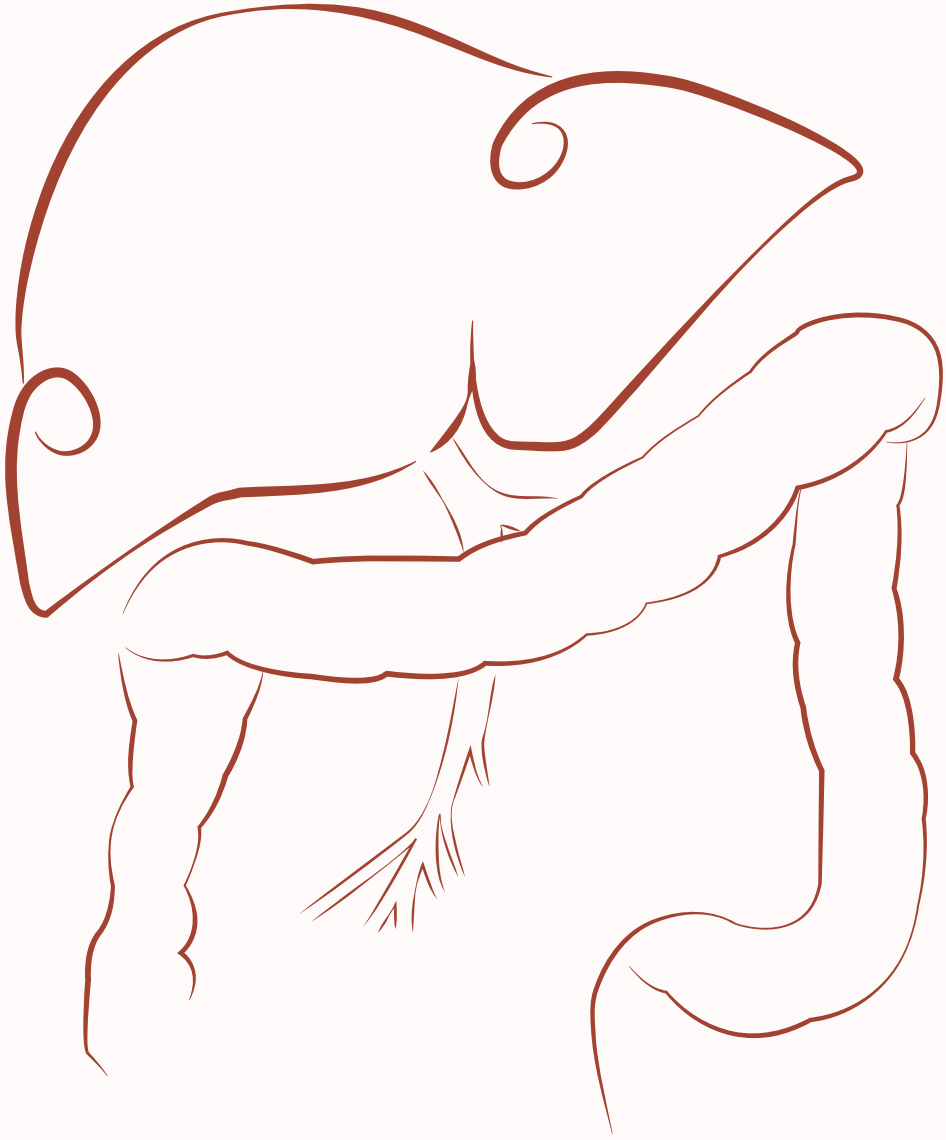


DISEASE RECURRENCE

AFTER RESECTION OF COLORECTAL CANCER

BORIS GALJART

Disease Recurrence After Resection of Colorectal Cancer

Boris Galjart

This thesis was partly realized due to the financial support of the Erasmus University and
the Department of Surgery, Erasmus Medical Centre.

Cover and layout design: Claire Galjart
Printing: ProefschriftMaken

ISBN: 978-94-6469-020-0

Disease Recurrence After Resection of Colorectal Cancer

Recidief ziekte na resectie van
het colorectaal carcinoom

Proefschrift

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

Prof. dr. A.L. Bredenoord

en volgens besluit van het College voor
Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 21 september 2022 om 15.30 uur

door

Boris Galjart
geboren te Rotterdam

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

Promotor	Prof. dr. C. Verhoef
Overige leden	Prof. dr. M. Koopman Prof. dr. P.J. Tanis Prof. dr. J.M. Klaase
Copromotor	Dr. D.J. Grünhagen

Table of contents

CHAPTER ONE

CHAPTER ONE

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Colorectal cancer epidemiology

Colorectal adenocarcinomas account for the large majority of colorectal cancers (CRCs) and develop from the glandular epithelial cells located in the colon and rectum. Ten percent of the global cancer burden is caused by CRC, as approximately 1.8 million patients are newly diagnosed with this disease on a yearly basis. Despite advances in the prevention, diagnosis and treatment of CRC, 900.000 patients die because of this malignancy each year.[1,2]

The molecular tumorgenesis of CRC has been studied since the 80's. Vogelstein and colleagues demonstrated that CRCs develop from benign adenomas to malignant carcinomas through several well described histological stages, due to the accumulation of genetic mutations.[3] Given that CRCs develop from pre-cancerous tumours, many Western countries have initiated nationwide screening programs to pursue early detection. These programs have caused the incidence of advanced CRC stages to decrease. Nevertheless, still 30 to 40 percent of Dutch patients diagnosed with CRC present with stage III or IV disease.[4] The likelihood of recurrence and long-term survival after resection of CRC is strongly related to disease stage. Among patients with resected stage II-III CRC, approximately 20 percent will be diagnosed with relapses, this being 70 percent in patients with resected stage IV CRC.[5,6]

Follow-up after surgery for non-metastatic colorectal cancer

As a considerable proportion of CRC patients will develop recurrent disease, most are offered routine oncological follow-up after resection. Follow-up schedules for CRC are carried out for several years after treatment and consist of clinical evaluations, sequential carcinoembryonic antigen (CEA) level measurements and frequent cross-sectional imaging (i.e. computed tomography, ultrasonography, chest radiographs). Visits are generally planned every three to six months. In addition, periodic endoscopic surveillance is performed within one year after surgery and every three to five years thereafter.[7-9]

Multimodality periodic oncological surveillance has been advocated for decades now and serves various purposes.[8,9] The main objective is to detect distant metastases and local regrowths as early as possible, in order to maximize the likelihood of salvage treatment. In addition, follow-up can be used to inform patients on their disease status and prognosis over time, and to detect and manage complications caused by CRC treatment. During follow-up visits, psychological and social support may be provided to optimize patient recovery and wellbeing.

The impact of follow-up on survival outcomes and treatment of recurrent disease has been studied in multiple randomized controlled trials, but remains debated nonetheless. In **Chapter 2** we therefore systematically reviewed and meta-analysed available literature on the impact of follow-up in five common solid tumours, including CRC, in order to reflect on its effectiveness.

Although several factors influence the risk and location of recurrences, a 'one size fits all' approach is currently applied with regards to the frequency and modalities used during follow-up.[8,9] A factor often thought to influence the effectiveness of follow-up diagnostics is pre-operative CEA level. Sequential CEA level measurements after resection are one of the main components of CRC follow-up, as risen CEA values after surgery are highly indicative of recurrent disease, especially with regards to hepatic metastases.[10] A considerable proportion of CRC patients, however, presents with low CEA values prior to surgery. Many physicians presume that CEA is a less sensitive biomarker in these patients and that imaging should be applied more frequently in this subgroup.[10] In Chapter 3 we compared different follow-up approaches (i.e. frequent versus infrequent imaging) in patients with low preoperative CEA values, in order to decide whether preoperative CEA may indeed be used to individualize follow-up practice.

Colorectal liver metastasis

Liver metastases are commonly diagnosed during CRC follow-up.[5,6] Approximately 15 percent of CRC patients presents with synchronous colorectal liver metastases (CRLM), while another 15 percent will develop hepatic metastases in the period thereafter.[11] Despite the hematogenic spread of cancer cells, patients with CRLM can still be cured, which is a major argument in favour of follow-up. About forty percent of patients is considered eligible for surgical therapy (i.e. resection and/or ablation), which results in a cure rate of approximately 20 percent.[11,12]

After resection of CRLM, surveillance is again initiated. During the follow-up period, seventy percent of CRLM patients develop recurrent disease. Most CRLM guidelines advocate a follow-up duration of at least five years, and sometimes longer. The large majority of recurrences, however, is diagnosed within the first three years after resection.[5,6] In **Chapter 4** we aimed to determine whether surveillance remains needed in patients being disease-free three years after resection of CRLM.

In order to reduce recurrence rates, several adjuncts to surgery have become available over time. Systemic chemotherapy regimens, which combine agents such as oxaliplatin, irinotecan and fluoropyrimidines (e.g. 5-FU), prolong survival in palliative patients.[13,14] Monoclonal antibodies, targeting the vascular endothelial growth factor (bevacizumab) or the epidermal growth factor (cetuximab, panitumumab) for instance, can be added to these regimens and further improve response rates in patients with metastatic CRC.[15,16] Systemic chemotherapy has also been applied in patients eligible for surgery. Three randomized studies showed improvements in disease-free survival, but could not demonstrate differences in overall survival.[17-19] Dutch guidelines therefore do not recommend the standard use of perioperative chemotherapy in patients with upfront resectable CRLM.

Another viable treatment option for patients with (borderline) resectable CRLM is hepatic arterial infusion chemotherapy. This treatment is currently being deployed within the Netherlands, but has been utilized in the United States for several decades. After fixating a catheter in the hepatic artery, high levels of chemotherapy are then continuously infused, often through a subcutaneous pump. The main objective of hepatic arterial infusion chemotherapy is to eradicate microscopic metastases within the liver prior to or after resection of CRLM. Several randomized controlled trials evaluating hepatic arterial infusion therapy have been performed.[20] The largest randomized controlled trial, in 156 patients, demonstrated superior two-year overall survival, although the long-term results did not significantly differ from systemic therapy alone.[21,22] A large retrospective single centre study however, showed a two year survival benefit after hepatic arterial infusion chemotherapy.[23]

Over the years many have attempted to identify factors related to the risk of recurrence and the effectiveness of (neo)adjuvant therapies. Fong's Clinical Risk Score is the most commonly used tool for this purpose, although it is not often used in clinical practice.[24] Retrospective studies have shown that systemic chemotherapy may be most effective in patients at high risk of recurrence, while hepatic arterial infusion chemotherapy seems most effective in patients at low to moderate oncological risk.[23,25] As the Clinical Risk Score has been developed in 1999, it does not include all modern-day risk factors, while it is quite simplistic from a statistical point of view. In **Chapter 5** we therefore aimed to create a novel model that predicts the risk of developing extrahepatic recurrence during follow-up. In addition, we set to determine whether these predictions can be used to guide the use of (neo)adjuvant therapies in CRLM patients.

Histopathological growth patterns of colorectal liver metastases

Next to Fong's Clinical Risk Score several other risk models have been developed over the years, in order to predict the risk of recurrence and probability of long-term survival. These models include various combinations of general patient characteristics (e.g. age) and characteristics of the primary tumour and CRLM (e.g. number and size of CRLM). Performance of such models is generally measured through Harrell's concordance index. The concordance index measures the number of pairs in which the survival was correctly predicted (i.e. patient A has a longer predicted and observed survival than patient B), out of all possible pairs in the database. A concordance index of 0.7 or higher indicates acceptable discriminatory capacity. At five years of follow-up after resection of CRLM, none of the risk models reached the 0.7 mark. This explains why these models are hardly used in clinical practice, and highlights the need for novel risk factors in CRLM patients.[26]

Histopathological growth patterns (HGPs) have emerged as a possible new risk factor for recurrence in patients with CRLM. A desmoplastic, replacement, and pushing type HGP have been described. In patients with desmoplastic type HGP, a rim of stromal tissue separates cancer cells from the normal liver parenchyma. In contrast to this phenotype stands the replacement type HGP, in which direct contact between tumour and liver cells is observed, as the tumour invades the normal liver parenchyma. The pushing type HGP is rarely described. The normal liver parenchyma is pushed aside by tumour cells, but no infiltration is present.[27]

Several biological differences between HGPs have been described. CRLM expressing a desmoplastic or pushing type HGP seem to derive their blood supply through sprouting angiogenesis, in which vessels are newly formed during tumour growth. This while replacement type CRLM co-opt pre-existing hepatic vasculature.[27,28] In addition, the desmoplastic HGP has been associated with increased infiltration of immune cells, something which is less often seen in CRLM with a replacement phenotype.[29,30] Given these biological differences, HGPs may be related to the risk of recurrence during follow-up for CRLM, and prognosis in general. In **chapter 6, 7, and 8** we aimed to unravel the relationship between HGPs and prognosis after resection of CRLM.

References

1 Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. May 2021;71(3):209-249.

2 Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. Apr 26 2014;383(9927):1490-1502.

