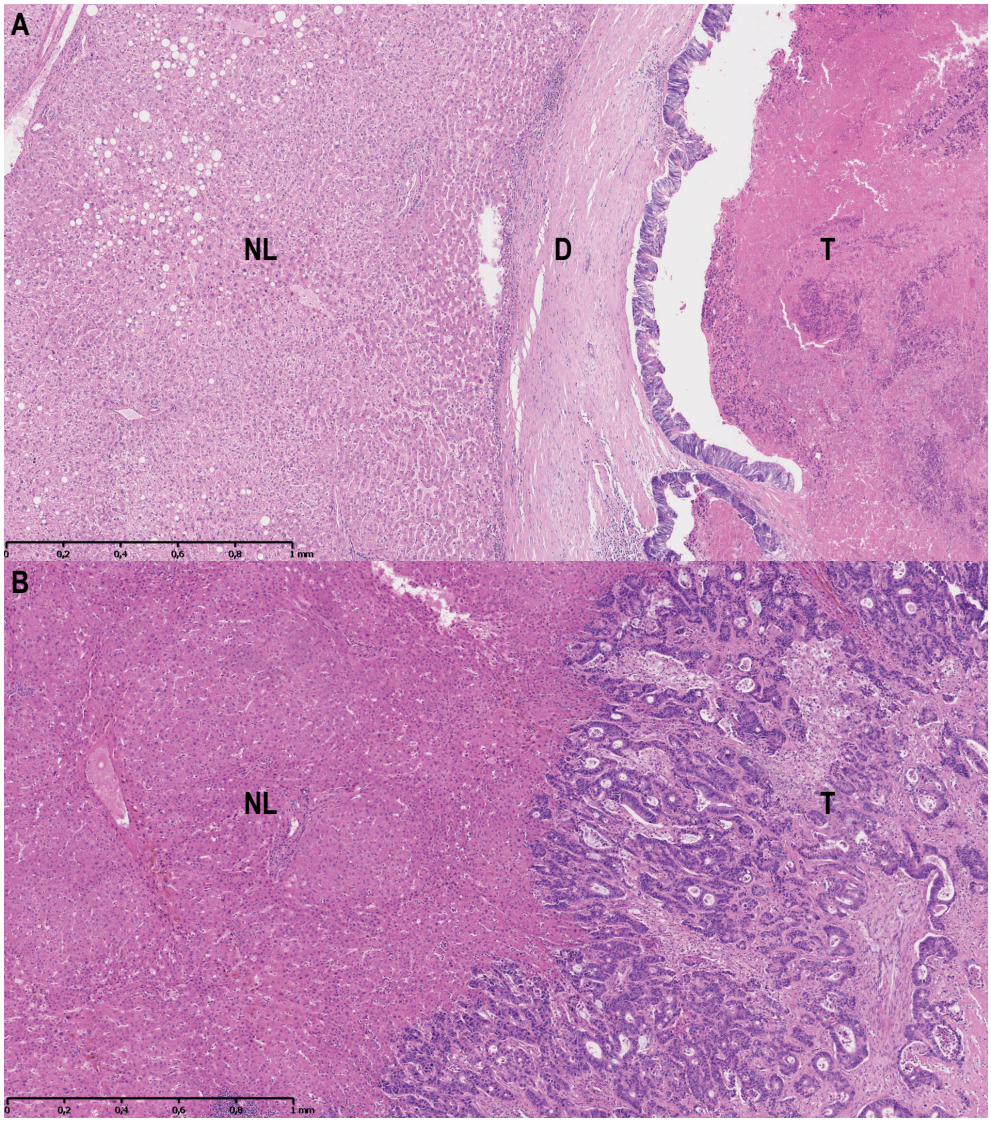

Figure 1A-B. 1A: Example of the dHGP. 1B: Example of the non-dHGP. Abbreviations: NL: normal liver; D: desmoplastic stroma; T: tumour core.

Statistical analysis

Categorical data are presented using absolute numbers with percentages and continuous data using medians with corresponding interquartile range (IQR). Differences in proportions were evaluated using the Chi-squared test. Medians were compared with the Mann-Whitney U test. Estimated median follow-up time for survivors was obtained using the reverse Kaplan-Meier method. Survival estimates were calculated using the Kaplan-Meier method. Survival estimates were computed until 60 months and were compared using the log-rank test. Uni- and multivariable binary logistic regression analysis was performed to investigate factors associated with R1 resection and factors associated with non-dHGP. Variables entered in the logistic regression model for positive margins were lymph node positivity of the primary tumour, the disease free interval between resection of the primary tumour and diagnosis of CRLM, number of CRLM, size of the largest CRLM, preoperative carcinoembryonic antigen (CEA) level, preoperative systemic and/or HAIP chemotherapy, known extra hepatic disease and HGP. Variables entered in the logistic regression model for any presence of non-dHGP were identical. Preoperative systemic and/or HAIP chemotherapy was included in the model due to the different proportional distribution of HGPs observed in patients treated with preoperative chemotherapy compared to chemonaive patients. [12] Logistic regression results were displayed using odds ratios (OR) and corresponding 95% confidence intervals (CI). To assess the prognostic value for OS, uni- and multivariable Cox proportional hazards regression analysis was performed. Potential multicollinearity in our Cox regression model was evaluated using the variance inflation factor (VIF).[21] The VIFs for all variables in the multivariable Cox regression were determined. A VIF below 4 indicates that no multicollinearity affecting the model exist, but should ideally be close to 1.[22] Proportional hazards regression results were displayed using hazard ratios (HR) and corresponding 95% CI. Statistical significance was defined as $\alpha < 0.05$. No imputation of missing data was applied. All analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL) and R version 3.5.1 (http://www.r-project.org).

Results

Patient characteristics

At the Erasmus MC Cancer Institute, a total of 971 patients underwent resection of CRLM between January 2000 – March 2015 and were evaluated for inclusion. At the Memorial Sloan Kettering Cancer Center, 1601 patients underwent resection of CRLM between January 2000 – December 2012 and were considered for eligibility. In total 1270 patients (49%) were excluded. The most common reason for exclusion was unavailability of H&E tissue sections (n=1055, 83%). A total of 1302 patients were included for analysis, of whom 170 (13%) had positive resection margins (figure 2).

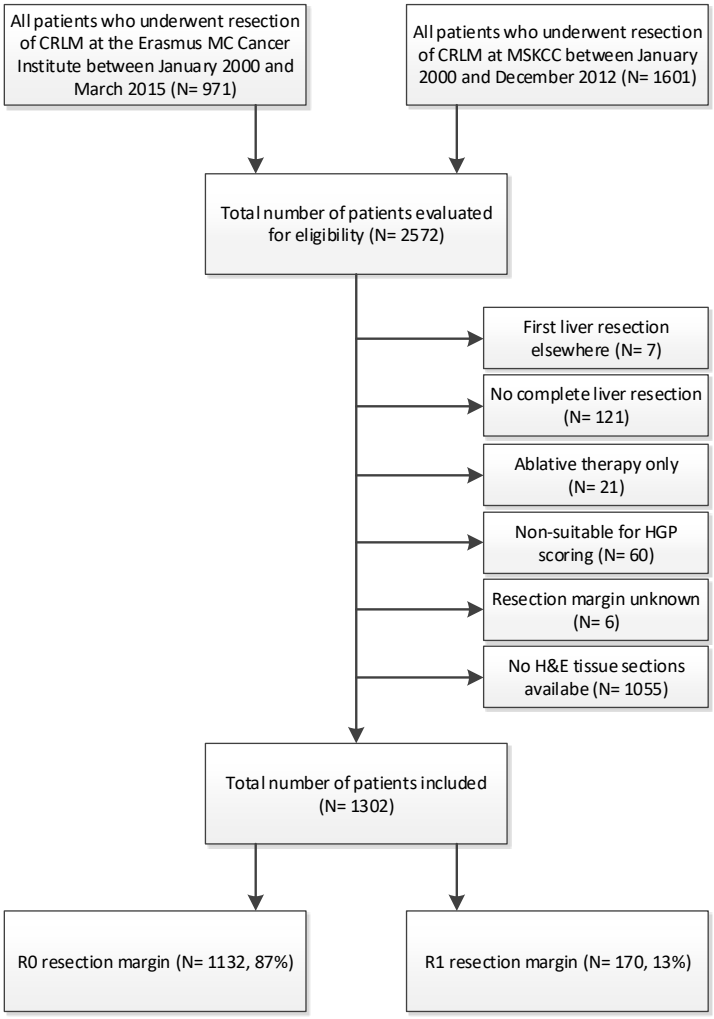

Figure 2. Flowchart of patient inclusion.

Non-dHGP versus dHGP

Baseline characteristics stratified by HGP are presented in supplementary table 1. Patients with non-dHGP had a higher proportion of lymph node positive primary tumours (62% versus 55%, p=0.039), a longer disease free interval (median 2 versus 0 months, p<0.001), larger CRLM (median 3.1 versus 2.5 cm, p<0.001) and higher preoperative CEA levels (median 14.3 versus 6.9 µg/L, p<0.001). The proportion of R1 resection margins was higher in the non-dHGP group (14% vs. 9%, p=0.007). Patients with non-dHGP less often received preoperative systemic chemotherapy without HAIP (54% versus 63%, p=0.004), less often received HAIP chemotherapy preoperatively (2% versus 5%, p=0.008) and less often received any perioperative HAIP chemotherapy (16% versus 21%, p=0.046).

On multivariable logistic regression analysis a node positive primary (OR [95%CI]: 1.53 [1.15-2.03], p=0.003) and larger CRLM (1.13 [1.06-1.20], p<0.001) were independently associated with a higher chance of non-dHGP, whereas preoperative systemic chemotherapy (0.54 [0.39-0.74], p<0.001) and preoperative HAIP chemotherapy (0.30 [0.15-0.62], p=0.001) were independently associated with a lower chance of finding non-dHGP on H&E tissue sections (supplementary table 2).

R0 versus R1 resection

Baseline characteristics stratified by resection margin status are presented in table 1.

The results of the uni- and multivariable logistic regression models on the presence of R1 resection are reported in table 2. Any observed non-dHGP (OR [95%CI]: 1.79 [1.11-2.87], p=0.016) and the number of CRLM (1.15 [1.08-1.23], p<0.001) were independently associated with a greater risk of an R1 resection.

Survival and resection margin in the context of HGPs

The median follow-up for survivors was 66 months (IQR: 46-100 months). During follow-up 677 patients (52%) died. The median OS of the total group was 58 months (IQR: 27-151). With an R0 resection, patients had a median OS of 64 months (IQR: 29-170), compared to a median of 37 months (IQR: 22-80) when an R1 resection was performed (overall log-rank: p<0.001, figure 3A). Median OS of patients with non-dHGP was 50 months (IQR: 25-114), while the median OS of patients dHGP was 80 months (IQR: 46-Not reached) (overall log-rank: p<0.001, figure 3B).

Table 1. Baseline characteristics stratified by resection margin status

		Total (N= 1302)	R0 resection (N= 1132, 87%)	R1 resection (N= 170, 13%)	P-value
Gender	Male	790 (60.7%)	685 (60.5%)	105 (61.8%)	0.755
	Female	512 (39.3%)	447 (39.5%)	65 (38.2%)	
Age	Median (IQR)	63 (55-71)	64 (55-71)	62 (55-67)	0.047*
Primary tumour					
Nodal status	N0	503 (39.9%)	442 (40.2%)	61 (37.4%)	0.496
	N+	759 (60.1%)	657 (59.8%)	102 (62.6%)	
	Missing	40 patients			
CRLM					
DFI in months	Median (IQR)	0 (0-17)	0 (0-17)	0 (0-15)	0.277
	Missing	*23 patients*			
Number of CRLM	Median (IQR)	2 (1-4)	2 (1-3)	3 (2-5)	<0.001*
Size of largest CRLM	Median (IQR)	3.0 (2.0-4.7)	3.0 (2.0-4.5)	3.3 (2.3-4.0)	0.028*
	Missing	*4 patients*			
Preoperative CEA (µg/L)	Median (IQR)	12.0 (4.4-40.8)	12.0 (4.4-35.5)	14.2 (4.3-58.0)	0.063
	Missing	*66 patients*			
CRS	Low	749 (60.8%)	674 (62.6%)	75 (48.1%)	<0.001*
	High	483 (39.2%)	402 (37.4%)	81 (51.9%)	
	Incomplete CRS	*70 patients*			
Preoperative systemic CTx without HAIP	No	575 (44.2%)	515 (45.5%)	60 (35.3%)	0.012*
	Yes	726 (55.8%)	616 (54.5%)	110 (64.7%)	
	Missing	*1 patient*			
Preoperative HAIP CTx	No	1263 (97.0%)	1101 (97.3%)	162 (95.3%)	0.161
	Yes	39 (3.0%)	31 (2.7%)	8 (4.7%)	
Any perioperative systemic CTx without HAIP	No	622 (47.8%)	549 (48.5%)	73 (42.9%)	0.173
	Yes	679 (52.2%)	582 (51.5%)	97 (57.1%)	
	Missing	*1 patient*			
Any perioperative HAIP CTx	No	1078 (82.8%)	937 (82.8%)	141 (82.9%)	0.957
	Yes	224 (17.2%)	195 (17.2%)	29 (17.1%)	
Extra Hepatic Disease	No	1138 (87.4%)	985 (87.0%)	153 (90.0%)	0.274
	Yes	164 (12.6%)	147 (13.0%)	17 (10.0%)	
HGP	Non-dHGP	997 (76.6%)	853 (75.4%)	144 (84.7%)	0.007*
	dHGP	305 (23.4%)	279 (24.6%)	26 (15.3%)	

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastases; CRS: clinical risk score; CTx: chemotherapy; DFI: disease free interval; dHGP: desmoplastic HGP; EMC: Erasmus Medical Centre; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; IQR: interquartile range; MSKCC: Memorial Sloan Kettering Cancer Center; N0: lymph node negative primary tumour; N+: lymph node positive primary tumour; non-dHGP: non desmoplastic HGP; R0: negative resection margin R1: positive resection margin; * indicates significant P-value

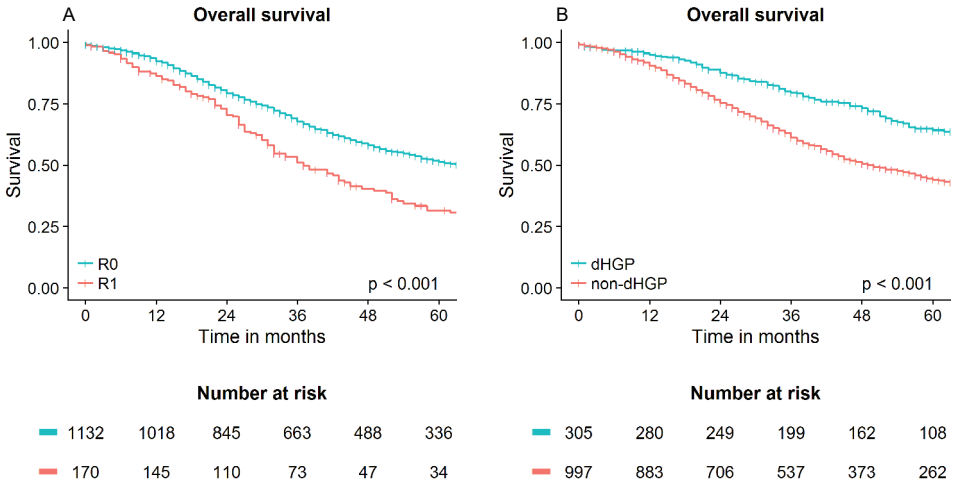

Figure 3A-B. Overall survival curves.

Table 2. Uni- and multivariable Logistic regression analysis of factors potentially associated with an R1 resection margin

	Univariable		Multivariable	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Node positive primary	1.125 [0.801-1.579]	0.497	1.072 [0.742-1.550]	0.710
DFI (cont.)	0.992 [0.982-1.002]	0.122	0.997 [0.986-1.009]	0.629
Number of CRLM (cont.)	1.189 [1.121-1.262]	<0.001*	1.153 [1.077-1.234]	<0.001*
Size CRLM (cont.)	1.043 [0.987-1.103]	0.131	1.043 [0.980-1.111]	0.184
Preoperative CEA (cont.)	1.000 [1.000-1.000]	0.939	1.000 [1.000-1.000]	0.744
Preoperative systemic CTx without HAIP	1.533 [1.096-2.144]	0.013*	1.202 [0.799-1.809]	0.376
Preoperative HAIP CTx	1.754 [0.792-3.882]	0.166	2.099 [0.863-5.105]	0.102
Extra hepatic disease	0.745 [0.438-1.265]	0.275	0.704 [0.395-1.254]	0.233
Non-dHGP	1.812 [1.168-2.810]	0.008*	1.787 [1.112-2.871]	0.016*

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastases; CTx: chemotherapy; DFI: disease free interval; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; non-dHGP: non-desmoplastic HGP; R1: positive resection margin; * indicates significant P-value

The results of the uni- and multivariable Cox regression analysis for OS are reported in table 3. All VIFs (data not shown) were below 1.5 indicating that there is no evidence of multicollinearity. After correction for well known risk factors and perioperative chemotherapy treatment strategy both R1 resection (HR [95%CI]: 1.41 [1.13-1.76], p=0.002) and non-dHGP (1.57 [1.26-1.95], p<0.001) remained independently associated with worse OS. Supplementary tables 3 and 4 display the uni- and multivariable Cox regression analyses for OS in patients with an R0 and an R1 resection margin, respectively.

Table 3. Univariable and multivariable Cox regression analysis Overall Survival total group

	Univariable		Multivariable	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection (cont.)	1.013 [1.006-1.019]	<0.001*	1.016 [1.008-1.023]	<0.001*
Node positive primary	1.464 [1.245-1.721]	<0.001*	1.455 [1.226-1.728]	<0.001*
DFI (cont.)	1.001 [0.997-1.005]	0.698	0.997 [0.993-1.002]	0.221
Number of CRLM (cont.)	1.055 [1.022-1.089]	0.001*	1.078 [1.039-1.118]	<0.001*
Size CRLM (cont.)	1.050 [1.027-1.074]	<0.001*	1.063 [1.035-1.091]	<0.001*
Preoperative CEA (cont.)	1.000 [1.000-1.000]	0.483	1.000 [1.000-1.000]	0.898
Any perioperative systemic CTx without HAIP	1.229 [1.056-1.430]	0.008*	0.788 [0.650-0.954]	0.015*
Any perioperative HAIP CTx	0.575 [0.460-0.719]	<0.001*	0.542 [0.413-0.710]	<0.001*
R1 resection CRLM	1.615 [1.316-1.981]	<0.001*	1.409 [1.129-1.759]	0.002*
Extrahepatic disease	1.784 [1.448-2.196]	<0.001*	1.731 [1.378-2.174]	<0.001*
Non-dHGP	1.814 [1.480-2.223]	<0.001*	1.569 [1.261-1.951]	<0.001*

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastases; CTx: chemotherapy; DFI: disease free interval; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; non-dHGP: non-desmoplastic HGP; R1: positive resection margin; * indicates significant P-value

Discussion

The current study demonstrates that the presence of any non-dHGP is independently associated with a higher incidence of positive resection margins in patients with resectable CRLM. In addition, an increasing number of CRLM is also independently associated with a higher risk of an R1 resection. These findings suggest that both technical aspects of the liver resection (e.g. the number of CRLM) and biology may play a role together in the obtainment of an R1 resection. Positive resection margins remain associated with worse survival in the context of HGPs.

It has been argued that the occurrence of positive resection margins may be a reflection of underlying tumour biology.[7-9] One of the potential explanations that has been suggested is the HGP.[7] The proposition that non-dHGP may put patients at a higher risk of positive resection margins is strengthened by the current report and recent literature. Brunner et al.[15] reported a decreased R1 resection rate among patients with "encapsulated" CRLM when compared to patients with "non-encapsulated" CRLM. Nearly three quarters of patients with an R1 resection displayed non-encapsulated CRLM. When one reviews the photographs of the H&E tissue sections presented in their paper, it appears that what the authors designate as "encapsulated" and "non-encapsulated" CRLM, may correspond respectively with dHGP and non-dHGP described by us and others.[10, 11] In that study, of the 121 only twenty patients (17%) with an R1 resection were observed and the ability to draw solid conclusions was limited. In the current study, 170 of the 1302 (13%) resected patients had an R1 resection, providing sufficient power to adequately correct for potential confounders. Increasing number of CRLM was also associated with greater risk of an R1 resection and is concordant with previous studies.[8, 23, 24] In addition, non-HGP was associated with positive margins, but R1 resections were also seen in patients with dHGP, suggesting that both technique and biology may play an important role in R1 resection margins.

Few small subsets of patients are known in whom resection margins hold no prognostic value after resection of CRLM.[9, 25-27] Patients with CRLM displaying non-dHGP had worse prognosis after resection of CRLM. This finding is in line with previous studies.[11, 12, 15, 17-19] With regard to the prognostic value of margin status there has been much debate. There are some studies that found no negative prognostic value of positive margins.[27] This lack of prognostic value of positive margins was also found by others: in patients with low or moderate disease burden[9, 28], in the era of modern systemic chemotherapy[9, 26] and in case of a good pathological response.[25] Although extensively studied and different definitions of a positive margin are handled in recent literature, most studies show a negative association of positive margins and survival after resection of CRLM.[8, 23, 25, 29, 30] The aforementioned illustrates that the prognostic value of both HGPs and positive margins have been reported before separately, but not within the scope of a single study. In the current study, after correction for the HGP and other clinicopathological variables, positive resection margins remained negatively associated with OS.

Subgroup analyses in the patients with an R1 resection showed that only the size of the largest CRLM was independently associated with worse survival. Interestingly, HAIP chemotherapy was not associated with improved survival in the R1 resection subgroup, whereas this was the strongest predictor for improved outcome in the R0 resection subgroup. This is in line with a previous propensity score matched cohort study that found no association between HAIP chemotherapy and improved survival in patients with an R1 resection.[31]

This is the first study to demonstrate a significant association between the HGP and the incidence of positive resection margins when correcting for potential confounders. As both the HGP and the resection margin are determined postoperatively, these findings are currently of little clinical relevance. This further underlines the urgent need for preoperative HGP assessment methods. Preoperative knowledge on the HGP could allow tailor-made (surgical) treatment strategies such as preoperative chemotherapy or wider resection margins in the case of non-dHGP CRLM. Several diagnostic tools are currently investigated for pre-operative HGP assessment. Examples include computational radiomics[32] and liquid biopsies (circulating tumour cells and cell-free DNA).[33-35] Future research should focus on finding a (non-invasive) preoperative surrogate marker for HGPs.

Limitations of the current study should be taken into account. HGP assessment was performed retrospectively. In 1052 potentially eligible patients no H&E tissue sections were available for HGP determination. This could have induced selection bias. Although the current study describes a fairly large number of patients with an R1 resection, subgroup analyses in 170 patients with an R1 resection might be prone to a type II statistical error. Another shortcoming of this study is the unavailability of (RAS) mutational status. The presence of RAS mutations has been associated with a higher chance of positive resection margins by Brudvik and colleagues using a definition of a <1 mm for positive resection margins.[36] The results of this study should be interpreted with caution due to sample size limitations (only 48 patients had a positive margin).

In conclusion both the presence of any non-dHGP and the number of CRLM are associated with a higher rate of positive resection margins. This suggests that not only technical aspects of the resection but also underlying tumour biology may influence the risk of a positive margin at hepatic resection for CRLM. Importantly, both positive resection margins and non-dHGP remain prognostic indicators for worse overall survival when taking both into consideration.

Figure captions

Figure 1A-B. 1A: Example of the dHGP. 1B: Example of the non-dHGP. Abbreviations: NL: normal liver; D: desmoplastic stroma; T: tumour core.

Figure 2. Flowchart of patient inclusion.

Fig. 3A-B. Overall survival curves.

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Supplementary materials

Supplementary table 1. Baseline characteristics stratified by HGP

		Total (N= 1302)	Non-dHGP (N= 997, 77%)	dHGP (N= 305, 23%)	P-value
Gender	Male	790 (60.7%)	606 (60.8%)	184 (60.3%)	0.887
	Female	512 (39.3%)	391 (39.2%)	121 (39.7%)	
Age	Median (IQR)	63 (55-71)	63 (55-71)	64 (54-71)	0.685
Primary tumour					
Nodal status	N0	503 (39.9%)	369 (38.3%)	134 (45.0%)	0.039*
	N+	759 (60.1%)	595 (61.7%)	164 (55.0%)	
	Missing	*40 patients*			
CRLM					
DFI in months	Median (IQR)	0 (0-17)	2 (0-18)	0 (0-11)	<0.001*
	Missing	23 patients			
Number of CRLM	Median (IQR)	2 (1-4)	2 (1-4)	2 (1-4)	0.464
	Missing	*5 patients*			
Size of largest CRLM	Median (IQR)	2.8 (1.8-4.5)	3.1 (2.2-5.0)	2.5 (1.5-4.0)	<0.001*
	Missing	33 patients			
Preoperative CEA (µg/L)	Median (IQR)	12.0 (4.4-40.8)	14.3 (4.9-48.3)	6.9 (3.0-23.3)	<0.001*
	Missing	*66 patients*			
CRS	Low	749 (60.8%)	579 (60.9%)	170 (60.5%)	0.908
	High	483 (39.2%)	372 (39.1%)	111 (39.5%)	
	Incomplete CRS	70 patients			
Preoperative systemic CTx without HAIP	No	575 (44.2%)	462 (46.4%)	113 (37.0%)	0.004*
	Yes	726 (55.8%)	534 (53.6%)	192 (63.0%)	
	Missing	*1 patient*			
Preoperative HAIP CTx	No	1263 (97.0%)	974 (97.7%)	289 (94.8%)	0.008*
	Yes	39 (3.0%)	23 (2.3%)	16 (5.2%)	
Any perioperative systemic CTx without HAIP	No	622 (47.8%)	484 (48.6%)	138 (45.2%)	0.306
	Yes	679 (52.2%)	512 (51.4%)	167 (54.8%)	
	Missing	*1 patient*			
Any perioperative HAIP CTx	No	1078 (82.8%)	837 (84.0%)	241 (79.0%)	0.046*
	Yes	224 (17.2%)	160 (16.0%)	64 (21.0%)	
Extra Hepatic Disease	No	1138 (87.4%)	866 (86.9%)	272 (89.2%)	0.285
	Yes	164 (12.6%)	131 (13.1%)	33 (10.8%)	
Resection margin	R0	1132 (86.9%)	853 (58.6%)	279 (91.5%)	0.007*
	R1	170 (13.1%)	144 (14.4%)	26 (8.5%)	

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastases; CRS: clinical risk score; CTx: chemotherapy; DFI: disease free interval; dHGP: desmoplastic HGP; EMC: Erasmus Medical Centre; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; IQR: interquartile range; MSKCC: Memorial Sloan Kettering Cancer Center; N0: lymph node negative primary tumour; N+: lymph node positive primary tumour; non-dHGP: non desmoplastic HGP; R0: negative resection margin; R1: positive resection margin; * indicates significant P-value

Supplementary table 2. Uni- and multivariable logistic regression analysis of factors potentially associated with the presence of any non-dHGP

	Univariable		Multivariable	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Node positive primary	1.318 [1.013-1.713]	0.040*	1.532 [1.153-2.034]	0.003*
DFI (cont.)	1.009 [1.001-1.017]	0.032*	1.003 [0.995-1.011]	0.493
Number of CRLM (cont.)	0.986 [0.932-1.042]	0.612	1.049 [0.981-1.123]	0.161
Size CRLM (cont.)	1.149 [1.079-1.223]	<0.001*	1.126 [1.055-1.202]	<0.001*
Preoperative CEA (cont.)	1.000 [1.000-1.001]	0.330	1.000 [1.000-1.000]	0.645
Preoperative systemic CTx without HAIP	0.680 [0.523-0.885]	0.004*	0.536 [0.390-0.737]	<0.001*
Preoperative HAIP CTx	0.427 [0.222-0.818]	0.010*	0.301 [0.145-0.622]	0.001*
Extra hepatic disease	1.247 [0.831-1.870]	0.286	1.227 [0.797-1.889]	0.353

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastases; CTx: chemotherapy; DFI: disease free interval; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; non-dHGP: non-desmoplastic HGP; R1: positive resection margin; * indicates significant P-value

Supplementary table 3. Cox regression analysis of all patients with R0 resection margin

	Univariable		Multivariable	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection (cont.)	1.013 [1.006-1.021]	<0.001	1.016 [1.007-1.024]	<0.001
Node positive primary	1.465 [1.229-1.747]	<0.001	1.489 [1.237-1.792]	<0.001
DFI (cont.)	1.001 [0.996-1.005]	0.740	0.996 [0.992-1.001]	0.137
Number of CRLM (cont.)	1.046 [1.009-1.084]	0.015	1.078 [1.035-1.122]	<0.001
Size CRLM (cont.)	1.046 [1.021-1.073]	<0.001	1.057 [1.027-1.087]	<0.001
Preoperative CEA (cont.)	1.000 [1.000-1.000]	0.518	1.000 [1.000-1.000]	0.708
Any perioperative CTx without HAIP	1.228 [1.041-1.450]	0.015	0.760 [0.618-0.934]	0.009
Any perioperative HAIP CTx	0.532 [0.414-0.682]	<0.001	0.472 [0.351-0.635]	<0.001
Extrahepatic disease	1.817 [1.452-2.273]	<0.001	1.724 [1.350-2.202]	<0.001
Non-dHGP	1.764 [1.420-2.191]	<0.001	1.596 [1.266-2.012]	<0.001

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastases; CTx: chemotherapy; DFI: disease free interval; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; non-dHGP: non-desmoplastic growth pattern; R0: negative resection margin; * indicates significant P-value

Supplementary table 4. Cox regression analysis of all patients with R1 resection margin

	Univariable		Multivariable	
Variable	**Hazard Ratio [95% CI]**	**P-value**	**Hazard Ratio [95% CI]**	**P-value**
Age at resection (cont.)	1.012 [0.993-1.032]	0.204	1.016 [0.994-1.039]	0.157
Node positive primary	1.338 [0.882-2.030]	0.170	1.527 [0.936-2.492]	0.090
DFI (cont.)	1.004 [0.992-1.017]	0.526	1.005 [0.988-1.023]	0.560
Number of CRLM (cont.)	1.042 [0.965-1.125]	0.290	1.079 [0.986-1.182]	0.099
Size CRLM (cont.)	1.067 [1.008-1.129]	0.025	1.087 [1.009-1.171]	0.027
Preoperative CEA (cont.)	1.000 [1.000-1.001]	0.714	1.000 [0.999-1.001]	0.662
Any perioperative CTx without HAIP	1.174 [0.804-1.713]	0.406	1.065 [0.608-1.865]	0.826
Any perioperative HAIP CTx	0.900 [0.542-1.492]	0.682	1.123 [0.537-2.351]	0.758
Extrahepatic disease	1.863 [1.052-3.300]	0.033	1.816 [0.959-3.439]	0.067
Non-dHGP	1.814 [0.993-3.316]	0.053	1.468 [0.745-2.892]	0.268

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastases; CTx: chemotherapy; DFI: disease free interval; HAIP: hepatic arterial infusion pump; HGP: histopathological growth pattern; non-dHGP: non-desmoplastic growth pattern; R1: positive resection margin; indicates significant P-value

Chapter 4

Salvage treatment for recurrences after first resection of colorectal liver metastases: the impact of histopathological growth patterns

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Clinical and Experimental Metastasis. 2019 Apr;36(2):109-118

Abstract

Background

The majority of patients recur after resection of colorectal liver metastases (CRLM). Patients with CRLM displaying a desmoplastic histopathological growth pattern (dHGP) have a better prognosis and lower probability of recurrence than patients with non-dHGP CRLM. The current study evaluates the impact of HGP type on the pattern and treatment of recurrences after first resection of CRLM.

Material and methods

A retrospective cohort study was performed, including patients with known HGP type after complete resection of CRLM. All patients were treated between 2000 and 2015. The HGP was determined on the CRLM resected at first partial hepatectomy. The prognostic value of HGPs, in terms of survival outcome, in the current patient cohort were previously published.

Results

In total 690 patients were included, of which 492 (71%) developed recurrent disease. CRLM displaying dHGP were observed in 103 patients (21%). Amongst patients with dHGP CRLM diagnosed with recurrent disease, more liver-limited recurrences were seen (43% vs. 31%, p=0.030), whereas patients with non-dHGP more often recurred at multiple locations (34% vs. 19%, p=0.005). Patients with dHGP CRLM were more likely to undergo curatively intended local treatment for recurrent disease (adjusted odds ratio: 2.37; 95% confidence interval (CI): [1.46-3.84]; p<0.001) compared to patients with non-dHGP.

Conclusions

The present study demonstrates that liver-limited disease recurrence after complete resection of CRLM is more often seen in patients with dHGP, whereas patients with non-dHGP more frequently experience multi-organ recurrence. Recurrences in patients with dHGP at first CRLM resection are more likely to be salvageable by local treatment modalities, but no prognostic impact of HGPs after salvage therapy for recurrent disease was found.

Introduction

After hepatic resection for colorectal liver metastases (CRLM) the majority of patients experiences recurrence of disease. Despite advances in the treatment of CRLM, recurrence rates reach up to 70%. [1-5] Approximately 40% of the patients with recurrent disease is again eligible for local treatment modalities. [4, 6-8] If disease biology allows the recurrence to be treated locally again, survival outcomes similar to the first local treatment of metastases are seen. [1, 4, 6-13] In case of a recurrence not amenable to local treatment prognosis is limited. [4, 7, 8, 13] In addition, clinical risk factors currently used for the prediction of prognosis after first hepatic resection for CRLM, have not proven equally useful in prognostication after repeat resection for recurrent CLRM. [14]

Histopathological growth patterns (HGPs) describe the transition border of CRLM to the normal liver parenchyma. [15] The assessment of HGPs has been standardised in international consensus guidelines [16] and multiple studies have reported the effect of HGPs on prognosis in patients with resectable CRLM. [16-22] We recently described the largest patient cohort to date and found that the desmoplastic HGP (dHGP) is associated with favourable overall survival, progression free survival compared to its non-desmoplastic counterpart (non-dHGP). [23] In the current study we aimed to identify in the same cohort of patients potential explanations for this survival difference. Differences in recurrence pattern (intra- versus extrahepatic) and/or treatment of recurrent disease (local versus systemic) might possibly account for the difference in survival outcomes between HGPs. Therefore, the current study investigates the pattern of first recurrence and the salvageability of recurrent disease after first partial hepatectomy for CRLM in the context of HGPs.