3 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. Jun 1 1990;61(5):759-767.

4 Breekveldt ECH, Lansdorp-Vogelaar I, Toes-Zoutendijk E, et al. Colorectal cancer incidence, mortality, tumour characteristics, and treatment before and after introduction of the faecal immunochemical testing-based screening programme in the Netherlands: a population-based study. Lancet Gastroenterol Hepatol. Jan 2022;7(1):60-68.

5 Qaderi SM, Galjart B, Verhoef C, et al. Disease recurrence after colorectal cancer surgery in the modern era: a population-based study. Int J Colorectal Dis. Nov 2021;36(11):2399-2410.

6 de Jong MC, Pulitano C, Ribero D, et al. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. Ann Surg. Sep 2009;250(3):440-448.

7 Meyerhardt JA, Mangu PB, Flynn PJ, et al. Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol. Dec 10 2013;31(35):4465-4470.

8 Argilés G, Tabernero J, Labianca R, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. Oct 2020;31(10):1291-1305.

9 Glynne-Jones R, Wyrwicz L, Tiret E, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. Jul 1 2017;28(suppl_4):iv22-iv40.

10 Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem. Apr 2001;47(4):624-630.

11 Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. Aug 2006;244(2):254-259.

12 Tomlinson JS, Jarnagin WR, DeMatteo RP, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol. Oct 10 2007;25(29):4575-4580.

13 de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol. Aug 2000;18(16):2938-2947.

14 Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med. Sep 28 2000;343(13):905-914.

15 Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. Jul 22 2004;351(4):337-345.

16 Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. Jun 3 2004;350(23):2335-2342.

17 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. Lancet Oncol. Nov 2013;14(12):1208-1215.

18 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. Lancet. Mar 22 2008;371(9617):1007-1016.

19 Kanemitsu Y, Shimizu Y, Mizusawa J, et al. Hepatectomy Followed by mFOLFOX6 Versus Hepatectomy Alone for Liver-Only Metastatic Colorectal Cancer (JCOG0603): A Phase II or III Randomized Controlled Trial. J Clin Oncol. Dec 1 2021;39(34):3789-3799.

20 Buisman FE, Filipe WF, Galjart B, et al. Adjuvant intra-arterial chemotherapy for patients with resected colorectal liver metastases: a systematic review and meta-analysis. HPB (Oxford). Nov 19 2021.

21 Kemeny N, Huang Y, Cohen AM, et al. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. N Engl J Med. Dec 30 1999;341(27):2039-2048.

22 Kemeny NE, Gonen M. Hepatic arterial infusion after liver resection. N Engl J Med. Feb 17 2005;352(7):734-735.

23 Groot Koerkamp B, Sadot E, Kemeny NE, et al. Perioperative Hepatic Arterial Infusion Pump Chemotherapy Is Associated With Longer Survival After Resection of Colorectal Liver Metastases: A Propensity Score Analysis. J Clin Oncol. Jun 10 2017;35(17):1938-1944.

24 Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. Sep 1999;230(3):309-318; discussion 318-321.

25 Ayez N, van der Stok EP, Grünhagen DJ, et al. The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator. Eur J Surg Oncol. Jul 2015;41(7):859-867.

26 Roberts KJ, White A, Cockbain A, et al. Performance of prognostic scores in predicting long-term outcome following resection of colorectal liver metastases. Br J Surg. Jun 2014;101(7):856-866.

27 Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. Oct 2001;195(3):336-342.

28 Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med. Nov 2016;22(11):1294-1302.

29 Brunner SM, Kesselring R, Rubner C, et al. Prognosis according to histochemical analysis of liver metastases removed at liver resection. Br J Surg. Dec 2014;101(13):1681-1691.

30 Höppener DJ, Nierop PMH, Hof J, et al. Enrichment of the tumour immune microenvironment in patients with desmoplastic colorectal liver metastasis. Br J Cancer. Jul 2020;123(2):196-206.

PART I

Individualize follow-up and treatment after surgery for (metastatic) colorectal cancer

CHAPTER TWO

CHAPTER TWO

FOLLOW-UP STRATEGY AND SURVIVAL FOR FIVE COMMON CANCERS: A META-ANALYSIS.

Boris Galjart, Diederik J. Höppener,
Joachim G.J.V. Aerts, Christiaan H. Bangma,
Cornelis Verhoef, Dirk J. Grünhagen

Published in the European Journal of Cancer

Abstract

Background: This meta-analysis aimed to evaluate the effectiveness of intensive follow-up after curative intent treatment for five common solid tumors, in terms of survival and treatment of recurrences.

Methods: A systematic literature search was conducted, identifying comparative studies on follow-up for colorectal, lung, breast, upper gastro-intestinal and prostate cancer. Outcomes of interest were overall survival (OS), cancer specific survival (CSS), and treatment of recurrences. Random effects meta-analyses were conducted, with particular focus on studies at low risk of bias.

Results: Fourteen out of 63 studies were considered to be at low risk of bias (8 colorectal, 4 breast, 0 lung, 1 upper gastro-intestinal, 1 prostate). These studies showed no significant impact of intensive follow-up on OS (hazard ratio, 95% confidence interval) for colorectal (0.99; 0.92-1.06), breast 1.06 (0.92-1.23), upper gastrointestinal (0.78; 0.51-1.19) and prostate cancer (1.00; 0.86-1.16). No impact on CSS (hazard ratio, 95% confidence interval) was found for colorectal cancer (0.94; 0.77-1.16). CSS was not reported for other cancer types. Intensive follow-up increased the rate of curative treatment (relative risk; 95% confidence interval) for colorectal cancer recurrences (1.30; 1.05-1.61), but not for upper gastro-intestinal cancer recurrences (0.92; 0.47-1.81). For the other cancer types, no data on treatment of recurrences was available in low risk studies.

Conclusion: For colorectal and breast cancer, high quality studies do not suggest an impact of intensive follow-up strategies on survival. Colorectal cancer recurrences are more often treated locally after intensive follow-up. For other cancer types evaluated, limited high quality research on follow-up is available.

Introduction

Most cancer survivors receive regular follow-up care after being treated with curative intent. Traditionally, follow-up is performed for a period of 5 years or longer for most types of solid tumors. Guidelines differ between tumor types, but generally advocate regular hospital visits, imaging, and serum tumor marker measurements when available.[1-4]

The main rationale behind oncologic follow-up is to detect metastases or novel primary tumors early, since prompt treatment of cancer relapses is deemed important for the likelihood of cure and survival. Next to this, follow-up can be used to address patients' needs with regards to psychosocial counselling, to evaluate treatment effects and complications, and to inform patients on their disease status and risk of recurrence.[5]

The debate surrounding oncological follow-up practices has existed for many years. It is associated with a considerable use of hospital resources and costs, may have impact on quality of life, while the effect of follow-up intensity on survival outcomes remains equivocal.[6, 7] Given that the number of cancer survivors will continue to grow,[8] improvements of follow-up practices should be pursued. Many studies evaluate the effectiveness of follow-up for individual tumors types, but a broad oncological perspective remains lacking.

We therefore sought to systematically assess and meta-analyze available literature on follow-up after curative intent treatment for five types of solid tumors (colorectal, lung, breast, upper gastro-intestinal, and prostate cancers) in order to determine the impact of different follow-up strategies on survival outcomes and treatment of recurrent disease.

Methods

Search strategy
This study was performed in line with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis, www.prisma-statement.org) guidelines. Pubmed/MEDLINE, Embase, Web of Science, the Cochrane database, and Google Scholar were systematically searched for studies published prior to the 12th of May 2020. The search terms are provided in supplementary table 1. Reference lists from eligible articles were also reviewed to identify additional publications.

Study selection
Screening for eligible studies was performed by two authors (BG, DH), independently. Studies were included when comparing follow-up approaches after curative intent treatment for colorectal, lung, breast, upper gastro-intestinal or prostate cancer, in light of overall survival (OS) or cancer-specific survival (CSS) outcomes. Treatment intent for recurrent disease (i.e. curative or palliative) was also evaluated. Both randomized controlled trials (RCTs) and observational studies (cohort and case-control) were considered. Inclusion was restricted to articles written in English. Non-original studies (e.g. reviews, editorials) were excluded, as were non-comparative studies and studies using simulation techniques (e.g. Markov modelling).

Data extraction and presentation
Data were extracted by two reviewers (BG, DH), independently. Studies were categorized based on the aspect of follow-up evaluated, being the frequency of testing, setting of follow-up (e.g. in-hospital or general practitioner), diagnostic modalities used, or a combination of the aforementioned

categories. Data on survival (hazard ratios (HR) including 95% confidence intervals (95%CI) for OS and CSS) and the probability of treatment with curative intent for recurrent disease (relative risk (RR) including 95%CI) were collected. When no ratios were reported, data were extracted from Kaplan-Meier figures, tables, and text. Multi-layered circle plots were created to visualize all aspects in relation to outcomes and the risk of bias.

Quality assessment

Quality assessment was performed by two reviewers (BG, DH), independently. The Cochrane tools ROBINS-I (for observational studies) and RoB2 (for randomized studies) were used.[9,10] Studies were considered to be at low risk of bias when qualified as either 'low' to 'moderate' using ROBINS-I, or as 'low risk' to 'some concerns' using RoB2.

Quantitative assessment

A random effects meta-analysis was conducted per tumor type and stratified for study risk of bias, using the generic inverse variance method (survival) or the Mantel-Haenszel method (treatment of recurrences). Methods described by Tierney et al. were applied to calculate log HRs and corresponding standard errors, in case these were not reported.[11] Both HRs and RRs were reported using the least intensive approach (e.g. lowest frequency, non-hospital setting) as a reference. In studies with multiple groups (i.e. >2 follow-up approaches), the most intensive approaches were combined to create a single pair-wise comparison with the least intensive approach, as recommended by the Cochrane Handbook.[12] The R Project for Statistical Computing version 4.1.0 (https://www.r-project.org/) was used for both the statistical analyses and visualization of the data (packages: meta (v4.18-1), ggplot2 (v3.3.2); circlize (v0.4.11)[13]).

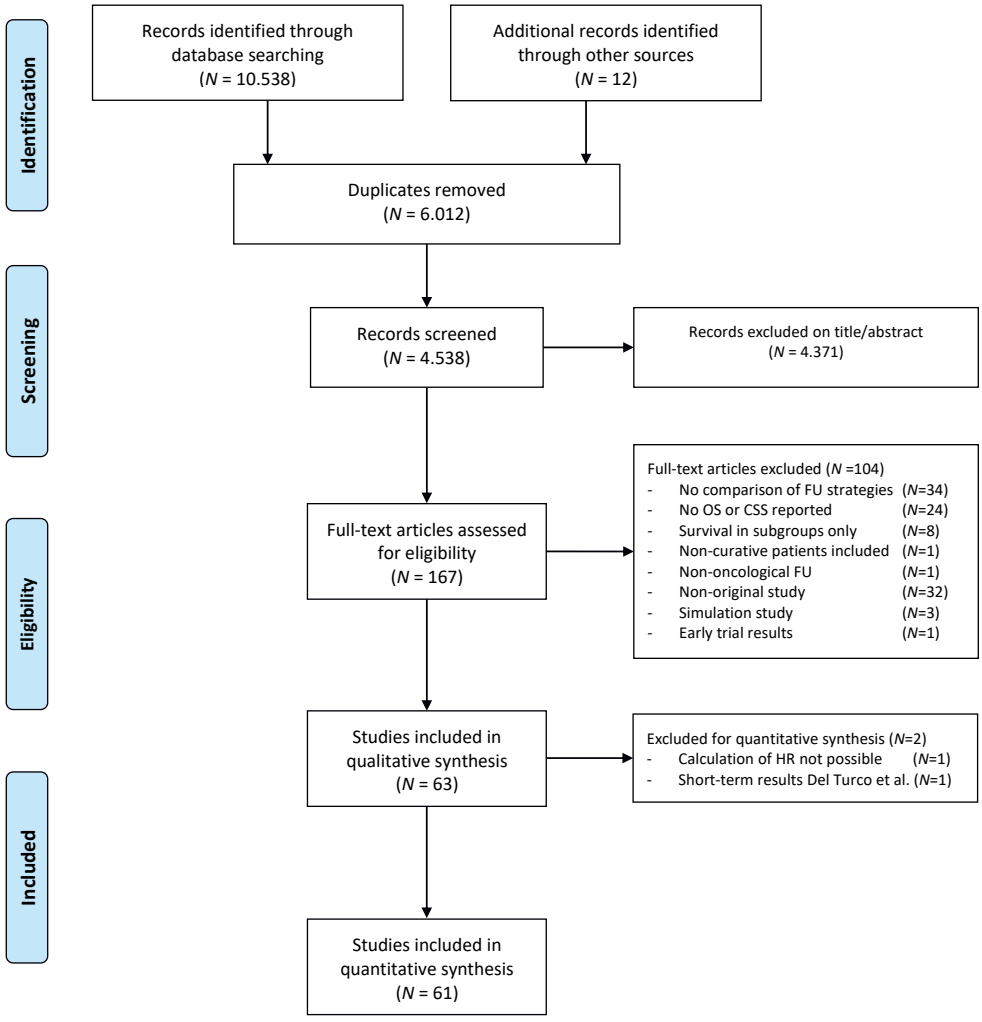


Figure 1: PRISMA flowchart.

RESULTS

The screening and selection process is illustrated in figure 1. After screening 4538 studies, 167 were screened full-text. Ultimately, 63 studies were deemed eligible for inclusion.[14-76] For quantitative analyses, 61 studies were eligible.

Study characteristics and outcomes

Figure 2 visualizes the studies obtained. Thirty-three original studies (52%) reported on the effect of follow-up in colorectal[14-46], 13 (21%) in lung[47-59], 11 (18%) in breast[60-70], five (8%) in upper gastrointestinal[71-75], and one (2%) in prostate cancer patients[76]. The majority of studies evaluated frequency of follow-up (N=38, 60%).[14, 16-21, 23-37, 39, 40, 42, 47, 50, 52, 53, 59, 66-71, 74, 76]. A total of 89.154 patients was included. Twenty-five RCTs (40%) were identified, including one long-term update[62], and comprised 12.458 patients in total.[14-17, 19-22, 32, 36, 38, 41-45, 54, 58, 61-65, 71, 75] Table 1 and 2 provide detailed overviews of the low and high risk of bias studies. Risk of bias assessment is provided in supplementary table 2A and B. Figure 3 visualizes outcomes per study. The results of the meta-analysis per tumor type, including stratified analyses are reported in table 3.

Colorectal cancer

The 33 colorectal cancer studies comprised 50.431 patients in total (table 1 and 2). Across all studies, intensive follow-up led to improved OS (HR 0.82, 95%CI 0.73-0.91) and an increased probability of curative intent treatment for recurrences (RR 1.60, 95%CI 1.21-2.11). An equally large, but non-significant, impact on CSS (HR 0.80, 95%CI 0.63-1.01) was observed. Considerable heterogeneity was present (I2 66 to 85% for the three outcomes) (table 3).

In the eight studies (24%) considered to be at low risk of bias, including seven RCTs[14-17, 19-21], no significant impact on OS (HR 0.99, 95%CI 0.92-1.06) and CSS (0.94, 95%CI 0.77-1.16) was observed with little to no heterogeneity (I2 7% and 0%). All low risk studies evaluated frequency of follow-up, of which three evaluated a symptom-based approach without use of diagnostics.[14, 15, 19] Primrose et al. also compared CT and CEA as diagnostic modalities during surveillance.[15] Although survival was not significantly impacted by follow-up strategy, intensive follow-up remained significantly associated with the probability of curative intent treatment for recurrences (RR 1.30, 95%CI 1.05-1.61) in low risk studies (I^2 28%).

Twenty-five studies (76%) were deemed to be at high risk of bias, the majority being observational (N=16, 64%).[22, 32, 36, 38, 41-45] Most high risk studies evaluated frequency of follow-up (N=18, 72%).[23-37, 39, 40, 42] Pooled effect estimates in high risk colorectal cancer studies were larger for all outcomes evaluated, with considerable heterogeneity (table 3).

Lung cancer

Within the thirteen lung cancer studies, 26.162 patients were included (table 2). All of the studies identified were considered to be at high risk of bias, including two RCTs.[54, 58] Five studies assessed frequency of follow-up[47, 50, 52, 53, 59], five the modalities used[49, 51, 54, 56, 57], one the setting in which follow-up was performed[58], and two evaluated multiple aspects[48, 55]. Follow-up did not significantly impact OS (HR 0.94, 95%CI 0.84-1.05) (table 3). Heterogeneity was moderate (I^2 49%). Only one study reported on CSS, in which no significant survival difference was obtained.[49] Intensive follow-up did not increase curative treatment rates (RR 1.34, 95%CI 0.82-2.20, I2 39%), as reported in four studies.[48-50, 52]

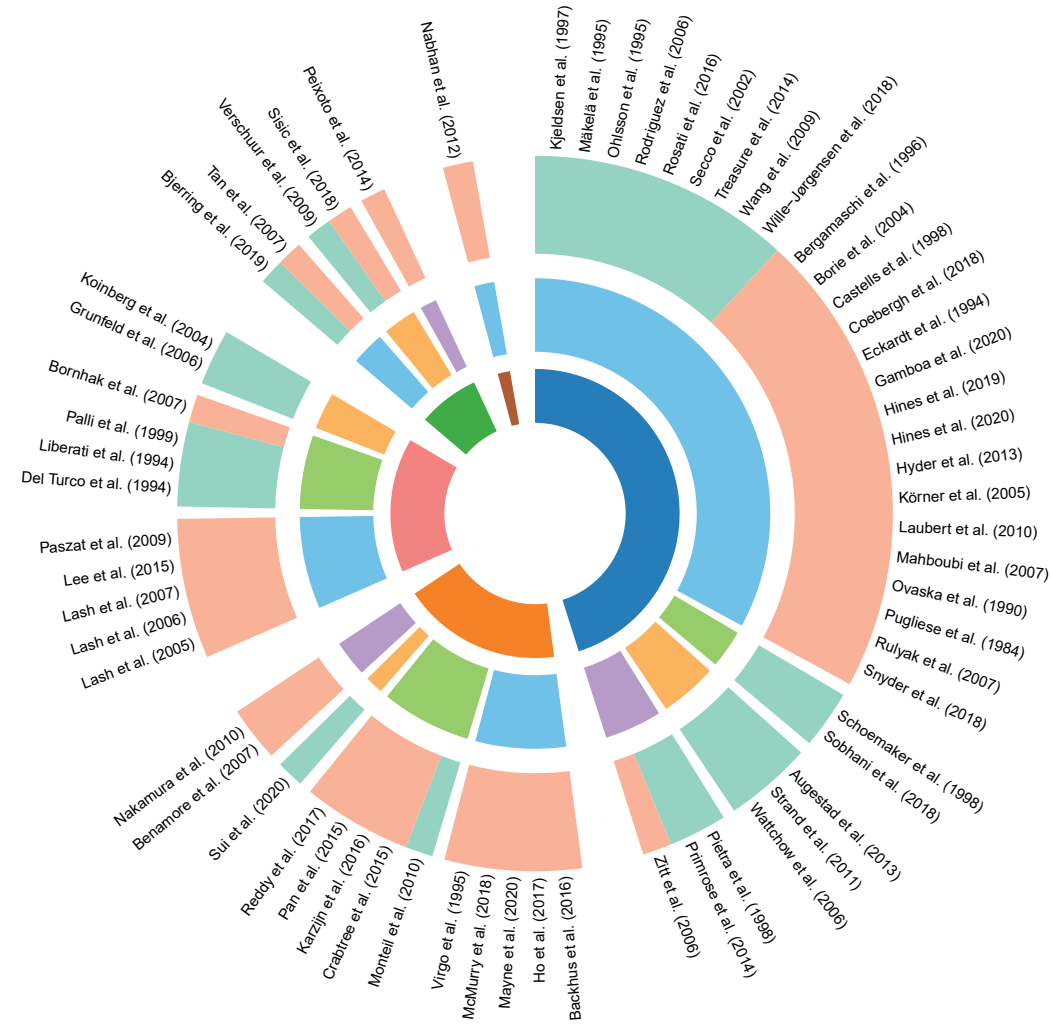


Figure 2: Multi-layered circle plot displaying all 63 included studies by cancer type (inner circle), aspect of follow-up investigated (middle circle), and study design (outer circle).

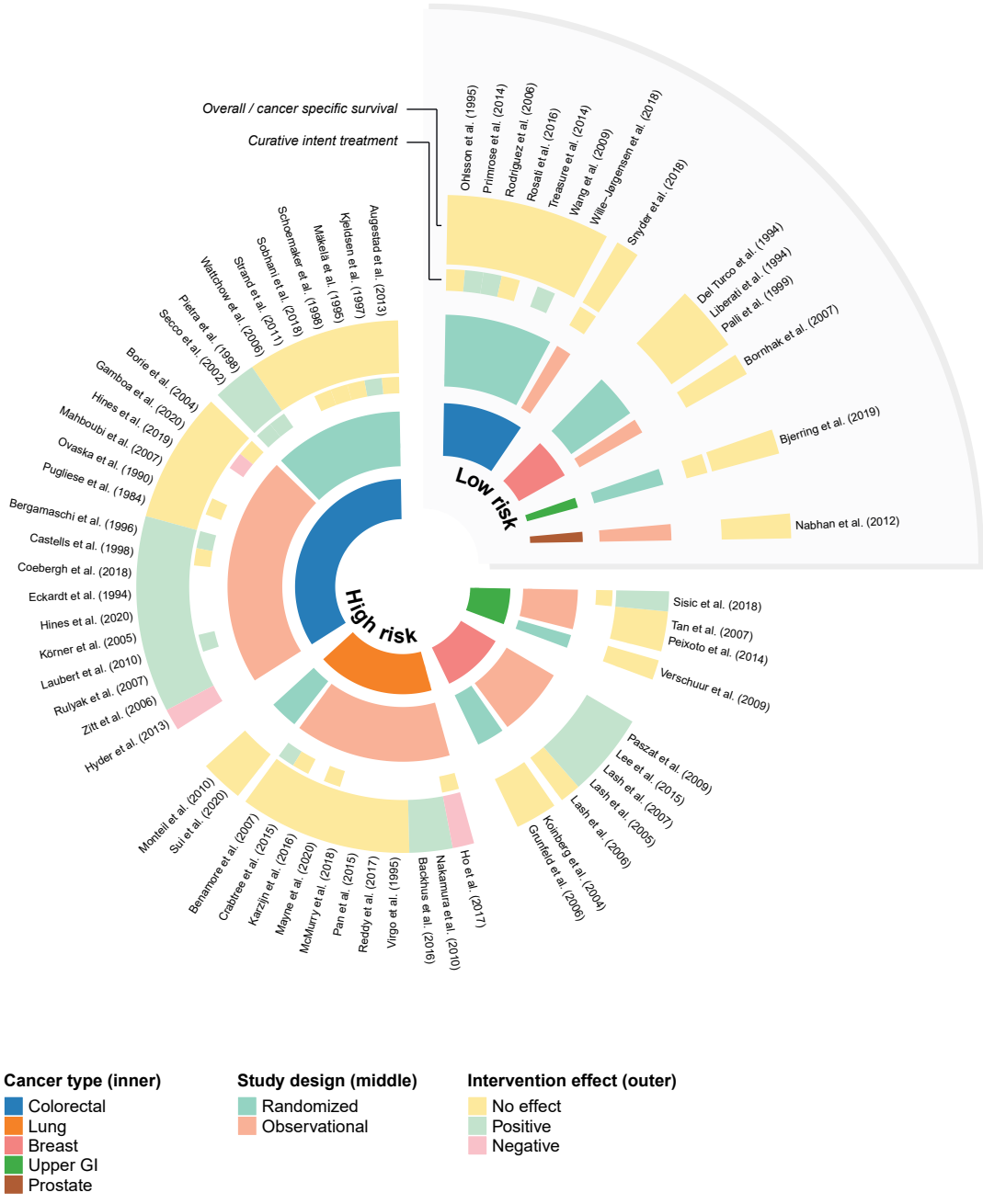


Figure 3: Multi-layered circle plot summarizing the reported effect in all 63 included studies, stratified by risk of bias. The inner circle represents cancer type, the middle circle study design, and the outer circle the effect of the intervention on overall or cancer-specific survival, and treatment intent.

Breast cancer
In total 10.585 breast cancer patients were included in eleven studies (table 1 and 2). Across all studies no significant impact of intensive follow-up on OS (HR 0.80, 95%CI 0.54-1.18) or CSS (HR 0.52, 95%CI 0.27-1.02) was observed (table 3). Heterogeneity was considerable for both outcomes (I2 92% and 94%). None of the studies reported on the (curative) treatment for local recurrence or metastatic disease.

Four studies (36%) were considered to be at low risk of bias, including two RCTs[61, 63], one long-term update of an RCT[62], and one prospective observational study[60]. All low risk studies compared different modalities used in the same frequency, generally every 3 to 6 months. When pooling the effects of individual studies, no impact on OS was observed (pooled HR 1.06, 95%CI 0.92-1.23), with no heterogeneity (I^2 0%). None observed significant additional value of using multiple diagnostics (e.g. liver ultrasonography, chest radiography, laboratory tests) next to clinical examinations and mammography's. None of the studies reported on CSS.