Methods

Patients

The current study was approved by the medical ethics committee of the Erasmus University Medical Centre Rotterdam (MEC 2018-1743). All consecutive patients that underwent first surgical treatment for CRLM between 2000 and 2015 at the Erasmus MC Cancer Institute were considered for inclusion. The prognostic value of HGPs, in terms of survival outcome, in the current patient cohort were previously published. [23] Patients selected for this study had to be completely free of all known macroscopic disease at some point following first resection of CRLM in order to be eligible for inclusion. A positive resection margin (R1) was defined as tumour cells (i.e. microscopic residual disease) at the resection margin and therefore patients with an R1 resection were eligible for inclusion. Patients with unknown HGP type were excluded.

Design and outcomes

Data on patient characteristics, primary tumour, CRLM and recurrence were extracted from a prospectively maintained database. H&E tissue sections were retrospectively analysed for HGP assessment. Disease free survival (DFS) was defined as the time in months between the first hepatic resection for CRLM and diagnosis of recurrence or death. Post-recurrence survival (PRS) was defined as the time in months between diagnosis of recurrence after first hepatic resection for CRLM and death. When alive patients were censored at date of last follow-up. Local therapy with curative intent was defined as resection, ablation and/or radiation therapy after which the patient was considered to be free of disease.

Treatment and follow-up after first partial hepatectomy

Perioperative chemotherapy for resectable CRLM is not standard of care in the Netherlands, since no OS benefit has been found in randomised setting. [24] Therefore preoperative chemotherapy at the Erasmus MC Cancer Institute is only considered in case of borderline resectable, more than four and/or synchronous CRLM. Some patients, however, received chemotherapy in referring hospitals prior to referral. Patients do not receive postoperative chemotherapy. Follow-up is performed up to five years after resection of CLRM. The follow-up consists of carcinoembryonic antigen (CEA) monitoring every three months for the entire follow-up duration and imaging every six months in the first three years and annually in the fourth and fifth year. In case of elevated CEA levels (>5 μg/L) or a rise in CEA levels (>25%) imaging is performed. When uncertainty with regard to the diagnosis of disease recurrence exists, biopsies are taken as confirmation. As with primary treatment for CRLM, treatment strategy for recurrent disease is established by a multidisciplinary board. The decision whether local therapies (resection, ablation, stereotactic body radiation) are considered beneficial for patients, depends on two factors: time to recurrence and localisation of recurrences.

Regarding time to recurrence, it was previously demonstrated that patients with a disease-free interval of less than six months again undergoing local treatment for the recurrence have poor survival outcomes. [25] Therefore, when patients present with recurrent disease within six months after resection of CRLM, patients first receive systemic chemotherapy before local therapy is considered. Systemic therapy normally consists of oxaliplatin- or irinotecan-based treatment regimens. Typically, three courses are administered followed by restaging and local therapy in case of partial response or stable disease. In case of progressive disease, patients are switched to second line chemotherapeutic regimens. When patients present with recurrent disease beyond six months after primary liver resection for CRLM and the lesions are treatable with local therapy, these patients are planned for local therapy accordingly. Again, no adjuvant chemotherapy is administered. Patients presenting with recurrent disease not eligible for local treatment receive palliative treatment.

Provided that the interval between first liver resection and recurrence is greater than 6 months, or less than 6 months, but at least stable disease after three courses of chemotherapy is observed, then localisation of recurrences is a decisive factor in the clinical decision making in these patients. The currently handled standard at our centre is, that when recurrent disease is liver-limited and it can be resected with sufficient remnant liver, local treatment of the colorectal liver metastases should be attempted. In addition, local treatment is deemed feasible when concurrent oligometastatic extrahepatic is present. When extrahepatic disease is present in >1 organ, local treatment is deemed futile.

HGP assessment

The HGPs were determined on the CRLM resected at the first hepatectomy. The HGP of CRLM describes the tumour-liver interface. Three different types of HGPs have been described; the desmoplastic (dHGP), the replacement (rHGP) and the rare pushing HGP (pHGP). [16] The latter two (rHGP and pHGP) can be taken together as non-dHGP, since recent findings indicate that patients with CRLM that display *any* proportion non-dHGP at the interface have impaired prognosis compared to patients with pure dHGP. [23] In this study, international consensus guidelines for HGP assessment of liver metastases were utilised to determine the HGPs. [16] HGP determination was jointly executed by at least three trained observers (PN, BG, DH, ES, RC, PV). The observers were blinded for clinical data and outcome during HGP assessment. Some CRLM display multiple HGPs, therefore the complete interface of all available H&E tissue sections of all CRLM in every patient were examined. Only if pure dHGP was observed, patients were categorised as such. All other patient displaying any non-dHGP were categorised as non-dHGP. In accordance with the consensus guidelines, not all tissue sections are suitable for HGP assessment. If less than twenty percent of the interface is assessable, if the tissue section is of insufficient quality or when no vital tumour is present, the HGP cannot be determined.

Statistical analysis

Categorical data were presented using counts and percentages. Continuous data were reported with medians and corresponding interquartile range (IQR). Differences in proportions were evaluated with the Chi-squared test. Medians were compared using the Mann-Whitney U test. Median follow-up time for survivors was estimated by means of the reversed Kaplan-Meier method. Survival estimates were obtained using the Kaplan-Meier method, computed until 60 months and compared with the log rank test. Uni- and multivariable Cox regression analysis was performed to correct for potential confounding. Results of the Cox regression analyses were expressed in hazard ratios (HR) and corresponding 95% confidence intervals (CI). Uni- and multivariable binary logistic regression analysis was performed to evaluate possible predictors for unsalvageable recurrence. Results of the logistic regression analyses were expressed in odds ratios (OR) and corresponding 95% CI. In both the binary logistic regression and the Cox univariable regression models, all variables potentially related to salvageability of recurrence and/or overall survival were considered. All variables with p-values <0.100 on univariable analysis were entered in the multivariable models. All statistical tests were two-sided and p-values <0.05 were considered statistically significant. All analyses were performed using SPSS version 24.0 (SPSS Inc., Chicago, IL) and R version 3.5.1 (http://www.r-project.org).

Results

Patients and disease free survival

During the study period 964 patients were treated surgically for CRLM at the Erasmus MC Cancer Institute. HGP determination was performed in 732 patients (76%). Patients were excluded due to: no (complete) resection of CRLM (n=100), missing H&E tissue sections (n=55), ablative therapy only (n=21) or non-suitable H&E tissue sections for HGP determination (n=56). Of these 732 patients, 690 were completely free of all known disease at some point following first resection of CRLM and were included in the study. Hence, 42 patients were excluded (n=24 primary tumour never resected after liver-first approach due to progressive metastatic disease, n=18 extrahepatic disease never treated locally).

Among the included patients, there were 173 (25%) with dHGP and 517 with non-dHGP (75%). Median follow-up for survivors was 76 months (IQR: 45-116). In total 492 patients (71%) had disease recurrence. A flowchart of the patient inclusion is displayed in figure 1. Baseline characteristics of all 690 patients compared for HGP are reported in table 1. At baseline there were several differences between patients with dHGP compared to patients non-dHGP, especially in terms of primary tumour characteristics (lymph node status and adjuvant treatment) and CRLM characteristics (disease-free interval, CEA, size of largest CRLM, resection margin and preoperative treatment).

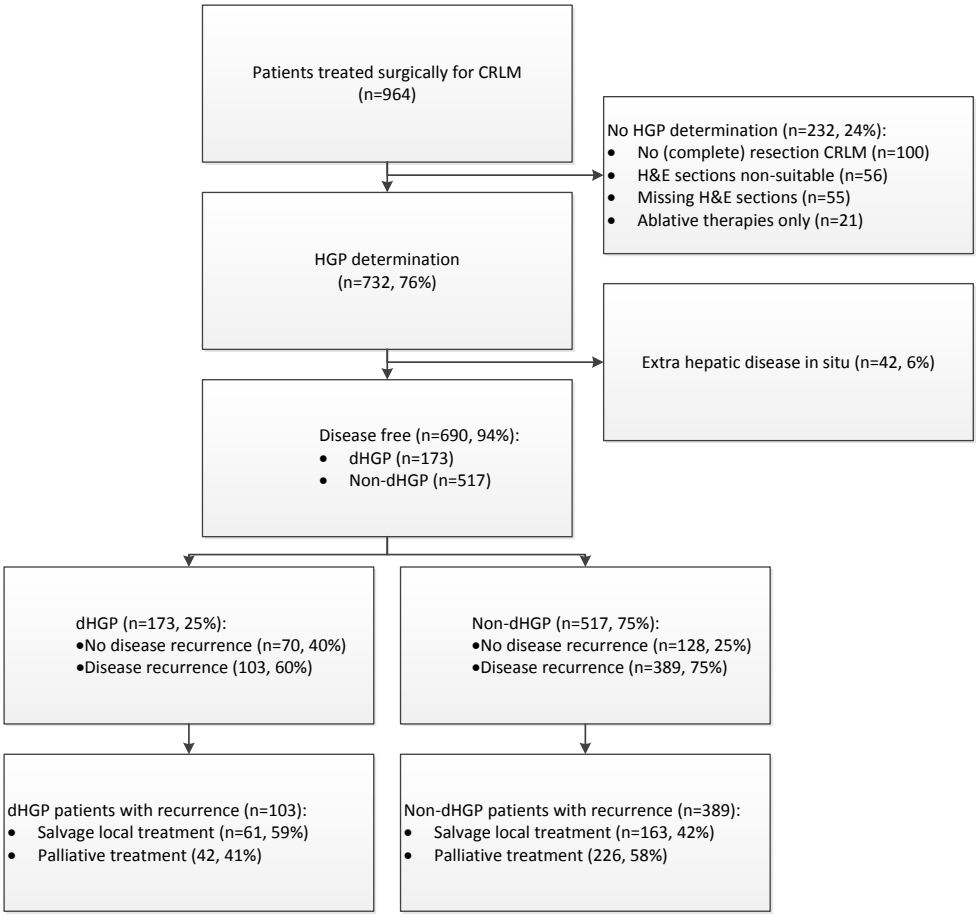


Figure 1. Flowchart of patient selection.

Table 1. Baseline characteristics of all patients stratified for HGP

		Total N=690	dHGP N=173	Non-dHGP N=517	P-value
Gender	Male	445 (65%)	109 (63%)	336 (65%)	0.637
	Female	245 (36%)	64 (37%)	181 (35%)	
Age	Median (IQR)	65 (58-71)	65 (56-72)	64 (58-71)	0.984
ASA	ASA I-II	617 (91%)	153 (89%)	464 (91%)	0.351
	ASA > II	63 (9%)	19 (11%)	44 (9%)	
	Missing	10 patients			
Primary tumour characteristics					
Location	Right-sided	116 (17%)	30 (17%)	86 (17%)	0.927
	Left-sided	302 (44%)	76 (44%)	226 (44%)	

		Total N=690	dHGP N=173	Non-dHGP N=517	P-value
	Rectum	256 (37%)	62 (36%)	194 (38%)	
	Double tumour	16 (2%)	5 (3%)	11 (2%)	
pTumour stage	pT0-2	134 (20%)	39 (23%)	95 (19%)	0.239
	pT3-4	546 (80%)	132 (77%)	414 (81%)	
	Missing	10 patients			
Nodal status	N0	270 (40%)	79 (47%)	191 (38%)	0.035*
	N+	407 (60%)	90 (53%)	317 (62%)	
	Missing	13 patients			
Adjuvant chemotherapy primary tumour	No	587 (85%)	160 (93%)	427 (83%)	0.002*
	Yes	103 (15%)	13 (8%)	90 (17%)	
CRLM characteristics					
Synchronous CRLM	No	329 (48%)	264 (51%)	65 (38%)	0.002*
	Yes	361 (52%)	253 (49%)	108 (62%)	
Disease-free interval (months)	Median (IQR)	2 (0-17)	0 (0-13)	5 (0-18)	0.006*
Number of CRLM	Median (IQR)	2 (1-4)	2 (1-4)	2 (1-4)	0.886
Size of largest CRLM (cm)	Median (IQR)	3.1 (2.0-4.5)	2.5 (1.8-4.2)	3.3 (2.3-4.8)	<0.001*
	Missing	2 patients			
Preoperative CEA (µg/L)	Median (IQR)	14.0 (4.7-50.0)	7.6 (3.2-30.0)	16.2 (5.1-53.0)	<0.001*
	Missing	28 patients			
Fong CRS	Low	408 (61%)	101 (61%)	307 (61%)	0.924
	High	262 (39%)	64 (39%)	198 (39%)	
	Incomplete CRS	20 patients			
Bilobar metastases	No	418 (61%)	106 (61%)	312 (60%)	0.830
	Yes	272 (39%)	67 (39%)	205 (40%)	
Preoperative CTx	No	365 (53%)	68 (39%)	297 (57%)	<0.001*
	Yes	325 (47%)	105 (61%)	220 (43%)	
Resection margin	R0	585 (85%)	158 (92%)	427 (83%)	0.004*
	R1	102 (15%)	14 (8%)	88 (17%)	
	Missing	3 patients			
Extra Hepatic Disease	No	629 (91%)	157 (91%)	472 (91%)	0.827

		Total N=690	dHGP N=173	Non-dHGP N=517	P-value
	Yes	61 (9%)	16 (9%)	45 (9%)	
Major liver resection	<3 complete segments	455 (66%)	122 (71%)	333 (64%)	0.142
	≥3 complete segments	235 (34%)	51 (30%)	184 (36%)	
Recurrence after first resection CRLM	No	198 (29%)	70 (40%)	128 (25%)	<0.001*
	Yes	492 (71%)	103 (60%)	389 (75%)	

Percentages do not always add up to 100% due to rounding. Abbreviations in alphabetical order: ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastases; CRS: clinical risk score; CTx: Chemotherapy; HGP: histopathological growth pattern; IQR: interquartile range; R1: irradical resection margin

Recurrence: survival, pattern and treatment

A smaller proportion of patients with dHGP had disease recurrence compared to patients with non-dHGP (60% vs. 75%). Median DFS of patients with dHGP was 17 months (IQR: 7-not reached) compared to 10 months (IQR: 5-28) in patients with non-dHGP. The DFS significantly differed between both groups (p<0.001, figure 2)

Table 2. Recurrence pattern

	Total (N=492)	dHGP (N=103)	Non-dHGP (N=389)	P-value
Intrahepatic only	166 (34%)	44 (43%)	122 (31%)	0.030*
Pulmonary only	104 (21%)	22 (21%)	82 (21%)	0.951
One other location only	70 (14%)	17 (17%)	53 (14%)	0.457
Local recurrence primary only	15 (3%)	3 (3%)	12 (3%)	
Peritoneal only	3 (1%)	1 (1%)	2 (1%)	
Distant lymph nodes only	26 (5%)	7 (7%)	19 (5%)	
Other location only	26 (5%)	6 (6%)	20 (5%)	
Two or more locations	152 (31%)	20 (19%)	132 (34%)	0.005*
Intrahepatic and pulmonary only	49 (10%)	10 (10%)	39 (10%)	
Intrahepatic and 1 other only	41 (8%)	3 (3%)	38 (10%)	
Pulmonary and 1 other only	25 (5%)	1 (1%)	24 (6%)	
Peritoneal and 1 other only	2 (1%)	0 (0%)	2 (1%)	
Multi organ (>2)	35 (7%)	6 (6%)	29 (8%)	
Treatment of recurrence with curative intent	224 (46%)	61 (59%)	163 (42%)	0.002*

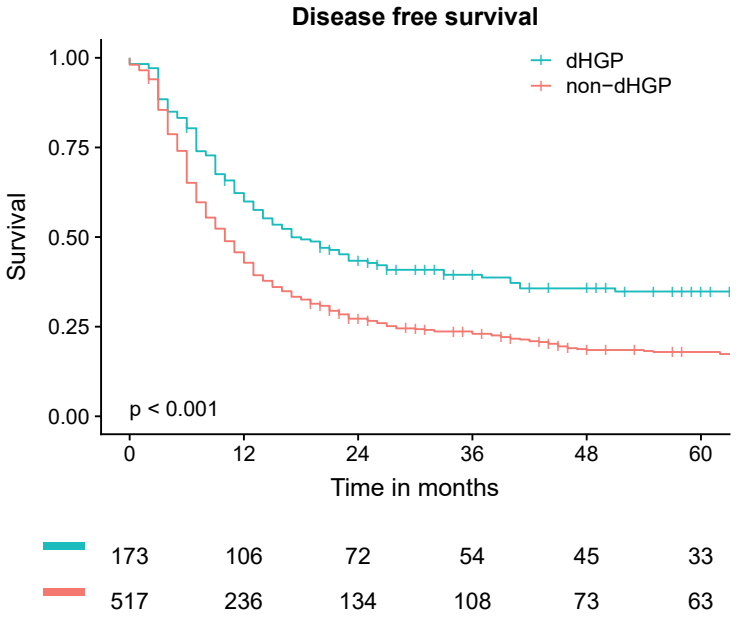


Figure 2. DFS after first hepatic resection for CRLM compared for HGP.

In total 492 patients had disease recurrence after first resection of CRLM. The median time to recurrence in these 492 patients with recurrent disease was 8 months (IQR: 5-14). This was 9 months (IQR: 6-14) in patients with dHGP compared to 8 months (IQR: 4-13 months) in patients with non-dHGP. At six months after first liver resection, 57% of patients with non-dHGP developing recurrences was disease-free, while 71% of patients with dHGP tumours developing recurrences was disease-free at this point in time. Data on the pattern of first recurrence stratified for HGP are reported in table 2. Patients with dHGP at first partial hepatectomy more often had an intrahepatic only recurrence (43% vs 31%, p=0.030) whereas patients with non-dHGP more often had a multi-organ (≥2) recurrence (34% vs 19%, p=0.005). Of all 492 patients with a recurrence, 224 (46%) were again treated with curative intent. Patients with dHGP were more often treated with curative intent for the recurrence (59% vs. 42%, p=0.002). After correction for potential confounders, dHGP at first partial hepatectomy remained a significant predictor for salvageable recurrence (OR: 2.37, p<0.001). Significant predictors negatively associated with salvageability were a right-sided primary tumour (OR: 0.36, p<0.001), a node positive primary tumour (OR: 0.57, p=0.008) and larger CRLM at first partial hepatectomy (OR: 0.92, p=0.036) (table 3).

As the higher rate of intrahepatic only recurrences in the dHGP group might explain the higher likelihood of curatively intended salvage treatment additional analyses have been performed, specifically excluding patients with intrahepatic recurrences only. We subsequently conducted the same multivariable logistic regression analysis as conducted previously and, despite excluding patients with liver-limited recurrences, still found a statistically significant association between dHGP and salvage treatment of the recurrence (adjusted OR: 3.16, p<0.001).

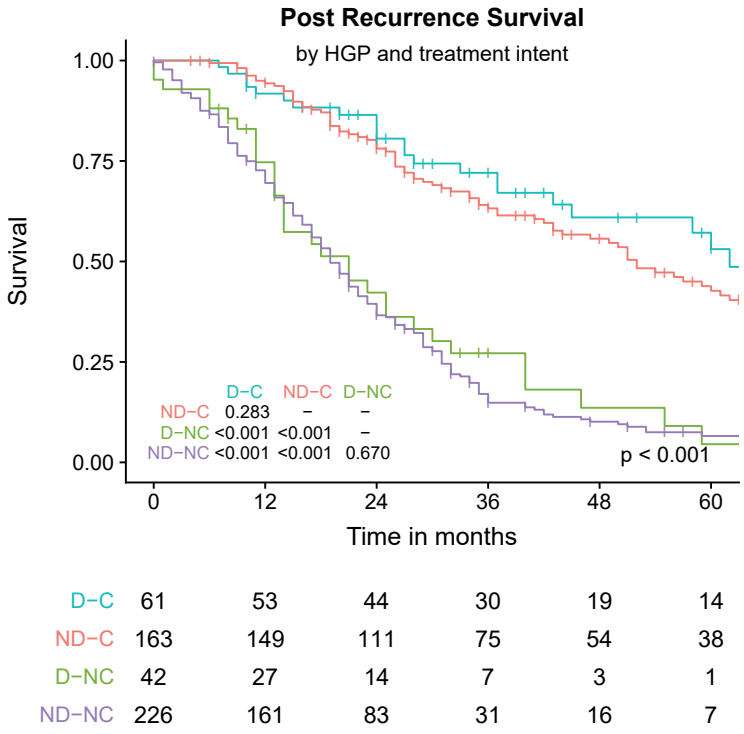
Table 3. Logistic regression for salvageable recurrence

	Univariable		Multivariable	
Variable	**Odds Ratio [95% CI]**	**P-value**	**Odds Ratio [95% CI]**	**P-value**
Age at resection CRLM (cont.)	0.986 [0.968-1.004]	0.122		
ASA > II	0.879 [0.470-1.642]	0.685		
Right-sided primary	0.416 [0.249-0.694]	0.001*	0.364 [0.211-0.628]	<0.001*
pT3-4	0.534 [0.334-0.855]	0.009*	0.686 [0.409-1.151]	0.153
Node positive primary	0.490 [0.336-0.715]	<0.001*	0.568 [0.375-0.860]	0.008*
Disease free interval (cont.)	1.011 [1.001-1.022]	0.037*	1.013 [1.003-1.024]	0.014*
Number of CRLM (cont.)	0.949 [0.880-1.023]	0.171		
Diameter largest CRLM (cont.)	0.932 [0.862-1.007]	0.076	0.915 [0.842-0.994]	0.036*
Preoperative CEA level (cont.)	1.000 [0.999-1.000]	0.270		
Preoperative chemotherapy	1.210 [0.849-1.727]	0.292		
R1 resection CRLM	0.971 [0.607-1.554]	0.903		
Extra hepatic disease	0.864 [0.483-1.545]	0.622		
Desmoplastic type tumours	2.014 [1.295-3.132]	0.002*	2.370 [1.462-3.840]	<0.001*

Abbreviations in alphabetical order: ASA: American Society of Anaesthesiologists; CEA: carcinoembryonic antigen; cont.: continuous; CRLM: colorectal liver metastases; R1: irradical resection margin

Post-recurrence survival

Median PRS after diagnosis of recurrence was 28 months (IQR: 15-59 months). Patients treated with curative intent had a median PRS of 56 months (IQR: 27-84 months) compared to 19 months (IQR: 11-32 months) for patients receiving palliative treatment (p<0.001). After stratification for treatment intent, no difference in PRS was observed between patients with dHGP and non-dHGP (both p-values >0.25, fig. 3).



D–C	61	53	44	30	19	14
ND–C	163	149	111	75	54	38
D–NC	42	27	14	7	3	1
ND–NC	226	161	83	31	16	7

Figure 3. PRS compared for HGP and treatment intent of the recurrence. (D-C: dHGP and curative intent, ND-C: Non-dHGP and curative intent, D-NC: dHGP and non-curative intent, ND-NC: Non-dHGP and non-curative intent)

Discussion

The current study demonstrates that patients with dHGP at first CRLM resection more often develop an intrahepatic only recurrence, whereas patients with non-dHGP more often experience multi-organ recurrence. Importantly, dHGP at first CRLM resection is independently associated with salvageable recurrences after first partial hepatectomy for CRLM. Prognosis after salvage treatment for recurrent disease is not impacted by HGP type determined at first resection of CRLM.

Unfortunately, the majority of patients develops a recurrence after curatively intended resection of CRLM. [1-12] The prognosis of patients with recurrent disease strongly depends on whether local treatment can still be performed. Disease load and tumour biology largely determine if local therapy is possible and beneficial. [4, 10, 12, 26] As this study shows, that recurrences in patients with dHGP at first CRLM resection are more likely to be salvageable, this potentially explains the observed outcome difference between patients with dHGP and non-dHGP. Several studies have suggested that dHGP is associated with favourable tumour characteristics and a lower recurrence rate. [16-23] The more favourable tumour behaviour of dHGP CRLM was further acknowledged in this study, as patients with dHGP at first CRLM resection more often experience intra-hepatic only recurrence, whereas patients with non-dHGP more often develop multi-organ metastases. This also partially explains why salvage therapy was more often performed in these patients, as repeat resection of isolated recurrences is often feasible. [1, 4, 6, 7, 9-12]. There were several differences observed at baseline between patients with dHGP compared to patients with non-dHGP in terms of clinical risk. Patients with non-dHGP had a greater proportion lymph node positive primaries, larger CRLM, and more often an R1 resection margin. These differences might also have attributed to the greater risk of multi-organ recurrences that are less likely salvageable with local treatment modalities in patients with non-dHGP. However, after correction for potentially confounding factors, dHGP remained significantly associated with salvageable recurrences. In addition, this study shows that patients with dHGP less often develop a recurrence and, if they do, the recurrence is also more often salvageable with local treatment modalities.

A frequently debated contraindication for local treatment of colorectal liver metastases is the simultaneous presence of extrahepatic disease. However, several recent (reviews of) retrospective series support resection of liver metastases and concurrent mono-organic extrahepatic disease in highly selected patients. [27-30] When extrahepatic disease is present in >1 organ, the benefit of local treatment seems questionable as it holds outcome similar to systemic treatment alone. [30] As we demonstrated that multi-organ metastasis are more often found in patients with non-dHGP, we believe that this also partially explains why salvage treatment is less often performed in these patients. Moreover, several studies have demonstrated that some localisations of (recurrent) metastases (e.g. liver and concurrent para-aortic lymph node metastases [31, 32]) are associated with poor survival outcomes after surgery. Therefore, local therapies are often not considered beneficial in

these patients. The true value of maximal tumour debulking in metastatic colorectal cancer will only be known after the completion of the ongoing ORCHESTRA trial (NCT01792934) in which patients are randomised between chemotherapy alone or the combination of chemotherapy and maximal tumour debulking.

The differences in recurrence patterns between HGP types might have implications for perioperative treatment. As patients with non-dHGP at first CRLM resection more often develop multi-organ recurrence, one could hypothesise that perioperative chemotherapy is more effective in these patients, since patients at high risk of (systemic) recurrence appear to benefit more from perioperative systemic treatment. [33, 34] Vice versa, patients with dHGP at first CRLM resection might benefit more from hepatic arterial infusion (HAI) chemotherapy as they are more likely to develop recurrences confined to the liver. This hypothesis is supported by the recent finding that patients with low clinical risk, and therefore are less likely to develop extrahepatic disease, appear to benefit the most from HAI chemotherapy whereas patients with extrahepatic disease do not seem to benefit from HAI chemotherapy. [35] Future studies should evaluate the effect of perioperative treatment in the context of HGPs.

As the scoring was performed jointly and the final HGP score was determined by consensus between all observers, no Kappa value for this specific study can be provided. However, in another recently submitted manuscript by our group we have found excellent Kappa indices (>0.9) for discrimination between dHGP and non-dHGP. [36]

This is the first paper demonstrating a significant association between distinct HGPs and differences in recurrence pattern in patients treated surgically for CRLM. Eefsen and colleagues [18] reported on the recurrence pattern in the context of HGPs but did not find an association. Importantly, the authors in that study applied an arbitrary cut-off value for the determination of the pre-dominant HGP. Recent insights have shown that the presence of any non-dHGP entails poor prognosis and no cut-off value for determination of the predominant HGP should be applied. [23] In addition, the number of patients with a recurrence in their study was limited and therefore a potential lack of power should also be considered. The current study handled no arbitrary cut-off value for pre-dominant HGP determination and describes a sufficiently large cohort, in which proper correction for confounding could be performed.

Most of the currently available risk factors for worse outcome after first resection of CRLM do not hold similar prognostic value when utilised for preoperative prognosis prediction at repeat resection of recurrent CRLM. [14] This indicates that there is a need for new prognostic markers in patients undergoing repeat partial hepatectomies for recurrent CRLM. This is the first study to evaluate the prognostic impact of HGPs of the CRLM resected at first liver resection for prognosis after repeat resection of CRLM. No difference in PRS was observed between patients with dHGP and non-dHGP. The reason that the HGP of the CRLM resected at first liver resection, rather than the HGP of recurrent CRLM resected at

repeat resection, were used in the current study was twofold. Firstly, if the HGP at first resection had proven to be prognostic after repeat resection it would have become not only a predictive marker for prognosis after first resection, but also a pre-salvage treatment marker for local treatment of the recurrence. Secondly, this cohort also describes patients with an extrahepatic recurrence without a concurrent hepatic recurrence and therefore no HGP of an recurrent CRLM could be utilised.

Recently RAS mutational status has also been associated with unsalvageable recurrences. [4] Unfortunately RAS and BRAF mutational status were unknown in the currently described patient cohort at time of resection. In an attempt to correct for this drawback, primary tumour location (right- vs. left-sided) was taken into account in the multivariable analysis. Right-sided tumours have been associated with the presence of KRAS [37, 38] and BRAF [37-40] mutations. Right-sidedness of the primary tumour was independently negatively associated with salvageability of recurrent disease in the present study. Despite correcting for primary tumour location (and thereby partially correcting for mutational status) HGP type remained statistically associated with salvageability of recurrent disease.

The limitations of the current study should be taken into account. Although data was extracted from a prospectively maintained database, HGP determination was performed retrospectively. Also, in 96 potentially eligible patients no HGP could be determined, which might have induced selection bias. The prognostic value of HGPs and their association with salvageability of recurrent disease after first resection of CRLM should therefore be validated, preferably in a prospective setting.

In conclusion, the present study confirms that over two thirds of patients develop a recurrence after primary resection of CRLM. Disease recurrence confined to the liver is more often seen in patients with dHGP at first CRLM resection whereas patients with non-dHGP more frequently develop multi-organ recurrence. Importantly, recurrences in patients with dHGP at first CRLM resection are more likely to be salvageable by local treatment modalities. HGPs determined at first CRLM resection had no prognostic value after salvage therapy for recurrent disease.

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Figure captions

Figure 1. Flowchart of patient selection.

Figure 2. DFS after first hepatic resection for CRLM compared for HGP.

Figure 3. PRS compared for HGP and treatment intent of the recurrence. (D-C: dHGP and curative intent, ND-C: Non-dHGP and curative intent, D-NC: dHGP and non-curative intent, ND-NC: Non-dHGP and non-curative intent)

Chapter 5

Are histopathological growth patterns predictive for the completion the liver-first approach for locally advanced rectal cancer and synchronous liver metastases?

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Adapted from European Journal of Surgical Oncology. 2019 Apr;45(4):591-596.

Abstract

Background

Patients with locally advanced rectal cancer (LARC) and synchronous liver metastases (sRLM) can be treated according to the liver-first approach. This study aimed to evaluate prognostic factors including histopathological growth patterns (HGPs) of the sRLM for completing treatment and in how many patients extensive lower pelvic surgery might have been omitted.

Methods

Retrospective analysis of all patients with LARC and sRLM treated at the Erasmus MC Cancer Institute according to the liver-first between 2003 and 2016.

Results

In total 129 consecutive patients were included. In 90 patients (70%) the liver-first was completed. Ten patients had a (near) complete response (ypT0-1N0) of their primary tumour. In 36 out of 39 patients *not* completing the liver-first protocol palliative rectum resection was withheld. dHGP was independently associated with a higher chance of completing the liver-first protocol (adjusted odds ratio (OR) [95% confidence interval (CI)]: 0.10 [0.01-0.80]; p=0.030). Subsequent analyses were performed in preoperatively available variables only. Optimal cut-offs for CEA level (53.15 µg/L), size (3.85 cm) and number (4) of RLMs were identified. A preoperative CEA level above 53.15 µg/L was an independent predictor for non-completion of the liver-first protocol (adjusted OR [95% CI]: 3.482 [1.451-8.372]; p=0.005).

Conclusion

Patients with CRLM displaying the dHGP have a higher chance of completing the liver-first protocol with curative intent. Nearly 40% patients with LARC and sRLM might be spared major pelvic surgery if the liver-first approach is applied.

Introduction

The liver-first approach – preoperative systemic chemotherapy followed by hepatic resection for colorectal liver metastases (CRLM) and resection of the primary tumour as last procedure – was first described in 2006. [1] This approach was initially considered for patients with advanced CRLM and a "normal" colorectal carcinoma (e.g. not locally advanced) because extensive metastases could not be treated in one session with the primary tumour. During the same period, our centre advocated the liver first approach for patients with locally advanced rectal cancer (LARC) and synchronous rectal liver metastases (sRLM). [2-4]

Low pelvic surgery after chemoradiotherapy (CRTx) is associated with considerable post-operative complications. This is a reason to treat the sRLM first, because postoperative morbidity of hepatic resections is generally low and patients who then have progressive disease may be spared the high morbidity of low pelvic surgery. Currently, only general prognostic factors and risk scores, such as the Fong criteria [5], are available to predict whether treatment will be completed. These criteria might not be sufficient for patients with LARC and sRLM. Recently, a new promising biomarker in patients with undergoing surgical treatment of CRLM has emerged. HGPs describe different patterns of tumour growth at the border of the CRLM where the tumour meets the normal liver parenchyma. [6] Based on prognosis two main HGP types can be distinguished: the desmoplastic (d) HGP and the non-desmoplastic. [7] In the dHGP the CRLM is separated from the liver parenchyma by a layer of desmoplastic stroma, whereas this desmoplastic capsule is absent in the non-dHGP. Patients with dHGP have superior prognosis compared to patients with non-dHGP. [6-15]

The liver-first approach also gives a good chance of an optimal pre-treatment (i.e. CRTx) of the LARC, hereby minimising the chance of a recurrence with also a chance on a (near) complete response. These patients could be treated with watchful waiting or other rectum sparing therapies and might only need extensive lower pelvic surgery in case of recurrence of disease.