Most of the seven high risk studies were observational (71%).[64, 65] In contrast to the low risk studies, all of the observational studies evaluated the frequency of follow-up, while the RCTs evaluated setting of follow-up. The randomized studies found no impact on OS when follow-up was performed by the family physician (HR 1.05, 95%CI 0.60-1.84) or the nurse practitioner (HR 1.22, 95%CI 0.58-2.57), compared to the standard hospital-based physician-led approach.[64, 65] The studies evaluating the frequency of follow-up all assessed the impact of receiving one or more diagnostic evaluations (i.e. mammography or multiple diagnostics) to a nihilistic approach.[66-70] All but one study (80%) found that any follow-up significantly improved survival. Pooled OS (HR 0.68, 95%CI 0.41-1.14) and CSS (HR 0.52, 95%CI 0.27-1.02) estimates were non-significant, and high heterogeneity was observed (I^2 91% and 94%).

Upper gastro-intestinal cancer
Five studies were identified in patients with upper gastro-intestinal cancers, including 1.273 patients in total (table 1 and 2). A significant benefit from intensive follow-up was observed, in terms of OS (HR: 0.79, 95%CI 0.66-0.95), with no heterogeneity (I^2 0%). Intensive follow-up was not significantly associated with treatment intent (RR 1.25, 95%CI 0.62-1.52, I2 50%). None of the studies reported on CSS (table 3).

Bjerring et al. conducted the only study in patients with upper-gastrointestinal cancers considered to be at low risk of bias, comparing imaging based to symptom based follow-up in patients with esophageal, gastric, and pancreatic cancer.[71] No significant difference in OS (HR 0.78, 95%CI 0.51-1.19), nor in the probability of being treated with curative intent for isolated locoregional disease (RR 0.92, 95%CI 0.47-1.81) could be demonstrated.

High risk studies were again mostly observational (75%)[72-74]. One study evaluated the frequency of follow-up[74], two the setting of follow-up[73, 75], and one study evaluated multiple aspects[72]. When pooling the high risk studies, intensive follow-up significantly improved OS (HR 0.79, 95%CI 0.65-0.97, I^2 0%) (table 3).

Table 1: Studies at low risk of bias.

Author (year)	N	Cancer type, stage	Follow-up duration	Intensive follow-up approach	Comparison (reference)
Ohlsson (1995) [14]	107	CRC, Dukes A-C	Range 5.5 - 8.8y	Clin exam/ CEA / CXR / proctoscopy : every 3m Y1-2, 6m Y3-4, 12m Y5 CT pelvis: every 6m Y1-2 Colonscopy: 3, 15, 30 and 60m	No organised follow-up
Primrose (2014) [15]	1202	CRC, Dukes A-C	Mean 3.7y	CEA-only: every 3m Y1-2,every 6m Y3-5 * CT-only: CT chest, abd, pelvis every 6m Y1-2, every 12m Y3-5 CT+CEA: protocols combined	No scheduled follow-up *
Rodriguez (2006) [16]	259	CRC, II - III	Median 49m and 45m	Clin exam / CEA: every 3m Y1-2, every 6m Y 3-5 Abd CT or US: every 6m Y1-2, every 12m Y3-5 CXR / colonoscopy: every 12m Y1-5	Clin exam / CEA: every 3m Y1-2, every 6m Y3-5 Colonoscopy: @ 12 and 36m **
Rosati (2016) [17]	1228	CRC, Dukes B2-C	Minimum 5y	Clin exam / CEA and CA 19-9: every 4m Y1-2, every 6m Y3-4, every 12m Y5 CXR / colonoscopy: every 12m Y1-5 Liver US: @ 4, 8, 12, 16, 24, 16, 48 and 60m	Clin exam / CEA: every 4m Y1-2, every 6m Y3-4, every 12m Y5 Colonoscopy: @ 12 and 48m Liver US: @ 4 and 16m
Snyder (2018) [18]	8529	CRC, I - III	Minimum 5y	High-frequency CT and CEA ***	Low-frequency CT and CEA ***
Treasure (2014) [19]	216	CRC, Dukes A-C	Minimum 18y	Second look surgery upon CEA rise Clin exam: every 3m Y1-2, every 6m Y3-5 CEA: every month Y1-3, every 3m Y4-5	No additional diagnostics upon CEA rise Clin exam: every 3m Y1-2, every 6m Y3-5
Wang (2009) [20]	326	CRC, Dukes A-C	Median 74m	Colonoscopy / clin exam/ CEA / abd CT or US / CXR: every 3m Y1, every 6m Y2-3, every 12m Y4-5	Colonoscopy: @ 6, 30 and 60 months Clin exam/ CEA / abd CT or US / CXR: every 3m Y1, every 6m Y2-3, every 12m Y4-5
Wille-Jørgensen (2018) [21]	2509	CRC, II - III	Median 5y	CT / CEA: @ 6, 12, 18, 24 and 36m	CT / CEA: @ 12 and 36m
Bornhak (2007) [60]	670	Breast, T1-4, N0-2	Almost all patients 5y	Clin exam / blood tests: every 3m Y1-3, every 6m Y4-5 Liver US / CXR: every 6m Y1-5 Mammography: every 6m Y1-3, every 12m Y4-5	Clin exam: every 3m Y1-3, every 6m Y4-5 Mammography: every 6m Y1-3, every 12m Y4-5
Del Turco (1994) [61] Palli (1999) [62]	1243	Breast, T1-4, N-/+	Almost all patients 10y	Clin exam: every 3m Y1-2, every 6 months Y3-5 Mammography: every 12m Y1-5 CXR / bone scan: every 6m Y1-5	Clin exam: every 3m Y1-2, every 6 months Y3-5 Mammography: every 12m Y1-5
Liberati (1994) [63]	1320	Breast, T1-3, N0-1	Median 71m	Clin exam / blood tests: every 3m Y1-2, every 6m Y3-5 Mammography / liver US / bone scan: every 12m Y1-5 CXR: every 6m Y1-5	Clin exam / blood test: every 3m Y1-2, every 6m Y3-5 Mammography: every 12m Y1-5
Bjerring (2019) [71]	183	GOJ, gastric, pancreatic, I-III	Almost all patients 2y	Clin exam / PET-CT / endoscopic US: every 3m Y1, every 6m Y2	Clin exam: every 3m Y1, every 6m Y2
Nabhan (2012) [76]	703	Prostate, I - III	Median 6.7y	PSA test within 2y after treatment	No PSA test within 2y after treatment

Abd = abdominal, CA 19-9 = carbohydrate antigen 19-9, CEA = carcinoembryonic antigen, clin exam = clinical examination, CRC = colorectal cancer, CT = computed tomography, CXR = chest x-ray, GOJ = gastro-oesophageal junction, m = months, MRI = magnetic resonance imaging, NR = not reported, NSCLC = non-small cell lung cancer, PET-CT = positron emission tomography – computed tomography, PSA = prostate specific antigen, US = ultrasound, w = weeks, y = years.

* Single chest, abdomen, and pelvis CT scan at 12 to 18months if requested at study entry by hospital clinician ; ** Only performed in patients at high risk for metachronous lesions (hereditary cancer, synchronous colorectal neoplasms); *** Classification of follow-up facility based. Expected number of scans/CEA measurements calculated through 2-level random intercept negative binomial model. High frequency facility defined as observed:expected ratio ≥1.

Prostate cancer

Only one study evaluating follow-up in terms of survival was identified in patients with prostate cancer (table 1).[76] The study was observational and considered to be at low risk of bias. Nahban et al. performed a two-year landmark analysis, evaluating the frequency of prostate specific antigen (PSA) testing after prostatectomy or radiotherapy. Frequent PSA testing within the first two years after treatment did not impact OS (table 3), neither in patients undergoing resection (HR 0.95, 95%CI 0.70-1.30), nor in patients receiving radiotherapy (HR 1.01, 95%CI 0.86-1.21) (combined HR 1.00, 95%CI 0.86-1.16).

Table 2: Studies at high risk of bias.

Author (year)	N	Cancer type, stage	Follow-up duration	Intensive follow-up approach	Comparison (reference)
Augestad (2013) [22]	110	Colon, Dukes A-C	58% completed 2y follow-up	Surgeon-led	General practitioner-led
Bergamaschi (1996) [23]	800	CRC, I-III	Minimum 60m	Clin exam / CEA / liver US: every 3m Y1, every 6m Y2-5 CXR: every 3m Y1, every 6m Y2, every 12m Y3-5 Endoscopy: every 12m Y1-5	Symptom-based
Borie (2004) [24]	231	CRC, Dukes A-C	NR	Clin exam: every 3m Y1-2, every 6m Y3-5 CEA / US: every 4-6m Y1-3, every 12m Y4-5 CXR: every 12m Colonoscopy: every 36m	Clin. Exam: at most every 6m Y1-5 CEA / US: at most every 12m Y1-3 CXR: at most every 12m Y1-2 Colonoscopy: at most every 36m
Castells (1998) [25]	199	CRC, I-III	Median 51m	Compliant (>70% adherence) to follow-up	Non-compliant (<70% adherence) to follow-up
Coebergh (2018) [26]	681	CRC, I-III	Median 68m for OS Median 34m for DFS	Intensive (≥ 3 follow-up moments in first year)	Minimal (≤ 2 follow-up moments in first year)
Eckardt (1994) [27]	212	CRC, Dukes A-C	Mean 91m and 94m	Full compliance to endoscopic follow-up	Non-compliance to endoscopic follow-up
Gamboa (2020) [28]	239	CRC, IV (peritoneal)	Median 17m	High frequency follow-up (every 2-4 months)	Low-frequency follow-up (every 6-12 months)
Hines (2019) [29]	17860	CRC, II-III	Median 9.0y and 9.6y	More adherent to guideline recommendations	Less adherent to guideline recommendations
Hines (2020) [30]	8783	CRC, I	Median > 10y	1 colonoscopy during follow-up or ≥2 colonoscopies during follow-up	No colonoscopy during follow-up
Hyder (2013) [31]	507	CRC, IV (liver)	NR	Follow-up with imaging (≥1 scan)	No imaging during follow-up
Kjeldsen (1997) [32]	597	CRC, Dukes A-C	NR	Clin exam / blood analysis without CEA / CXR / colonoscopy: every 6m Y1-3, every 12m Y4-5, every 5y Y6-15	Clin exam / blood analysis without CEA / CXR / colonoscopy:every 5y Y1-15
Körner (2005) [33]	314	CRC, Dukes A-C	Median 66m	Guideline follow-up	No follow-up
Laubert (2010) [34]	1469	CRC, I-IV	Median 70m	Intensive follow-up (>70% adherence) or Minimal follow-up (<70% adherence)	No follow-up
Mahboubi (2007) [35]	389	CRC, I-IV	NR	Regular (≥1 per 6 months) follow-up at GP or Occasional (<1 per 6 months) follow-up at GP	No follow-up
Mäkelä (1995) [36]	106	CRC, Dukes A-C	NR	Clin exam / CEA / CXR: every 3m Y1-2, every 6m Y3-5 Liver US: every 6m Y1-5 CT abd / colonoscopy: every 12m Y1-5 Sigmoidoscopy*: every 3m Y1-5	Clin exam / CEA / CXR / sigmoidoscopy*: every 3m Y1-2, every 6m Y3-5 Barium enema: every 12m Y1-5
Ovaska (1990) [37]	507	CRC, Dukes A-C	85% 5y	Clin exam / CEA / sigmoidoscopy: @3, 6, 12, 18, 24, 36, 48 and 60m CXR / colography: every 6m Y1, every 12m Y2-5	No follow-up
Pietra (1998) [38]	207	CRC, Astler-Coller B1-C2	NR	Clin exam / CEA / US: every 3m Y1-2, every 6m Y3-4, every 12m thereafter CXR / CT / colonoscopy: every 12m Y1-5	Clin exam / CEA / US: every 6m Y1, every 12m Y2-5 CXR / colonoscopy: every 12m Y1-5
Pugliese (1984) [39]	177	CRC, Dukes B-C	Median 33m	Clin exam / blood tests - later including CEA: every 3m Y1-2, every 6m Y3-5 CXR / liver US / colonoscopy: every 6m Y1-2, every 12m Y3-5	No follow-up
Rulyak (2007) [40]	1002	CRC, 0-III	Median 3.6y	≥1 colonoscopies during follow-up	No colonoscopy during follow-up

Schoemaker (1998) [41]	325	CRC, Dukes A-C	94% 60m	Clin exam / CEA: every 3m Y1-2, every 6m Y3-5 CXR / liver CT / colonoscopy: every 12m Y1-5	Clin exam / CEA: every 3m Y1-2, every 6m Y3-5
Secco (2002) [41]	358	CRC, Dukes A-C	Median 61.5m (high-risk) 42m (low-risk)	High risk patients Clin exam/ CEA: every 3m Y1-2, every 4m Y3, every 6m Y4-5 Abd and pelvic US: every 6m 1-3Y, every 12m Y4-5 CXR / rectosigmoidoscopy*: every 12m Y1-5 Low risk patients Clin exam/ CEA: every 6m Y1-2, every 12m Y3-5 Abd and pelvic US: every 6m 1-2Y, every 12m Y3-5 CXR: every 12m Y1-5 Rectosigmoidoscopy*: every 12m Y1-2, every 24m Y3-5	Minimal follow-up not clearly defined
Sobhani (2018) [43]	239	CRC, II-IV	NR	Follow-up with whole body CT and PET-CT: every 6m Y1-3	Follow-up with whole body CT: every 6m Y1-3
Strand (2011) [44]	110	CRC, I-IV	NR	Surgeon-led	Nurse-led
Wattchow (2006) [45]	203	CRC, Dukes A-C	87% 2y	Surgeon-led	General practitioner-led
Zitt (2006) [46]	430	CRC, I-IV	Mean 49m	Clin exam / CEA: every 3m Y1-2, every 6m Y3-5 CXR + abd US / CT + colonscopy: every 6m Y1-5 (alternating per 6m) Rectoscopy @ 3, 6 and 9 months or Non-standardised follow-up in hospital or at GP	No follow-up
Backhus (2016) [47]	18406	NSCLC, I-II	NR	Clinical visitation within 4-8m after treatment	No clinical visitation within 4-8m after treatment
Benamore (2007) [48]	75	NSCLC, III	Median 77m and 44m	Follow-up within clinical trial Clin exam/ blood analysis / CXR: every 2/3m Y1-2, every 6m thereafter CT chest and upper abd / MRI brain: every 6m Y1-3, every 12m thereafter	Non-trial follow-up Clin exam/ blood analysis / CXR: every 3m Y1-2/3, every 6m Y3/4-5
Crabtree (2015) [49]	554	NSCLC, I	NR	Routine CT-based follow-up	Routine CXR-based follow-up
Ho (2017) [50]	263	NSCLC, IB-II	NR	Frequency CT and/or clinical visits per or above guidelines	Frequency CT and/or clinical visits below guidelines
Karzijn (2016) [51]	73	NSCLC, I-II	NR	Follow-up with CT imaging	Follow-up with CXR only
Mayne (2020) [52]	187	NSCLC, IA	Median 36m and 56.4m	Early (6 ± 3 months) start of CT surveillance	Late (12 ± 3 months) start of CT surveillance
McMurry (2018) [53]	4463	NSCLC, I-III	Minimum 60m	3 months CT-imaging interval	6 or 12 months CT-imaging interval
Monteil (2010) [54]	69	NSCLC, I-IIIA	Median 25m and 29m	PET-CT / Brain CT	Brain, chest and upper abd CT / abd US / bone scintigraphy **
Nakamura (2010) [55]	1398	NSCLC, I-III	Median 79m	Follow-up by chest physician Clin exam / CXR: every 3-4m Y1-3 CT: every 6m Y1-3	Follow-up by thoracic surgeon Clin exam / CXR: every 3-4m Y1-3

Pan (2015) [56]	92	NSCLC, IIB-IIIB	Median 23m	Follow-up with CT imaging and a single PET-CT @ 9m	Follow-up with CT imaging only
Reddy (2017) [57]	200	NSCLC, III	Median 59.4m	Follow-up with PET-CT and CT alternating in Y1-2	Follow-up with CT only
Sui (2020) [58]	200	NSCLC, I-III	85% 5y	WeChat app-based education and rehabilitation program - including disease related education (once a week for 12w), rehabilitation exercise guidance (once a week for 40w), daily activity supervision (once a week for 12m), and psychosocial support (every 2 weeks for 12m)	Regular rehabilitation program
Virgo (1995) [59]	182	Lung (subtype NR), I-IIIA	Mean 3.3y	Intensive follow-up, any of the following criteria: ≥4 visits and/or blood tests and/or CXR per year, ≥1 CT per year, or any bronchoscopy and/or sputum cytology	Non-intensive follow-up (none of the criteria met)
Grunfeld (2006) [64]	968	Breast, early stage	Median 3.5y	Guideline follow-up carried out by cancer specialist	Guideline follow-up carried out by family physician
Koinberg (2004) [65]	264	Breast, I-II	NR	Physician-led Clin exam: every 3m Y1-2, every 6m Y3-5, and yearly thereafter Mammography: every 12m Y1-5	Nurse-led Clin exam: on-demand Mammography: every 12m Y1-3, screening-programme thereafter
Lash (2005) [66]	303	Breast, I-II	Median 7.4y	Number of consecutive years of guideline surveillance	(continuous analysis, no separate comparison group)
Lash (2006) [67]	334	Breast, I-IIIA	NR	One or more mammograms during follow-up	No mammograms during follow-up
Lash (2007) [68]	812	Breast, I-II	NR	Number surveillance mammograms received	(continuous analysis, no separate comparison group)
Lee (2015) [69]	3770	Breast, I-III	Median 7.1y	Clin exam / blood analysis / mammography / CXR / breast, abd and pelvic US / bone scans: every 3-6m Y1-5, and yearly thereafter	Control group of patients lost to follow-up after adjuvant therapy
Paszat (2009) [70]	901	Breast, I-II	Median 141m and 29m	≥1 surveillance mammography	No surveillance mammography
Peixoto (2014) [72]	292	Gastroesophageal, I-III	NR	Oncologist follow-up with Clin exam or Blood analysis or Imaging or endoscopy	Discharge to general practitioner
Sisic (2018) [73]	587	Gastroesophageal, I-III	Median 60.5m and 68.5m	Follow-up in cancer centre Clin exam / CT abd or abd US and endoscopy (alternating): every 3m Y1-2, every 6m Y3-4, every 12m Y5	Individual follow-up by other physicians
Tan (2007) [74]	102	Gastric, I-IV	Mean 3.4y	>1 CT scans per year	≤1 CT scans per year
Verschuur (2009) [75]	109	Gastroesophageal, I-IV	98% 1y	Physician-led follow-up	Nurse-led follow-up

Abd = abdominal, CA 19-9 = carbohydrate antigen 19-9, CEA = carcinoembryonic antigen, clin exam = clinical examination, CRC = colorectal cancer, CT = computed tomography, CXR = chest x-ray, GOJ = gastro-oesophageal junction, m = months, MRI = magnetic resonance imaging, NR = not reported, NSCLC = non-small cell lung cancer, PET-CT = positron emission tomography – computed tomography, PSA = prostate specific antigen, US = ultrasound, w = weeks, y = years.

*For rectal and sigmoid cancer patients only. ** Only in patients with symptoms possibly related to bone metastases.

Table 3. Meta-analysis per tumor type.

Cancer type	N	OS HR [95%CI]	I^2	N	CSS HR [95%CI]	I^2	N	Curative treatment RR [95%CI]	I^2
All studies									
Colorectal	30	0.82 [0.73-0.91] *	85%	11	0.80 [0.63-1.01]	84%	19	1.60 [1.21-2.11] *	66%
Lung	12	0.94 [0.84-1.05]	49%	1	1.45 [0.84-2.5]	NA	4	1.34 [0.82-2.20]	39%
Breast	8	0.80 [0.54-1.18]	92%	3	0.52 [0.27-1.02]	94%	-	-	-
Upper GI	5	0.79 [0.66-0.95] *	0%	-	-	-	2	1.25 [0.62-2.52]	50%
Prostate	1	1.00 [0.86-1.16]	NA	-	-	-	-	-	-
Low risk of bias									
Colorectal	8	0.99 [0.92-1.06]	7%	3	0.94 [0.77-1.16]	0%	6	1.30 [1.05-1.61] *	28%
Lung	-	-	-	-	-	-	-	-	-
Breast	3 **	1.06 [0.92-1.23]	0%	-	-	-	-	-	-
Upper GI	1	0.78 [0.51-1.19]	NA	-	-	-	1	0.92 [0.47-1.81]	NA
Prostate	1	1.00 [0.86-1.16]	NA	-	-	-	-	-	-
High risk of bias									
Colorectal	22	0.75 [0.65-0.86] *	87%	8	0.74 [0.54-1.02]	88%	13	1.77 [1.06-2.94] *	74%
Lung	12	0.94 [0.84-1.05]	49%	1	1.45 [0.84-2.5]	NA	4	1.34 [0.82-2.20]	39%
Breast	5	0.68 [0.41-1.14]	91%	3	0.52 [0.27-1.02]	94%	-	-	-
Upper GI	4	0.79 [0.65-0.97] *	0%	-	-	-	1	1.73 [0.84-3.59]	NA
Prostate	-	-	-	-	-	-	-	-	-

*Statistically significant result

** Long-term updates 62 from the study by Del Turco et al. 61 were used for the meta-analysis in breast cancer patients.

CI = confidence interval, CSS = cancer specific survival, HR = hazard ratio, N = number of studies reporting on outcome, NA = not applicable, OS = overall survival, RR = relative risk, Upper GI = upper gastrointestinal.

Discussion

This meta-analysis provides a general overview on the impact of follow-up strategies on survival and treatment outcomes for five common tumor types. We found that for tumors other than colorectal and breast cancer, little to no high quality evidence is available to formulate evidence based follow-up guidelines upon. The impact of different follow-up strategies on CSS and treatment for recurrences could only be evaluated for colorectal cancer, as these outcomes were hardly reported in high quality studies for the other types of cancer.

When pooling the eight available colorectal cancer studies at low risk of bias, no significant OS (HR 0.99, 95%CI 0.92-1.06) or CSS (0.94, 95%CI 0.77-1.16) benefit was observed after intensive surveillance (i.e. more frequent imaging). These results are consistent with previous meta-analyses which only included RCTs.[77, 78] Other meta-analyses did find a survival benefit in terms of OS, but none regarding CSS.[79-84] In line with our results, all available meta-analyses evaluating colorectal cancer follow-up found that intensive follow-up increases the probability of receiving curative intent treatment for recurrent disease, leading to several hypotheses. The consistently higher curative intent treatment rates suggest that intensive follow-up after colorectal cancer surgery successfully meets its main objective (i.e. early detection of relapses to increase treatment possibilities). Nevertheless, this does not translate in a survival benefit at a population level. The cure rate of approximately twenty percent after local treatment for metastatic colorectal disease shows the need for some form of follow-up.[85] Both intensive and less intensive follow-up approaches (e.g. mostly based on sequential CEA measurements) may however both be equally able to identify those patients that will benefit most from local therapies. Other factors (e.g. pre-existing tumor biology, host immune response) than the timely detection of recurrences may ultimately have a larger impact on survival after colorectal cancer resection.[86]

In breast cancer, the low risk studies strongly suggest that frequent, multimodality follow-up (i.e. including MRI, bone scans, and laboratory assessments) does not provide benefits for patients in terms of survival, compared to a mammography based approach (HR 1.06, 95%CI 0.92-1.23 for OS). Low-frequency imaging surveillance using mammograms is being advocated by the majority of guidelines[87, 88], but has mostly been compared to symptom-based follow-up in observational studies prone to bias (HR 0.68 (95%CI 0.41-1.14) for OS, 0.52 (95%CI 0.27-1.02) for CSS).[64-70] The effectiveness of annual mammography surveillance therefore remains questionable, especially since none of the breast cancer studies report data on the (curative) treatment of recurrent disease or novel primary tumors. Despite the lack of evidence, a mammography frequency similar to that of most breast cancer screening programs (e.g. every 1-3 years)[89] seems acceptable from both a medical and an economic point of view. Interestingly, no high quality data is available on the relationship between follow-up strategy and the other outcomes evaluated in this study (i.e. CSS, treatment intent). Such data would provide additional insights regarding follow-up in this population.

The quality of the 13 identified lung cancer and the 5 upper-gastrointestinal cancer studies was mostly poor, with 100% and 80% of studies being at high risk of bias. A potential OS benefit was observed in upper-gastrointestinal cancers studies (HR 0.79, 95%CI 0.66-0.95). For both tumors, most guidelines either refrain from advising on the frequency of diagnostic or clinical evaluations, or continue to advise imaging, blood tests, and clinical evaluations every 3-6 months during the first years after surgery.[90-95] This meta-analysis shows that any policy making with regards to follow-up for these cancer types is not based on robust evidence. Given the outcomes in breast and colorectal cancer, no large effect is to be expected from frequent multimodality follow-up. So while high quality studies are formally needed to evaluate effectiveness of intensive follow-up in lung and upper gastro-intestinal cancers, combining survival with other relevant endpoints (e.g. quality of

life, cost-effectiveness) should be considered to maximize return of investment.