The aim of the current study was twofold: to evaluate currently available prognostic factors including HGPs in patients treated for LARC and sRLM according to the liver-first protocol and to evaluate in how many patients extensive lower pelvic surgery might have been omitted when treated according to this approach for LARC and sRLM.

Material and methods

This is a retrospective analysis of a prospectively maintained patient database, consisting of all patients who underwent resection for RLM in a tertiary referral centre in the Netherlands. The database comprises of multiple perioperative and clinicopathological characteristics of both primary rectal cancer and RLM. The current study was approved by the medical ethics committee of the Erasmus University Medical Centre Rotterdam (MEC-2018-1031).

Patients and treatment approach

Since 2003 all patients presented at our centre with LARC and sRLM are treated according to the liver-first approach. All consecutive patients between 2003 and 2016 were included in the current study. LARC was defined as tumour >5 cm, expected distance of <2 mm to mesorectal fascia or ingrowth of adjacent organ (T4) on MRI or lymph node positive tumour meaning 1 lymph node >8 mm or 4 lymph nodes > 5 mm on CT scan or MRI). Patients described in previous publications by this group were also included in the study. [2, 4] Treatment for all patients was assessed in a multidisciplinary team (MDT). After systemic treatment with chemotherapy radiological tumour response was assessed. If no disease-progression was observed, laparotomy and liver resection were performed first. After liver surgery, neoadjuvant (C)RTx was administered after consultation again by the MDT. After finishing (C)RTx, patients were re-staged by CT Thorax/Abdomen and low pelvic MRI. Surgery of the primary tumour was performed as last stage. Surgery was planned 6-10 weeks after neoadjuvant (C)RTx. [16] Complications were categorised according to the Clavien-Dindo classification. [17]

Pre-operative chemotherapy

CT-scan of thorax and abdomen and CEA levels assessed the response to pre-operative chemotherapy after two or three cycles. Response was defined as decrease in tumour size and CEA levels. In patients scheduled for resection, the interval between the last course of chemotherapy and liver surgery was at least four weeks. Bevacizumab was excluded from the last course of chemotherapy to ensure that the interval between the last course of bevacizumab and surgery was at least six weeks.

Liver resection

The pathological response was categorized as complete response (CR) when no vital tumour cells were found, as partial response (PR) when both vital tumour cells and treatment effects were found and as stable disease (SD) when merely vital tumour cell and no treatment effect was observed.

HGP determination and categorisation

HGPs were determined in accordance with recently published international guidelines for HGP determination. [6] All available archival haematoxylin and eosin (H&E) stained tissue sections were evaluated while the observers were blinded for all other clinicopathological variables and outcome. As it may occur that multiple HGP types are observed on the same

tissue section, each tissue section was assessed for the HGP and scored using percentages. When multiple tissue section of one metastasis were available, these scores were averaged in order to obtain the metastasis level score. When multiple metastases were present and had tissue sections available, metastasis level scores were averaged in order to obtain patient level HGP score. In accordance with recent findings [7], patients were categorised as dHGP when 100% pure dHGP was seen. If any other proportion of non-dHGP was found on any of the evaluated H&E tissue sections, patients were categorised non-dHGP (i.e. <100% dHGP) accordingly.

Statistical analysis

Categorical data are presented as absolute numbers and percentages. Continuous data are presented as medians (and interquartile ranges (IQR)) or means (with standard deviations (SD)). Different proportions between groups were tested using the Chi-squared test. Medians were compared using the Mann-Whitney U test. The Kaplan-Meier method was used to estimate survival. Follow-up was estimated using the reverse Kaplan-Meier method. Overall survival (OS) was considered the time between the date of resection of the sRLM and the date of death. Patients were censored when alive at last follow-up date. Uni- and multivariable binary logistic regression analysis was performed to evaluate prognostic factors for the completion of the liver-first protocol and Odds Ratios (OR) for these factors were calculated. All variables with p-values below 0.05 on univariable analysis were included in the multivariable analysis. Receiver operating characteristic (ROC) analysis was used to identify the optimal cut-off points of the continuous variables (preoperative CEA, number and size of sRLM). The area under the curve (AUC) was used to determine the discriminatory performance of the logistic regression model. P values below 0.05 were considered significant. All analyses were performed using SPSS (SPSS version 24.0, Inc., IBM Corporation, Chicago, Ill., USA) and R version 3.5.1 (http://www.r-project.org).

Results

There were 152 patients with LARC and sRLM treated at our centre during the study period. In principle, all patients with LARC and sRLM, who are referred to our centre are treated according to the liver-first protocol since 2003. However, over the years there have been some exceptions. We identified 23 patients with LARC and sRLM who were not treated according to the liver-first protocol. The reasons for these exceptions are listed in figure 1.

Table 1. Baseline characteristics

		Total (N=129)	Completed LF (N=90, 70%)	Not completed LF (N=39, 30%)	p-value
Gender	Male	92 (71.3%)	69 (76.7%)	23 (59.0%)	0.041*
	Female	37 (28.7%)	21 (23.3%)	16 (41.0%)	
Age	Median (IQR)	62 (56-68)	63 (56-69)	62 (56-67)	0.565
ASA	ASA I-II	116 (89.9%)	78 (86.7%)	38 (97.4%)	0.062
	ASA > II	13 (10.1%)	12 (13.3%)	1 (2.6%)	
RLM characteristics					
Number of RLM	1 tumour	25 (19.4%)	17 (18.9%)	8 (20.5%)	0.830
	>1 tumour	104 (80.6%)	73 (81.1%)	31 (79.5%)	
Size of largest RLM	≤ 5 cm	106 (82.2%)	79 (87.8%)	27 (69.2%)	0.011*
	>5 cm	23 (17.8%)	11 (12.2%)	12 (30.8%)	
Preoperative CEA	≤ 200 µg/L	112 (91.1%)	81 (95.3%)	31 (81.6%)	0.014*
	>200 µg/L	11 (8.9%)	4 (4.7%)	7 (18.4%)	
	Missing	*6 patients*			
Bilobar metastasis	No	112 (91.1%)	81 (95.3%)	31 (81.6%)	0.018*
	Yes	11 (8.9%)	4 (4.7%)	7 (18.4%)	
EHD known preoperatively	No	110 (85.3%)	79 (87.8%)	31 (79.5%)	0.222
	Yes	19 (14.7%)	11 (12.2%)	8 (20.5%)	
Resection margin	R0	102 (91.1%)	82 (95.3%)	20 (76.9%)	0.004*
	R1	10 (8.9%)	4 (4.7%)	6 (23.1%)	
	Missing	*17 patients*			
HGP	dHGP	30 (28.8%)	28 (37.3%)	2 (6.9%)	0.002*
	Non-dHGP	74 (71.2%)	47 (62.7%)	27 (93.1%)	
	Missing	*25 patients*			

LF = liver first protocol; IQR = interquartile range; ASA = American society of anaesthesiologists; Physical Status Classification System; RLM = rectal liver metastases; CEA = Carcinoembryonic antigen; EHD = extrahepatic disease; R0 = Negative; R1 = Positive; HGP = Histopathological growth pattern; dHGP = Desmoplastic HGP; * = significant p-value

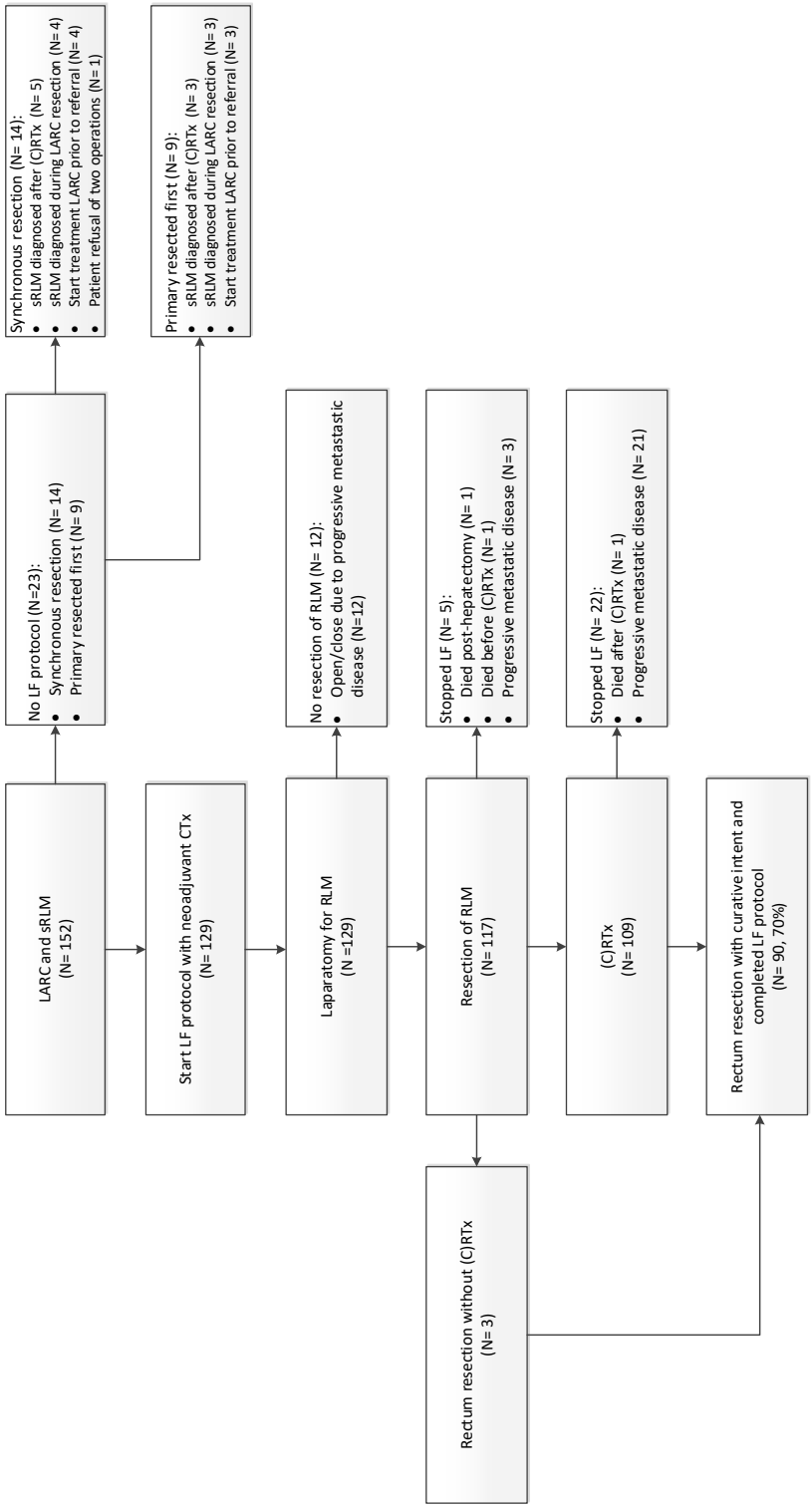


Figure 1. Flowchart of the clinical course of the 129 patients

In total, 129 patients with LARC and sRLM were treated according to the liver-first protocol and included in the current study. Baseline characteristics are displayed in table 1. A flowchart of the clinical course of these 129 patients is presented in figure 1.

Pre-operative chemotherapy and response of the liver metastases

In accordance with the liver-first protocol, all 129 patients received pre-operative chemotherapy (median 4 cycles (IQR: 3-6)). Patients predominantly received capox (N= 104, 81%). Other treatment regimens included folfox (N=13, 10%), folfiri (N= 7, 8%), capecitabine (N= 2, 2%), irinotecan (N=2, 2%) and folfirinox (N= 1, 1%). Of one patient the type of chemotherapy was unknown. In 34 patients (26%) bevacizumab was added to the regimen. After chemotherapeutic treatment 5 patients (4%) had a complete radiological response, while 102 patients (79%) had responded partially and 20 patients (16%) had stable disease. Two patients (2%) had growth of their metastases despite systemic treatment, but were treated surgically nonetheless.

Surgical treatment, pathological response and HGP of the liver metastases

In total 117 of the 129 patients were treated surgically for RLM. In twelve patients (9%) RLMs were not resected due to intra-operatively discovered unexpected progression of metastatic disease. Of the 129 patients that underwent laparotomy for intended surgical treatment of sRLM 121 (94%) had no or only mild complications (Clavien-Dindo grade 0-2) and 8 patients (6%) had severe complications (Clavien-Dindo grade >2), of whom one patient (1%) died postoperatively. Histopathological evaluation of the liver tumours showed pathological PR in 84 patients (72%), CR in 12 patients (10%) and SD in 5 patients (5%). In 15 patients (13%) there was no pathological response evaluation available, due to treatment with ablative therapy only (N= 5) or it was not reported in the pathology reports (N= 10). As a resection specimen is a prerequisite for HGP determination, the 117 patients that underwent resection of their sRLM were potentially eligible for HGP determination. In the 12 patients with a CR of the sRLM HGP assessment was not possible. In 1 patients H&E slides were unavailable. Therefore, the HGP could be determined in 104 out of the 117 resected patients (89%) of whom 30 (29%) had sRLM displaying 100% pure dHGP.

Table 2. Treatment for primary tumour if not resected curatively

	N=39 (%)
Palliative rectum resection	3 (7.7%)
Palliative (C)RTx and colostomy	9 (23.1%)
Colostomy	2 (5.1%)
Palliative (C)RTx	13 (33.3%)
Rectal stenting	1 (2.6%)
None or palliative CTx and/or pain medication only	10 (25.6%)
Died post hepatectomy	1 (2.6%)

(C)RTx = (chemo)radiotherapy; CTx= chemotherapy

Rectal cancer

In 39 of the 129 patients (30%) the liver first protocol could not be completed. As stated, twelve patients did not undergo liver resection. In five patients sRLM were resected, but did not start with (C)RTx due to progressive metastatic disease or interim death. In the remaining 22 patients, 21 revealed progressive metastatic disease at restaging between liver and rectal surgery and one of them died before rectal surgery. In these 21 patients the median time between liver resection and restaging that revealed progressive metastatic disease was 3 months (IQR: 3.0-4.5). The treatment given regarding their primary tumour is displayed in table 2.

Table 3. Uni- and multivariable binary logistic regression analysis for the non-completion of LF

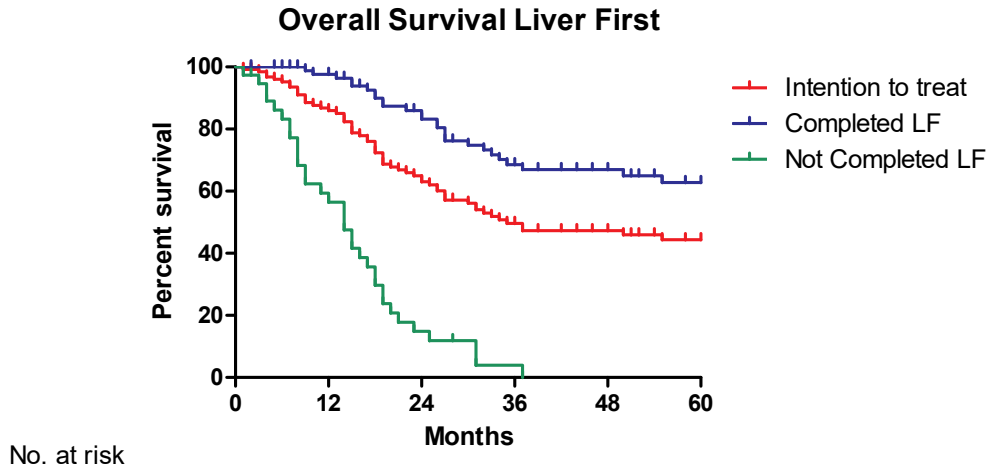
Variables	Univariable		Multivariable	
	OR [95% CI]	p-value	OR [95% CI]	p-value
Male gender	0.438 [0.196-0.977]	0.044*		
Age (cont.)	1.009 [0.971-1.048]	0.657		
ASA > II	0.171 [0.021-1.364]	0.096		
Number of RLM >1	0.902 [0.353-2.309]	0.830		
Size of metastasis >5 cm	3.192 [1.263-8.070]	0.014*	2.099 [0.579-7.601]	0.259
CEA >200	4.573 [1.251-16.717]	0.022*		
Bilobar RLM	1.883 [0.849-4.175]	0.120		
Pre-operative EHD	1.853 [0.681-5.043]	0.227		
R1 resection	6.150 [1.584-23.873]	0.009*	3.639 [1.255-14.898]	0.072
dHGP	0.124 [0.027-0.563]]	0.007	0.099 [0.012-0.797]	0.030*

LF= liver first protocol; OR = odds ratio; ASA= American society of anesthesiologists; CRLM= colorectal liver metastases; CEA= carcinoembryonic antigen; EHD = extra hepatic disease; * = significant p-value

In 90 patients (70%) surgery of the rectum with curative intent was performed and the liver-first protocol was completed. Of these 90 patients, 78 (87%) did not experience any signs of obstruction that needed additional procedures. In eleven patients (12%) there was the need for a colostomy (5 prior to and 6 during the liver-first protocol) and in one patients (1%) a rectal stent was placed. Of the 90 patients that completed the treatment trajectory 77 (86%) had no or only mild complications (Clavien-Dindo grade 0-2) and 13 patients (14%) had severe complications (Clavien-Dindo grade >2), but no postoperative mortality was observed. Nine patients (10%) had a pathological complete response of the primary tumour and one patient had an ypT1N0 tumour.

Follow-up and survival

Median follow-up of survivors was 58 months (IQR: (30 – 86 months)). Median OS of the complete intention to treat group was 35 months (IQR: 18 – 92 months). Median OS in the 90 patients that completed the liver-first protocol was not reached at five years. For the 39 patients that did not complete the liver-first protocol the median OS was 14 months (IQR: 8 – 19 months). The Kaplan-Meier curves are presented in figure 2.



No. at risk						
Intention to treat	129	101	67	44	35	28
Completed LF	90	81	62	43	35	28
Not compl. LF	39	20	5	1	0	0

Figure 2. Kaplan-Meier graphs for OS

Prognostic factors for non-completion of the liver-first protocol

Twenty-eight out of the 30 patients (93%) with pure dHGP sRLM completed the liver-first approach with curative intent, compared to 47 out of the 74 patients (63%) with non-dHGP sRLM (p=0.002). The only variable significantly associated with the non-completion of the liver-first protocol on multivariable analysis was the presence of non-dHGP (OR: 0.10 [0.01-0.80]; p=0.030; table 3). However, as the HGP and the resection margin status are only available after liver resection we also performed logistic regression analyses without these variables and thereby evaluating only the preoperatively available variables. This analysis showed no significant association between any of the tested variables and not completing the liver-first protocol. The subsequently performed ROC analysis identified the optimal cut-offs for the continuous variables: preoperative CEA (53.15 µg/L), size (3.85 cm) and number (4) of RLMs. The use of optimal cut-offs slightly improved performance of the logistic regression model, as the AUC increased from 0.699 to 0.713. The improved logistic regression model showed that patients with CEA levels above 53.15 µg/L have a higher odds for non-completion of the liver-first protocol (OR: 3.482 [1.451-8.372]; p=0.005; table 4). However, seventeen patients out of the 36 patients with a CEA level of >53.15 µg/L still completed the treatment sequence.

Table 3. Uni- and multivariable binary logistic regression analysis of preoperatively available variables for the non-completion of LF

Variables	**Univariable**		**Multivariable**	
	OR [95% CI]	**P-value**	**OR [95% CI]**	**P-value**
Male gender	0.438 [0.196-0.977]	0.044*	0.524 [0.216-1.271]	0.153
Age (cont.)	1.009 [0.971-1.048]	0.657		
ASA > II	0.171 [0.021-1.364]	0.096		
Number of RLM >1	0.902 [0.353-2.309]	0.830		
Size of metastasis >5 cm	3.192 [1.263-8.070]	0.014*	2.456 [0.917-6.578]	0.074
CEA > 200	4.573 [1.251-16.717]	0.022*	3.742 [0.968-14.464]	0.056
Bilobar RLM	1.883 [0.849-4.175]	0.120		
Pre-operative EHD	1.853 [0.681-5.043]	0.227		

Improved uni- and multivariable binary logistic regression analysis of preoperatively available variables with optimal cut-offs for the non-completion of LF

Variables	**Univariable**		**Multivariable**	
	OR [95% CI]	**P-value**	**OR [95% CI]**	**P-value**
Male gender	0.438 [0.196-0.977]	0.044*	0.481 [0.194-1.190]	0.113
Age (cont.)	1.009 [0.971-1.048]	0.657		
ASA > II	0.171 [0.021-1.364]	0.096		
Number of RLM >4	1.625 [0.751-3.518]	0.218		
Size of metastasis >3.85 cm	2.251 [1.021-4.962]	0.044*	1.470 [0.593-3.642]	0.405
CEA > 53.15	4.000 [1.746-9.162]	0.001*	3.482 [1.451-8.372]	0.005*
Bilobar RLM	1.883 [0.849-4.175]	0.120		
Pre-operative EHD	1.853 [0.681-5.043]	0.227		

LF= liver first protocol; OR = odds ratio; ASA= American society of anesthesiologists; CRLM= colorectal liver metastases; CEA= carcinoembryonic antigen; EHD = extra hepatic disease; * = significant p-value

Discussion

The current study presents the results of the largest series of patients treated for rectal cancer and sRLM according to the liver-first protocol to date. Patients with CRLM displaying the non-dHGP have a higher chance of non-completion of the liver-first protocol. New cut-off threshold for several well-known preoperatively assessable risk factors that improve prognostication in patients with sRLM were identified. Most importantly, it demonstrated that in 92% (36 out of 39) of the patients not completing the liver-first protocol extensive pelvic surgery was eventually not necessary. Another ten patients had responded so well to the preoperative CTx and (C)RTx (ypT0N0 N= 9 and ypT1N0 N= 1) that rectum preservation could have been an option. This adds up to 36% (46 out of 129 patients) of the total group in whom omission of extensive rectal surgery could have been considered.

In this and other series describing the liver-first approach, approximately 30-40% of patients do not complete the full liver-first treatment protocol. [1, 2, 4, 18, 19] In order to define in which patients local treatment is desirable, this study evaluated prognostic factors for completion of the liver-first protocol. With regard to prognosis in patients with colorectal liver metastases several risk scores have been proposed [5, 20-22], of which the Fong score is most often utilised. [5] HGPs are a fairly new biomarker in patients undergoing resection of CRLM and their assessment was recently standardised in international guidelines. [6] In the present study HGPs were the only variable significantly associated with the non-completion of the liver-first protocol on multivariable analysis. In this study one significant prognostic variable that is preoperatively available with regard to completion of the protocol was found, namely CEA levels above 53.15 μg/L. This might be useful in counselling patients, yet cannot be used to withhold therapy according the liver first protocol as seventeen patients out of the 36 patients with a CEA level of >53.15 μg/L still completed the treatment sequence. No literature is available specifically describing prognostic factors for the non-completion of the treatment sequence in patients treated for sRLM, therefore external validation of the results of this study is warranted. Further research is needed to identify new biomarkers that can improve patient stratification and selection before starting the liver-first protocol. In addition, as HGPs at this moment can only be determined postoperatively, future studies should focus on evaluating possibilities of preoperative HGP determination. If HGP scores would be assessable preoperatively, they might be utilised in patient selection for the liver-first approach and personalised treatment strategies for CRLM patients in general. That being said, the currently existing inability of preoperative HGP determination does not entirely preclude clinical applicability of HGPs in the clinical decision making. The HGP can be determined after the operation that is least prone to morbidity – the liver resection. Nearly all patients with dHGP completed the liver-first protocol with curative intent, in contrast to less than two thirds of patients with non-dHGP. This indicates that treating physicians could take the HGP in consideration when selecting patients for the operation which is most prone to morbidity – the TME after (C)RTx. In case of non-dHGP sRLM one might apply a higher threshold for selecting patients for TME as it is more likely that these patients experience systemic disease progression. Contrastingly, one might go to greater lengths than usual to

perform a TME on patients with dHGP as it is less likely that systemic disease progression occurs. [12]

Regardless of HGP, patients with LARC and sRLM are at high risk of disease progression and futile extensive pelvic surgery. Therefore, the liver-first approach could be the optimal approach in patients with sRLM, especially as it increases the possibilities for rectum sparing strategies. TAMIS or watchful waiting could be considered if a clinical (near) complete response is seen, as it is oncological safe to preserve the rectum in selected cases. However, should TAMIS be performed and if histopathology reveals a >ypT1N0 tumour, local and systemic recurrence is lurking and completing major excision is recommended. [23-29] Several studies have shown that pelvic surgery for rectal cancer is associated with high morbidity rates, resulting in long-term complications. [30, 31] However, in these studies stage IV patients are being disregarded. By applying the liver-first approach pre-eminently those patients are selected out who will not have any survival advantage from major surgery and can therefore be saved from this kind of surgery. Therefore, it is remarkable that nearly all attention for rectum sparing therapies goes out to patients with stage I and II (sometimes stage III) rectal cancer, since these patients experience relatively good oncological outcome and survival rates. [26, 28, 32, 33]

The majority (70%) of patients treated according to this protocol can be treated with curative intent. Similar results have been shown in multiple other studies. [1, 2, 4, 18] Recently, it was acknowledged by an intention-to-treat analysis, that no differences in completion rate between the classical approaches and the liver-first approach are observed, showing that up to 35% of patients does not complete the full treatment trajectory irrespective of the chosen treatment approach. [34] In addition, no differences have been demonstrated in the literature between the three treatment sequences (liver-first, bowel-first or synchronous resection) in terms of OS, disease free survival or postoperative complication rates. [18, 35-39] However, no randomised controlled trial comparing the three sequences has been performed and therefore the currently available literature might subject to selection bias.

A proportion of incurable patients with the primary tumour in situ require additional surgical treatment nonetheless, due to obstruction, perforation or pain. [40, 41] In this study, three patients not completing the liver-first protocol ultimately underwent rectum excision. In addition, systemic chemotherapy induces rapid symptom relief in patients with high-risk rectal cancer. [42] This, combined with the fact that most patients in the current study did not need a surgical intervention, implies that it is relatively safe not to resect the primary rectal tumour. A recent systematic review and a meta-analysis comparing non-resection and resection in patients with unresectable stage IV CRC show similar complication and symptom rates in both groups, which validates the currently obtained results. [40, 41] The systematic reviews failed to find a survival benefit. [40, 43] However, in contrast, a meta-analysis [41] and a nationwide population-based study did. [44] It seems as if there will only be certainty about whether or not the resection of the primary tumour is beneficial for overall survival in the case of unresectable metastases when the results of an ongoing

randomised controlled trial (CAIRO 4) will be published. [45] Considering the fact that symptom rates are comparable between resected and non-resected patients and a survival benefit, if any, remains to be proven, the liver-first protocol is a reasonable approach in patients with synchronous RLM and rectal cancer.

This study has several limitations that should be acknowledged. This is a retrospective analysis of selected patients in a single institution. It should also be taken into account that some patients start the liver-first protocol, but have evident progression under chemotherapy and are therefore excluded from liver surgery, as limited yield should be expected from surgical treatment in case of disease progression during chemotherapeutic treatment.[46] Since the currently used database consists of patients who underwent laparotomy for intended surgical treatment of sRLM, patients that stopped the liver-first protocol before resection of the RLMs were not included in this study. Therefore, it should be given consideration that a small proportion of patients that initially started the liver first protocol was not included in the analysis, which could have affected the results obtained.

Conclusion

The current study has shown that in this series patients with CRLM displaying the dHGP have a higher chance of completing the liver-first protocol with curative intent. Although a preoperatively available predictor (CEA>53.15 μg/L) for the non-completion of the liver-first protocol was found as well, this cannot be used to exclude patients from the liver-first protocol as the majority of patients with CEA levels of >53.15 μg/L still underwent resection of both the primary tumour and the hepatic metastasis with curative intent. Furthermore, over one-third of patients could be spared from extensive lower pelvic surgery. In patients not completing the liver-first protocol extensive pelvic surgery was ultimately not necessary in 92% of the cases and a substantial proportion of patients could have been candidates for rectal preserving therapies.

Figure captions

Figure 1. Flowchart of the clinical course of the 129 patients

Figure 2. Kaplan-Meier graphs for OS

Table 1. Baseline characteristics of patients treated by the liver first protocol

Table 2. Primary tumour not resected curatively

Table 3. Results of the logistic regression analyses of all variables

Table 4. Results of the logistic regression analyses of preoperatively available variables

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Chapter 6

Preoperative systemic chemotherapy alters the histopathological growth patterns of colorectal liver metastases

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Accepted for publication in in The Journal of Pathology: Clinical Research

Abstract

Histopathological growth patterns (HGPs) are a reliable, reproducible, and strong prognostic biomarker that can be assessed on H&E of resected colorectal liver metastases (CRLM). Assessment estimates the relative fraction of the tumour-liver interface for each of three growth patterns; the desmoplastic HGP reflects good prognosis. Whether preoperative chemotherapy affects the HGP is currently unclear. The present international multicentre study evaluates this in an original cohort of 877 consecutive patients treated in the Netherlands, an external validation cohort of 1203 consecutive patients treated in the USA, and a post-hoc analysis from the phase III randomised controlled EORTC 40983 trial (n=70). All patients underwent resection of CRLM with or without preoperative systemic chemotherapy. Trial patients were randomised between perioperative chemotherapy and resection or resection alone. HGPs were determined according to consensus guidelines and compared for preoperative treatment status. Three separate tumour regression gradings were available for the trial cohort. These were correlated with HGP stratified for treatment arm. In the original cohort the average presence of desmoplastic HGP was 43% for chemo-naive versus 67% for preoperatively treated patients ($p<0.001$). A significant association between chemotherapy and desmoplastic HGP was found on multivariable analysis (β[95%CI]: 24.53 [18.27;30.79], $p<0.001$). In the validation cohort the average presence of desmoplastic HGP was 40% for chemo-naive versus 63% for preoperatively treated patients ($p<0.001$). This association remained on multivariable analysis (β[95%CI]: 23.94 [18.48;29.39], $p<0.001$). In the EORTC 40983 trial the average desmoplastic HGP presence was 33% in the resection arm versus 61% in the chemotherapy arm ($p=0.005$). Chemotherapy was independently associated with an increase in desmoplastic HGP (β[95%CI]: 25.01 [3.77-46.25], $p=0.022$). All three tumour regression gradings were significantly associated with the desmoplastic HGP in the chemotherapy arm (all $p<0.03$). None were associated in the resection arm (all $p>0.11$). Preoperative chemotherapy induces histopathological changes that alter the HGP of CRLM.

Introduction

Histopathological growth patterns (HGPs) describe distinct phenotypes of tumour growth at the transition zone between pre-existing liver parenchyma and colorectal liver metastases (CRLM). [1] HGPs have been associated with prognosis in patients undergoing resection of CRLM. [1-9] The determination of HGPs has been standardised in international guidelines. [1] Three main HGP phenotypes are recognised: the replacement, the pushing and the desmoplastic type HGP (figure 1A-C). [1, 10] Based on prognosis, a dichotomy can be made. Patients with any observed non-desmoplastic HGP (i.e. any pushing or replacement HGP) have worse survival outcomes compared to patients with pure desmoplastic HGP.[5] This difference in survival was less apparent for patients treated with preoperative systemic chemotherapy.[5] Furthermore, higher proportions of the desmoplastic HGP were observed in pre-treated patients. These results suggest that preoperative chemotherapy may affect the HGP and raises questions regarding the assessment and value of this biomarker after preoperative systemic treatment. These results require external validation.

The European Organization for Research and Treatment of Cancer (EORTC) intergroup study 40983 randomised controlled phase III trial compared surgery alone to surgery combined with perioperative systemic chemotherapy in patients with resectable CRLM. [11, 12]

This study evaluates the effect of preoperative systemic chemotherapy on the HGPs of CRLM in an original cohort of consecutive patients undergoing resection in the Netherlands, a similar external validation cohort of patients treated in the USA, and in a post-hoc analysis of a subset from the EORTC 40983 randomised controlled clinical trial.

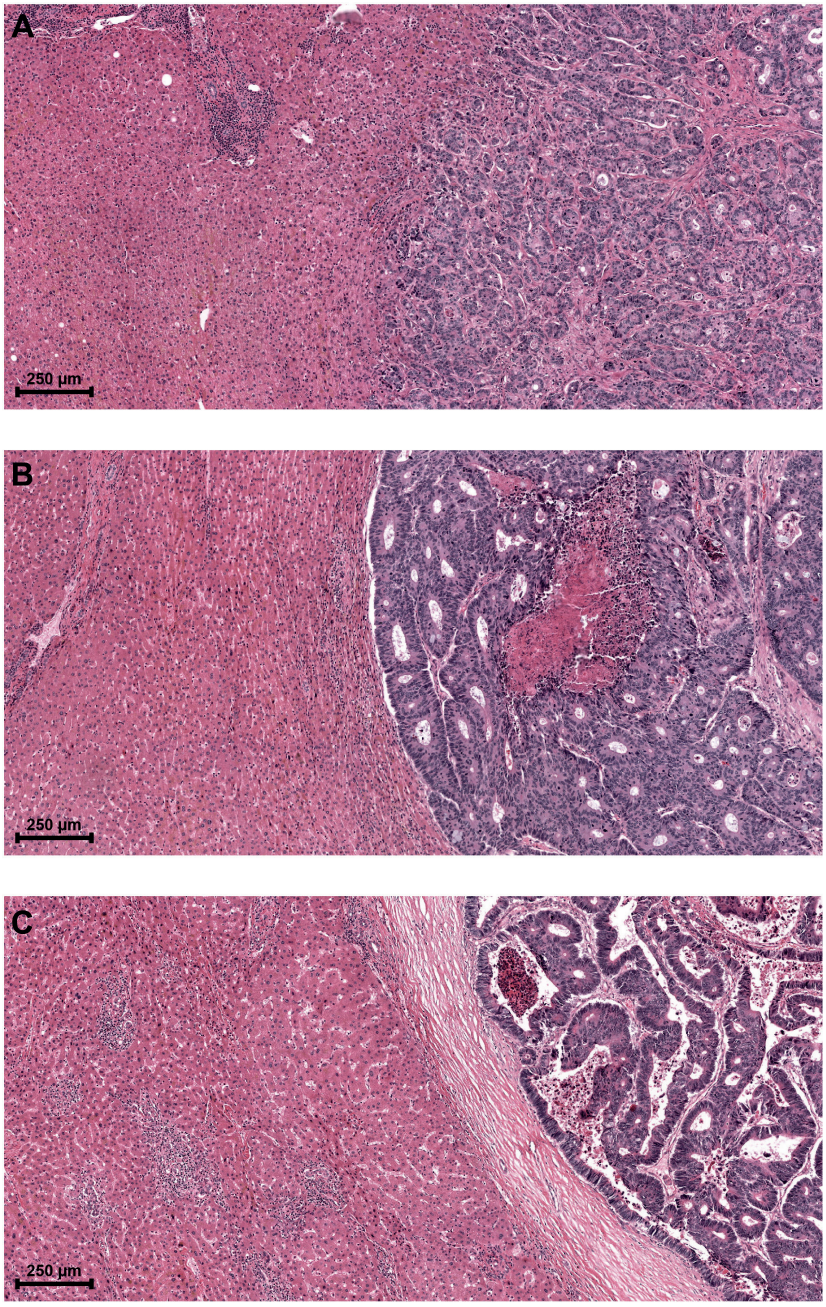

Figure 1A-C. Examples of the distinct histopathological growth patterns (HGPs). 1A. Example of replacement type HGP in which tumour cells "replace" hepatocytes and infiltrate the liver parenchyma with direct tumour-liver cell contact. 1B. Example of pushing type HGP in which the liver parenchyma is "pushed" aside but is not infiltrated. No direct tumour-liver cell contact is present. 1C. Example of desmoplastic type HGP, in which the tumour is separated from the liver parenchyma by a desmoplastic capsule. No direct tumour-liver cell contact is present.