For prostate cancer only one study was identified. Follow-up for prostate cancer differs from other types of cancer, as the strategy solely relies on serum tumor marker measurements (i.e. PSA). Intensive prostate cancer follow-up thus remains relatively non-intensive, when compared to follow-up for other cancers. In addition, serum PSA measurements are relatively cheap and can easily be performed in a general practitioner setting. As only 1-10% of deaths in prostate cancer patients relate to cancer progression, little may be expected from a highly frequent oncological follow-up program in this population as a whole, at least in terms of a survival benefit.[96] After a median follow-up of 6.7 years, Nabhan et al. indeed found no impact of frequent PSA testing during the first two years, neither after resection (HR 0.95, 95%CI 0.70-1.30) nor radiotherapy (HR 1.01, 95%CI 0.86-1.21). None of the follow-up guidelines for prostate cancer make recommendations on the actual frequency of follow-up, but all advocate a frequency depending on patient characteristics and preference. A more frequent approach may be applied in patients needing reassurance and vice versa. Such an approach may also be suitable in patients with other types of cancer, especially when a reliable serum tumor marker is available (e.g. CEA in colorectal cancer and gastric cancer). Dutch and Scandinavian national guidelines for colorectal cancer are currently moving in this direction, with only one or two scheduled imaging procedures advocated during follow-up.[97-99] The lack of evidence favoring such an approach in non-colorectal cancer patients remains however.

Multiple studies comparing different diagnostic modalities in multiple types of cancer were identified in this review, including several high quality RCTs.[15, 61-63] None of these studies demonstrated that the addition of more sensitive diagnostics actually improves survival outcomes, while they are associated with increasing health costs. During follow-up, adding and combining several different diagnostics apparently does not provide the expected survival benefit. Given the currently presented lack of evidence it might be worthwhile to reconsider the frequent and combined use of multiple diagnostics during follow-up, especially in colorectal cancer patients. Importantly, it has to be stressed that these results do not declare oncological follow-up practices futile, and certainly do not propose a nihilistic, symptom-based, follow-up approach for all cancer patients. However, a change of approach may be necessary and beneficial. For instance, out-of-hospital follow-up (or at least partly), in close collaboration with the general practitioner, could be an appealing alternative from a patient wellbeing and economic point of view.[100, 101] Both of these outcomes should play a major role in deciding which type of follow-up is most appropriate for cancer patients. Adequate (meta-analytic) data on quality of life and cost-effectiveness is currently lacking, but one could assume an impact of follow-up on both. Anxiety, patient satisfaction, costs, but also physical wellbeing and post treatment pain are all important aspects that should be taken into account when evaluating different follow-up approaches.

This meta-analysis should be evaluated in light of its limitations. Few studies with long-term follow-up (i.e. ten years or more) were identified. Long-term updates from high quality follow-up studies should be pursued, as the impact of an increased curative intent treatment rate for recurrences may not be visual yet after an initial five years of follow-up. In addition, many of the studies lacked statistical power to detect survival differences between follow-up approaches, and one could argue that the same may apply to the currently performed meta-analyses.

Conclusion

This meta-analysis provides a broad perspective on the available evidence with regards to oncological follow-up after curative intent treatment for common solid cancers. It shows that little high quality data is available for tumors other than colorectal and breast cancer. Amongst the high quality studies identified, intensive follow-up approaches do not seem to prolong survival, despite resulting in high curative intent treatment rates for colorectal cancer.

References

1 Cardoso R, Coburn NG, Seevaratnam R, Mahar A, Helyer L, Law C, et al. A systematic review of patient surveillance after curative gastrectomy for gastric cancer: a brief review. Gastric Cancer. 2012;15 Suppl 1:S164-7.

2 Swinnen J, Keupers M, Soens J, Lavens M, Postema S, Van Ongeval C. Breast imaging surveillance after curative treatment for primary non-metastasised breast cancer in non-high-risk women: a systematic review. Insights Imaging. 2018;9:961-70.

3 van der Stok EP, Spaander MCW, Grünhagen DJ, Verhoef C, Kuipers EJ. Surveillance after curative treatment for colorectal cancer. Nat Rev Clin Oncol. 2017;14:297-315.

4 Schmidt-Hansen M, Baldwin DR, Hasler E. What is the most effective follow-up model for lung cancer patients? A systematic review. J Thorac Oncol. 2012;7:821-4.

5 Brandenbarg D, Berendsen AJ, de Bock GH. Patients' expectations and preferences regarding cancer follow-up care. Maturitas. 2017;105:58-63.

6 Edelman MJ, Meyers FJ, Siegel D. The utility of follow-up testing after curative cancer therapy. A critical review and economic analysis. J Gen Intern Med. 1997;12:318-31.

7 Guy GP, Jr., Ekwueme DU, Yabroff KR, Dowling EC, Li C, Rodriguez JL, et al. Economic burden of cancer survivorship among adults in the United States. J Clin Oncol. 2013;31:3749-57.

8 Surveillance Research Program DoCCaPS, National Cancer Institute. Cancer Treatment & Survivorship. Facts & Figures 2019-2021. 2019.

9 Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. Bmj. 2016;355:i4919.

10 Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj. 2011;343:d5928.

11 Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007;8:16.

12 Higgins JP, Green S, Collaboration C. Cochrane Handbook for Systematic Reviews of Interventions: The Cochrane Collaboration; 2011.

13 Gu Z, Gu L, Eils R, Schlesner M, Brors B. circlize Implements and enhances circular visualization in R. Bioinformatics. 2014;30:2811-2.

14 Ohlsson B, Breland U, Ekberg H, Graffner H, Tranberg KG. Follow-up after curative surgery for colorectal carcinoma. Randomized comparison with no follow-up. Dis Colon Rectum. 1995;38:619-26.

15 Primrose JN, Perera R, Gray A, Rose P, Fuller A, Corkhill A, et al. Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. Jama. 2014;311:263-70.

16 Rodríguez-Moranta F, Saló J, Arcusa A, Boadas J, Piñol V, Bessa X, et al. Postoperative surveillance in patients with colorectal cancer who have undergone curative resection: a prospective, multicenter, randomized, controlled trial. J Clin Oncol. 2006;24:386-93.

17 Rosati G, Ambrosini G, Barni S, Andreoni B, Corradini G, Luchena G, et al. A randomized trial of intensive versus minimal surveillance of patients with resected Dukes B2-C colorectal carcinoma. Ann Oncol. 2016;27:274-80.

18 Snyder RA, Hu CY, Cuddy A, Francescatti AB, Schumacher JR, Van Loon K, et al. Association Between Intensity of Posttreatment Surveillance Testing and Detection of Recurrence in Patients With Colorectal Cancer. Jama. 2018;319:2104-15.

19 Treasure T, Monson K, Fiorentino F, Russell C. The CEA Second-Look Trial: a randomised controlled trial of carcinoembryonic antigen prompted reoperation for recurrent colorectal cancer. BMJ Open. 2014;4:e004385.

20 Wang T, Cui Y, Huang WS, Deng YH, Gong W, Li CJ, et al. The role of postoperative colonoscopic surveillance after radical surgery for colorectal cancer: a prospective, randomized clinical study. Gastrointest Endosc. 2009;69:609-15.

21 Wille-Jørgensen P, Syk I, Smedh K, Laurberg S, Nielsen DT, Petersen SH, et al. Effect of More vs Less Frequent Follow-up Testing on Overall and Colorectal Cancer-Specific Mortality in Patients With Stage II or III Colorectal Cancer: The COLOFOL Randomized Clinical Trial. Jama. 2018;319:2095-103.

22 Augestad KM, Norum J, Dehof S, Aspevik R, Ringberg U, Nestvold T, et al. Cost-effectiveness and quality of life in surgeon versus general practitioner-organised colon cancer surveillance: a randomised controlled trial. BMJ Open. 2013;3.

23 Bergamaschi R, Arnaud JP. Routine compared with nonscheduled follow-up of patients with "curative" surgery for colorectal cancer. Ann Surg Oncol. 1996;3:464-9.

24 Borie F, Daurès JP, Millat B, Trétarre B. Cost and effectiveness of follow-up examinations in patients with colorectal cancer resected for cure in a French population-based study. J Gastrointest Surg. 2004;8:552-8.

25 Castells A, Bessa X, Daniels M, Ascaso C, Lacy AM, García-Valdecasas JC, et al. Value of postoperative surveillance after radical surgery for colorectal cancer: results of a cohort study. Dis Colon Rectum. 1998;41:714-23; discussion 23-4.

26 Coebergh van den Braak RRJ, Lalmahomed ZS, Büttner S, Hansen BE, Ijzermans JNM, Group MS. Nonphysician Clinicians in the Follow-Up of Resected Patients with Colorectal Cancer. Dig Dis. 2018;36:17-25.

27 Eckardt VF, Stamm H, Kanzler G, Bernhard G. Improved survival after colorectal cancer in patients complying with a postoperative endoscopic surveillance program. Endoscopy. 1994;26:523-7.

28 Gamboa AC, Zaidi MY, Lee RM, Speegle S, Switchenko JM, Lipscomb J, et al. Optimal Surveillance Frequency After CRS/HIPEC for Appendiceal and Colorectal Neoplasms: A Multi-institutional Analysis of the US HIPEC Collaborative. Ann Surg Oncol. 2020;27:134-46.

29 Hines RB, Jiban MJH, Specogna AV, Vishnubhotla P, Lee E, Zhang S. The association between post-treatment surveillance testing and survival in stage II and III colon cancer patients: An observational comparative effectiveness study. BMC Cancer. 2019;19:418.

30 Hines RB, Jiban MJH, Specogna AV, Vishnubhotla P, Lee E, Troy SP, et al. Surveillance Colonoscopy in Older Stage I Colon Cancer Patients and the Association With Colon Cancer-Specific Mortality. Am J Gastroenterol. 2020;115:924-33.

31 Hyder O, Dodson RM, Mayo SC, Schneider EB, Weiss MJ, Herman JM, et al. Post-treatment surveillance of patients with colorectal cancer with surgically treated liver metastases. Surgery. 2013;154:256-65.

32 Kjeldsen BJ, Kronborg O, Fenger C, Jørgensen OD. A prospective randomized study of follow-up after radical surgery for colorectal cancer. Br J Surg. 1997;84:666-9.

33 Körner H, Söreide K, Stokkeland PJ, Söreide JA. Systematic follow-up after curative surgery for colorectal cancer in Norway: a population-based audit of effectiveness, costs, and compliance. J Gastrointest Surg. 2005;9:320-8.

34 Laubert T, Bader FG, Oevermann E, Jungbluth T, Unger L, Roblick UJ, et al. Intensified surveillance after surgery for colorectal cancer significantly improves survival. Eur J Med Res. 2010;15:25-30.

35 Mahboubi A, Lejeune C, Coriat R, Binquet C, Bouvier AM, Béjean S, et al. Which patients with colorectal cancer are followed up by general practitioners? A population-based study. Eur J Cancer Prev. 2007;16:535-41.

36 Mäkelä JT, Laitinen SO, Kairaluoma MI. Five-year follow-up after radical surgery for colorectal cancer. Results of a prospective randomized trial. Arch Surg. 1995;130:1062-7.

37 Ovaska J, Järvinen H, Kujari H, Perttilä I, Mecklin JP. Follow-up of patients operated on for colorectal carcinoma. Am J Surg. 1990;159:593-6.

38 Pietra N, Sarli L, Costi R, Ouchemi C, Grattarola M, Peracchia A. Role of follow-up in management of local recurrences of colorectal cancer: a prospective, randomized study. Dis Colon Rectum. 1998;41:1127-33.

39 Pugliese V, Aste H, Saccomanno S, Bruzzi P, Bonelli L, Santi L. Outcome of follow-up programs in patients previously resected for colorectal cancer. Tumori. 1984;70:203-8.

40 Rulyak SJ, Lieberman DA, Wagner EH, Mandelson MT. Outcome of follow-up colon examination among a population-based cohort of colorectal cancer patients. Clin Gastroenterol Hepatol. 2007;5:470-6; quiz 07.

41 Schoemaker D, Black R, Giles L, Toouli J. Yearly colonoscopy, liver CT, and chest radiography do not influence 5-year survival of colorectal cancer patients. Gastroenterology. 1998;114:7-14.

42 Secco GB, Fardelli R, Gianquinto D, Bonfante P, Baldi E, Ravera G, et al. Efficacy and cost of risk-adapted follow-up in patients after colorectal cancer surgery: a prospective, randomized and controlled trial. Eur J Surg Oncol. 2002;28:418-23.

43 Sobhani I, Itti E, Luciani A, Baumgaertner I, Layese R, André T, et al. Colorectal cancer (CRC) monitoring by 6-monthly 18FDG-PET/CT: an open-label multicentre randomised trial. Ann Oncol. 2018;29:931-7.

44 Strand E, Nygren I, Bergkvist L, Smedh K. Nurse or surgeon follow-up after rectal cancer: a randomized trial. Colorectal Dis. 2011;13:999-1003.

45 Wattchow DA, Weller DP, Esterman A, Pilotto LS, McGorm K, Hammett Z, et al. General practice vs surgical-based follow-up for patients with colon cancer: randomised controlled trial. Br J Cancer. 2006;94:1116-21.

46 Zitt M, Mühlmann G, Weiss H, Kafka-Ritsch R, Oberwalder M, Kirchmayr W, et al. Assessment of risk-independent follow-up to detect asymptomatic recurrence after curative resection of colorectal cancer. Langenbecks Arch Surg. 2006;391:369-75.