Material and methods

The current study was performed according to the STROBE guidelines for cohort studies and approved by the medical ethics committee of the Erasmus University Medical Centre Rotterdam (MEC 2018-1743). [13] A waiver for renewed written informed consent was granted .

Original cohort
All consecutive patients undergoing first resection of CRLM between January 2000 and February 2019 at the Erasmus MC Cancer Institute (Rotterdam, the Netherlands) were evaluated for eligibility. Part of this cohort was previously described by Galjart et al. [5] In accordance with the previous study, patients with incomplete resection, treated by ablation only, or in whom the HGP could not be determined were excluded. Patient characteristics, primary tumour and CRLM characteristics, treatment details, follow-up, and disease recurrence were extracted from a prospectively maintained database.

External validation cohort
All consecutive patients undergoing first resection of CRLM between January 2000 and January 2019 at the Memorial Sloan Kettering Cancer Center (MSKCC)(New York City, NY, USA) were considered for inclusion in the external validation cohort. Similar exclusion criteria were applied. Additionally, patients receiving preoperative hepatic arterial infusion pump (HAIP) chemotherapy were excluded as this study evaluates the relationship between HGPs and preoperative *systemic* chemotherapy. Data regarding patient characteristics, primary tumour and CRLM characteristics, treatment details, follow-up, and disease recurrence were also extracted from a prospectively maintained database.

Randomised patient cohort
A subset of patients from the EORTC 40983 trial (NCT00006479) of whom digitalised H&E tissue sections were available were included for post-hoc analysis. This subset of patients has been described previously. [14] The details of the original trial including its short- and long-term results are reported elsewhere.[11, 12] In summary, the EORTC 40983 trial randomised 364 patients with up to four resectable CRLM between either perioperative chemotherapy and resection (CTx arm), or resection only (Rx arm). Perioperative chemotherapy consisted of the FOLFOX4 regimen with six planned preoperative and six planned postoperative cycles. [15]

HGP determination
Determination of HGPs was done in accordance with international consensus guidelines. [1] Assessment was performed by light microscopy on all available haematoxylin and eosin (H&E) stained tissue sections from all resected CRLM and blinded for outcome, preoperative treatment status, and all other clinicopathological patient characteristics. Assessment was performed by trained observers (PN, DH, ES, BG) together/in consultation with a dedicated HGP pathologist (PV).[1] For the EORTC 40983 trial patients assessment was performed on

digitalised H&E tissue sections[14] by trained observers (PN, DH, BG) and a dedicated HGP pathologist (PV) separately. Discordant cases were subsequently reviewed by all observers together (PN, DH, BG, PV) to achieve consensus. As multiple HGPs can be present in a single tumour, the entire tumour-liver interface on each slide was examined. During assessment, the relative fraction of the total length of the interface of desmoplastic, replacement and/or pushing HGP is estimated and expressed as percentage. Herein each proportion of the interface representing 5% or more was taken into account. Metastasis level estimates were calculated with equal weights assigned to individual tissue sections. The final patient level HGP scores were subsequently calculated with equal weights assigned to individual metastases. The average presence of each distinct HGP observed was determined in each of the three cohorts and stratified for preoperative treatment status. The proportional distribution of distinct HGPs was displayed graphically and stratified for preoperative treatment status, in which the horizontal axis represented individual patients and the vertical axis the corresponding observed proportion of each distinct HGP at the tumour-liver interface. The average presence of each distinct HGP is represented by its surface area. HGP determination was not performed if no viable tumour was present, in case of inadequate tissue preservation of H&E tissue section(s), or if less than 20% of the tumour-liver interface was assessable.[1] In accordance with previous findings, patients were classified as either pure desmoplastic HGP (i.e. 100% desmoplastic HGP), or non-desmoplastic HGP (any replacement and/or pushing HGP).[5, 16] A simplified decision tree, adapted with permission from van Dam et al.[1], to determine the HGP on a patient level based on this clinically relevant distinction is provided in figure 2. With regard to preoperative treatment stratification: patients in the original cohort and the external validation cohort who received any systemic chemotherapy within six months prior to CRLM resection – with the exception of capecitabine as radiosensitiser in the treatment for rectal cancer – were considered preoperatively treated. In addition, several examples of the desmoplastic HGP with and without preoperative systemic chemotherapy were selected and were evaluated in a descriptive manner.

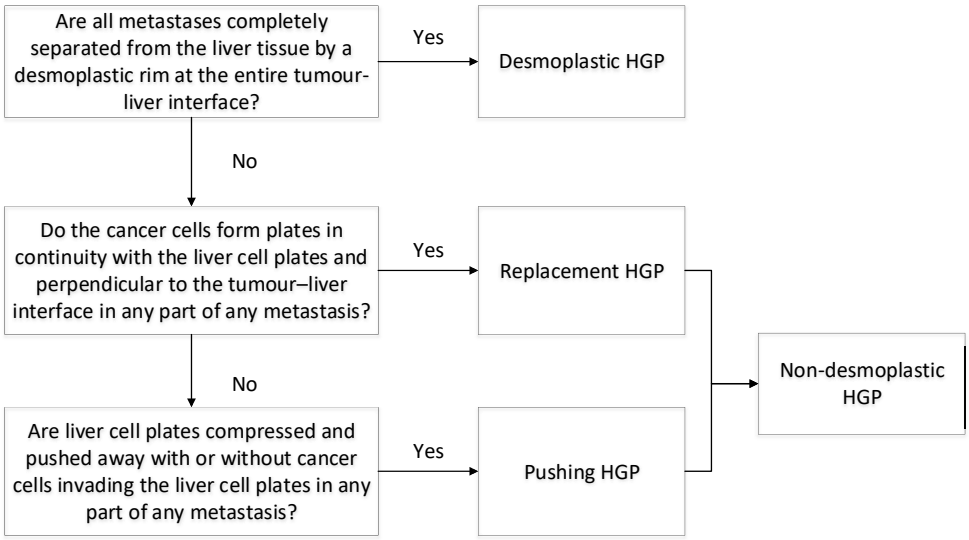

Figure 2. Simplified decision tree to determine the growth patterns of liver metastases based on the key histopathological characteristics. Adapted with permission from van Dam et al.[1]

Tumour regression gradings

For the subset of the EORTC 40983 trial three separate tumour regression gradings were available; the Mandard tumour regression grade (TRG)[17], the mean percentage of tumour cells according to Blazer et al.[18], and the histological tumour regression according to Rubbia-Brandt[19]. These three tumour regression gradings were all determined prior to the conception of this study, by an independent senior pathologist (CJ) not involved in HGP assessment, and blinded for treatment arm and patient outcome. The Mandard TRG recognises five grades: 1 absence of cancer cells replaced by abundant fibrosis, 2 rare residual cancer cells scattered throughout abundant fibrosis; 3 increase in the number of cancer cells but fibrosis remains predominant; 4 residual cancer outgrowing fibrosis; and 5 absence of regressive changes.[17] The method described by Blazer et al. assesses pathological response to preoperative chemotherapy in patients with colorectal liver metastasis by semi-quantitatively estimating the percentage of viable tumour in relation to tumour surface area.[18] The histological tumour regression according to Rubbia-Brandt is an adaptation of the Mandard TRG and recognises three grades of tumour regression in colorectal liver metastases: no histological tumour regressive or response changes (NHR); partial histological tumour response (PHR); and major or complete histological tumour response (MjHR).[19] Tumour regression according to all three grading systems was correlated with HGP stratified for treatment arm.

Statistical analysis

Categorical data are reported using absolute numbers and corresponding percentages and continuous data using medians with corresponding interquartile ranges (IQR). Proportional differences were evaluated with the Chi-squared test. Differences in medians between two groups were assessed using the Mann–Whitney U test. The average presence of distinct HGPs was compared across preoperative treatment status by means of a parametric t-test. To evaluate whether preoperative chemotherapy was associated with the observed proportion of the desmoplastic HGP at the interface, uni- and multivariable linear regression analyses were performed and expressed using the β coefficient with corresponding 95% confidence intervals (CI). Additional uni- and multivariable linear regression models were computed in a combined cohort of all patients with available data on *APC*, *KRAS*, *NRAS*, and *BRAF* mutational status, as well as microsatellite instability (MSI) status. The association between tumour regression and the desmoplastic HGP was assessed in the trial cohort for each of the three gradings and in each treatment arm separately by multivariable logistic regression. Results are graphically displayed using scatter plots with corresponding regression line and are reported using the β coefficient with corresponding 95% confidence intervals (CI). The reversed Kaplan-Meier method was applied to estimate the median follow-up time for survivors. Overall survival (OS) was defined as the time in months from date of resection until the date of death. When alive, patients were censored at date of last follow-up. Kaplan-Meier analysis was used to determine survival estimates which were compared by means of the log-rank test. Uni- and multivariable Cox regression analysis for OS was performed in the original and the external validation cohort to correct for potential confounding. In these cohorts, survival analyses on the HGP stratified by preoperative chemotherapy have previously been performed and were therefore not repeated.[5, 16, 20] Results of the Cox regression analyses were expressed using hazard ratios (HR) and corresponding 95% CIs. For the EORTC 40983 trial subset the OS difference between treatment arms was estimated and compared to the long-term results of the entire trial (expressed as HR with corresponding 95%CI).[12] In an attempt to assess differences between pre-treated and chemo-naive patients with a desmoplastic HGP (i.e. 100% desmoplastic), clinicopathological factors and OS were compared between these subgroups in a combined cohort of all available patients. All analyses were performed using R version 4.1.0 (http://www.r-project.org).

Results

Original cohort

At the Erasmus MC Cancer Institute 1257 patients were treated surgically for CRLM between January 2000 and February 2019. Patients were excluded due to incomplete resection of CRLM (n=133), ablative therapy only (n=33), and unsuitable or unavailable H&E tissue sections for HGP determination (n=214). The remaining 877 (70%) patients were included for analysis. Preoperative systemic chemotherapy was administered to 462 patients (53%). Baseline patient characteristics stratified by preoperative treatment are presented in table 1. A graphical display of the distinct HGPs stratified for preoperative treatment status is shown in figure 3. The average presence of desmoplastic HGP observed at the interface was 43% in chemo-naive versus 67% in preoperatively treated patients (p<0.001, figure 3D). Preoperative systemic chemotherapy was independently associated with a higher proportion of desmoplastic HGP observed (adjusted β [95%CI]: 24.57 [18.28; 30.87], p<0.001, table 2). On multivariable analysis, a non-desmoplastic HGP was associated with an adjusted HR [95%CI] for OS of 1.56 [1.23-1.98] (p<0.001, supplementary table 1).[5, 16]

External validation

During the study period 2550 patients were treated surgically for CRLM at the MSKCC and were potentially eligible for inclusion. Patients were excluded due to any preoperative HAIP chemotherapy (n=202), incomplete resection of CRLM (n=84), ablative therapy only (n=14), unsuitable or unavailable H&E tissue sections for HGP determination (n=1042), and missing clinical information on in- and exclusion criteria (n=5). In total, 1203 (47%) patients were included for analysis. Preoperative systemic chemotherapy was administered to 793 patients (66%). Baseline characteristics compared for preoperative treatment are presented in table 1. A graphical display of the distinct HGPs stratified for preoperative treatment status is shown in figure 4. The average presence of desmoplastic HGP observed at the interface was 40% in chemo-naive patients versus 63% in preoperatively treated patients (p<0.001, figure 4D). On multivariable analysis preoperative chemotherapy was significantly associated with a higher proportion of desmoplastic HGP (adjusted β [95%CI]: 24.18 [18.70; 29.66], p<0.001, table 2). A non-desmoplastic HGP was associated with an adjusted HR [95%CI] for OS of 1.75 [1.29-2.37] (p<0.001, supplementary table 2).[16, 20]

Table 1. Baseline characteristics of all three cohorts stratified by preoperative treatment status

		Original cohort Erasmus MC Cancer Institute			External validation cohort MSKCC			Randomised patient cohort EORTC 40983 trial		
		Preoperative chemotherapy			Preoperative chemotherapy			Treatment arm		
		No	Yes		No	Yes		Rx arm	CTx arm	
		n = 462 (%)	n = 415 (%)	p-value*	n = 410 (%)	n = 793 (%)	p-value*	n = 40 (%)	n = 30 (%)	p-value*
Age at resection CRLM - *(median [IQR])*		66.0 [59.2. 73.0]	63.0 [56.0. 69.0]	<0.001	62.0 [52.0. 72.0]	57.0 [48.0. 66.0]	<0.001	67.5 [59.8. 72.0]	65.0 [58.5. 71.8]	0.536
Gender	Male	298 (65)	270 (65)	0.863	228 (56)	452 (57)	0.645	22 (55)	19 (63)	0.484
	Female	164 (35)	145 (35)		182 (44)	341 (43)		18 (45)	11 (37)	
Primary tumour location	Right-sided	80 (18)	65 (16)	0.655	141 (36)	204 (27)	0.003	8 (20)	8 (27)	0.768
	Left-sided	195 (43)	175 (43)		175 (44)	331 (45)		15 (38)	11 (37)	
	Rectal	175 (39)	170 (41)		80 (20)	207 (28)		16 (40)	11 (37)	
	Missing	*12 (3)*	*5 (1)*		*14 (3)*	*51 (6)*		*1** (2)*	*0 (0)*	
Adjuvant CTx for primary	No	369 (80)	383 (93)	<0.001	140 (56)	256 (56)	0.967	30 (75)	23 (77)	0.872
	Yes	92 (20)	28 (7)		109 (44)	198 (44)		10 (25)	7 (23)	
	Missing	*1 (0)*	*4 (1)*		*161 (39)*	*339 (43)*		-	-	
(y)pT-stage	0	6 (1)	15 (4)	0.004	0 (0)	7 (1)	<0.001	0 (0)	0 (0)	0.823
	1	11 (2)	4 (1)		21 (5)	10 (1)		1 (3)	0 (0)	
	2	74 (16)	47 (12)		46 (12)	65 (9)		4 (10)	4 (13)	
	3	327 (72)	259 (69)		263 (67)	462 (66)		30 (77)	23 (77)	
	4	38 (8)	51 (14)		60 (15)	153 (22)		4 (10)	3 (10)	
	Missing	*6 (1)*	*39 (9)*		*20 (5)*	*96 (12)*		*1 (2)*	*0 (0)*	
(y)pN-stage	0	196 (43)	139 (37)	0.202	185 (46)	246 (32)	<0.001	17 (44)	13 (43)	0.893
	1	170 (38)	151 (40)		155 (38)	307 (40)		17 (44)	12 (40)	
	2	87 (19)	83 (22)		64 (16)	213 (28)		5 (13)	5 (17)	
	Missing	9 (2)	42 (10)		6 (1)	27 (3)		1 (2)	0 (0)	
Differentiation grade	pCR	5 (2)	13 (4)	0.189	-	-		0 (0)	0 (0)	0.733
	G1	6 (2)	6 (2)		-	-		9 (23)	7 (23)	
	G2	267 (93)	266 (89)		-	-		28 (72)	20 (67)	

		Original cohort Erasmus MC Cancer Institute			External validation cohort MSKCC			Randomised patient cohort EORTC 40983 trial		
		Preoperative chemotherapy			Preoperative chemotherapy			Treatment arm		
		No	Yes		No	Yes		Rx arm	CTx arm	
		n = 462 (%)	n = 415 (%)	p-value*	n = 410 (%)	n = 793 (%)	p-value*	n = 40 (%)	n = 30 (%)	p-value*
	G3	9 (3)	15 (5)		-	-		2 (5)	3 (10)	
	Missing	*175 (38)*	*115 (28)*		*410 (100)*	*793 (100)*		*1 (2)*	*0 (0)*	
Disease-free interval in months - *(median [IQR])*		11.0 [0.0. 22.8]	0.0 [0.0. 0.5]	<0.001	12.0 [0.0. 24.8]	0.0 [0.0. 5.0]	<0.001	5.8 [0.0. 14.5]	0.0 [0.0. 14.8]	0.334
Number of CRLM - *(median [IQR])*		1.0 [1.0. 2.0]	3.0 [1.0. 5.0]	<0.001	1.0 [1.0. 2.0]	2.0 [1.0. 4.0]	<0.001	1.0 [1.0. 2.0]	2.0 [1.0. 3.0]	0.096
Diameter of largest CRLM in cm - *(median [IQR])*		3.2 [2.1. 4.8]	2.2 [1.3. 3.7]	<0.001	3.0 [2.1. 5.0]	2.5 [1.6. 4.2]	<0.001	3.0 [2.5. 4.6]	2.5 [2.0. 3.6]	0.100
Preoperative CEA in µg/L - *(median [IQR])*		11.0 [4.1. 29.0]	19.0 [5.3. 74.0]	<0.001	9.8 [3.8. 31.2]	9.5 [3.9. 35.3]	0.577	7.4 [2.3. 23.9]	17.2 [4.8. 58.5]	0.078
Clinical risk score	Low-risk (0-2)	331 (75)	177 (48)	<0.001	286 (74)	346 (47)	<0.001	27 (69)	15 (52)	0.142
	High-risk (3-5)	111 (25)	190 (52)		100 (26)	393 (53)		12 (31)	14 (48)	
	Missing	*20 (4)*	*48 (12)*		*24 (6)*	*54 (7)*		*1 (2)*	*1 (3)*	
Extrahepatic disease	No	429 (93)	350 (84)	<0.001	362 (88)	651 (82)	0.005	38 (95)	27 (90)	0.421
	Yes	33 (7)	65 (16)		48 (12)	142 (18)		2 (5)	3 (10)	
Resection margin status	R0	410 (90)	329 (80)	<0.001	368 (91)	675 (86)	0.009	37 (92)	29 (97)	0.457
	R1	48 (10)	84 (20)		35 (9)	109 (14)		3 (8)	1 (3)	
	Missing	*4 (1)*	*2 (0)*		*7 (2)*	*9 (1)*		-	-	
Histopathological growth pattern	Desmoplastic	85 (18)	125 (30)	<0.001	53 (13)	185 (23)	<0.001	7 (18)	8 (27)	0.355
	Non-desmoplastic	377 (82)	290 (70)		357 (87)	608 (77)		33 (82)	22 (73)	
APC	Wildtype	11 (46)	8 (24)	0.075	21 (15)	53 (16)	0.737	-	-	
	Mutant	13 (54)	26 (76)		118 (85)	271 (84)		-	-	
	Missing	*438 (95)*	*381 (92)*		*271 (66)*	*469 (59)*		*40 (100)*	*30 (100)*	
KRAS	Wildtype	42 (63)	65 (56)	0.345	151 (58)	360 (56)	0.633	-	-	
	Mutant	25 (37)	52 (44)		109 (42)	279 (44)		-	-	

		Original cohort Erasmus MC Cancer Institute			External validation cohort MSKCC			Randomised patient cohort EORTC 40983 trial		
		Preoperative chemotherapy			Preoperative chemotherapy			Treatment arm		
		No	Yes		No	Yes		Rx arm	CTx arm	
		n = 462 (%)	n = 415 (%)	p-value*	n = 410 (%)	n = 793 (%)	p-value*	n = 40 (%)	n = 30 (%)	p-value*
	Missing	*395 (85)*	*298 (72)*		*150 (37)*	*154 (19)*		*40 (100)*	*30 (100)*	
NRAS	Wildtype	36 (95)	65 (97)	0.558	204 (96)	510 (95)	0.791	-	-	
	Mutant	2 (5)	2 (3)		9 (4)	25 (5)		-	-	
	Missing	*424 (92)*	*348 (84)*		*197 (48)*	*258 (33)*		*40 (100)*	*30 (100)*	
BRAF	Wildtype	56 (95)	102 (100)	0.022	226 (96)	563 (96)	0.663	-	-	
	Mutant	3 (5)	0 (0)		10 (4)	21 (4)		-	-	
	Missing	*403 (87)*	*313 (75)*		*174 (42)*	*209 (26)*		*40 (100)*	*30 (100)*	
MSI status	MSS	62 (97)	71 (95)	0.523	116 (97)	284 (98)	0.314	-	-	
	MSI	2 (3)	4 (5)		4 (3)	5 (2)		-	-	
	Missing	*398 (86)*	*340 (82)*		*290 (71)*	*504 (64)*		*40 (100)*	*30 (100)*	

Percentages of categorical variables are reported accross valid cases only (i.e. missing are not considered).
*P-values are calculated accross valid cases only (i.e. missing are not considered).
**Multiple primary tumours.
Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; CTx: chemotherapy; EORTC: European Organization for the Research and Treatment of Cancer; G: grade; IQR: interquartile range; MSI: microsatellite instable; MSKCC: Memorial Sloan Kettering Cancer Center; MSS: microsatellite stable; pCR: pathological complete response; R0: negative resection margin (>0mm margin); R1: positive resection margin; Rx: resection.

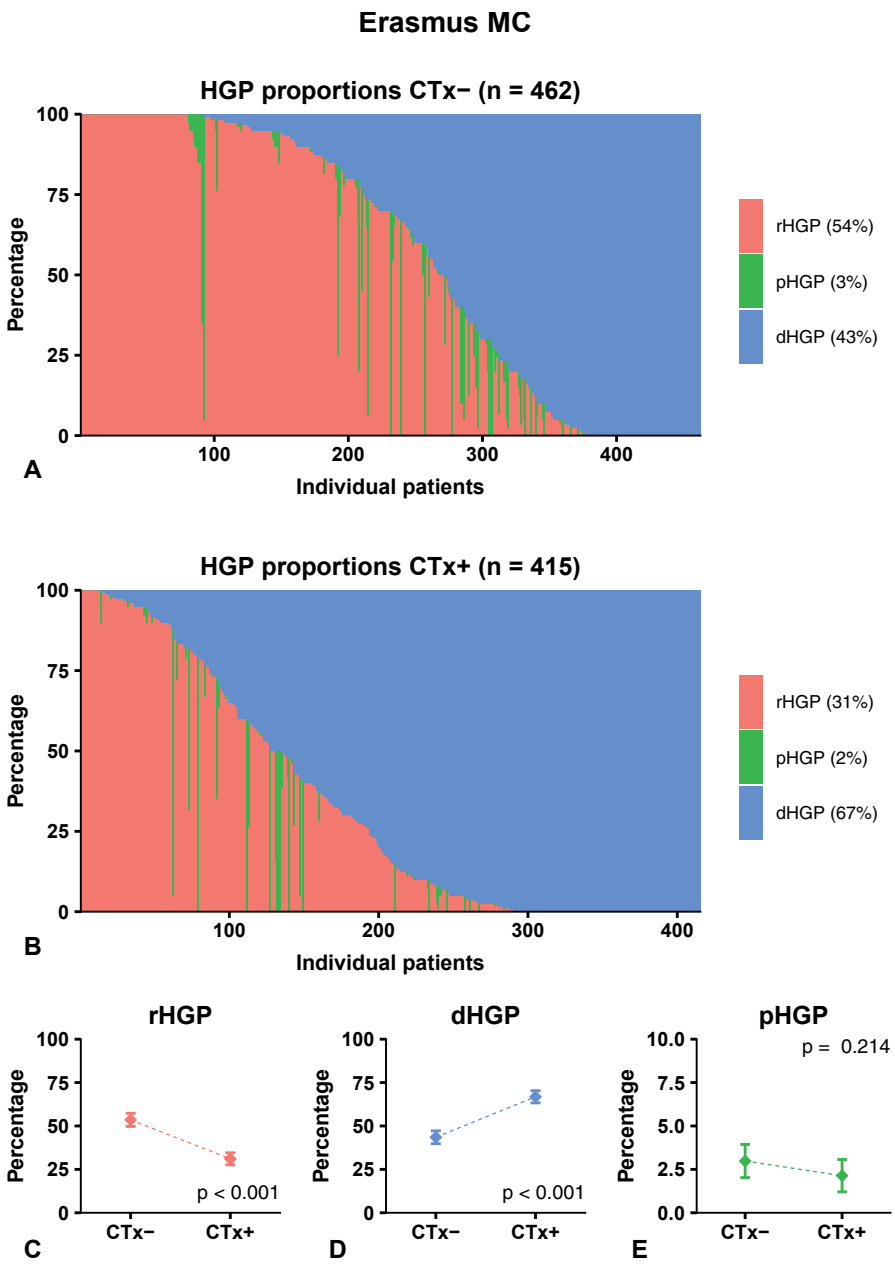

Figure 3A-D. Distribution of histopathological growth patterns (HGPs) in the original cohort of the Erasmus MC cancer institute stratified for preoperative treatment status. 1A. Distribution of HGPs in the chemo-naive cohort. 1B. Distribution of HGPs in the preoperatively treated cohort. 1C-D. Average observed proportion of replacement type HGP (1C) and desmoplastic type HGP (1D) in chemo-naive patients compared to preoperatively treated patients.

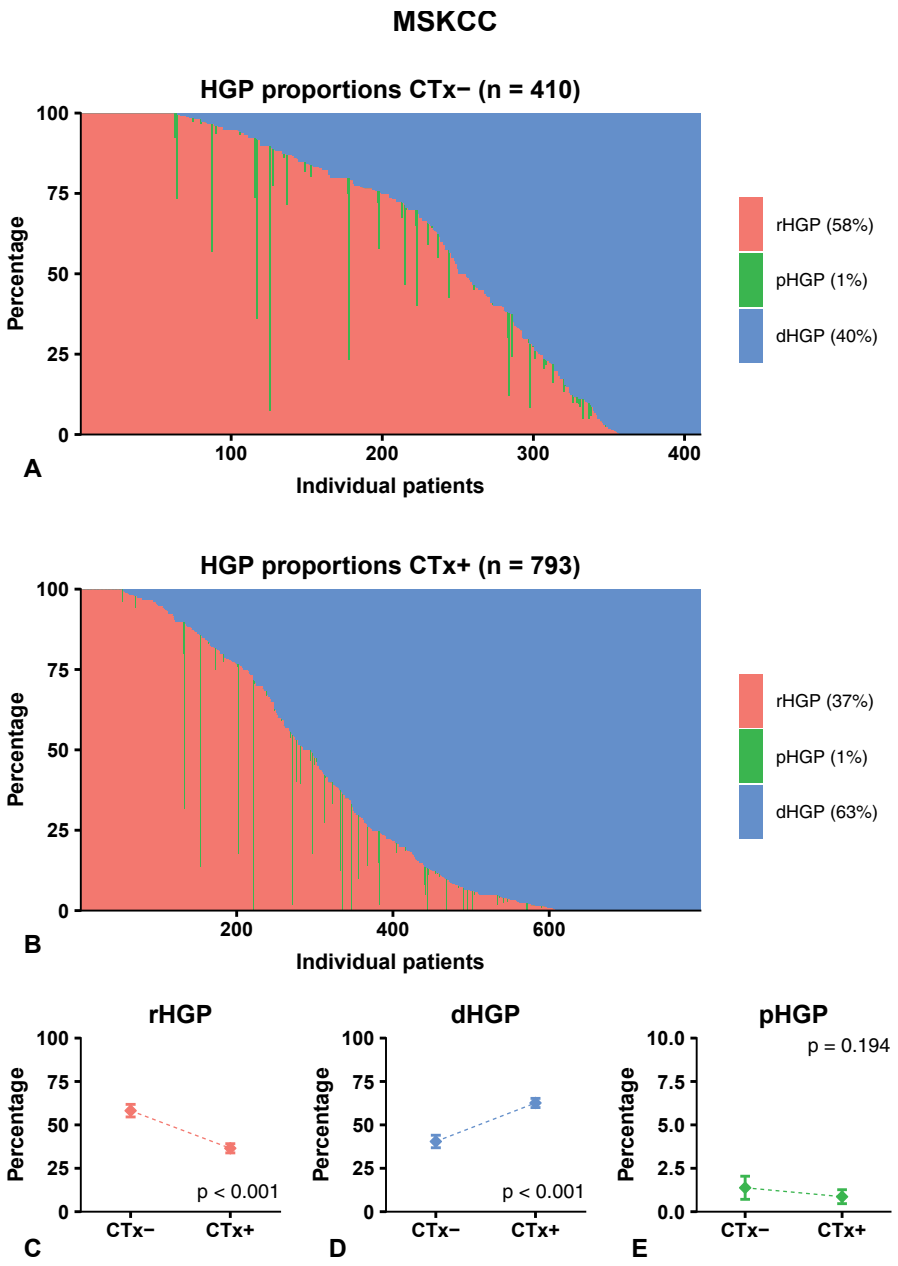

Figure 4A-D. Distribution of histopathological growth patterns (HGPs) in the external validation cohort of the Memorial Sloan Kettering Cancer Center (MSKCC) stratified for preoperative treatment status. 2A. Distribution of HGPs in the chemo-naive cohort. 2B. Distribution of HGPs in the preoperatively treated cohort. 2C-D. Average observed proportion of replacement type HGP (2C) and desmoplastic type HGP (2D) in chemo-naive patients compared to preoperatively treated patients.

Randomised patient cohort

Digital H&E tissue sections of 70 patients were obtained and the HGP was subsequently scored, of whom 40 patients were treated in the Rx only arm. In total, 112 digitalised H&E tissue sections were reviewed. Baseline characteristics compared for treatment arm are displayed in table 1. No significant differences were found and baseline characteristics were comparable to those of the original trial cohort. [11, 12] In addition, OS did not differ between treatment arms (HR [95%CI]: 0.79 [0.42;1.48], p=0.46), and was similar to the published long-term results of the original trial (HR [95%CI]: 0.88 [0.68;1.14], p=0.34). [12]

A graphical display of the distinct HGPs stratified for treatment arm is shown in figure 5. The average presence of desmoplastic HGP observed at the interface was 33% in the Rx only arm versus 61% in the CTx arm (p=0.005, figure 5D). Preoperative systemic chemotherapy was independently associated with a higher proportion of desmoplastic HGP (adjusted β [95%CI]: 23.29 [1.78; 44.79], p=0.034, table 2).

Within the Rx only arm no associations were found between either the Mandard TRG, mean percentage of tumour cells, or Rubbia Brandt tumour regression grade and the observed percentage of desmoplastic HGP (all p>0.11, figure 6 A-C & supplementary table 3). In the CTx only arm increased levels of tumour regression based on either the Mandard TRG, mean percentage of tumour cells, or Rubbia Brandt tumour regression grade were all significantly associated with increase in desmoplastic HGP (all p<0.04, figure 6 D-F & supplementary table 3).

The median follow-up for survivors [IQR] was 103 months [93-120] during which 41 patients (59%) died. Reported 5-year OS rates in the Rx only arm were 83% versus 51% for patients with a pure desmoplastic HGP compared to patients with a non-desmoplastic HGP (supplementary figure 1, overall log-rank: p=0.16). In the CTx arm 5-year OS rates were 63% versus 59% for patients with a pure desmoplastic HGP compared to patients with a non-desmoplastic HGP (supplementary figure 1, overall log-rank: p=0.99).

Table 2. Uni- and multivariable linear regression analysis in all three cohorts for association with the desmoplastic HGP

	Original cohort - Erasmus MC Cancer Institute			
	Univariable		**Multivariable (n = 725)**	
	β [95%CI]	**p-value**	**β [95%CI]**	**p-value**
Primary tumour location - *right-sided vs left-sided or rectal*	1.26 [-5.92; 8.44]	0.731	0.74 [-6.59; 8.06]	0.843
Primary tumour T-stage - *(y)pT 0-4**	-2.65 [-6.45; 1.14]	0.170	-1.05 [-5.12; 3.01]	0.611
Primary tumour nodal status - *(y)pN 0-2**	-5.51 [-9.15; -1.87]	0.003	-5.49 [-9.42; -1.56]	0.006
Disease-free interval - *months**	-0.24 [-0.40; -0.09]	0.002	-0.04 [-0.21; 0.14]	0.664
Number of CRLM*	1.33 [0.28; 2.38]	0.013	-0.15 [-1.38; 1.07]	0.806
Diameter of largest CRLM - *cm**	-2.48 [-3.54; -1.41]	<0.001	-1.23 [-2.37; -0.09]	0.035
Preoperative CEA level - *100 µg/L**	-0.24 [-1.10; 0.61]	0.577	-0.75 [-1.60; 0.11]	0.087
Preoperative chemotherapy - *yes vs no*	23.30 [18.18; 28.42]	<0.001	24.57 [18.28; 30.87]	<0.001
	External validation cohort - MSKCC			
	Univariable		**Multivariable (n = 899)**	
	β [95%CI]	**p-value**	**β [95%CI]**	**p-value**
Primary tumour location - *right-sided vs left-sided or rectal*	-1.51 [-6.46; 3.45]	0.550	2.67 [-2.68; 8.03]	0.328
Primary tumour T-stage - *(y)pT 0-4**	-1.36 [-4.75; 2.04]	0.433	-2.21 [-5.95; 1.54]	0.247
Primary tumour nodal status - *(y)pN 0-2**	-2.48 [-5.42; 0.45]	0.097	-6.19 [-9.50; -2.87]	<0.001
Disease-free interval - *months**	-0.27 [-0.39; -0.15]	<0.001	-0.12 [-0.28; 0.04]	0.149
Number of CRLM*	1.12 [0.20; 2.04]	0.017	0.02 [-1.08; 1.12]	0.977
Diameter of largest CRLM - *cm**	-2.11 [-2.87; -1.35]	<0.001	-1.53 [-2.38; -0.68]	<0.001
Preoperative CEA level - *100 µg/L**	-0.08 [-0.35; 0.19]	0.556	-0.09 [-0.35; 0.18]	0.511
Preoperative chemotherapy - *yes vs no*	22.19 [17.69; 26.69]	<0.001	24.18 [18.70; 29.66]	<0.001
	Randomised patient cohort - EORTC 40983 trial			
	Univariable		**Multivariable (n = 68)**	
	β [95%CI]	**p-value**	**β [95%CI]**	**p-value**
Primary tumour location - *right-sided vs left-sided or rectal*	3.85 [-19.91; 27.62]	0.747	3.56 [-21.14; 28.26]	0.774
Primary tumour T-stage - *(y)pT 0-4**	-4.69 [-23.85; 14.47]	0.627	-0.86 [-21.15; 19.43]	0.933
Primary tumour nodal status - *(y)pN 0-2**	-4.84 [-19.05; 9.37]	0.499	-5.71 [-20.83; 9.42]	0.453
Disease-free interval - *months**	0.15 [-0.48; 0.77]	0.645	0.09 [-0.56; 0.73]	0.785
Number of CRLM*	5.86 [-2.46; 14.18]	0.165	4.12 [-4.65; 12.89]	0.351
Diameter of largest CRLM - *cm**	-2.13 [-4.86; 0.61]	0.126	-1.66 [-4.74; 1.43]	0.288
Preoperative CEA level - *100 µg/L**	0.03 [-0.05; 0.11]	0.425	0.02 [-0.06; 0.11]	0.597
Treatment arm - *CTx vs Rx arm*	27.97 [8.95; 46.98]	0.005	23.29 [1.78; 44.79]	0.034

* Entered as continuous variable
Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; CTx: chemotherapy; EORTC: European Organization for the Research and Treatment of Cancer; HGP: histopathological growth pattern; MSKCC: Memorial Sloan Kettering Cancer Center; Rx: resection.

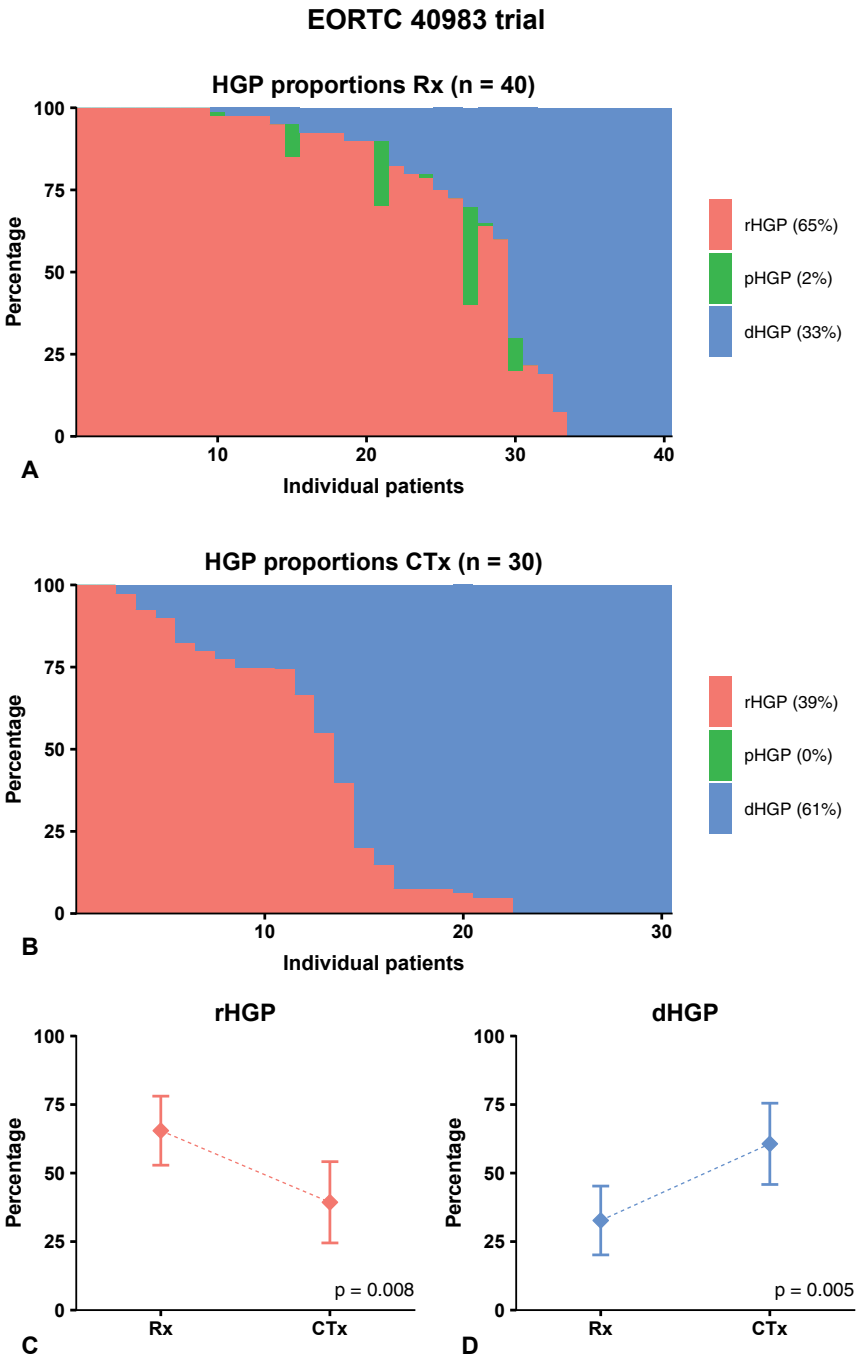

Figure 5A-D. Distribution of HGPs in the EORTC 40983 trial stratified for preoperative treatment status. 3A. Distribution of histopathological growth patterns (HGPs) in the resection only arm. 3B. Distribution of HGPs in the preoperatively treated arm. 3C-D. Average observed proportion of replacement type HGP (3C) and desmoplastic type HGP (3D) in chemo-naive patients compared to preoperatively treated patients

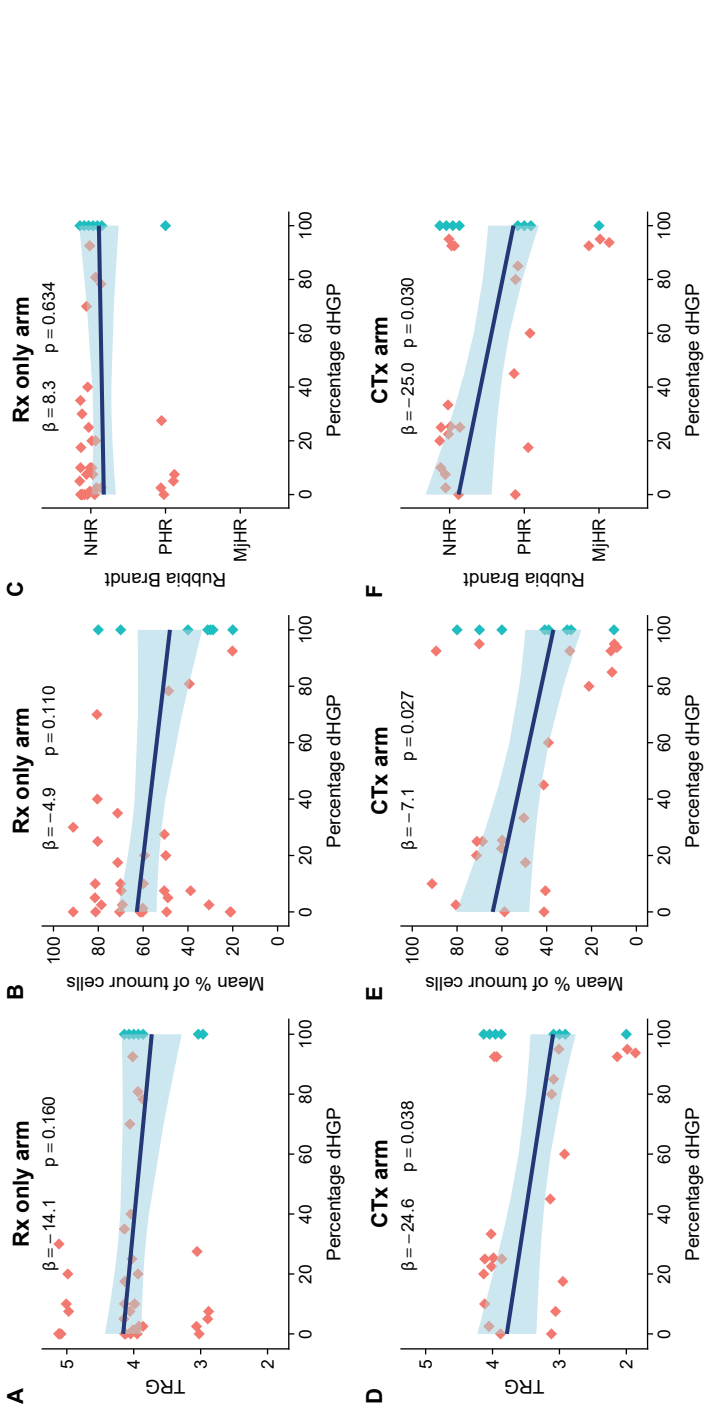

Figure 6A-F. Results of the multivariable linear regression models investigating three separate gradings of tumour regression in patients randomized to either perioperative chemotherapy with resection (D-F) or resection only (A-C) within the EORTC 40983 phase III trial. The dots resemble individual patients; dark-grey dots represent a non-desmoplastic and light-grey dots a desmoplastic phenotype, respectively. The regression line represents the association for one of three tumour regression gradings with the desmoplastic HGP (dHGP) on multivariable analysis, with the ribbon representing the 95% confidence interval of the estimate. TRG: tumour regression grade; NHR: no histological response; PHR: partial histological response; MjHR: major histological response.

Genetics data

The uni- and multivariable linear regression models within the combined cohort of all patients with available genetics data showed that MSI was significantly associated with an increased proportion of desmoplastic HGP at the interface (adjusted β [95%CI]: 39.97 [13.59; 66.34], p=0.003, table 3). No significant associations existed between APC, KRAS, NRAS, or BRAF mutational status and the proportion of desmoplastic HGP (all p-values > 0.6, table 3). When correcting for genetic risk factors, preoperative chemotherapy remained independently associated with a higher proportion of desmoplastic HGP (adjusted β [95%CI]: 19.83 [10.85; 28.82], p<0.001).

Comparing chemo-naive and pre-treated desmoplastic patients

Clinicopathological characteristics compared between all chemo-naive and pre-treated desmoplastic patients (i.e. 100% desmoplastic HGP) are provided in table 4 (combined cohort). In comparison to chemo-naive patients, pre-treated desmoplastic patients were younger, had more advanced (y)pT&N stage, a shorter disease-free interval, more CRLM, and a higher preoperative serum CEA (table 4). The size of the largest CRLM measured at pathological examination was however significantly smaller for the pre-treated desmoplastic patients (table 4). Concerning genetic risk factors, no significant differences were observed.

Chemo-naive desmoplastic patients had a significantly longer OS compared to the pre-treated desmoplastic patients, with 5-year (95%CI) OS of 74% (67-83%) compared to 60% (54-66%) (p=0.004; figure 7). This difference remained on multivariable analysis, with an adjusted HR for OS of 1.78 [1.16-2.74]; p=0.008 (supplementary table 4) for pre-treated desmoplastic versus chemo-naive desmoplastic patients.

Table 3. Uni- and multivariable linear regression analysis for the association with the desmoplastic HGP in a combined cohort of patients with available genetics data

	Univariable		Multivariable (n = 725)	
	β [95%CI]	**p-value**	**β [95%CI]**	**p-value**
Primary tumour location - *right-sided vs left-sided or rectal*	-0.61 [-4.64; 3.43]	0.767	2.46 [-6.57; 11.49]	0.593
Primary tumour T-stage - *(y)pT 0-4**	-1.91 [-4.41; 0.60]	0.136	-1.77 [-6.88; 3.33]	0.495
Primary tumour nodal status - *(y)pN 0-2**	-3.71 [-5.99; -1.43]	0.001	-8.59 [-13.76; -3.42]	0.001
APC - *mutant vs wildtype*	0.82 [-7.40; 9.03]	0.845	0.81 [-10.14; 11.75]	0.885
KRAS - *mutant vs wildtype*	3.70 [-1.02; 8.41]	0.124	-0.55 [-8.38; 7.28]	0.890
NRAS - *mutant vs wildtype*	8.83 [-3.65; 21.31]	0.165	5.33 [-15.06; 25.73]	0.607
BRAF - *mutant vs wildtype*	3.31 [-10.09; 16.71]	0.628	5.45 [-15.41; 26.31]	0.608
MSI - *MSI vs MSS*	20.82 [1.89; 39.75]	0.031	39.97 [13.59; 66.34]	0.003
Disease-free interval - *months**	-0.26 [-0.35; -0.17]	<0.001	-0.26 [-0.54; 0.03]	0.076
Number of CRLM*	1.21 [0.52; 1.91]	<0.001	1.66 [0.15; 3.17]	0.031
Diameter of largest CRLM - *cm**	-2.23 [-2.85; -1.61]	<0.001	-1.34 [-2.96; 0.27]	0.103
Preoperative CEA level - *100 µg/L**	-0.09 [-0.35; 0.17]	0.479	0.31 [-0.15; 0.77]	0.191
Preoperative chemotherapy - *yes vs no*	22.00 [18.68; 25.32]	<0.001	19.83 [10.85; 28.82]	<0.001

* Entered as continuous variable

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; CTx: chemotherapy; HGP: histopathological growth pattern; MSI: microsatellite instable; MSS: microsatellite stable; Rx: resection.

Desmoplastic HGP examples with and without preoperative chemotherapy

In figure 8 panels A-J several examples of CRLM with a desmoplastic HGP are presented. Panels A-E pertain to resected CRLM of chemo-naive patients, and in panels F-J CRLM resected from preoperatively treated patients are displayed. As delineated by international consensus guidelines the desmoplastic HGP can exhibit several distinguishing features like the presence of a rim of desmoplastic stroma separating the metastasis from the liver parenchyma (prerequisite), which is often accompanied by a (dense) lymphocytic infiltrate around this stroma.[1, 21] Moreover, the composition of the tumour cells does not mimic the architectural pattern of the liver parenchyma. Almost all of the aforementioned histopathological features apply to all panels in figure 8. Despite these general similarities, a closer observation reveals some apparent morphological differences. Varying degrees tumour-regression are present in the preoperatively treated CRLM. For example, in panels F-G a Mandard TRG of respectively 2 and 3 is seen. While these examples formally meet the conditions to be classified as desmoplastic HGP (i.e. separation of the metastasis from the liver tissue by a desmoplastic rim), few vital tumour cells are present. Therefore it is unknown whether the currently observed morphology represents the “original” (prior to systemic chemotherapy) metastasis morphology, since such an assessment (HGP pre-chemotherapy) is currently impossible. In panel H we observe a metastasis that formally classifies as desmoplastic (i.e. separation of the liver parenchyma and the metastasis by desmoplasia), however the tumour cells in the periphery of the metastases are organised in trabecular-like (plate-like) pattern resembling liver parenchyma, albeit accompanied by relatively obvious inter-plate fibrosis. This plate-like pattern is often observed in the replacement HGP type. Again, since pre-chemotherapy morphology of this metastasis is unknown it is at this moment unachievable to assess with certainty whether chemotherapy induced this increased intercellular fibrosis and desmoplasia surrounding the metastasis. Panels I and J display CRLM with a “classic” desmoplastic phenotype, although the higher amount of necrosis in I might be regarded as the impact of the pre-operative chemotherapy.

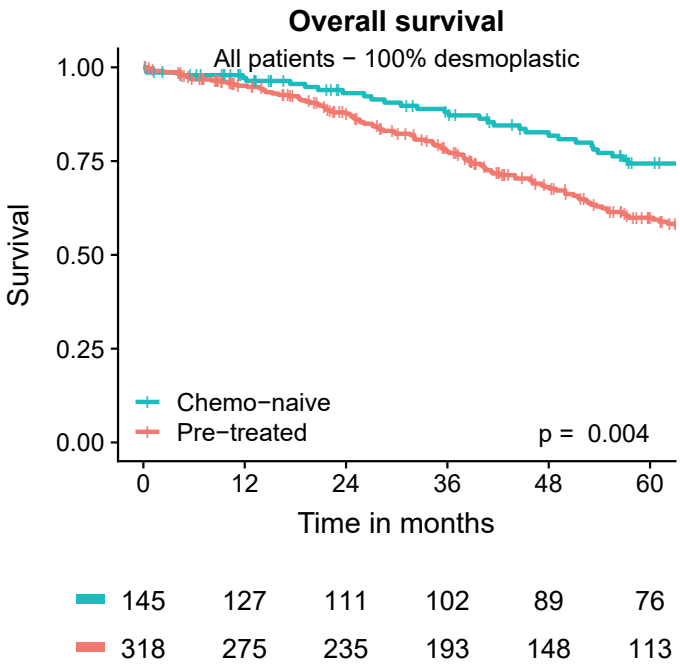

Figure 7. Kaplan-Meier overall survival curves stratified by pre-operative treatment status in a combined cohort of only desmoplastic patients (i.e. 100% desmoplastic histopathological growth pattern).

Table 4. Clinicopathological characteristics of desmoplastic patients only (i.e. 100% desmoplastic HGP) stratified by preoperative treatment status (combined cohort)

			Desmoplastic patients only		
			Preoperative chemotherapy		
			No	**Yes**	
		missing (%)	**n = 145 (%)**	**n = 318 (%)**	**p-value**
Age at resection CRLM - *(median [IQR])*			67.0 [56.0. 75.0]	61.0 [51.0. 68.0]	<0.001
Gender	Female		87 (60)	206 (65)	0.322
	Male		58 (40)	112 (35)	
Primary tumour location	Right-sided	*21 (5)*	31 (22)	82 (27)	0.560
	Left-sided		64 (46)	129 (43)	
	Rectal		44 (32)	92 (30)	
(y)pT-stage	0	*31 (7)*	1 (1)	15 (5)	<0.001
	1		5 (4)	2 (1)	
	2		29 (20)	25 (9)	
	3		97 (68)	190 (66)	
	4		10 (7)	58 (20)	
(y)pN-stage	0	*13 (3)*	84 (58)	119 (39)	<0.001
	1		46 (32)	116 (38)	
	2		14 (10)	71 (23)	
Disease-free interval in months - *(median [IQR])*		*5 (1)*	9.0 [0.0. 22.0]	0.0 [0.0. 1.0]	<0.001
Number of CRLM - *(median [IQR])*		*3 (1)*	1.0 [1.0. 2.0]	2.0 [1.0. 4.0]	<0.001
Diameter of largest CRLM in cm - *(median [IQR])*		*15 (3)*	2.3 [1.5. 3.5]	1.8 [1.1. 3.0]	0.002
Preoperative CEA in µg/L - *(median [IQR])*		*37 (8)*	4.8 [2.6. 11.5]	8.0 [3.1. 29.9]	<0.001
APC	Wildtype	*364 (79)*	3 (18)	15 (18)	0.950
	Mutant		14 (82)	67 (82)	
KRAS	Wildtype	*256 (55)*	17 (52)	94 (54)	0.791
	Mutant		16 (48)	80 (46)	
NRAS	Wildtype	*293 (63)*	25 (93)	135 (94)	0.713
	Mutant		2 (7)	8 (6)	
BRAF	Wildtype	*277 (60)*	28 (97)	149 (95)	0.704
	Mutant		1 (3)	8 (5)	
MSI status	MSS	*343 (74)*	27 (93)	86 (95)	0.779
	MSI		2 (7)	5 (5)	

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; IQR: interquartile range; MSI: microsatellite instable; MSS: microsatellite stable.

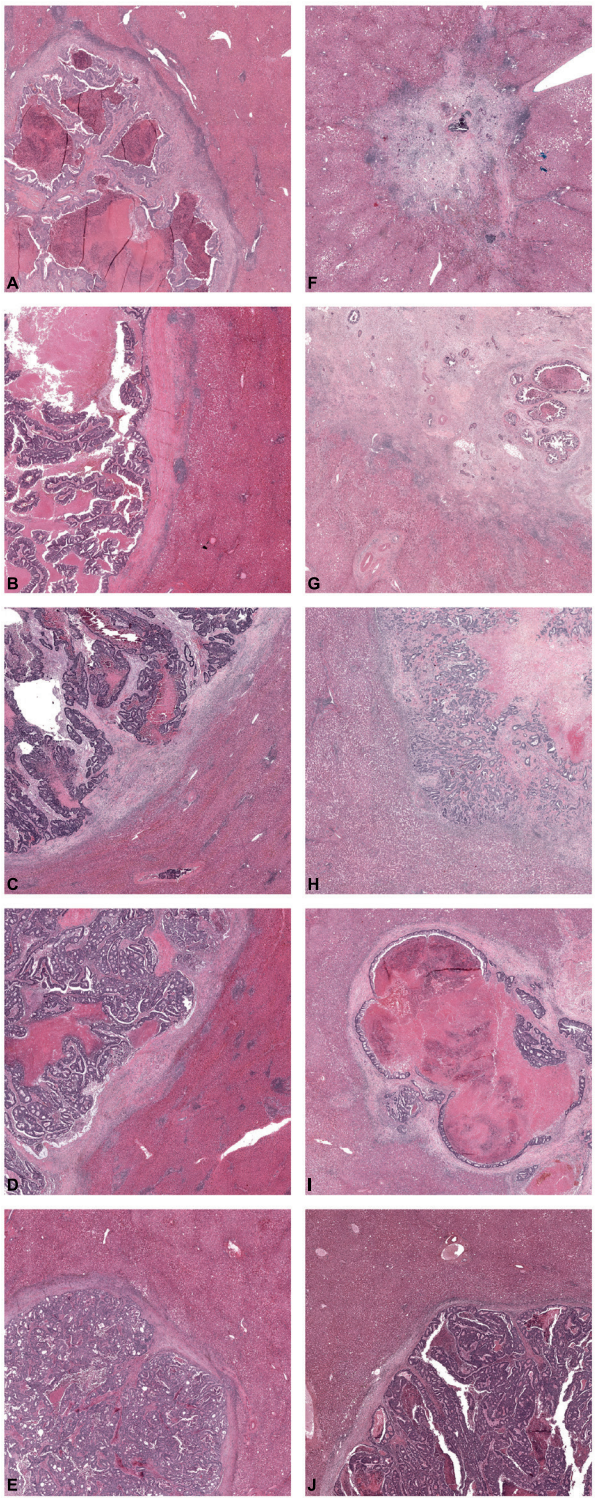

Figure 8A-J. Representative examples of resected CRLM exhibiting a desmoplastic HGP in chemo-naive (A-E) and pre-treated (D-J) patients respectively.

Discussion

In all three cohorts described in this study, preoperative chemotherapy was associated with a higher proportion of desmoplastic HGP observed at the interface. These results were obtained in an original cohort and validated in both an independent retrospective external validation cohort, as well as in a post-hoc analysis of the prospective randomised controlled EORTC 40983 clinical trial.

We previously demonstrated a significant difference in HGPs between chemo-naive and preoperatively treated patients undergoing surgical treatment of CRLM.[5] The value of those findings was limited at the time, as it was the only study describing a difference and it was based on retrospective data from a single centre. One other study has described a modest difference in the observed percentage of distinct HGPs after preoperative treatment with chemotherapy and bevacizumab.[4] Similar to the results of the current study, a higher percentage of desmoplastic HGP was reported after preoperative treatment, albeit not significant, but this can likely be attributed to the limited sample size. The current study has addressed both shortcomings of these two previous papers; large sample sizes and multiple external validation cohorts, including a subset of a randomised controlled trial. And indeed, in the current study we were able to confirm an increase in desmoplastic HGP observed after preoperative chemotherapy in three independent cohorts. In addition, we demonstrate within the randomised controlled trial that pathology gradings designed for quantifying tumour regression as a result of therapy were associated with higher proportions of the desmoplastic HGP for the pre-treated patients only. Herein it is important to note that these gradings were determined prior to the conception of the current study, and that determination was performed blinded for treatment arm by an independent senior pathologist not involved in the HGP scoring presented. Therefore this presents the optimal method to assess such an association, for if we now were to determine these gradings this cannot be done independent of HGP as they are determined – and therefore visible – on the same H&E slides. These results strongly suggest HGP phenotype alteration by chemotherapy. This should be taken into account in future studies and/or guidelines regarding HGPs of CRLM, since desmoplastic growth induced by chemotherapy may be a distinct phenotype, with considerable biological and clinical differences with the naturally occurring desmoplastic growth pattern.

This alteration in growth pattern phenotype after pre-operative treatment could occur in at least two ways; either the chemotherapy agents really induces desmoplasia in a proportion of non-desmoplastic liver metastasis, or relatively more desmoplastic lesions remain after chemotherapy . In an attempt to determine which explanation is more likely, we compared the clinicopathological factors between chemo-naive and preoperatively treated desmoplastic patients only. In this comparison, the pre-treated desmoplastic patients had more advanced (y)pT&N stage, as well as more metastases in general, and a higher preoperative serum CEA, all traits that have previously been associated with non-desmoplastic metastases.[5, 22] It has to be noted however that these findings are at severe

risk of selection bias, since patients with a more advanced disease are more likely to receive preoperative systemic chemotherapy. Nevertheless, comparing OS between these two groups showed that the pre-treated desmoplastic patients had worse survival compared to the chemo-naive desmoplastic patients, even after correction for these baseline differences. This is in line with previous reports, which either demonstrate a marginally prognostic value for HGPs in pre-treated patients[5], or a prognostic value that is less pronounced compared to that in chemo-naive patients.[16] To address selection bias, we attempted to validate these findings in the randomised cohort presented, as the EORTC 40983 trial randomised between perioperative chemotherapy and upfront resection. While the current post-hoc analysis was severely underpowered to find significant survival differences, the observed survival estimates did resemble those reported previously.[5, 20] In addition to this difference in prognosis, a recent study demonstrated that adjuvant systemic chemotherapy resulted in a survival benefit for the chemo-naive non-desmoplastic patients only, hinting at differences in chemo-sensitivity between the untreated growth patterns.[20] Our results and the literature therefore suggest more a dilution of the preoperatively treated "pure desmoplastic" population by a more aggressive former non-desmoplastic tumour type component.

Systemic chemotherapy has indeed been associated with alterations in gene expression in CRLM [23], the immune infiltrate of CRLM [14, 23], the immune response in malignancies in general [24, 25], and tumoural fibrosis or necrosis in CRLM.[18, 19] The explanation for the conversion of non-desmoplastic to desmoplastic HGP as a consequence of preoperative systemic chemotherapy might therefore also (partially) lie in these associations. The problem currently faced however is that assessment of the HGPs is only possible after systemic therapy and subsequent resection. There is currently no way to assess whether a patient was desmoplastic prior to the start of chemotherapy, or that a "desmoplastic-like" pattern was induced, and consequently also which mechanism may have induced this change. Future attempts should therefore focus on the pre-treatment or pre-operative determination of the HGPs, with recent reports showing promise for a medical imaging approach.[26]

There is as of yet no clear consensus on the biology behind the prognostic value of HGPs itself. Some potential explanatory factors are the differences in vascular architecture of CRLM [27, 28], the variance of immune infiltrate in and around CRLM [2, 21], and the up-regulation of signalling pathways of cell motility and invasiveness of cancer cells in the replacement HGP.[4] Concerning genetics, our recent external validation study did associate the desmoplastic HGP with MSI, but not with the known genetic risk factors KRAS and BRAF.[16] Here we again report similar results. MSI was associated with an increased proportion of the desmoplastic HGP, while no such associations existed for APC, KRAS, NRAS, or BRAF mutations. A potential explanation for the association between MSI tumours and the desmoplastic HGP might be that MSI tumours are hypermutated, and therefore present more potential neoantigens to targets for T cells, resulting in a higher probability for a (partially) successful anti-cancer T-cell response.[29, 30] It was demonstrated by us and others that the microenvironment of desmoplastic HGP is indeed enriched with T-cells.[2, 31] This association between the

desmoplastic HGP and MSI is especially of interest since MSI tumours represent an actionable target for immunotherapy in metastatic colorectal cancer.[32] Future investigations aimed at validating these findings, and identify other potential genetic associations related to the HGPs therefore seem warranted.

Limitations of the current study should be taken into account. HGP determination was performed retrospectively. Furthermore, a complete pathological response to preoperative chemotherapy makes HGP assessment impossible. This means that the patients with the most favourable response to chemotherapy (i.e. Mandard TRG 1), albeit rare, were excluded. Complete pathological response to chemotherapy is associated with fibrosis on histopathological examination, excluding these patients therefore makes it likely that conversion to desmoplastic HGP may be underestimated in the current study. Only a subset of patients from the randomised EORTC 40983 trial were available for post-hoc analysis, which may have introduced selection bias. Baseline characteristics and survival outcomes of the currently presented subset were however comparable to those found in the original trial. [11, 12] This suggests that this subset is a proper representation of the EORTC 40983 trial population. The risk of selection bias rather could apply to the external validation cohort, as it represents a retrospective, non-randomised cohort.

The results of the current study strongly suggest that systemic chemotherapy induces histopathological changes that lead to an increase of desmoplastic HGP as recognised by international consensus guidelines. As it is currently impossible to assess the HGP prior to chemotherapy treatment, we can at present not determine whether this increase is resembling actual change of underlying biology, or is a limitation of the current HGP assessment algorithm after systemic preoperative chemotherapy. The limited evidence currently available may however favour the latter. This should be taken into account in future studies and/or guidelines regarding HGPs of CRLM.

Figure captions

Figure 1A-C. Examples of the distinct histopathological growth patterns (HGPs). 1A. Example of replacement type HGP in which tumour cells "replace" hepatocytes and infiltrate the liver parenchyma with direct tumour-liver cell contact. 1B. Example of pushing type HGP in which the liver parenchyma is "pushed" aside but is not infiltrated. No direct tumour-liver cell contact is present. 1C. Example of desmoplastic type HGP, in which the tumour is separated from the liver parenchyma by a desmoplastic capsule. No direct tumour-liver cell contact is present.

Figure 2. Simplified decision tree to determine the growth patterns of liver metastases based on the key histopathological characteristics. Adapted with permission from van Dam et al.[1]

Figure 3A-D. Distribution of histopathological growth patterns (HGPs) in the original cohort of the Erasmus MC cancer institute stratified for preoperative treatment status. 1A. Distribution of HGPs in the chemo-naive cohort. 1B. Distribution of HGPs in the preoperatively treated cohort. 1C-D. Average observed proportion of replacement type HGP (1C) and desmoplastic type HGP (1D) in chemo-naive patients compared to preoperatively treated patients.

Figure 4A-D. Distribution of histopathological growth patterns (HGPs) in the external validation cohort of the Memorial Sloan Kettering Cancer Center (MSKCC) stratified for preoperative treatment status. 2A. Distribution of HGPs in the chemo-naive cohort. 2B. Distribution of HGPs in the preoperatively treated cohort. 2C-D. Average observed proportion of replacement type HGP (2C) and desmoplastic type HGP (2D) in chemo-naive patients compared to preoperatively treated patients.

Figure 5A-D. Distribution of HGPs in the EORTC 40983 trial stratified for preoperative treatment status. 3A. Distribution of histopathological growth patterns (HGPs) in the resection only arm. 3B. Distribution of HGPs in the preoperatively treated arm. 3C-D. Average observed proportion of replacement type HGP (3C) and desmoplastic type HGP (3D) in chemo-naive patients compared to preoperatively treated patients.

Figure 6A-F. Results of the multivariable linear regression models investigating three separate gradings of tumour regression in patients randomized to either perioperative chemotherapy with resection (D-F) or resection only (A-C) within the EORTC 40983 phase III trial. The dots resemble individual patients; dark-grey dots represent a non-desmoplastic and light-grey dots a desmoplastic phenotype, respectively. The regression line represents the association for one of three tumour regression gradings with the desmoplastic HGP (dHGP) on multivariable analysis, with the ribbon representing the 95% confidence interval of the estimate. TRG: tumour regression grade; NHR: no histological response; PHR: partial histological response; MjHR: major histological response.

Figure 7. Kaplan-Meier overall survival curves stratified by pre-operative treatment status in a combined cohort of only desmoplastic patients (i.e. 100% desmoplastic histopathological growth pattern).
Figure 8A-J. Representative examples of resected CRLM exhibiting a desmoplastic HGP in chemo-naive (A-E) and pre-treated (D-J) patients respectively.

Supplementary material

Supplementary table 1. Erasmus MC uni- and multivariable Cox regression analysis for overall survival.

Supplementary table 2. MSKCC uni- and multivariable Cox regression analysis for overall survival.

Supplementary table 3. Multivariable linear regression analysis on the relationship between tumour regression gradings and the percentage of desmoplastic HGP within the EORTC 40983 treatment arms.