47 Backhus LM, Farjah F, Liang CK, He H, Varghese TK, Jr., Au DH, et al. Imaging surveillance and survival for surgically resected non-small-cell lung cancer. J Surg Res. 2016;200:171-6.

48 Benamore R, Shepherd FA, Leighl N, Pintilie M, Patel M, Feld R, et al. Does intensive follow-up alter outcome in patients with advanced lung cancer? J Thorac Oncol. 2007;2:273-81.

49 Crabtree TD, Puri V, Chen SB, Gierada DS, Bell JM, Broderick S, et al. Does the method of radiologic surveillance affect survival after resection of stage I non-small cell lung cancer? J Thorac Cardiovasc Surg. 2015;149:45-52, 3 e1-3.

50 Ho C, Siegfried J, Remo K, Laskin J. Adherence to surveillance guidelines in resected NSCLC: Physician compliance and impact on outcomes. Lung Cancer. 2017;112:176-80.

51 Karzijn R, Alberts L, Kelder JC, Hofman FN, El Sharouni SY, Kastelijn EA, et al. Post-treatment Surveillance for Stage I and II Non-small Cell Lung Cancer: Impact on Clinical Outcome. Anticancer Res. 2016;36:5413-8.

52 Mayne NR, Mallipeddi MK, Darling AJ, Jeffrey Yang CF, Eltaraboulsi WR, Shoffner AR, et al. Impact of Surveillance After Lobectomy for Lung Cancer on Disease Detection and Survival. Clin Lung Cancer. 2020;21:407-14.

53 McMurry TL, Stukenborg GJ, Kessler LG, Colditz GA, Wong ML, Francescatti AB, et al. More Frequent Surveillance Following Lung Cancer Resection Is Not Associated With Improved Survival: A Nationally Representative Cohort Study. Ann Surg. 2018;268:632-9.

54 Monteil J, Vergnenègre A, Bertin F, Dalmay F, Gaillard S, Bonnaud F, et al. Randomized follow-up study of resected NSCLC patients: conventional versus 18F-DG coincidence imaging. Anticancer Res. 2010;30:3811-6.

55 Nakamura R, Kurishima K, Kobayashi N, Ishikawa S, Goto Y, Sakai M, et al. Postoperative follow-up for patients with non-small cell lung cancer. Onkologie. 2010;33:14-8.

56 Pan Y, Brink C, Schytte T, Petersen H, Wu YL, Hansen O. Planned FDG PET-CT Scan in Follow-Up Detects Disease Progression in Patients With Locally Advanced NSCLC Receiving Curative Chemoradiotherapy Earlier Than Standard CT. Medicine (Baltimore). 2015;94:e1863.

57 Reddy JP, Tang C, Shih T, Kim B, Kim C, Nguyen QN, et al. Influence of Surveillance PET/CT on Detection of Early Recurrence After Definitive Radiation in Stage III Non-small-cell Lung Cancer. Clin Lung Cancer. 2017;18:141-8.

58 Sui Y, Wang T, Wang X. The impact of WeChat app-based education and rehabilitation program on anxiety, depression, quality of life, loss of follow-up and survival in non-small cell lung cancer patients who underwent surgical resection. Eur J Oncol Nurs. 2020;45:101707.

59 Virgo KS, McKirgan LW, Caputo MC, Mahurin DM, Chao LC, Caputo NA, et al. Post-treatment management options for patients with lung cancer. Ann Surg. 1995;222:700-10.

60 Bornhak S, Heidemann E, Herschlein HJ, Simon W, Merkle E, Widmaier G, et al. Symptom-oriented follow-up of early breast cancer is not inferior to conventional control. Results of a prospective multicentre study. Onkologie. 2007;30:443-9.

61 Rosselli Del Turco M, Palli D, Cariddi A, Ciatto S, Pacini P, Distante V. Intensive diagnostic follow-up after treatment of primary breast cancer. A randomized trial. National Research Council Project on Breast Cancer follow-up. Jama. 1994;271:1593-7.

62 Palli D, Russo A, Saieva C, Ciatto S, Rosselli Del Turco M, Distante V, et al. Intensive vs clinical follow-up after treatment of primary breast cancer: 10-year update of a randomized trial. National Research Council Project on Breast Cancer Follow-up. Jama. 1999;281:1586.

63 Impact of follow-up testing on survival and health-related quality of life in breast cancer patients. A multicenter randomized controlled trial. The GIVIO Investigators. Jama. 1994;271:1587-92.

64 Grunfeld E, Levine MN, Julian JA, Coyle D, Szechtman B, Mirsky D, et al. Randomized trial of long-term follow-up for early-stage breast cancer: a comparison of family physician versus specialist care. J Clin Oncol. 2006;24:848-55.

65 Koinberg IL, Fridlund B, Engholm GB, Holmberg L. Nurse-led follow-up on demand or by a physician after breast cancer surgery: a randomised study. Eur J Oncol Nurs. 2004;8:109-17; discussion 18-20.

66 Lash TL, Clough-Gorr K, Silliman RA. Reduced rates of cancer-related worries and mortality associated with guideline surveillance after breast cancer therapy. Breast Cancer Res Treat. 2005;89:61-7.

67 Lash TL, Fox MP, Silliman RA. Reduced mortality rate associated with annual mammograms after breast cancer therapy. Breast J. 2006;12:2-6.

68 Lash TL, Fox MP, Buist DS, Wei F, Field TS, Frost FJ, et al. Mammography surveillance and mortality in older breast cancer survivors. J Clin Oncol. 2007;25:3001-6.

69 Lee JY, Lim SH, Lee MY, Kim H, Kim M, Kim S, et al. Impact on Survival of Regular Postoperative Surveillance for Patients with Early Breast Cancer. Cancer Res Treat. 2015;47:765-73.

70 Paszat L, Sutradhar R, Grunfeld E, Gainford C, Benk V, Bondy S, et al. Outcomes of surveillance mammography after treatment of primary breast cancer: a population-based case series. Breast Cancer Res Treat. 2009;114:169-78.

71 Bjerring OS, Fristrup CW, Pfeiffer P, Lundell L, Mortensen MB. Phase II randomized clinical trial of endosonography and PET/CT versus clinical assessment only for follow-up after surgery for upper gastrointestinal cancer (EUFURO study). Br J Surg. 2019;106:1761-8.

72 Peixoto RD, Lim HJ, Kim H, Abdullah A, Cheung WY. Patterns of surveillance following curative intent therapy for gastroesophageal cancer. J Gastrointest Cancer. 2014;45:325-33.

73 Sisic L, Strowitzki MJ, Blank S, Nienhueser H, Dorr S, Haag GM, et al. Postoperative follow-up programs improve survival in curatively resected gastric and junctional cancer patients: a propensity score matched analysis. Gastric Cancer. 2018;21:552-68.

74 Tan IT, So BY. Value of intensive follow-up of patients after curative surgery for gastric carcinoma. J Surg Oncol. 2007;96:503-6.

75 Verschuur EM, Steyerberg EW, Tilanus HW, Polinder S, Essink-Bot ML, Tran KT, et al. Nurse-led follow-up of patients after oesophageal or gastric cardia cancer surgery: a randomised trial. Br J Cancer. 2009;100:70-6.

76 Nabhan M, Kim SP, Shah ND, Bagniewski SM, Shi Q, Karnes RJ, et al. The relationship of the intensity of posttreatment prostate-specific antigen surveillance and prostate cancer outcomes: results from a population-based cohort. Mayo Clin Proc. 2012;87:540-7.

77 Jeffery M, Hickey BE, Hider PN. Follow-up strategies for patients treated for non-metastatic colorectal cancer. Cochrane Database Syst Rev. 2019;9:CD002200.

78 Mokhles S, Macbeth F, Farewell V, Fiorentino F, Williams NR, Younes RN, et al. Meta-analysis of colorectal cancer follow-up after potentially curative resection. Br J Surg. 2016;103:1259-68.

79 Jones RP, Jackson R, Dunne DF, Malik HZ, Fenwick SW, Poston GJ, et al. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. Br J Surg. 2012;99:477-86.

80 Pita-Fernández S, Alhayek-Aí M, González-Martín C, López-Calviño B, Seoane-Pillado T, Pértega-Díaz S. Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis. Ann Oncol. 2015;26:644-56.

81 Renehan AG, Egger M, Saunders MP, O'Dwyer ST. Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trials. Bmj. 2002;324:813.

82 Rosen M, Chan L, Beart RW, Jr., Vukasin P, Anthone G. Follow-up of colorectal cancer: a meta-analysis. Dis Colon Rectum. 1998;41:1116-26.

83 Tjandra JJ, Chan MK. Follow-up after curative resection of colorectal cancer: a meta-analysis. Dis Colon Rectum. 2007;50:1783-99.

84 Zhao Y, Yi C, Zhang Y, Fang F, Faramand A. Intensive follow-up strategies after radical surgery for nonmetastatic colorectal cancer: A systematic review and meta-analysis of randomized controlled trials. PLoS One. 2019;14:e0220533.

85 Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol. 2007;25:4575-80.

86 Cady B. Basic principles in surgical oncology. Arch Surg. 1997;132:338-46.

87 Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol. 2019;30:1194-220.

88 Runowicz CD, Leach CR, Henry NL, Henry KS, Mackey HT, Cowens-Alvarado RL, et al. American Cancer Society/American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline. J Clin Oncol. 2016;34:611-35.

89 Independent UKPoBCS. The benefits and harms of breast cancer screening: an independent review. Lancet. 2012;380:1778-86.

90 Postmus PE, Kerr KM, Oudkerk M, Senan S, Waller DA, Vansteenkiste J, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28:iv1-iv21.

91 Schneider BJ, Ismaila N, Aerts J, Chiles C, Daly ME, Detterbeck FC, et al. Lung Cancer Surveillance After Definitive Curative-Intent Therapy: ASCO Guideline. J Clin Oncol. 2020;38:753-66.

92 Lordick F, Mariette C, Haustermans K, Obermannová R, Arnold D, Committee EG. Oesophageal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2016;27:v50-v7.

93 Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D, et al. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2016;27:v38-v49.

94 Parker C, Castro E, Fizazi K, Heidenreich A, Ost P, Procopio G, et al. Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31:1119-34.

95 Bekelman JE, Rumble RB, Chen RC, Pisansky TM, Finelli A, Feifer A, et al. Clinically Localized Prostate Cancer: ASCO Clinical Practice Guideline Endorsement of an American Urological Association/American Society for Radiation Oncology/Society of Urologic Oncology Guideline. J Clin Oncol. 2018;36:3251-8.

96 Robinson D, Garmo H, Lissbrant IF, Widmark A, Pettersson A, Gunnlaugsson A, et al. Prostate Cancer Death After Radiotherapy or Radical Prostatectomy: A Nationwide Population-based Observational Study. Eur Urol. 2018;73:502-11.

97 Danish Colorectal Cancer Group. Danish Colorectal Cancer Group website. http://dccg.dk/aarsrapporter-2. 2017.

98 Swedish National Board of Health and Welfare. National guidelines in Sweden. http://www.socialstyrelsen.se/nationellariktlinjerforbrostprostatatjocktarmsochandtarmscancervard. . 2017.

99 Richtlijn colorectaal carcinoom. www.richtlijnendatabase.nl. 2020.

100 Vos JAM, Duineveld LAM, Wieldraaijer T, Wind J, Busschers WB, Sert E, et al. Effect of general practitioner-led versus surgeon-led colon cancer survivorship care, with or without eHealth support, on quality of life (I CARE): an interim analysis of 1-year results of a randomised, controlled trial. Lancet Oncol. 2021;22:1175-87.

101 Qaderi SM, Vromen H, Dekker HM, Stommel MWJ, Bremers AJA, de Wilt JHW. Development and implementation of a remote follow-up plan for colorectal cancer patients. Eur J Surg Oncol. 2020;46:429-32.

Supplementary table 1: Search term and results.