Supplementary table 4. Uni- and multivariable Cox regression analysis for overall survival in desmoplastic patients only (combined cohort).

Supplementary figure 1. Kaplan-Meier overall survival curves stratified by histopathological growth pattern in the resection only (Rx) arm and perioperative chemotherapy (CTx) arm of the EORTC 40983 trial

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Supplementary materials

Supplementary table 1. Erasmus MC uni- and multivariable Cox regression analysis for overall survival

	Univariable		Multivariable (n=715)	
	HR [95%CI]	**p-value**	**HR [95%CI]**	**p-value**
Age at resection	1.01 [1.01-1.02]	<0.001	1.02 [1.01-1.03]	<0.001
ASA classification - *>II vs I-II*	1.19 [0.92-1.56]	0.19	1.17 [0.88-1.56]	0.29
Primary tumour location - *right-sided vs left-sided or rectal*	1.20 [0.97-1.50]	0.10	1.28 [1.01-1.62]	0.04
Primary tumour T-stage - *(y)pT 3-4 vs (y)pT 0-2*	1.29 [1.04-1.61]	0.02	1.03 [0.81-1.31]	0.82
Primary tumour nodal status - *N+ vs N0*	1.53 [1.28-1.83]	<0.001	1.49 [1.22-1.82]	<0.001
Disease-free interval - *months*	0.99 [0.99-1.00]	0.02	0.99 [0.99-1.00]	0.08
Number of CRLM	1.07 [1.04-1.10]	<0.001	1.07 [1.03-1.11]	<0.001
Diameter of largest CRLM - *cm*	1.04 [1.01-1.07]	0.01	1.05 [1.01-1.09]	0.01
Preoperative CEA level - *µg/L*	1.00 [1.00-1.00]	0.37	1.00 [1.00-1.00]	0.32
Preoperative chemotherapy - *yes vs no*	1.23 [1.04-1.44]	0.01	1.18 [0.96-1.46]	0.12
HGP - *non-desmoplastic vs desmoplastic*	1.73 [1.41-2.12]	<0.001	1.56 [1.23-1.98]	<0.001

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; HGP: histopathological growth patterns.

Supplementary table 2. MSKCC uni- and multivariable Cox regression analysis for overall survival

	Univariable		Multivariable (n=863)	
	HR [95%CI]	**p-value**	**HR [95%CI]**	**p-value**
Age at resection	1.01 [1.00-1.02]	0.02	1.01 [1.00-1.02]	0.05
ASA classification - *>II vs I-II*	1.21 [0.99-1.47]	0.06	1.14 [0.89-1.45]	0.30
Primary tumour location - *right-sided vs left-sided or rectal*	1.35 [1.11-1.65]	<0.01	1.38 [1.09-1.75]	<0.01
Primary tumour T-stage - *(y)pT 3-4 vs (y)pT 0-2*	1.39 [1.01-1.90]	0.04	1.06 [0.74-1.52]	0.74
Primary tumour nodal status - *N+ vs N0*	1.44 [1.18-1.76]	<0.001	1.51 [1.17-1.94]	<0.01
Disease-free interval - *months*	1.00 [0.99-1.01]	0.93	1.00 [0.99-1.00]	0.44
Number of CRLM	1.07 [1.03-1.10]	<0.001	1.09 [1.05-1.14]	<0.001
Diameter of largest CRLM - *cm*	1.06 [1.04-1.09]	<0.001	1.08 [1.04-1.11]	<0.001
Preoperative CEA level - *µg/L*	1.00 [1.00-1.00]	0.91	1.00 [1.00-1.00]	0.69
Preoperative chemotherapy - *yes vs no*	1.51 [1.23-1.85]	<0.001	1.77 [1.35-2.32]	<0.001
Adjuvant systemic chemotherapy - *yes vs no*	0.63 [0.52-0.77]	<0.001	0.89 [0.67-1.18]	0.42
Adjuvant HAIP chemotherapy - *yes vs no*	0.71 [0.58-0.87]	<0.001	0.72 [0.55-0.94]	0.02
HGP - *non-desmoplastic vs desmoplastic*	1.74 [1.34-2.26]	<0.001	1.75 [1.29-2.37]	<0.001

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; HAIP: hepatic arterial infusion pump chemotherapy; HGP: histopathological growth pattern; MSKCC: Memorial Sloan Kettering Cancer Center.

Supplementary table 3. Multivariable linear regression analysis on the relationship between tumour regression gradings and the percentage of desmoplastic HGP within the EORTC 40983 treatment arms

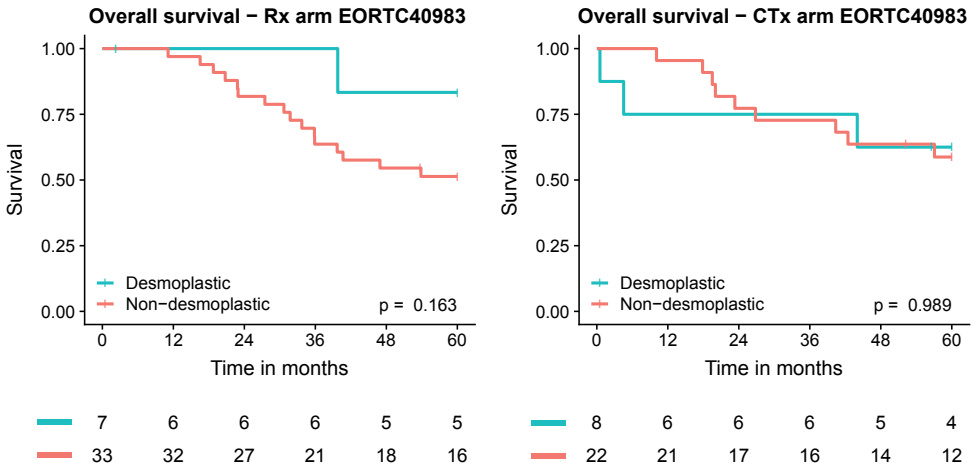
	Rx only arm		CTx arm	
Mandard tumour regression grade	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**
Primary tumour T-stage - *(y)pT 1-4**	-5.21 [-28.14; 17.72]	0.647	10.20 [-23.69; 44.09]	0.541
Primary tumour nodal status - *(y)pN 0-2**	-5.72 [-24.04; 12.59]	0.530	-1.02 [-24.64; 22.61]	0.930
Number of CRLM*	7.45 [-2.66; 17.56]	0.143	-5.83 [-21.88; 10.23]	0.462
Mandard tumour regression grade - *per 1 grade up*	-14.15 [-34.17; 5.88]	0.160	-24.60 [-47.76; -1.43]	0.038
Mean percentage of tumour cells	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**
Primary tumour T-stage - *(y)pT 1-4**	-2.62 [-25.34; 20.10]	0.816	4.82 [-27.68; 37.33]	0.762
Primary tumour nodal status - *(y)pN 0-2**	-6.36 [-24.46; 11.73]	0.480	2.93 [-20.88; 26.73]	0.802
Number of CRLM*	6.96 [-3.06; 16.98]	0.167	-3.40 [-18.53; 11.73]	0.647
Mean percentage of tumour cells - *per 10% up*	-4.92 [-11.02; 1.18]	0.110	-7.09 [-13.33; -0.85]	0.027
Rubbia Brandt histologic regression grade	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**
Primary tumour T-stage - *(y)pT 1-4**	-3.73 [-27.21; 19.74]	0.748	11.07 [-22.66; 44.81]	0.505
Primary tumour nodal status - *(y)pN 0-2**	-6.55 [-25.33; 12.23]	0.483	-2.34 [-25.73; 21.05]	0.838
Number of CRLM*	7.35 [-3.03; 17.73]	0.159	-5.21 [-20.86; 10.44]	0.499
Rubbia Brandt histologic regression grade - *per grade*	8.34 [-26.89; 43.56]	0.634	-24.96 [-47.36; -2.56]	0.030

* Entered as continuous variable
Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; HGP: histopathological growth pattern.

Supplementary table 4. Uni- and multivariable Cox regression analysis for overall survival in desmoplastic patients only (combined cohort)

	Univariable		Multivariable (n=364)	
	HR [95%CI]	**p-value**	**HR [95%CI]**	**p-value**
Age at resection	1.03 [1.01-1.04]	<0.001	1.03 [1.01-1.05]	<0.001
Primary tumour location - *right-sided vs left-sided or rectal*	1.26 [0.90-1.75]	0.18	1.10 [0.76-1.60]	0.61
Primary tumour T-stage - *(y)pT 3-4 vs (y)pT 0-2*	1.18 [0.81-1.74]	0.39	1.00 [0.64-1.55]	1.00
Primary tumour nodal status - *N+ vs N0*	1.40 [1.04-1.90]	0.03	1.67 [1.16-2.40]	<0.01
Disease-free interval - *months*	1.00 [1.00-1.01]	0.38	1.01 [0.99-1.02]	0.31
Number of CRLM	1.11 [1.05-1.18]	<0.001	1.13 [1.05-1.21]	<0.001
Diameter of largest CRLM - *cm*	1.00 [0.94-1.06]	1.00	1.00 [0.93-1.07]	0.95
Preoperative CEA level - *µg/L*	1.00 [1.00-1.00]	0.38	1.00 [1.00-1.00]	0.64
Preoperative chemotherapy - *yes vs no*	1.60 [1.15-2.21]	<0.01	1.78 [1.16-2.74]	<0.01

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis.



Supplementary figure 1. Kaplan-Meier overall survival curves stratified by histopathological growth pattern in the resection only (Rx) arm and perioperative chemotherapy (CTx) arm of the EORTC 40983 trial.

Chapter 7

The desmoplastic histopathological growth pattern marks good prognosis after resection of colorectal liver metastasis: a post-hoc analysis in two randomised controlled trials

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Submitted

Abstract

Introduction

Based on the morphology of the tumour-liver boundary resected colorectal liver metastases (CRLM) can be classified into two main histopathological growth patterns (HGP). Retrospective cohort studies have repeatedly demonstrated that the desmoplastic HGP is associated with good survival outcomes compared to the non-desmoplastic HGP after resection of CRLM. The aim of this study is to validate this finding within a prospective cohort of patients included in randomised controlled trials.

Methods

CRLM resection specimens collected in two phase III randomized controlled trials (New EPOC & EORTC 40983) were used. HGP assessment was performed retrospectively on haematoxylin & eosin stained slide images according to consensus guidelines and blinded for patient characteristics, treatment, and outcome. Patients were classified as desmoplastic if all CRLM exhibited only desmoplastic growth, and as non-desmoplastic otherwise. Overall (OS) and progression-free (PFS) survival were estimated using Kaplan-Meier analysis and multivariable Cox regression.

Results:

In total 222 trial-patients were included. A desmoplastic phenotype was observed on histopathology in 56 (25%). Desmoplastic patients had significantly longer OS and PFS compared to non-desmoplastic patients, with 5-year estimates of 66% versus 51% for OS ($p=0.04$), and 45% versus 21% for PFS ($p<0.001$), respectively. On multivariable analysis, a desmoplastic phenotype was an independent prognostic factor (adjusted hazard ratio [95%CI]) for both OS (0.58 [0.35-0.95], $p=0.03$) and PFS (0.48 [0.32-0.74], $p<0.001$).

Conclusion

This study confirms a desmoplastic HGP as a marker for good prognosis in patients undergoing resection of CRLM within prospective randomized controlled trials.

Introduction

Resection of colorectal cancer liver metastasis (CRLM) has become well established within the treatment of advanced colorectal cancer (CRC). While more than two-thirds of patients will generally experience cancer recurrence, surgery for CRLM does offer the potential of long-term cure, which is achieved in roughly a quarter of patients.[1, 2] Given the high rate of cancer recurrences, many have focussed on predicting prognosis to improve patient selection and ultimately prevent futile surgeries. To this end, multiple studies have sought to identify novel clinical and genetic predictors [3-6], and morphological classifications of histopathology have also emerged.[7, 8]

Distinct histopathological growth patterns (HGP) are recognised at the CRLM tumour to liver parenchyma boundary.[9] In the standardized assessment of HGPs haematoxylin and eosin (H&E) stained resection specimens are used to estimate the relative presence of the desmoplastic and non-desmoplastic growth patterns. The two largest retrospective cohort studies to date demonstrated favourable prognosis for patients with a fully desmoplastic phenotype, and all patients exhibiting any non-desmoplastic growth had a more than two-fold risk of death and cancer recurrence. [10, 11] Differences in tumour vascularisation, cancer-cell motility, and enrichment in the tumour microenvironment have been observed between the different HGPs and provide some biological evidence for their prognostic impact.[7, 12-17]

Retrospective studies are at risk of several types of bias. Therefore, validation of HGPs as a prognostic marker after resection of CRLM in prospective cohorts remains needed. In order to do so, data from two prospective randomized controlled trials (New EPOC & EORTC 40983) [18-21] were combined to confirm the prognostic impact of HGPs after resection of CRLM.

Material and methods

The present study was approved by the medical ethics committee of the Erasmus University Medical Centre Rotterdam (MEC 2018-1743). A waiver for renewed written informed consent was granted.

New EPOC

The New-EPOC randomised controlled trial allocated 257 KRAS exon-2 wild-type patients with (suboptimally) resectable CRLM to perioperative systemic chemotherapy with or without cetuximab, an anti-epidermal growth factor receptor (EGFR) antibody. The chemotherapeutic regimens consisted of CAPOX, FOLFOX or FOLFIRI and was administered for 12 weeks preoperatively, and also 12 weeks postoperatively. Response to preoperative chemotherapy was assessed according to the Response Evaluation Criteria In Solid Tumours (RECIST). Surveillance was performed every three months for the first two years and every 6 months for a further 3 years until progression or death. Follow-up consisted of clinical examination, chest-abdomen-pelvis computed tomography (CT), and laboratory assessments including serum carcinoembryonic antigen (CEA) levels. All 208 trial participants who actually underwent resection of CRLM in the primary analysis of the long-term results were deemed eligible for inclusion.[21] Those with available digitalised haematoxylin and eosin (H&E) stained tissue sections of the resected CRLM suitable for HGP assessment were included. The short- and long-term results of the New EPOC trial have been published. [20, 21]

EORTC 40983

The European Organization for Research and Treatment of Cancer (EORTC) intergroup study 40983 (NCT00006479) prospective randomised controlled trial allocated 364 patients with four or less resectable CRLM to either surgery alone or surgery combined with perioperative systemic chemotherapy. Twelve cycles of the FOLFOX4 regimen were administered as perioperative chemotherapy equally divided pre- and postoperatively.[22] For the perioperative chemotherapy arm, response to preoperative chemotherapy was assessed according to RECIST. Surveillance consisted of chest radiography, abdominal ultrasound or CT scan, and serum CEA determination. Patients were followed-up every 3 months in the first two years following treatment and every 6 months thereafter. For a subset of 82 trial patients H&E resection specimens have previously been collected for post-hoc analysis.[23] All patients in this subset for whom the HGP could be determined were included. The details and short- and long-term results of the original trial have been published. [18, 19]

HGP assessment

All HGP determination was performed retrospectively, blinded for patient characteristics, treatment, and outcome, and according to international guidelines.[9] Digital slide images were reviewed by multiple trained observers (PN, DH, BG) and an expert in the field of HGPs (PV). The assessment algorithm is described in detail in international consensus guidelines. [9] In summary, three HGP phenotypes are recognized: the desmoplastic, replacement, and

pushing HGP. As these can appear in conjunction, the relative presence of each phenotype at the tumour-liver boundary is estimated (expressed in %) on H&E stained slides of resected CRLM. Patients were classified as desmoplastic if only the desmoplastic type HGP was observed in all available slides (i.e. 100% desmoplastic), and as non-desmoplastic if any HGP other than the desmoplastic type (i.e. replacement and/or pushing) was observed in any slide (i.e. <100% desmoplastic).[10, 11] There was no minimum slide requirement for HGP assessment. In accordance with the international guidelines the HGP was however not determined in case of complete pathological response following preoperative treatment (i.e. no vital tumour), if less than one fifth of the total tumour-liver interface was assessable, or if the quality of the tissue section was deemed insufficient for reliable assessment (e.g. tissue tear at the invasive margin). [9] The HGP scores were stored at a third party prior to release of the data to ensure transparency and guarantee blinding for patient characteristics, treatment, and outcome.

Statistical analysis

The main outcome of the study was overall survival, defined as the time in months from the date of randomisation until the date of death. Besides OS, progression-free survival was evaluated. PFS was defined as the time in months from the date of randomisation until the date of disease recurrence, disease progression, or death, whichever occurred first. Survival outcomes were compared using Kaplan-Meier analysis and uni- and multivariable Cox proportional hazards regression analysis. Survival estimates are reported as five year survival rates with corresponding 95% confidence interval (CI) and were compared using the log-rank test, or the Peto & Peto modification of the Gehan-Wilcoxon test in case of crossing survival curves.[24] The results of the Cox regression models are expressed in hazard ratio (HR) with corresponding 95% CI. Median follow-up for survivors was determined using the reverse Kaplan-Meier method. Given the worse OS observed for the patients treated with cetuximab within the New EPOC trial, the effect of cetuximab on OS was compared in desmoplastic and non-desmoplastic patients separately for the New EPOC trial patients using Kaplan-Meier analysis. In addition, effect modification between the HGP and trial cohort, perioperative chemotherapy, and cetuximab was investigated for both OS and PFS using interaction terms for the entire cohort.[25] For all patients treated with preoperative chemotherapy the response according to RECIST, and the relative 'sum of all lesions' after preoperative chemotherapy was compared. The 'sum of all lesions' was defined as the cumulative diameter of all measurable lesions on imaging. The relative 'sum of all lesions' was calculated by dividing the measurement after completion of preoperative chemotherapy by the measurement prior to the start of preoperative chemotherapy. Categorical data are presented as counts with corresponding percentages and continuous data as medians with corresponding interquartile ranges (IQR). Proportional differences were compared by the chi-squared test and differences in medians by the Mann–Whitney U test. Statistical significance was defined as an $\alpha<0.05$. All analyses were performed using R version 4.0.3 (http://www.r-project.org).

Results

Of the 208 patients who underwent liver resection within the New-EPOC trial, digitalised H&E tissue sections were available for 159 (76%). For 11 (22%) of the 49 patients without digitalised H&E slides no residual tumour was reported at pathology. A total of 165 slides were reviewed of which 158 were deemed suitable for HGP assessment. Out of the 159 potentially eligible patients the HGP could be determined in 152 (96%) with a median (range) slide number of 1 (1-2) per patient. Reasons for the HGP not being determined were no or insufficient tumour-liver interface present for 6 patients and insufficient quality for assessment in 1. Of the 152 included patients 79 (52%) received perioperative chemotherapy only (CTx only), and 73 (48%) received perioperative chemotherapy combined with cetuximab (CTx + cetuximab). Baseline characteristics stratified by treatment arm were comparable to that of the original trial population (supplementary table 1). The median (IQR) follow-up for survivors in the 152 New EPOC trial patients was 69 (59-81) months following randomisation. The proportion of patients with a desmoplastic HGP did not differ between treatment arms (CTx only: 24% vs. CTx + cetuximab: 30%, p=0.40, supplementary table 1).

Within the subset of 82 patients of the EORTC 40983 trial clinical data and digitalised H&E slide images were available for 70. A total of 108 slides were reviewed of which 103 were deemed suitable for HGP assessment. In 4 slides the HGP could not be determined due to the absence of a tumour-liver interface, and for 1 slide the quality was insufficient. Despite these 5 unsuitable slide images the HGP could be determined for all 70 patients with a median (range) slide number of 1 (1-7) per patient. Of these 70 patients, 30 (43%) were treated with perioperative chemotherapy. Baseline characteristics stratified by treatment arm were comparable to that of the original trial population (sup. table 2). The median (IQR) follow-up for survivors in the 70 EORTC 40983 trial patients was 106 (93-121) months following randomisation. The proportion of patients with a desmoplastic HGP did not differ between treatment arms (perioperative chemotherapy: 27% vs. resection only: 18%, p=0.35, supplementary table 2).

The combined cohort comprised of 222 patients. A graphical display of the distinct HGP distribution is shown in figure 1. Fifty-six patients (25%) exhibited a desmoplastic phenotype (i.e. 100% desmoplastic) on histopathology. This did not differ between trial cohorts (EORTC 40983: 15 [21%] vs. New EPOC: 41 [27%], p=0.38). Baseline characteristics compared for desmoplastic versus non-desmoplastic phenotype are presented in table 1. Besides higher preoperative serum CEA levels in non-desmoplastic patients (p=0.04, table 1), no differences in baseline or treatment characteristics reached statistical significance (table 1).

Table 1. Baseline characteristics compared for desmoplastic versus non-desmoplastic phenotype

			Desmoplastic	Non-desmoplastic	
		missing (%)	n = 56 (%)	n = 166 (%)	p-value
Age at resection CRLM - *(median [IQR])*			63.0 [59.0. 68.0]	65.5 [59.0. 72.0]	0.08
Gender	Male		35 (62)	107 (64)	0.79
	Female		21 (38)	59 (36)	
Primary tumour location	Left-sided		47 (84)	133 (80)	0.53
	Right-sided		9 (16)	33 (20)	
T-stage	pT 0-2	*8 (4)*	8 (15)	16 (10)	0.33
	pT 3-4		46 (85)	144 (90)	
N-stage	N0	*8 (4)*	20 (37)	58 (36)	0.92
	N+		34 (63)	102 (64)	
Number of CRLM - *(median [IQR])*			2.0 [1.0. 3.0]	2.0 [1.0. 3.0]	0.24
Diameter of largest CRLM in mm - *(median [IQR])*		*3 (1)*	30.0 [20.0. 46.0]	33.0 [23.0. 50.0]	0.24
Disease-free interval in months - *(median [IQR])*		*2 (1)*	1.0 [0.0. 12.0]	2.0 [0.0. 14.8]	0.19
Preoperative CEA in µg/L - *(median [IQR])*		*5 (2)*	6.4 [3.0. 21.4]	14.1 [4.7. 40.8]	0.04
Perioperative chemotherapy	No		7 (12)	33 (20)	0.21
	Yes		49 (88)	133 (80)	
Perioperative cetuximab	No		34 (61)	115 (69)	0.24
	Yes		22 (39)	51 (31)	
Resection margin involved	No		50 (89)	137 (83)	0.23
	Yes		6 (11)	29 (17)	
Extrahepatic disease	No		52 (93)	162 (98)	0.10
	Yes		4 (7)	4 (2)	
Chemotherapy regimen*	CAPOX	*41 (19)*	8 (16)	19 (14)	0.94
	FOLFIRI		5 (10)	13 (10)	
	FOLFOX		36 (73)	100 (76)	

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; IQR: interquartile range. *For the perioperatively treated patients

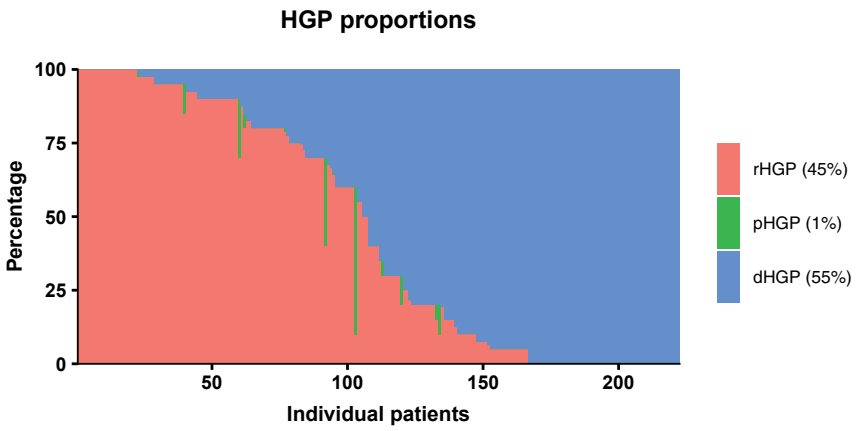

Figure 1. Distribution of the replacement (r), pushing (p), and desmoplastic (d) histopathological growth pattern (HGP) for all 222 patients.

Table 2. Uni- and multivariable Cox-proportional hazards models for overall and progression-free survival

	Overall survival (n=207)				Progression-free survival (n=207)			
	Univariable		Multivariable		Univariable		Multivariable	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
Cohort - *New EPOC vs EORTC 40983*	1.02 [0.69-1.51]	0.92	0.90 [0.47-1.73]	0.76	0.90 [0.65-1.26]	0.54	0.88 [0.52-1.47]	0.62
Right-sided primary - *yes vs no*	1.52 [0.98-2.35]	0.06	1.98 [1.22-3.22]	<0.01	1.21 [0.83-1.76]	0.33	1.43 [0.95-2.14]	0.09
N-stage - *N+ vs N0*	1.37 [0.92-2.04]	0.13	1.26 [0.83-1.91]	0.29	1.32 [0.94-1.83]	0.11	1.23 [0.87-1.74]	0.23
Disease-free interval - *months*	0.99 [0.97-1.00]	0.08	0.98 [0.97-1.00]	0.06	0.99 [0.98-1.00]	0.05	0.99 [0.98-1.00]	0.09
Number of CRLM	0.96 [0.83-1.12]	0.64	1.00 [0.84-1.18]	0.98	1.00 [0.88-1.14]	0.97	1.01 [0.88-1.16]	0.91
Diameter of largest CRLM - *mm*	1.00 [1.00-1.01]	0.59	1.00 [0.99-1.00]	0.24	1.00 [1.00-1.00]	0.93	1.00 [0.99-1.00]	0.29
Preoperative CEA - *µg/L*	1.00 [1.00-1.00]	0.26	1.00 [1.00-1.00]	0.16	1.00 [1.00-1.00]	0.42	1.00 [1.00-1.00]	0.51
Resection margin involved - *yes vs no*	2.09 [1.36-3.22]	<0.001	2.28 [1.39-3.74]	<0.01	1.84 [1.24-2.73]	<0.01	1.93 [1.23-3.03]	<0.01
Perioperative chemotherapy - *yes vs no*	0.89 [0.57-1.40]	0.62	0.69 [0.36-1.35]	0.28	0.85 [0.57-1.27]	0.43	0.82 [0.46-1.45]	0.50
Perioperative cetuximab - *yes vs no*	1.33 [0.91-1.95]	0.15	1.50 [0.91-2.48]	0.11	1.01 [0.73-1.41]	0.93	1.04 [0.69-1.56]	0.86
HGP - *desmoplastic vs non-desmoplastic*	0.64 [0.41-1.01]	0.06	0.58 [0.35-0.95]	0.03	0.51 [0.35-0.76]	<0.001	0.48 [0.32-0.74]	<0.001

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CI: confidence interval; CRLM: colorectal liver metastasis; HGP: histopathological growth pattern; HR: hazard ratio

The median (IQR) follow-up for survivors in the combined cohort was 78 (63-96) months following randomisation, during which 170 (74%) patients experienced disease progression and 120 (52%) died. Patients with a desmoplastic phenotype had significantly longer OS, with 5-year (95%CI) estimates of 66% (54-80) versus 51% (44-60) (Peto & Peto: p=0.04, figure 2A). Similarly PFS was significantly longer for the patients with a desmoplastic phenotype, with 5-year (95%CI) estimates of 45% (34-61) versus 21% (16-28) (Log-rank: p<0.001, figure 2B). Results of the uni- and multivariable OS and PFS Cox proportional hazards regression analyses are reported in table 2. A desmoplastic phenotype was an independent marker (adjusted HR [95%CI]) for good OS outcome (0.58 [0.35-0.95], p=0.03), and good PFS outcome (0.48 [0.32-0.74], p<0.001).

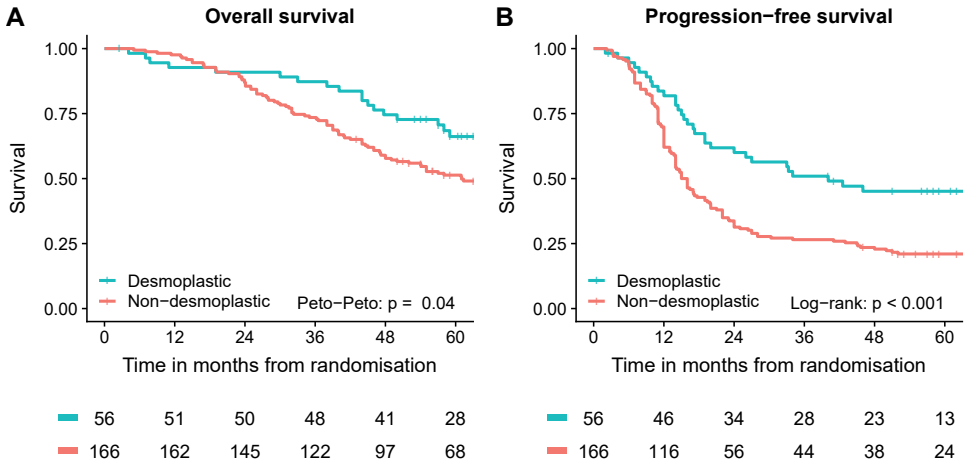

Figure 2. Kaplan-Meier survival curves for overall (A) and progression-free (B) survival by histopathological growth pattern.

No significant differences in overall survival were found between the no cetuximab and cetuximab arms of the New EPOC trial within either the non-desmoplastic (5-year OS 58% vs 37%, p=0.07, supplementary figure 1A) or the desmoplastic patients (5-year OS 68% vs 60%, p=0.77, supplementary figure 1B), respectively. No effect modification could be demonstrated between the HGP and trial cohort, use of perioperative chemotherapy, and the addition of cetuximab for either OS or PFS (OS all p>0.6, PFS all p>0.3, supplementary table 3) within the entire cohort. For the 182 trial patients treated with perioperative chemotherapy the response according to RECIST and the relative 'sum of all lesions' after preoperative chemotherapy compared for HGP is shown in table 3. Response according to RECIST (i.e. complete or partial) was seen in 77% (n=36) of patients with a desmoplastic HGP compared to 71% (n=94) for non-desmoplastic (p=0.55, table 3). Similarly, progressive disease according to RECIST was seen in 2% (n=1) of desmoplastic patients versus 4% (n=3) in non-desmoplastic (p=0.59, table 3). The relative 'sum of all lesions' after preoperative

chemotherapy was smaller for desmoplastic vs. non-desmoplastic patients with a median [IQR] of 0.5 [0.4-0.7] vs. 0.6 [0.4-0.8], respectively, but this difference did not reach statistical significance (p=0.06, table 3). The proportion of non-desmoplastic patients with a relative 'sum of all lesions' greater or equal to 1 (i.e. no change or an increase in the cumulative size of all lesions on imaging after preoperative chemotherapy) was significantly higher than in the desmoplastic patients (18% [n=23] vs. 4% [n=2], p=0.03, table 3). The waterfall plot in figure 3 displays the relative change (i.e. delta) in the 'sum of all lesions' for all patients. All patients with a relative change of 0.25 (i.e. 25% increase) or more were all non-desmoplastic.

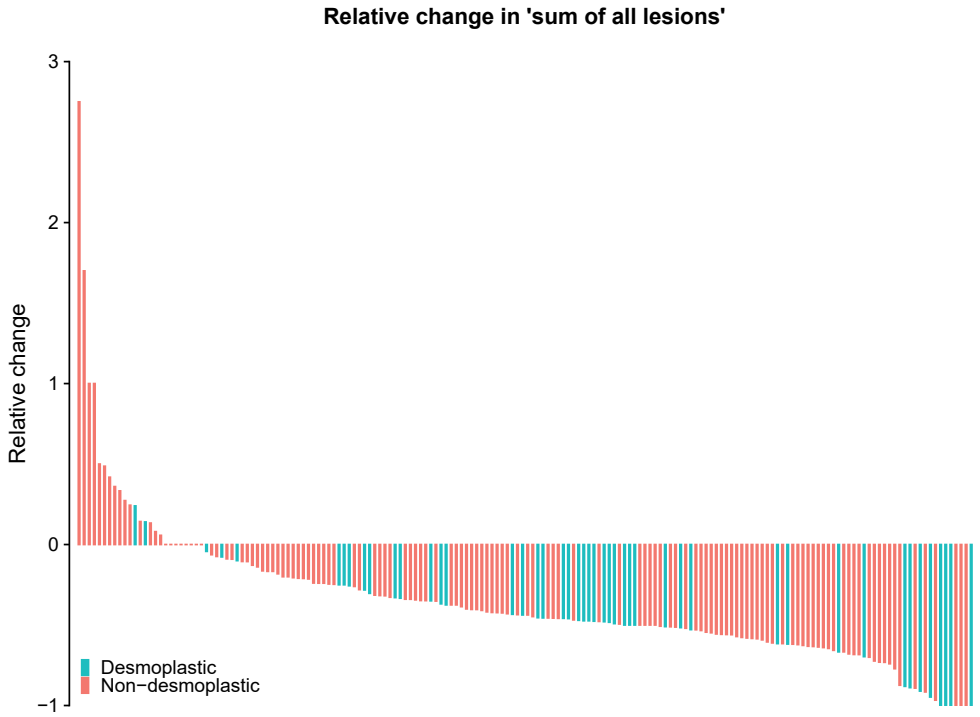

Figure 3. Waterfall plot resembling the relative change in the sum of all lesions on preoperative imaging after preoperative chemotherapy for the 182 trial patients treated with perioperative systemic therapy.

Table 3. Response to preoperative chemotherapy stratified by histopathological growth pattern

			Desmoplastic	**Non-desmoplastic**	
		missing (%)	**n = 49 (%)**	**n = 133 (%)**	**p-value**
Response according to RECIST	Complete response	*3 (2)*	2 (4)	2 (2)	0.66
	Partial response		34 (72)	92 (70)	
	Stable disease		10 (21)	34 (26)	
	Progressive disease		1 (2)	4 (3)	
Response (complete or partial)	No	*3 (2)*	11 (23)	38 (29)	0.48
	Yes		36 (77)	94 (71)	
Progressive disease	No	*3 (2)*	46 (98)	128 (97)	0.75
	Yes		1 (2)	4 (3)	
Relative 'sum of all lesions' - *median [IQR]*		*6 (3)*	0.5 [0.4. 0.7]	0.6 [0.4. 0.8]	0.06
Relative 'sum of all lesions' ≥ 1	No	*6 (3)*	44 (96)	107 (82)	0.03
	Yes		2 (4)	23 (18)	

Abbreviations in alphabetical order: CRLM: colorectal liver metastasis; IQR: interquartile range; RECIST: response evaluation criteria in solid tumours.

Discussion

The current study demonstrates that a desmoplastic phenotype is independently associated with good survival outcomes in patients with resected CRLM in a pooled post-hoc analysis of two prospective phase III randomized controlled trials. Patients with a desmoplastic phenotype had a two-fold risk reduction for death and cancer recurrence.

The prognostic value of HGPs has been shown in multiple retrospective studies with varying sample sizes, HGP classification cut-offs, use of systemic treatments, and length of patient follow-up. [9, 10, 12, 16, 26-31] In the first large retrospective cohort of 732 patients investigating HGPs and prognosis an approximate two-fold risk reduction was demonstrated for patients with a purely desmoplastic HGP compared to patients with any non-desmoplastic HGP, but only in chemo-naive patients.[10] The largest study to date investigating HGPs and prognosis described 780 patients and externally validated HGPs as a marker for good survival following resection of CRLM.[11] In this external validation study a desmoplastic phenotype was again associated with an approximate two-fold risk reduction for death and cancer recurrence following surgical resection of CRLM, but now for both the chemo-naive as well as pre-treated patients. While many reports are thus available, a major limitation of all studies investigating HGPs and prognosis so far has been their retrospective nature. Now with the results of the current study confirming this two-fold risk reduction of death and cancer recurrence for patients with a desmoplastic phenotype in a pooled cohort of two randomized controlled phase III clinical trials, HGPs have for the first time been associated with prognosis within a prospective cohort of patients.

Despite the prospective nature of the cohort, not unlike many of the retrospective studies published before, use and type of systemic treatment varied in this study. But whereas in a retrospective design this might be due to patient selection and therefore prone to selection bias, in the current cohort this variation is a result of the trial designs. The EORTC 40983 trial randomised between upfront resection or perioperative chemotherapy and resection[18, 19], and the New-EPOC trial between perioperative chemotherapy and resection without or with the addition of cetuximab.[20, 21] As a result, the current cohort describes a heterogeneous group of 4 homogenous treatment strategies. Of the 222 patients undergoing resection of CRLM described in the current study 40 received no perioperative systemic treatment, 30 received twelve cycles of FOLFOX4 chemotherapy, 79 received oxaliplatin or irinotecan combined with fluorouracil, and 79 received oxaliplatin or irinotecan combined with fluorouracil and cetuximab. Interpreting results therefore should consider these different treatments. The EORTC 40983 reported a significantly longer PFS for the perioperative treatment arm in the per-protocol analysis, whereas PFS in the intention to treat analysis and OS in general did not differ between treatment arms.[18, 19] The New-EPOC trial demonstrated a significantly worse OS for the patients treated with cetuximab.[20, 21] Another important aspect of this heterogeneity in treatment is that for the great majority (n=182, 82%) HGP assessment was performed in liver and metastasis tissue exposed to systemic chemotherapy. This is relevant as there is increasing evidence

to suggest an interplay between HGP phenotype and chemotherapy. For one preoperative systemic chemotherapy leads to an increase in the desmoplastic phenotype.[10] While this might seem favourable, the prognostic impact may be less after pre-treatment.[10] Also, HGPs have been shown predictive for postoperative systemic chemotherapy efficacy, but only in the preoperatively chemo-naive patients.[32] Prognosis and HGP can therefore only be assessed in light of systemic (chemo)therapy. We tried to investigate this in our current analyses using interaction terms for both perioperative chemotherapy and cetuximab to test for possible effect modification. None of the interaction terms for either OS or PFS however reached statistical significance. We also investigated the effect of cetuximab on OS stratified for HGP within the New EPOC trial patients only but failed to demonstrate a difference in treatment effect of cetuximab between desmoplastic or non-desmoplastic patients. It has to be noted that the sample size of this current cohort lacks statistical power to properly investigate associations between HGP and individual treatment arms of both randomized trials, increasing the likelihood of a type II statistical error. And as far as prognosis of the different treatment strategies of this cohort is concerned, our results demonstrated a desmoplastic phenotype as a marker for good outcome in multivariable regression models with perioperative chemotherapy and cetuximab entered as separate confounders.

Response to preoperative chemotherapy measured in the cumulative size of all lesions on imaging and according to RECIST was also investigated for all patients of both trials receiving preoperative chemotherapy. Given that an increase in desmoplastic HGP is observed following preoperative chemotherapy, one might hypothesize response to chemotherapy would more often be seen in the patients with a desmoplastic HGP, and vice-versa disease progression would more often be seen in the patients with a non-desmoplastic HGP.[10] Although response according to RECIST was more often seen in the patients with a desmoplastic HGP, and the cumulative size of their lesions was relatively smaller after preoperative chemotherapy, none of these differences reached statistical significance. No change or an increase in the cumulative size of all lesions on imaging after preoperative chemotherapy was however significantly associated with a non-desmoplastic HGP. Moreover, all patients in whom a 25% or greater increase in the cumulative size of all lesions was observed were all non-desmoplastic, with some even showing a doubling or even tripling in cumulative size. These results suggest that progression during preoperative chemotherapy might be indicative of a non-desmoplastic HGP. Herein it is important to note that the current study only included patients who actually underwent resection of their CRLM, since a resection specimen remains a prerequisite to reliably determine the HGP. Patients who were thus not resected due to progressive disease under preoperative chemotherapy are not represented in the current analyses. Similarly, complete pathological response to preoperative chemotherapy renders HGP assessment impossible, meaning patients with the most favourable response are also not accounted for. While no patients were excluded due to no residual tumour on H&E within the selection of patients eligible for HGP assessment, 22% of the patients with unavailable H&E slides of the New EPOC trial were reported to have no residual tumour on pathology.

Another important aspect of the nature of this present cohort is that the New-EPOC trial, which comprises 70% of the study, only included KRAS exon-2 wildtype patients. KRAS mutation status is an established biomarker for primary and metastatic CRC.[6, 33] Specifically within the treatment of CRLM, KRAS-mutants (approximately 30-40% of patients) have worse outcome following surgery, in part attributed to a predisposition for early extrahepatic recurrences.[34-37] Some have even associated KRAS with positive resection margins, need for anatomical resection, and reduced local control after ablation.[38-40] While the exact number of KRAS mutants is unknown, (KRAS was not determined in the EORTC 40983 trial) this must be low compared to other cohorts (approximately 12% given a 40% mutation rate in the 70 patients of the EORTC 40983 trial). From this two things can be inferred. First, as the proportion of patients with a desmoplastic and a non-desmoplastic phenotype correspond largely to both large previous retrospective series which did not select patients based on KRAS status, HGP phenotype is likely independent of KRAS.[10, 11] Second, the results of the current study mostly apply to KRAS wildtype patients, meaning it can be an overestimation, underestimation, or correct estimation depending on if and how KRAS and the HGPs may or may not interact. This highlights the need for an in depth analysis of genetic alterations and the HGPs of CRLM. Besides KRAS, such an analysis can elaborate on the relationship with other established biomarkers as well, for instance BRAF mutations and microsatellite instability, both of which were not available for the current cohort.

Despite these several and important limitations and considerations of this post-hoc analysis, this study also has multiple strengths. The prospective nature of the patient cohort minimizes selection bias. The median follow-up for survivors was 7.5 years with all patients undergoing comparable and regularly scheduled follow-up according to a predefined schedule. Moreover data collection on progression and survival was collected prospectively during the entire follow-up period, including standardized assessments of response to preoperative chemotherapy. An important step in the establishment of HGPs as a biomarker for prognosis after resection of CRLM is made by this 'prospective' validation. Considering the low intra- and intertumoural heterogeneity and excellent intraobserver agreement of HGPs, this histopathology marker has proven reliable, relevant, and robust.[41] As such future efforts should aim to unravel underlying biology. Several reports have associated the desmoplastic HGP with an increase in both peritumoural and intratumoural infiltration of T-lymfocytes, specifically CD8+ cytotoxic T-cells.[13, 14, 16] Besides T-cells, the desmoplastic HGP has also been associated with a B-cell signature.[13, 42] These results may suggest an interactivity between the HGP of CRLM and the immune system. This is strengthened by the notion that HGPs are also present in other primary and secondary liver malignancies, with favourable prognosis repeatedly reported for the desmoplastic phenotype.[43-45] These patterns may thus reflect a pan-cancer phenotype of (intrahepatic) immunity. To better understand this, future studies could focus on immune activation and functionality of T-cell populations in relation with HGPs. Besides immunology, as explained above, genetic association studies using DNA or RNA sequencing techniques will also provide valuable information. As for potential clinical implications of HGPs in the treatment of CRLM, for now only postoperative implementation is possible due to the necessity of a resection specimen for determination.

This remains a hurdle for widespread clinical implementation, but also here advancements are being made. Machine learning techniques on MRI are especially promising.[46] Another possibility might be to look at primary CRC histology, which has been associated with HGP morphology.[47]

In conclusion this study confirms for the first time a desmoplastic phenotype as a marker for good survival outcomes in patients undergoing resection of CRLM within prospective randomized controlled trials. The results of this study solidify the association between HGPs and prognosis following surgical resection of CRLM. Our study also suggests that disease-progression during preoperative chemotherapy might be associated with a non-desmoplastic HGP. Given the compelling evidence of the association between HGPs and prognosis implementation of this marker in the standardized histopathology assessment of resected CRLM seems warranted.

Figure captions

Figure 1. Distribution of the replacement (r), pushing (p), and desmoplastic (d) histopathological growth pattern (HGP) for all 222 patients.

Figure 2. Kaplan-Meier survival curves for overall (A) and progression-free (B) survival by histopathological growth pattern.

Figure 3. Waterfall plot resembling the relative change in the sum of all lesions on preoperative imaging after preoperative chemotherapy for the 182 trial patients treated with perioperative systemic therapy.
Supplementary figure 1: Kaplan-Meier overall survival curves for non-desmoplastic (A) and desmoplastic (B) patients of the New EPOC trial by treatment arm.

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Supplementary materials

Supplementary table 1. Baseline characteristics of the New-EPOC trial patients compared for treatment arm

			No cetuximab	**Cetuximab**	
		missing (%)	**n = 79 (%)**	**n = 73 (%)**	**p-value**
Age at resection CRLM - *(median [IQR])*			64.0 [58.0. 69.0]	64.0 [60.0. 69.0]	0.84
Gender	Male		50 (63)	51 (70)	0.39
	Female		29 (37)	22 (30)	
Primary tumour location	Left-sided		69 (87)	57 (78)	0.13
	Right-sided		10 (13)	16 (22)	
T-stage	pT 0-2	*7 (5)*	12 (16)	3 (4)	0.02
	pT 3-4		64 (84)	66 (96)	
N-stage	N0	*7 (5)*	25 (32)	23 (34)	0.86
	N+		52 (68)	45 (66)	
Number of CRLM - *(median [IQR])*			2.0 [1.0. 3.0]	2.0 [1.0. 3.0]	0.55
Diameter of largest CRLM in mm - *(median [IQR])*		*3 (2)*	35.0 [20.0. 47.0]	33.5 [24.0. 55.0]	0.23
Disease-free interval in months - *(median [IQR])*			2.0 [0.5. 15.0]	1.0 [0.0. 12.0]	0.10
Preoperative CEA in µg/L - *(median [IQR])*		*5 (3)*	14.0 [3.9. 39.0]	12.9 [5.0. 25.0]	0.70
Resection margin involved	No		66 (84)	55 (75)	0.21
	Yes		13 (16)	18 (25)	
Extrahepatic disease	No		78 (99)	71 (97)	0.51
	Yes		1 (1)	2 (3)	
Chemotherapy regimen	CAPOX	*1 (1)*	14 (18)	13 (18)	0.51
	FOLFIRI		7 (9)	11 (15)	
	FOLFOX		57 (73)	49 (67)	
Histopathological growth pattern	Desmoplastic		19 (24)	22 (30)	0.40
	Non-desmoplastic		60 (76)	51 (70)	

Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; IQR: interquartile range.

Supplementary table 2. Baseline characteristics of the EORTC 40983 trial patients compared for treatment arm

			Surgery alone	**Surgery with perioperative chemotherapy**	
		missing (%)	**n = 40 (%)**	**n = 30 (%)**	**p-value**
Age at resection CRLM - *(median [IQR])*			67.5 [59.8. 72.0]	65.0 [58.5. 71.8]	0.54
Gender	Male		22 (55)	19 (63)	0.48
	Female		18 (45)	11 (37)	
Primary tumour location	Left-sided		32 (80)	22 (73)	0.51
	Right-sided		8 (20)	8 (27)	
T-stage	pT 0-2	*1 (1)*	5 (13)	4 (13)	0.95
	pT 3-4		34 (87)	26 (87)	
N-stage	N0	*1 (1)*	17 (44)	13 (43)	0.98
	N+		22 (56)	17 (57)	
Number of CRLM - *(median [IQR])*			1.0 [1.0. 2.0]	2.0 [1.0. 3.0]	0.10
Diameter of largest CRLM in mm - *(median [IQR])*			30.0 [24.5. 45.5]	25.0 [20.2. 36.5]	0.10
Disease-free interval in months - *(median [IQR])*		*2 (3)*	5.8 [0.0. 14.5]	0.0 [0.0. 14.8]	0.33
Preoperative CEA in µg/L - *(median [IQR])*			7.4 [2.3. 23.9]	17.2 [4.8. 58.5]	0.08
Resection margin involved	No		37 (92)	29 (97)	0.46
	Yes		3 (8)	1 (3)	
Extrahepatic disease	No		38 (95)	27 (90)	0.42
	Yes		2 (5)	3 (10)	
Histopathological growth pattern	Desmoplastic		7 (18)	8 (27)	0.35
	Non-desmoplastic		33 (82)	22 (73)	

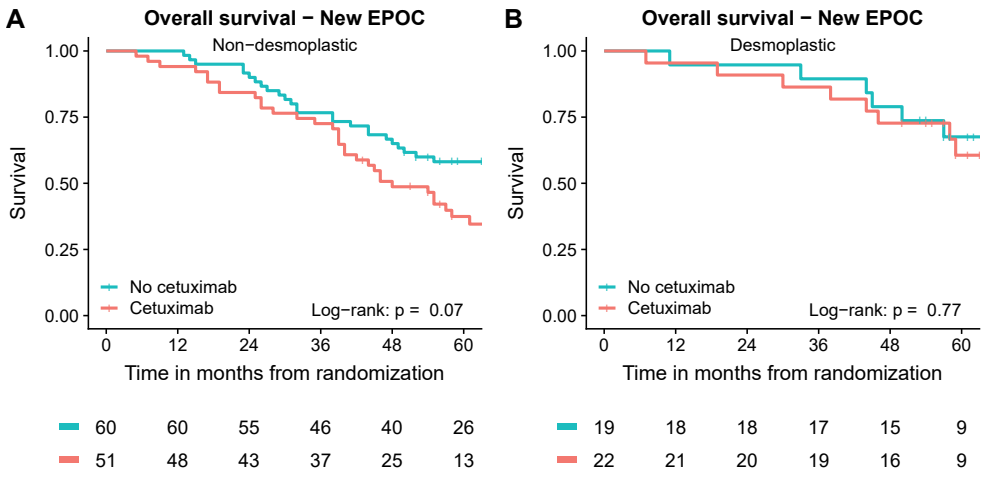
Abbreviations in alphabetical order: CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; IQR: interquartile range.

Supplementary table 3. Multivariable Cox-proportional hazards models with test for interaction for overall and progression-free survival

		Overall survival					
		Variable 1		Variable 2		Variable 1 * Variable 2	
Variable 1	**Variable 2**	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**
HGP - *desmoplastic vs non-desmoplastic*	**Cohort** - *New EPOC vs EORTC 40983*	**1.47 [0.65-3.32]**	**0,35**	**0.98 [0.40-2.39]**	**0,96**	**1.09 [0.41-2.92]**	**0,86**
HGP - *desmoplastic vs non-desmoplastic*	**Perioperative chemotherapy** - *yes vs no*	**2.00 [0.60-6.68]**	**0,26**	**1.19 [0.35-4.03]**	**0,78**	**0.74 [0.20-2.71]**	**0,65**
HGP - *desmoplastic vs non-desmoplastic*	**Perioperative cetuximab** - *yes vs no*	**1.47 [0.82-2.62]**	**0,20**	**1.15 [0.50-2.66]**	**0,75**	**1.27 [0.49-3.25]**	**0,62**

		Progression-free survival					
		Variable 1		Variable 2		Variable 1 * Variable 2	
Variable 1	**Variable 2**	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**	**HR [95%CI]**	**p-value**
HGP - *desmoplastic vs non-desmoplastic*	**Cohort** - *New EPOC vs EORTC 40983*	**1.47 [0.74-2.93]**	**0,27**	**0.68 [0.32-1.45]**	**0,32**	**1.48 [0.64-3.43]**	**0,36**
HGP - *desmoplastic vs non-desmoplastic*	**Perioperative chemotherapy** - *yes vs no*	**3.07 [0.93-10.15]**	**0,07**	**1.39 [0.42-4.58]**	**0,59**	**0.59 [0.17-2.09]**	**0,41**
HGP - *desmoplastic vs non-desmoplastic*	**Perioperative cetuximab** - *yes vs no*	**1.94 [1.18-3.18]**	**<0.01**	**1.04 [0.50-2.14]**	**0,92**	**1.03 [0.46-2.31]**	**0,95**

Abbreviations in alphabetical order: HGP: histopathological growth pattern



Supplementary figure 1. Kaplan-Meier overall survival curves for non-desmoplastic (A) and desmoplastic (B) patients of the New EPOC trial by treatment arm.

PART III

General discussion, future perspectives, summaries and appendices

Chapter 8

General discussion, summary and future perspectives

General discussion, summary and future perspectives

Metastatic spread to the liver is often seen in colorectal cancer (CRC) patients. In about one third of all CRC patients, colorectal liver metastases (CRLM) are found at some point in time. [1-4] A part of this patient population is ineligible for surgical/local treatment of the CRLM and only treatments aimed life prolongation remain. [1-4]

In practically all types of disease including, but not limited to, (solid) malignancies prognostic and predictive markers are of great relevance in order to adequately inform patients, personalise treatment and prevent undertreatment but –equally important– overtreatment as well. *Prognostic markers* distinguish a subset of patients within a population with a distinct course of a certain disease (without the influence of treatment) from the entire population with that disease. This is in contrast to *predictive markers*, which are to be used to define a subset within an entire disease population that will or will not respond to a certain treatment. Ideally – and not rarely – biomarkers in solid malignancies hold both prognostic and predictive value. Since the adoption of liver surgery as mainstay in the treatment of patients with resectable CRLM treating physicians have attempted to predict the potential benefit of a metastasectomy. However, prognosis prediction for patients undergoing hepatic resection for CRLM remains a contemporary problem. To that end several prognostication tools have been constructed. [1-12] For these prognostication tools also the term "clinical risk scores (CRS)" is utilised. As the name implies, these scores evaluate clinical characteristics of the patients, the primary tumour and CRLM (e.g. nodal status of the primary tumour, number and size of the CRLM) to determine the risk for disease recurrence and/or death. Although these CRS may have seemed promising when first described, their reproducibility and therefore clinical applicability proved limited. [12, 13] The most commonly utilised CRS is the one developed by Fong and colleagues. [1] The limited value of currently available CRS in general is underlined in several ways. Firstly, the most commonly used CRS [1] was first described over two decades ago and thus evaluated patients operated for CRLM even before that time and may therefore not adequately reflect patients treated presently. Secondly, the reproducibility of the distinct CRS in external validation cohorts proved limited. [13] Lastly, it was demonstrated that a considerable proportion of patients with a low clinical risk according to the CRS rapidly experience disease recurrence after CRLM resection, whereas an important proportion of patients with high clinical risk survives at ten years and can be considered cured. [14] These findings together corroborate that the existing prognostication tools are of insufficient reliability to be used in clinical decision making. In addition, the aforementioned inconsistencies in prognostic discriminatory value of the existing CRS and other prognostication tools in patients with CRLM have prompted researchers to search for new biomarkers. The past two decades several novel biomarkers in CRC patients have described. Amongst others, mutational status of *RAS* or *BRAF* genes have been suggested as prognostic and predictive biomarkers in patients treated surgically for CRLM. [15-19] In about 37% of CRC patients RAS mutations are seen and BRAF in approximately 5%. [20-22] Patients with RAS or BRAF mutations undergoing resection of CRLM have worse prognosis than their counterparts with RAS and BRAF wild-type. [15-19] In addition, monoclonal

antibodies against the epidermal growth factor receptor (EGFR) only are effective in KRAS wild-type patients. [23] This indicates that RAS mutational status holds both prognostic and predictive value. This finding has led to a modified CRS with the incorporation of the RAS mutational status, which outperformed the traditional CRS in terms of prognostic value. [15] Due to its low incidence, little is known with regard to the relevance of a BRAF mutation in patients undergoing resection for CRLM. Some have questioned the benefit of CRLM resection and therefore its feasibility in patients with BRAF mutations due to the poor survival of patients treated with systemic chemotherapy in palliative setting [24, 25] and in (meta-analysis of) small series with limited follow-up of patients with BRAF mutations undergoing CRLM resection. However, it was recently demonstrated in a large multicentre study long-term overall survival can be achieved in patients with BRAF mutated CRLM. [26] Despite these recent advances in knowledge about genetic biomarkers in CRLM patients none of the established CRS or genetic biomarkers currently impacts the decision making in the surgical treatment of patients with resectable CRLM. This underlines the need for novel prognostic and predictive biomarkers that are a proper reflection of tumour biology in patients with (potentially) resectable CRLM.

As liver resection for CRLM became accepted as standard of care towards the end of the previous millennium, higher quantities of CRLM tissue became available as more resections were performed. In contrast to the decades before then, as the presence of CRLM was often posed as a contraindication for surgical treatment. Around this period it became noted by several unrelated groups that different patterns of tumour growth could be distinguished when reviewing CRLM under the microscope. [27, 28] Different nomenclature was used in various parts of the world, but essentially describe the same types of growth patterns (GP). Nagashima and colleagues first described several different types of GPs: the invasive GP (subdivided in infiltrative and expansive), the marginal fibrosis GP and the lymphocytic infiltration GP. As the name implies, in the infiltrative GP liver plates are directly infiltrated by tumour cells. The expansive GP indicates that the tumour expands within the liver without the presence separating tissue, but does not infiltrate. In the marginal fibrosis GP the metastasis is separated from the liver parenchyma by fibrosis. The lymphocytic infiltration GP was given if copious amounts of lymphocytes and other inflammatory cells were seen around the metastasis. The infiltrative patterns was associated with worse prognosis after CRLM resection. [27] Shortly thereafter, the currently most often utilised terms were first described consisting of the desmoplastic (d) histopathological growth pattern (HGP), the replacement (r) HGP and the pushing (p) HGP. [28] HGPs describe the manner of growth of a CRLM at transition border from metastasis to liver parenchyma. In dHGP metastases are separated from the liver parenchyma by a fibrotic capsule consisting of desmoplastic stroma and a dense lymphocytic infiltrate is practically always present. The architecture of the liver parenchyma is not “preserved” and these metastases are dependent on neoangiogenesis for their blood supply. [28] No direct contact between hepatocytes and tumour cells is observed. The rHGP owes its name to the fact that tumour cells “replace” hepatocytes while conserving the reticulin network of the parenchyma and thereby preserving the architecture of the liver. The rHGP is characterised by minimal neoangiogenesis, instead

blood supply is acquired by means of vessel co-option. This means that the existing liver sinusoidal blood vessels falls victim to hostile takeover by the metastasis that thereby bypasses the need for newly formed vasculature. Intimate direct cell-cell contact is seen between hepatocytes and cancer cells. The pHGP describes a pattern of growth in which the liver cell plates are pushed aside, but infiltrative growth and desmoplastic stroma are absent. Some studies have suggested that dHGP is associated with superior prognosis when compared to replacement- and pushing-type tumours (i.e. non-desmoplastic type tumours (non-dHGP)). These non-dHGP tumours have been linked to aggressive tumour biology (e.g. increased cancer cell motility, non-angiogenic growth [29]) and reduced infiltration of CD8+ immune cells [30], resulting in poor prognosis after resection of CRLM. [29-34] Since the first description of HGPs they have been associated with prognostic value in various cohorts. [29-35] These studies were of varying quality as sample size often was limited, as was the length of patient follow-up. In addition, these studies did not adequately differentiate between patients that were and were not treated preoperatively with systemic chemotherapy. Preoperative chemotherapy may influence the type of HGP observed, which could have biased the outcomes. Most importantly, they were executed before any consensus existed with regard to the manner of HGP determination. While HGPs of CRLM had been described for nearly two decades, consensus on how to systematically and uniformly assess them was lacking until recently. International consensus guidelines have provided a framework for HGP assessment in a uniform and replicable manner. [36] The aforementioned indicates that the need existed for a large study on HGPs, adequately stratified for preoperative treatment and corrected for other known risk factors with long-term follow-up while the HGPs are determined in a replicable manner. An effort was made in **Chapter 1** of the current thesis to fulfil this need. The study not only confirmed the prognostic value of HGPs in chemo-naive patients, but also showed that the presence, rather than its abundance, of *any* non-dHGP is sufficient to indicate impaired prognosis in patients with resected CRLM. This means that patients with pure dHGP have a relatively good prognosis compared to all other patients with (a proportion of) non-dHGP, making HGPs an “on/off phenomenon”. No additional prognostic impact of an increasing percentage of non-dHGP was observed. All studies regarding HGPs previously utilised an arbitrary cut-off point (e.g. >50% or >75%) to determine the “predominant” HGP. Similarly, the recent consensus paper advocates the 50% cut-off point for this purpose. However, given the findings of our study the results of all previous HGP studies, including those of the consensus paper, should be re-evaluated and future studies should take into account this on/off phenomenon rather than using arbitrary cut-off values. Importantly, the prognostic value in patients preoperatively treated with systemic chemotherapy was reduced while the presence of dHGP was higher in this subset of patients. This suggests that chemotherapy is either associated with a change in growth pattern or at least with a different patient selection. As the HGP currently can only be determined postoperatively, the possibility of truly evaluating the HGP within the same patient pre-and post-chemotherapy remains elusive at present. Apart from prognostic value, HGPs seem to hold predictive value as well since it was recently shown that HGPs might be utilised to predict the effectivity of systemic chemotherapy. [37] Preoperative knowledge of the HGP would enable researchers to evaluate the conversion by chemotherapy

hypothesis and clinicians to take the HGP into account when considering the administering of preoperative chemotherapy. This underlines the need for methods to preoperatively determine the HGP. In addition to the search for a less or non-invasive surrogate for HGP determination, validation of our findings should be sought for, preferably in randomised setting. Currently the only predictive biomarker approved in CRLM patients is the RAS mutational status, which is utilised for determining whether benefit is to be expected from administering anti-EGFR inhibitors. There is no such guiding instrument for regular systemic chemotherapy and fulfilment of this vacancy would have enormous clinical impact. The HGP has shown potential to fulfil this need. If the predictive value of HGPs could be validated prior to the discovery of non-invasive surrogates for HGP determination, the Dutch practice of only administering chemotherapy preoperatively when indicated and the indications on itself should be reconsidered.

The fact that HGPs possess both prognostic and predictive value makes them a promising biomarker within the field of CRLM treatment. However, prognostic and predictive characteristics are not the only necessities for a reliable and applicable biomarker. Knowledge on the replicability, learnability and its heterogeneity is also vital. In **Chapter 2** these essential biomarker characteristics have been evaluated and HGPs were found to exhibit little heterogeneity and can be determined with a high diagnostic accuracy, making them a reliable and replicable histological biomarker. HGPs can be determined on ordinary haematoxylin and eosin stained tissue sections. This indicates no additional staining is required compared to routine pathology investigation of CRLM resection specimens. HGP determination has recently been standardised. [36] The current chapter demonstrates that untrained researchers without prior pathology experience can rapidly learn to score the HGP reliably with a high diagnostic accuracy and experienced pathologist even more so. This in combination with the fact that no additional resources are needed to determine HGPs, makes them an ideal candidate to be included in routine pathology assessment of CRLM resection specimens.

The current thesis describes several efforts to evaluate HGPs in terms of clinical applicability in patients undergoing surgical treatment of CRLM. One of them handles about a prognostic factor that has been the subject of discussion for decades within the field of CRLM surgery: the hepatic resection margin. Positive margins (i.e. tumours cells present at the resection margin) have been suggested to be a reflection of underlying tumour biology rather than surgical technique. [38-40] As the non-dHGP has been demonstrated to reflect tumour biology of resectable CRLM, it was hypothesised in **Chapter 3** that patient with non-dHGP were at higher risk of positive resection margins. This hypothesis could be confirmed, but an increasing number of CRLM was also associated with a higher positive margins risk suggesting that not only tumour biology, but surgical technique as well may influence the risk of positive margins during CRLM resection. Preoperative determination of HGPs would clear the road for several personalised treatment possibilities in CRLM treatment. Amongst others, the surgical plan could be adapted according to an increased risk of an irradical resection seen in non-dHGP CRLM. Moreover, additional therapies aimed at treating

occult metastases in the form of systemic or localised chemotherapeutic treatments could be utilised more frequently and more specifically in these patients as well. On the other hand, others might be spared from these additional treatments and their side-effects when the a priori chance of occult metastases is limited. This is obviously not limited to occult metastases at the resection margin, but also the case in patients with occult distant and/or CRLM at time of first liver resection. In addition, this higher risk for positive margins might also impact other local treatment strategies for CRLM including ablative therapies. This has already been demonstrated for KRAS mutational status. [41] Deduced from the higher positive margin rates in non-dHGP CRLM, a larger ablation zone might be justified in patients with non-dHGP CRLM. Despite advances in the treatment of CRLM the past few decades, the majority of patients experience recurrent disease with recurrences rates reaching over 70%. [14, 42-45] In an attempt to clarify the survival differences between CRLM patients with different HGPs, **Chapter 4** describes the pattern of recurrence after first CRLM resection and the salvageability of the recurrence stratified for HGP. Patients with non-dHGP at first CRLM resection more often experienced multi-organ recurrence which were also less likely to be salvageable with local treatment modalities compared to their dHGP counterparts. In contrast to the resection margin, in this case the HGP is known which indicates that it can already be used to determine whether additional treatment might be beneficial. When dHGP is observed, localised treatment directed at occult disease in the liver might be considered as dHGP is associated with liver only recurrent disease. Additional systemic chemotherapy might be considered in case of non-dHGP as more often recurrent disease often is multifocal. Importantly, localised chemotherapeutic treatment strategies seem less favourable in case of non-dHGP CRLM for the same reason. The results with regard to recurrence pattern after first resection of CRLM and positive margins need validation, but are nonetheless promising in the sense that they might indicate future clinical applicability of HGPs in the management of patients with CRLM.

In patients with CRLM present at time of diagnosis of the primary tumour the dilemma of what to treat first emerges. An option is the liver-first approach – preoperative systemic chemotherapy followed by hepatic resection for CRLM and resection of the primary tumour as final procedure. [46-48] A proportion of patients does not complete the treatment sequence with curative intent. [46-51] **Chapter 5** evaluated whether the non-completion of the liver-first treatment might be predicted. dHGP was found to be a strong predictor for the completion of the liver-first protocol with curative intent. As the liver resection is the first stage of this two-staged approach the HGP might be taken into account when considering the final stage: the lower pelvic surgery after chemoradiotherapy with its high morbidity rates. In case dHGP is observed at the liver resection, it might be justified to be more aggressive in order to complete the sequence, whereas a more conservative approach might be logical in case non-dHGP is seen. Importantly, the HGP might allow clinicians to inform patients more adequately with regard to the a prior chance of completion of the entire treatment sequence.

In **Chapter 6** the potential influence of systemic preoperative chemotherapy on HGPs was studied by evaluating the distribution of the HGPs stratified for preoperative systemic treatment status in an original cohort, an external validation cohort and in a post-hoc analysis of a subset from the EORTC 40983 randomised controlled clinical trial. [52, 53] The results of this study support the HGP conversion by preoperative chemotherapy hypothesis postulated in **Chapter 1**. Previous studies, including the consensus paper, regarding the HGP did not adequately differentiate patients based on preoperative treatment status. The results in this chapter suggest that the dHGP after chemotherapy might be a different entity than the dHGP observed in chemo-naive patients. This might have induced erroneous categorisation of patients in previous work, possibly explaining the reduced prognostic discriminatory value after chemotherapy described in **Chapter 1**. This new insight should be taken into account in future research regarding HGPs. Lastly, in **Chapter 7** the prognostic value HGPs was evaluated by means of a post-hoc analysis of the two prospective randomised controlled trials, the EPOC and the New EPOC trial [52-55] in patients who received perioperative chemotherapy.

Future perspectives

HGPs of CRLM have been described for over 15 years [28], their assessment has recently been standardised [36] and their prognostic value has been demonstrated in the current thesis and previous research. [29-36] In addition, there is evidence to suggest that HGPs harbour predictive value as well. [37] Several issues need to be addressed before clinical applicability of HGPs in the treatment of CRLM patients becomes reality. The consensus paper [36] marked the beginning of standardised HGP assessment, making HGPs easily reproducible and readily available. The current thesis, however, has consequences for parts of the consensus paper. For instance, the results of **Chapter 1** indicate that no cut-off for the determination of the predominant HGP should be used. In addition, The results of **Chapters 1 and 6** suggest that there might be an effect of preoperative chemotherapy on the HGP observed. It appears that the proportion of dHGP is higher after chemotherapy, which might be explained by a conversion by chemotherapy. Obviously, validation of the results presented here are in need of validation. However, these findings together corroborate that it might be considered to re-evaluate and possibly update the consensus taking into account the results described in the current thesis. Several efforts are made to validate the predictive and prognostic value of HGPs in prospective setting. The gold standard for the validation of their predictive value would be a randomised controlled trial including patients with non-dHGP CRLM and randomly allocating them to receive postoperative systemic chemotherapy after CRLM resection or standard of care being follow-up only. Moreover, clinical applicability of HGPs should also be further evaluated in existing clinical trials. For instance in the ongoing PUMP trial. [56] The current thesis demonstrates that patients with dHGP CRLM at first liver resection more often develop recurrences confined to the liver. This could indicate that these patients would benefit most from therapies directed solely at disease in the liver such as hepatic arterial infusion pump chemotherapy. Apart from validation, a truly important hurdle to take is that resection, at present, is a prerequisite for HGP determination. Preoperative knowledge of the HGP would allow more personalised treatment for CRLM patients. For example, patients with non-dHGP could be treated with preoperative chemotherapy or a wider resection margin could be aimed for in these patients as we know they are at higher risk for positive margins. In addition, knowledge of the HGP without resection would enable clinicians to also tailor treatment using HGPs in patients ineligible for CRLM resection. Several options for HGP determination without resection are currently being explored. Examples include computational radiomics [57] and liquid biopsies [58] such as circulating tumour cells and cell-free DNA. [59-61] In addition, although its applicability for CRLM remains to be elucidated, the “electronic nose” has shown promising results for becoming a non-invasive, diagnostic tool for the detection of CRC. [62] Future research not only should focus on finding an preoperative or non-invasive surrogate marker for HGPs, but should also aim to unravel the underlying biological mechanisms and genetic basis of HGPs. To that end, tumour and hepatic tissue (also specifically sampled for the HGP) was prospectively collected from all patients who provided informed consent and underwent resection of CRLM at the Erasmus MC Cancer Institute or one of the other participating centres throughout Europe. The tissue was subsequently whole genome

sequenced and is currently being evaluated whether the genetic basis that underlies the HGP can be unravelled. Genetic pathways potentially important for HGPs and prognosis prediction in CRLM patient will subsequently be evaluated in animal studies in the near future. If the genetic basis of distinct HGPs would be exposed, this could be used in the search for non-invasive surrogate markers for HGPs. Ultimately, this would hopefully lead to a biomarker with true potential to influence clinical decision making in CRLM patients and thereby optimising treatment strategies.

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Chapter 9

Dutch summary / Nederlandse samenvatting

Nederlandse samenvatting

Uitzaaiingen naar de lever worden vaak gezien in patiënten met een colorectaal carcinoom (CRC). In ongeveer een derde van alle CRC patiënten is er op een gegeven moment sprake van zogeheten colorectale levermetastasen (CRLM). Een gedeelte van deze patiënten komt niet in aanmerking voor chirurgie c.q. lokale behandeling van de CRLM en slechts behandelingen met als doel levensverlenging resteren dan. [1-4]

Toen tegen het einde van het vorige millennium leverresectie voor CRLM als standaardzorg werd geaccepteerd, kwamen grotere hoeveelheden CRLM weefsel beschikbaar naarmate er meer resecties werden uitgevoerd. In tegenstelling tot de decennia daarvoor, omdat de aanwezigheid van CRLM vaak werd gebruikt als een contra-indicatie voor chirurgische behandeling. Rond deze periode werd opgemerkt door verschillende niet-verwante groepen dat verschillende patronen van tumorgroei konden worden onderscheiden wanneer CRLM onder de microscoop werden bekeken. [5, 6] Verschillende nomenclatuur werd gebruikt in verschillende delen van de wereld, maar beschrijft in wezen dezelfde soorten groeipatronen (GP). Nagashima en collega's beschreven eerst verschillende typen GP's: het invasieve GP (onderverdeeld in infiltratief en expansief), het marginale fibrose GP en het lymfocytaire infiltratie GP. Zoals de naam al indiceert, worden in het infiltratieve GP leverplaten direct geïnfiltreerd door tumorcellen. Het expansieve GP geeft aan dat de tumor in de lever uitzet zonder de aanwezigheid van scheidend weefsel, maar niet infiltreert. Bij het marginale fibrose GP wordt de metastase door fibrose gescheiden van het leverparenchym. Met het lymfocytaire infiltratie GP werd bedoeld dat er grote hoeveelheden lymfocyten en andere ontstekingscellen rond de metastase werden gezien. De infiltratieve patronen waren geassocieerd met een slechtere prognose na CRLM-resectie. [5] Kort daarna werden de momenteel het meest gebruikte termen voor het eerst beschreven, bestaande uit het desmoplastische (d) histopathologische groeipatroon (HGP), het "replacement" (r) HGP en het "pushing" (p) HGP. [6] HGPs beschrijven de groeiwijze van een CRLM aan overgang van metastase naar leverparenchym. Bij het dHGP worden metastasen gescheiden van het leverparenchym door een fibrotische capsule bestaande uit desmoplastisch stroma en is praktisch altijd een dicht lymfocyten infiltraat aanwezig. De architectuur van het leverparenchym wordt niet "behouden" en deze metastasen zijn voor hun bloedtoevoer afhankelijk van neoangiogenese. [6] Er wordt geen direct contact tussen hepatocyten en tumorcellen waargenomen. Het rHGP dankt zijn naam aan het feit dat tumorcellen hepatocyten "vervangen" terwijl ze het reticuline-netwerk van het parenchym behouden en daardoor de architectuur van de lever behouden. Het rHGP wordt gekenmerkt door minimale neoangiogenese, in plaats daarvan wordt bloedtoevoer verkregen door middel van coöptatie van bloedvaten. Dit betekent dat de bestaande sinusoïdale bloedvaten van de lever het slachtoffer worden van een vijandige overname door de metastase die daarmee de behoefte aan nieuw gevormd vaatstelsel omzeilt. Intiem direct cel-celcontact wordt gezien tussen hepatocyten en kankercellen. Het pHGP beschrijft een groeipatroon waarbij de levercelplaten opzij worden gedrukt, maar infiltratieve groei en desmoplastisch stroma ontbreken. Sommige studies hebben gesuggereerd dat dHGP geassocieerd is met

een superieure prognose in vergelijking met tumoren van het vervangende en pushing-type (i.e. non-desmoplastische tumoren (non-dHGP)). Deze niet-dHGP-tumoren zijn in verband gebracht met agressieve tumorbiologie (e.g. verhoogde motiliteit van kankercellen, niet-angiogene groei [7]) en verminderde infiltratie van CD8 + immuuncellen [8], wat resulteert in een slechte prognose na resectie van CRLM. [7-12] Sinds de eerste beschrijving van HGPs zijn ze in verschillende cohorten in verband gebracht met prognostische waarde.[7-13] Deze onderzoeken waren van wisselende kwaliteit, aangezien de steekproefomvang vaak beperkt was, evenals de duur van de follow-up van de patiënt. Bovendien maakten deze onderzoeken geen adequaat onderscheid tussen patiënten die preoperatief wel en niet werden behandeld met systemische chemotherapie. Preoperatieve chemotherapie zou het waargenomen type HGP kunnen beïnvloeden, wat de uitkomsten zou kunnen aantasten. Bovendien werden deze studies uitgevoerd voordat er een consensus bestond over de manier waarop HGP word bepaald. Hoewel HGPs van CRLM al bijna twee decennia werden beschreven, ontbrak tot voor kort consensus over hoe ze systematisch en uniform konden worden beoordeeld. Internationale consensusrichtlijnen hebben een kader geboden voor HGP-beoordeling op een uniforme en repliceerbare manier. [14] Het bovenstaande geeft aan dat er behoefte bestond aan een groot onderzoek naar HGPs, adequaat gestratificeerd voor preoperatieve behandeling en gecorrigeerd voor andere bekende risicofactoren met langdurige follow-up, terwijl de HGPs op een repliceerbare manier worden bepaald. In **Hoofdstuk 1** van dit proefschrift is een poging gedaan om aan deze behoefte te voldoen. De studie bevestigde niet alleen de prognostische waarde van HGPs bij chemo-naïeve patiënten, maar toonde ook aan dat de *aanwezigheid*, in plaats van de *hoeveelheid*, van non-dHGP voldoende is om een verminderde prognose te indiceren bij patiënten met gereseceerde CRLM. Dit betekent dat patiënten met een zuivere dHGP een relatief goede prognose hebben in vergelijking met alle andere patiënten met (een deel) non-dHGP, waardoor HGPs een “aan/uit-fenomeen” zijn. Er werd geen additionele prognostische impact van een toenemend percentage non-dHGP waargenomen. Alle studies met betrekking tot HGPs gebruikten voorheen een willekeurig afkappunt (bijv.> 50% of> 75%) om het “dominante” HGP te bepalen. Evenals de recente consensus voor het afkappunt van 50% voor dit doel heeft gepleit. Gezien de bevindingen van onze studie moeten de resultaten van alle eerdere HGP onderzoeken, inclusief die van de consensus, opnieuw worden geëvalueerd en toekomstige studies moeten rekening houden met dit aan/uit-fenomeen in plaats van met willekeurige afkapwaarden. Belangrijk is dat de prognostische waarde bij patiënten die preoperatief werden behandeld met systemische chemotherapie was verminderd, terwijl de aanwezigheid van dHGP hoger was in deze subgroep van patiënten. Dit suggereert dat chemotherapie ofwel wordt geassocieerd met een verandering in het groeipatroon of op zijn minst met een andere patiënten selectie. Aangezien het HGP momenteel alleen postoperatief kan worden bepaald, blijft de mogelijkheid om de HGP echt te evalueren bij dezelfde patiënt pre- en post-chemotherapie momenteel niet mogelijk. Afgezien van prognostische waarde, lijken HGPs ook een voorspellende waarde te hebben, aangezien onlangs werd aangetoond dat HGPs kunnen worden gebruikt om de effectiviteit van systemische chemotherapie te voorspellen. [15] Preoperatieve kennis van de HGP zou onderzoekers in staat stellen om de conversie door chemotherapiehypothese te evalueren en clinici om rekening te houden

met de HGP bij het overwegen van preoperatieve chemotherapie. Dit onderstreept de behoefte aan methoden om preoperatief het HGP te bepalen. Naast de zoektocht naar een minder of niet-invasieve surrogaat voor HGP bepaling, moet er gezocht worden naar validatie van onze bevindingen, bij voorkeur in een gerandomiseerde setting. Momenteel is de enige voorspellende biomarker die is goedgekeurd voor CRLM patiënten de RAS-mutatiestatus, die wordt gebruikt om te bepalen of er voordeel te verwachten is van het toedienen van anti-EGFR-remmers. Er bestaat niet zo'n sturend instrument voor reguliere systemische chemotherapie en vervulling van deze vacature zou een enorme klinische impact hebben. Het HGP heeft het potentieel getoond om in deze behoefte te voorzien. Als de voorspellende waarde van HGPs kan worden gevalideerd voorafgaand aan de ontdekking van niet-invasieve surrogaten voor HGP bepaling, moeten de Nederlandse praktijk om chemotherapie alleen preoperatief toe te dienen wanneer geïndiceerd en de indicaties op zichzelf worden heroverwogen.

Het feit dat HGPs zowel prognostische als voorspellende waarde bezitten, maakt ze een veelbelovende biomarker op het gebied van CRLM behandeling. Echter, prognostische en voorspellende kenmerken zijn niet de enige vereisten voor een betrouwbare en toepasbare biomarker. Kennis over de repliceerbaarheid, leerbaarheid en de heterogeniteit ervan zijn ook essentieel. In **Hoofdstuk 2** zijn deze essentiële biomarker karakteristieken geëvalueerd. Er werd gevonden dat HGPs weinig heterogeniteit vertonen en kunnen worden bepaald met een hoge diagnostische nauwkeurigheid, waardoor ze een betrouwbare en repliceerbare histologische biomarker zijn. HGPs kunnen worden bepaald op gewone met hematoxyline en eosine gekleurde weefselcoupes. Dit geeft aan dat er geen aanvullende kleuring nodig is in vergelijking met routinematig pathologisch onderzoek van CRLM resectiepreparaten. De HGP bepaling is onlangs gestandaardiseerd. [36] Het huidige hoofdstuk laat zien dat ongetrainde onderzoekers zonder voorafgaande pathologie-ervaring snel kunnen leren om het HGP betrouwbaar te scoren met een hoge diagnostische nauwkeurigheid en een ervaren patholoog zelfs nog meer. Dit in combinatie met het feit dat er geen extra middelen nodig zijn om HGPs te bepalen, maakt ze een ideale kandidaat om te worden opgenomen in routinematige pathologiebeoordeling van CRLM resectiepreparaten.

Dit proefschrift beschrijft verschillende pogingen om HGPs te evalueren in termen van klinische toepasbaarheid bij patiënten die een chirurgische behandeling van CRLM ondergaan. Een van hen behandelt een prognostische factor die al decennia onderwerp van discussie is binnen het gebied van CRLM chirurgie: de resectiemarge. Er wordt gesuggereerd dat positieve marges (i.e. als tumorcellen aanwezig zijn bij de resectiemarge) een weerspiegeling zijn van de onderliggende tumorbiologie in plaats van de chirurgische techniek. [16-18] Aangezien is aangetoond dat het non-dHGP de tumorbiologie van resectabele CRLM weerspiegelt, werd in **Hoofdstuk 3** de hypothese getest dat patiënten met non-dHGP een hoger risico liepen op positieve resectiemarges. Deze hypothese kon worden bevestigd, maar een toenemend aantal CRLM werd ook geassocieerd met een hoger risico op positieve resectiemarges, wat suggereert dat niet alleen de tumorbiologie, maar ook de chirurgische techniek het risico op positieve marges tijdens CRLM-resectie kan

beïnvloeden. Preoperatieve bepaling van HGPs zou de weg vrijmaken voor verschillende gepersonaliseerde behandelingsmogelijkheden bij CRLM-behandeling. Het chirurgische plan zou onder meer kunnen worden aangepast aan een verhoogd risico op een irradicale resectie bij niet-dHGP CRLM. Bovendien zouden aanvullende therapieën gericht op het behandelen van occulte metastasen in de vorm van systemische of gelokaliseerde chemotherapeutische behandelingen vaker en ook meer specifiek bij deze patiënten kunnen worden toegepast. Aan de andere kant kunnen anderen worden gespaard van deze aanvullende behandelingen en hun bijwerkingen wanneer de a priori kans op occulte metastasen beperkt is. Dit is uiteraard niet beperkt tot occulte metastasen aan de resectiemarge, maar ook het geval bij patiënten met occulte afstand en/of CRLM op het moment van eerste leverresectie. Bovendien kan dit hogere risico op positieve marges ook van invloed zijn op andere lokale behandelingsstrategieën voor CRLM, waaronder ablatieve therapieën. Dit is al aangetoond voor de KRAS-mutatiestatus. [19] Afgeleid van de hogere proportie positieve resectiemarges in non-dHGP CRLM, zou een grotere ablatiezone gerechtvaardigd kunnen zijn bij patiënten met non-dHGP CRLM. Ondanks de vooruitgang in de behandeling van CRLM in de afgelopen decennia, ervaart de meerderheid van de patiënten recidief ziekte met een recidiefpercentage van meer dan 70%. [20-24] In een poging om de verschillen in overleving tussen CRLM-patiënten met verschillende HGPs op te helderen, beschrijft **Hoofdstuk 4** het patroon van recidief na de eerste CRLM resectie en de behandelbaarheid van het recidief gestratificeerd voor HGP. Patiënten met non-dHGP bij de eerste CRLM resectie hadden vaker een recidief van meerdere organen, die ook minder vaak te behandelen waren met lokale behandelingsmodaliteiten met een curatieve intentie in vergelijking met hun dHGP-tegenhangers. In tegenstelling tot de resectiemarge is in dit geval het HGP bekend, wat aangeeft dat hiermee al kan worden bepaald of aanvullende behandeling zinvol kan zijn. Wanneer dHGP wordt waargenomen, kan plaatselijke behandeling gericht op occulte ziekte in de lever worden overwogen, aangezien dHGP wordt geassocieerd met terugkeer van ziekte beperkt tot de lever. Aanvullende systemische chemotherapie kan worden overwogen in het geval van non-dHGP, aangezien recidiverende ziekte vaker multifocaal is. Belangrijk is dat lokale chemotherapeutische behandelingsstrategieën om dezelfde reden minder gunstig lijken in het geval van non-dHGP CRLM. De resultaten met betrekking tot het recidiefpatroon na de eerste resectie van CRLM en positieve resectiemarges behoeven validatie, maar zijn niettemin veelbelovend in die zin dat ze kunnen duiden op toekomstige klinische toepasbaarheid van HGPs bij de behandeling van patiënten met CRLM.

Bij patiënten met CRLM die aanwezig waren op het moment van diagnose van de primaire tumor, doet zich het dilemma voor wat als eerste moet worden behandeld. Een optie is de “liver-first” benadering - preoperatieve systemische chemotherapie gevolgd door hepatische resectie voor CRLM en resectie van de primaire tumor als laatste procedure. [25-27] Een deel van de patiënten voltooit de behandelreeks niet met curatieve intentie. [25-30] **Hoofdstuk 5** evalueerde of de niet-voltooiing van de liver-first behandeling zou kunnen worden voorspeld. dHGP bleek een sterke voorspeller te zijn voor de voltooiing van het liver-first protocol met curatieve intentie. Aangezien de leverresectie de eerste fase is van deze tweetraps benadering, kan het HGP in aanmerking worden genomen bij het overwegen van

de laatste fase: de kleine bekkenoperatie na chemoradiotherapie met de bijkomende hoge morbiditeitscijfers. In het geval dat dHGP wordt waargenomen bij de leverresectie, kan het gerechtvaardigd zijn om agressiever te zijn om de sequentie te voltooien, terwijl een meer conservatieve benadering logisch kan zijn in het geval dat non-dHGP wordt gezien. Belangrijk is dat de HGP clinici in staat zou kunnen stellen om patiënten adequater te informeren over de kans op voltooiing van de volledige behandelsequentie.

In **Hoofdstuk 6** werd de potentiële invloed van systemische preoperatieve chemotherapie op HGPs bestudeerd door de verdeling van de HGPs gestratificeerd voor preoperatieve systemische behandelingsstatus in een origineel cohort, een extern validatiecohort en in een post-hoc analyse van een subgroep uit de EORTC 40983 te evalueren. gerandomiseerde gecontroleerde klinische studie. [31, 32] De resultaten van deze studie ondersteunen de HGP conversie door middel van preoperatieve chemotherapie-hypothese zoals gepostuleerd in **Hoofdstuk 1**. Eerdere studies, inclusief de consensus paper, met betrekking tot de HGP lieten patiënten niet adequaat differentiëren op basis van preoperatieve behandelingsstatus. De resultaten in dit hoofdstuk suggereren dat de dHGP na chemotherapie een andere entiteit kan zijn dan de dHGP die wordt waargenomen bij chemo-naïeve patiënten. Dit zou kunnen hebben geleid tot een verkeerde categorisering van patiënten in eerdere studies, wat mogelijk de verminderde prognostische discriminerende waarde verklaart na chemotherapie beschreven in **Hoofdstuk 1**. Met dit nieuwe inzicht moet rekening worden gehouden in toekomstig onderzoek naar HGPs. Ten slotte wordt in **Hoofdstuk 7** de prognostische waarde van HGPs geëvalueerd door middel van een post-hoc analyse van de twee prospectieve gerandomiseerde gecontroleerde studies [31-34] in patiënten die perioperatieve chemotherapie kregen.

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Chapter 10

Appendices

Scientific output

2021 Histopathological growth patterns and survival after resection of colorectal liver metastasis: an external validation study
Höppener DJ, Galjart B, **Nierop PMH**, Buisman FE, van der Stok EP, Coebergh van den Braak RRJ, van Amerongen MJ, Balachandran VP, Jarnagin WR, Kingham TP, Doukas M, Shia J, Nagtegaal ID, Vermeulen PB, Groot Koerkamp B, Grünhagen DJ, de Wilt JHW D'Angelica MI, Verhoef C.
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PhD Portfolio

Name PhD student	P.M.H. Nierop
Erasmus MC department	Surgery
Division	Surgical Oncology
PhD Period	January 2017 – December 2019
Title thesis	Histopathological growth patterns of colorectal liver metastases: A clinical evaluation
Promotor	Prof. Dr. C. Verhoef
Copromotor	Dr. D.J. Grünhagen
Date defense thesis	01-09-2021

PhD Training

Oral presentations	Year	ECTS
Timing of systemic chemotherapy in the surgical treatment of colorectal liver metastases: a propensity score matched analysis. 39th ESSO Congress, Rotterdam, The Netherlands.	2019	1.0
Systemic chemotherapy for CRLM: The predictive value of HGPs and recurrence patterns. Liver Metastases Research Network congress, Valencia, Spain.	2019	1.0
Histopathological growth patterns and positive margins after resection of colorectal liver metastases. NVvH Chirurgendagen, Veldhoven, The Netherlands.	2018	1.0
Histopathological growth patterns and positive margins after resection of colorectal liver metastases. 13th IHPBA World Congress, Geneva, Switzerland.	2018	1.0
Desmoplastic histopathological growth pattern as a prognostic marker of improved outcome after resection of colorectal liver metastases. 13th IHPBA World Congress, Geneva, Switzerland.	2018	1.0
Clinical implications of histopathological growth patterns. Liver Metastases Research Network congress, Montreal, Canada.	2018	1.0
Histopathological growth patterns update: The Erasmus MC Experience. Liver Metastases Research Network congress, Rotterdam, The Netherlands.	2017	1.0
The liver-first approach for locally advanced rectal cancer and synchronous liver metastases. 12th Biennial E-AHPBA Congress, Mainz, Germany.	2017	1.0

Poster presentations	Year	ECTS
Salvage treatment for recurrences after first resection of colorectal liver metastases: the impact of histopathological growth patterns. 38th ESSO Congress, Budapest Hungary.	2018	0.5
Histopathological growth patterns and positive margins after resection of colorectal liver metastases. 38th ESSO Congress, Budapest Hungary.	2018	0.5
The liver-first approach for locally advanced rectal cancer and synchronous liver metastases. 8th European Multidisciplinary Colorectal Cancer Congress, Amsterdam, The Netherlands.	2016	0.5

Courses	Year	ECTS
Biostatistics 1, NIHES.	2018	5.7
BROK (Basiscursus regelgeving Klinisch Onderzoek), NFU.	2018	1.5
Research Integrity Course, Erasmus MC.	2018	0.3
OpenClinica Training.	2018	0.5
Survival Analysis Course, Molmed.	2017	0.6

Teaching	Year	ECTS
Supervising master thesis (2x)	2017-2019	4.0

(Inter)national conferences	Year	ECTS
39th ESSO Congress, Rotterdam, The Netherlands.	2019	0.9
Liver Metastases Research Network, Valencia, Spain.	2019	0.6
NVvH Chirurgendagen, Veldhoven, The Netherlands.	2019	0.6
38th ESSO Congress, Budapest Hungary.	2018	0.9
13th IHPBA World Congress, Geneva, Switzerland.	2018	1.2
Liver Metastases Research Network, Montreal, Canada.	2018	0.6
NVvH Chirurgendagen, Veldhoven, The Netherlands.	2018	0.6
Liver Metastases Research Network, Rotterdam, The Netherlands.	2017	0.6
12th Biennial E-AHPBA Congress, Mainz, Germany.	2017	1.2
NVvH Chirurgendagen, Veldhoven, The Netherlands.	2017	0.6
8th European Multidisciplinary Colorectal Cancer Congress, Amsterdam, The Netherlands.	2016	0.9

Other	Year	ECTS
Organising the Liver Metastases Research Network congress.	2017	2.0
Organising the 20th Wondcongres.	2019	2.0

Acknowledgements

Het schrijven van een proefschrift is een gezamenlijke inspanning en het boek dat voor u ligt, is hierop zeker geen uitzondering. De totstandkoming van dit proefschrift is mogelijk gemaakt dankzij inzet, hulp, steun, kennis, geduld en vertrouwen van velen. Ik wil enkelen graag in het bijzonder bedanken.

Geachte promotor, prof. dr. C. Verhoef, beste Kees, de manier waarop jij iedereen om je heen weet te motiveren en te inspireren is ongekend en heb ik echt ongelooflijk veel respect voor. De meest gebruikte uiterst simpele edoch effectieve motivational quote “Werken!” in combinatie met een ram op de deur zullen mij altijd bijblijven. Evenals je feilloze geheugen voor percentages, oneindig goede humeur, de humor, de barbecues, potten pingpong, congressen etc. etc. Jouw gave om iedereen het gevoel te geven echt onderdeel van het team te zijn is ongelooflijk en kan menigeen een voorbeeld aan nemen. Heel veel dank voor de fantastische tijd en begeleiding!

Geachte copromotor, dr. D.J. Grünhagen, beste Dirk, heel veel dank voor de begeleiding, je vlijmscherpe blik en de gezelligheid de afgelopen jaren. Ik heb ontzettend veel waardering voor alle tijd en moeite die je in mij en dit proefschrift hebt gestoken. Ik heb heel veel van je geleerd en ik hoop dat in de toekomst te mogen blijven doen. Dank!

Geachte leden van de promotiecommissie, ik wil u allen hartelijk danken voor het beoordelen van dit proefschrift. Daarnaast kijk ik er zeer naar uit om met u van gedachten te wisselen over de inhoud.

Paranimfen, beste Roeland en Jan. Wat is het ongelooflijk mooi dat jullie mij samen bijstaan tijdens de verdediging van mijn proefschrift! Roeland, samen vanaf de Hildegaert via het Erasmiaans naar Groningen en vele jaren nadien nog steeds zulke goede maten is echt heel bijzonder. De dingen die wij samen hebben meegemaakt zijn er te veel om hier te benoemen en, misschien nog wel belangrijker, het merendeel is ook niet voor herhaling op papier vatbaar. Ik weet zeker dat er nog vele jaren gaan volgen! Jan, onze verstandshouding begon als Groninger – Rotterdammer, vervolgens collega’s, maar ging al heel snel over tot echte maten. Samen “verbannen” worden naar de G-Flat in plaats van op de A-Gang mogen zitten, was eigenlijk een geluk bij een ongeluk. Daar is de basis van onze promoties zeker niet gelegd, maar des te meer die van onze vriendschap. Dank voor alles en ik weet zeker dat er nog vele mooie herinneringen zullen volgen!

Beste (oud) Daniël chirurgen, dr. Rothbarth, dr. Koppert, dr. Madsen, dr. van Ginhoven, dr. Burger, dr. Dassen, dr. Ayez, dr. Grotenhuis, beste Joost, Linetta, Eva, Tessa, Pim, Anneriet, Ninos en Brechtje. Heel veel dank voor de tijd en moeite die jullie in de studies hebben gestoken en voor de gezelligheid.

Mw. Eichholtz, beste Marianne, de woorden: “Ik weet misschien nog wel iemand bij de oncologische chirurgie” waren het begin van dit proefschrift. Heel veel dank voor de introductie bij Kees.

Eric, dank voor het beste advies ooit: De kans om in de Daniël onderzoek te mogen doen onder begeleiding van Kees en Dirk moet je grijpen!

Boris, elkaar begeleiden als masteronderzoeker is toch wel iets heel erg moois. Veel dank voor de introductie in de wondere wereld van de CRLM database en de statistiek.

Huppy, Diederik, eigenlijk zou er op de voorkant van dit boek moeten staan: Mede mogelijk gemaakt door D.J. Höppener. Zonder jouw hulp was het gegarandeerd niet afgekomen, heel veel dank! Daarnaast heel veel dank voor de mooie tijd die we hebben gehad tijdens al onze internationale HGP scoor avonturen.

Tube, Florian, jouw toewijding voor ons werk en onderzoek zijn bewonderenswaardig. Dank voor alle hulp en gezelligheid!

Dr. Vermeulen, beste Peter, dank voor de vruchtbare samenwerking en voor alle tijd die u in onze gezamenlijk projecten heeft gestoken. Ik hoop van harte dat we in de toekomst nog veel meer geheimen van de HGPs mogen ontrafelen!

Coebergh, dank voor de prettige samenwerking en voor het scoren van de eerste 700+ HGPs samen met Eric, Boris en Peter.

Coauteurs, veel dank voor de hulp, tijd, moeite, data, ideeën, geduld, vertrouwen en vooral de samenwerking.

Secretariaat van de Daniël/OGC en in het bijzonder Sandra, bedankt voor alle hulp en gezelligheid.

Daniël maatjes, de Daniël en de A-Gang zoals wij hen kennen zijn niet meer, maar des te levendiger zijn de geweldige herinneringen eraan! De potten pingpong, de lunches, de barbecues, de winteruitjes, de congressen; ik zou het zo nog een keer willen doen. Heel veel dank allemaal voor de geweldige tijd.

Flex 21-OGC, na de “turbulente samensmelting” en nadat de flexwerkplekken stilletjes waren veranderd in vaste plekken en het stof voldoende was neergedaald hebben we mijns inziens een heerlijke tijd gehad met zijn allen op Na-21. De congressen, de lunches en de borrels waren top.

Pien, dank voor alle steun, rondjes rennen, ritjes naar de Daniël en voor de hulp in de kliniek de afgelopen anderhalf jaar in het Ikazia. De SS Rotterdam gaat gebeuren!

Dr. Groot Koerkamp, beste Bas, dank voor je hulp met de stukken en je bereidheid om te helpen zorgen dat Diederik en ik onderzoek konden doen in het MSKCC.

Dear dr. D'Angelica, thank you for the collaboration and the opportunity to perform research at your department. Your devotion to patient care and research are admirable.

Dear professor Primrose, thank you for the collaboration. Your enthusiasm for research is contagious!

EORTC, dr. Mauer, dr. Julie, thank you for kindly providing the data of the EORTC 40893 trial and the collaboration.

Chirurgen, plastisch chirurgen en assistenten van het Ikazia Ziekenhuis. Ik voel me echt als een vis in het water bij jullie. In het bijzonder dr. den Hoed en dr. Vles, Ted en Wouter, dank voor alles. Ik ben blij en trots dat ik mijn opleiding bij jullie mag vervolgen.

Wondcongresvrienden, het is een eer om dit prachtige evenement drie jaar lang samen met jullie te organiseren!

EVM & SJ, Chris, Dirk, Olivier, Teng, Ernest, Laurens, Sjoerd en Roeland, ondanks het feit dat ik de maandag na een EVM weekend gegarandeerd lichtelijk verzwakt aan mijn week begin, hebben de ontelbare mooie dingen die we de afgelopen jaren hebben gedaan, ervoor gezorgd dat ik de nodige afleiding had. Het blijft bijzonder dat we na bijna 20 jaar nog steeds zulke goede maten zijn. Ik hoop dat er nog vele EVM weekenden zullen volgen. PS ik krijg nog steeds een vakantie van jullie.

De Claes, Kees en Olivier, dank dat jullie me hebben opgevangen toen ik terug kwam in Rotterdam. Ik heb zwaar genoten op de Claes gedurende de eerste periode van mijn onderzoek.

Hildegaert boys, Jesse, Sebas, Friso, Bas, Alexander, Robert en Roeland, vanaf groep 1 vrienden. De frequentie van het contact fluctueert door de jaren heen, maar desondanks still going strong. Dank voor jullie vriendschap.

Van Dijk, Dijkers, ik waardeer zeer dat onze van Dijk aangelegenheden en vriendschappen voortduren ondanks het feit dat ik de "vreemde eend in de bijt" ben die in Rotterdam is gaan wonen. Opdat er nog vele mooie dingen mogen volgen.

JC R-Max, ondanks het feit dat ik op de helaas schaarse clubaangelegenheden meer tijd kwijt ben aan mezelf te verdedigen waarom ik geen plastisch chirurg wordt dan aan uitleggen waar mijn onderzoek over gaat, vind ik het toch heel mooi om het resultaat met jullie te delen.

Lieve Laura, Niels, Jeroen, Marjanne, omi en oma, heel veel dank voor jullie steun, interesse, mooie avonden, weekenden en vakanties door de jaren heen. Marjanne, nogmaals heel hartelijk dank voor de fantastische schildering.

Lieve oma en Bommi, wat bijzonder dat jullie mijn proefschrift kunnen zien. Ik zou willen dat opa en opi het ook hadden kunnen zien. Heel veel dank voor alles.

Lieve Marijne, Maud en Lex, hoe vier zo verschillende personen tegelijkertijd zo veel op elkaar kunnen lijken, blijft mij verbazen. Ik ben ongelooflijk trots en gelukkig om jullie als zussen en broer te hebben.

Lieve papa en mama, het is cliché, maar daardoor niet minder waar: Zonder jullie steun was dit proefschrift er nooit gekomen. Jullie onvoorwaardelijke liefde, steun en vertrouwen ben ik jullie eeuwig dankbaar voor.

Lieve Britt, wij weten allebei dat mijn dank aan jou zo groot is, dat een dankwoord daar geen eer aan doet. Dank voor al jouw geduld, steun, afleiding en vertrouwen, maar bovendien thank you for being you. Je bent geweldig!

About the author

Pieter Maarten Harry Nierop was born on August 30th 1991 in Rotterdam, the Netherlands, where he grew up as the eldest of four. He graduated in 2009 from the Erasmiaans Gymnasium after which he started medical school at the University of Groningen. During medical school he worked at the liver transplant unit of the University Medical Centre Groningen. This is where his interest for the field of surgery originated. This interest grew during his clinical internships into the ambition to become a surgeon. For his final year of medical school he returned to Rotterdam. After obtaining his medical degree in December 2016 Maarten started in January 2017 as a PhD candidate at the Department of Surgical Oncology at the Erasmus MC Cancer Institute (prof. dr. C. Verhoef and dr. D.J. Grünhagen) focusing on the histopathological growth patterns of colorectal liver metastases resulting in this thesis. During his PhD Maarten was given the opportunity to perform research at several international centres including the Ruprecht Karl University of Heidelberg, Heidelberg, Germany and at the Memorial Sloan Kettering Cancer Center, New York City, New York, USA. In January 2020 Maarten started as a resident at the Department of Surgery of the Ikazia Hospital in Rotterdam and in January 2021 he subsequently started his general surgery training (dr. P.T. den Hoed).