Performed on the 12th of May 2020

Summary table:

Database	*References*	*After de-duplication*
Embase.com	3659	3599
Medline (Ovid)	3190	277
Web of Science	2841	284
Cochrane Central	648	275
Google Scholar	200	91
Total	**10538**	**4526**

Embase.com: 3659

('breast cancer'/exp OR 'breast tumor'/de OR 'prostate cancer'/exp OR 'prostate tumor'/de OR 'large intestine cancer'/exp OR 'large intestine tumor'/exp OR 'bronchus tumor'/exp OR 'lung cancer'/exp OR 'lung tumor'/de OR 'esophagus cancer'/exp OR 'esophagus tumor'/de OR 'pancreas cancer'/exp OR 'pancreas tumor'/de OR 'stomach cancer'/exp OR 'stomach tumor'/de OR (((breast* OR mamma* OR prostat* OR colorect* OR colon* OR rect* OR anal* OR anus OR large-intestin* OR cecum* OR cecal* OR bronch* OR lung* OR pulmonar* OR esophag* OR oesophag* OR pancrea* OR stomach* OR gastric*) NEAR/3 (cancer* OR tumor* OR tumour* OR neoplas* OR carcino* OR adenocarcino*))):ab,ti,kw) AND ('follow up'/mj/de OR (followup* OR follow-up* OR surveillance* OR following-up OR monitoring*):ti) AND ('postoperative period'/exp OR (postoperativ* OR post-operativ* OR postsurg* OR post-surg* OR posttreatment* OR post-treatment* OR posttherap* OR post-therap* OR ((after*) NEAR/3 (surg* OR curative* OR treatment* OR therap* OR gastrectom* OR prostatectom* OR pancreatectom* OR resect*))):ab,ti,kw) NOT [conference abstract]/lim AND [English]/lim

Medline (Ovid): 3190

(exp Breast Neoplasms/ OR exp Prostatic Neoplasms/ OR exp Cecal Neoplasms/ OR exp Colorectal Neoplasms/ OR bronchus tumor/ OR exp Lung Neoplasms/ OR exp Esophageal Neoplasms/ OR exp Pancreatic Neoplasms/ OR Stomach Neoplasms/ OR (((breast* OR mamma* OR prostat* OR colorect* OR colon* OR rect* OR anal* OR anus OR large-intestin* OR cecum* OR cecal* OR bronch* OR lung* OR pulmonar* OR esophag* OR oesophag* OR pancrea* OR stomach* OR gastric*) ADJ3 (cancer* OR tumor* OR tumour* OR neoplas* OR carcino* OR adenocarcino*))).ab,ti,kf.) AND (* Follow-Up Studies/ OR (followup* OR follow-up* OR surveillance* OR following-up OR monitoring*).ti.) AND (Postoperative Period/ OR (postoperativ* OR post-operativ* OR postsurg* OR post-surg* OR posttreatment* OR post-treatment* OR posttherap* OR post-therap* OR ((after*) ADJ3 (surg* OR curative* OR treatment* OR therap* OR gastrectom* OR prostatectom* OR pancreatectom* OR resect*))).ab,ti,kf.) NOT (letter* OR news OR comment* OR editorial* OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt. AND english.lg.

Web of Science: 2841

TS=(((((breast* OR mamma* OR prostat* OR colorect* OR colon* OR rect* OR anal* OR anus OR large-intestin* OR cecum* OR cecal* OR bronch* OR lung* OR pulmonar* OR esophag* OR oesophag* OR pancrea* OR stomach* OR gastric*) NEAR/2 (cancer* OR tumor* OR tumour* OR neoplas* OR carcino* OR adenocarcino*)))) AND ((postoperativ* OR post-operativ* OR postsurg* OR post-surg* OR posttreatment* OR post-treatment* OR posttherap* OR post-therap* OR ((after*) NEAR/2 (surg* OR curative* OR treatment* OR therap* OR gastrectom* OR prostatectom* OR pancreatectom* OR resect*))))) AND TI=((followup* OR follow-up* OR surveillance* OR following-up OR monitoring*)) AND DT=(Article OR Review) AND LA=English

Cochrane Central: 648

((((breast* OR mamma* OR prostat* OR colorect* OR colon* OR rect* OR anal* OR anus OR large-intestin* OR cecum* OR cecal* OR bronch* OR lung* OR pulmonar* OR esophag* OR oesophag* OR pancrea* OR stomach* OR gastric*) NEAR/3 (cancer* OR tumor* OR tumour* OR neoplas* OR carcino* OR adenocarcino*))):ab,ti,kw) AND ((followup* OR follow-up* OR surveillance* OR following-up OR monitoring*):ti) AND ((postoperativ* OR post-operativ* OR postsurg* OR post-surg* OR posttreatment* OR post-treatment* OR posttherap* OR post-therap* OR ((after*) NEAR/3 (surg* OR curative* OR treatment* OR therap* OR gastrectom* OR prostatectom* OR pancreatectom* OR resect*))):ab,ti,kw)

Google Scholar: *top 200*

“breast|prostate|colorectal|lung|pulmonary|esophageal|oesophageal|pancreas|stomach cancer|tumor|tumour|neoplasm|carcinoma” intitle:followup|surveillance|“follow up”|monitoring postoperative|postsurgery|“after|post surgery|treatment|therapy”

Supplementary table 2A: ROBINS-I risk of bias assessment

Author	Tumor type	D1 Confounding*	1.1 Is there potential for confounding?	1.2 Analyse based on splitting participants' FU time based on intervention?	1.4 Appropriate correction for confounding?	1.5 Were confounding domains measured validly and reliably?	1.6 Controlling for post-intervention variables?	D2 Bias of selection of participants	2.1 Selection of particip. based on characteristics observed after intervention?	2.2 Post-intervention variables that influenced selection associated with intervention?	2.3 Post-intervention variables influenced selection also influenced by outcome?	2.4 Do start of follow-up and start of intervention coincide?	2.5 Adjustment techniques used that correct for the presence of selection biases?	D3 Bias in classification of intervention**	3.1 Intervention groups clearly defined?	3.2 Information to define intervention groups recorded at the start?	3.3 Classification of intervention status affected by knowledge of the outcome?	D4 Bias due to deviations from intervention	4.1 Were there deviations from the intended intervention?	4.2 Were these deviations unbalanced between groups?	D5 Bias due to missing data	5.1 Were outcome data available for nearly all patients?	5.2 Exclusion due to missing interv status?	5.3 Exclusion due to missing data other than intervention?	5.4 Reasons for missing data similar across interventions?	5.5 Results robust to the presence of missing data?	D6 Bias in measurements of outcomes	6.1 Outcome measure influenced by knowledge of the intervention?	6.2 Outcome assessors aware of the intervention?	6.3 Methods of outcome assessment comparable across intervention groups?	6.4 Systematic errors in measurement of the outcome related to intervention?	D7 Bias in selection of reported results	7.1 Multiple outcome measurements?	7.2 Multiple analyses of intervention-outcome realationship?	7.3 Different subgroups analysed?	Overall bias assessment
Bergamaschi et al. (1996)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Low	Y	Y	N	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	Moderate	N	N	N	Critical
Borie et al. (2004)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	N	Y	N	Moderate	N	N	N	Critical
Castells et al. (1998)	CRC	Serious	Y	N	PN	Y	N	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	Moderate	PN	Y	Y	NI	PY	NI	NI	NI	NI	NI	Moderate	N	N	N	Serious
Coebergh et al. (2018)	CRC	Serious	Y	N	PN	Y	PY	Moderate	PY	PY	PY	NA	N	Serious	PN	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	N	Y	N	Moderate	N	N	N	Serious
Eckardt et al. (1994)	CRC	Moderate	Y	N	PN	Y	N	Low	N	N	N	NA	NA	Serious	PN	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Serious
Gamboa et al. (2020)	CRC	Serious	Y	N	PN	Y	N	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	NI	NI	Y	NI	NI	NI	NI	NI	NI	NI	NI	Moderate	N	N	N	Serious
Hines et al. (2019)	CRC	Low	Y	N	Y	Y	N	Serious	N	N	N	NA	N	Serious	Y	N	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	NI	Y	N	Moderate	N	N	N	Serious
Hines et al. (2020)	CRC	Low	Y	N	Y	Y	N	Serious	N	N	N	NA	N	Serious	Y	N	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	NI	Y	N	Moderate	N	N	N	Serious
Hyder et al. (2013)	CRC	Serious	Y	N	PY	Y	N	Low	N	N	N	NA	NA	Serious	Y	N	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	NI	Y	N	Moderate	N	N	N	Serious
Körner et al. (2005)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Critical	N	N	PY	NA	NA	NA	NI	NI	NI	NI	NI	NI	Critical	PY	Y	NI	NI	Moderate	N	N	N	Critical
Laubert et al. (2010)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	Low	Y	N	N	NA	Y	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Mahboubi et al. (2007)	CRC	Moderate	Y	N	Y	Y	N	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Serious
Ovaska et al. (1990)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	Y	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Pugliese et al. (1984)	CRC	Critical	Y	N	N	NA	NA	Moderate	N	N	N	NA	N	Serious	PN	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Rulyak et al. (2007)	CRC	Moderate	Y	N	Y	Y	Y	Moderate	N	N	N	NA	PY	Serious	Y	N	PY	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	NI	Y	N	Moderate	N	N	N	Serious
Snyder et al. (2018)	CRC	Low	Y	N	Y	Y	N	Low	N	N	N	NA	NA	Low	Y	PY	N	NI	NI	NI	Low	Y	N	N	Y	Y	Low	N	NI	Y	N	Moderate	N	N	N	Moderate
Zitt et al. (2006)	CRC	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Backhus et al. (2016)	Lung	Moderate	Y	N	Y	Y	N	Moderate	N	PY	PY	NA	NA	Serious	Y	N	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	NI	Y	N	Serious	Y	N	Y	Serious
Benamore et al. (2007)	Lung	Critical	Y	N	N	NA	NA	Moderate	N	N	N	NA	NA	Moderate	Y	Y	N	Moderate	Y	Y	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Crabtree et al. (2015)	Lung	Low	Y	N	Y	Y	N	Serious	PY	PY	PY	NA	PY	Serious	N	N	Y	NA	NA	NA	Serious	N	Y	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Serious
Ho et al. (2017)	Lung	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	Low	Y	N	N	NI	Y	Low	N	N	Y	N	Moderate	N	N	N	Critical
Karzijn et al. (2016)	Lung	Serious	Y	N	PN	Y	N	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Serious
Mayne et al. (2020)	Lung	Critical	Y	N	N	NA	NA	Moderate	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	Low	Y	N	N	Y	Y	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
McMurry et al. (2018)	Lung	Low	Y	N	Y	Y	N	Serious	PY	N	N	NA	PN	Serious	N	N	Y	NA	NA	NA	Low	Y	N	N	Y	Y	Low	N	N	Y	N	Moderate	N	N	N	Serious
Nakamura et al. (2010)	Lung	Critical	Y	N	PY	N	Y	Low	N	N	N	NA	NA	Critical	N	Y	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Serious	Y	N	Y	Critical
Pan et al. (2015)	Lung	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Low	Y	Y	N	Serious	Y	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	Moderate	N	N	N	Critical
Reddy et al. (2017)	Lung	Serious	Y	N	Y	Y	N	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	Moderate	N	N	N	Serious
Virgo et al. (1995)	Lung	Critical	Y	N	N	NA	NA	Moderate	N	N	N	NA	N	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Bornhak et al. (2007)	Breast	Moderate	Y	N	Y	PN	N	Low	N	NA	NA	NA	NA	Low	PY	N	Y	NA	NA	NA	Moderate	PN	NI	Y	PN	N	NI	NI	NI	NI	NI	Moderate	N	N	N	Moderate
Lash et al. (2007)	Breast	Serious	Y	N	PY	Y	Y	Serious	Y	Y	Y	NA	PN	Serious	Y	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	N	PY	N	Moderate	N	N	N	Serious
Lash et al. (2005)	Breast	Serious	Y	N	PY	Y	Y	Serious	Y	Y	Y	NA	PN	Serious	Y	N	Y	NA	NA	NA	Low	Y	N	N	N	NA	Low	N	N	PY	N	Moderate	N	N	N	Serious
Lash et al. (2006)	Breast	Serious	Y	N	PY	Y	Y	Serious	Y	Y	Y	NA	PN	Serious	Y	N	Y	NA	NA	NA	Low	Y	N	N	N	NA	Low	N	N	PY	N	Moderate	N	N	N	Serious
Lee et al. (2015)	Breast	Moderate	Y	N	Y	Y	Y	Moderate	N	PY	PY	NA	N	Serious	N	N	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	Moderate	Y	NI	PY	N	Moderate	N	N	N	Serious
Paszat et al. (2009)	Breast	Moderate	Y	N	Y	Y	Y	Moderate	N	N	Y	NA	Y	Serious	Y	N	Y	NA	NA	NA	Serious	N	N	Y	N	NI	Low	N	N	PY	N	Moderate	N	N	N	Serious
Peixoto et al. (2014)	Upper GI	Moderate	Y	N	Y	Y	Y	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Low	N	N	Y	N	Moderate	N	N	N	Serious
Sisic et al. (2018)	Upper GI	Serious	Y	N	Y	Y	N	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Serious
Tan et al. (2007)	Upper GI	Critical	Y	N	N	NA	NA	Low	N	N	N	NA	NA	Serious	N	N	Y	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Critical
Nabhan et al. (2012)	Prostate	Low	Y	N	Y	Y	N	Low	N	N	N	NA	NA	Moderate	Y	PY	N	NA	NA	NA	NI	NI	NI	NI	NI	NI	Moderate	PY	NI	Y	N	Moderate	N	N	N	Moderate

CRC = colorectal cancer, FU = follow-up, N = no, NA = not applicable, NI = no information, PN = probably no, PY = probably yes, SC = some concerns, Upper GI = upper gastro-intestinal, Y = yes

* D1 - 1.3, 1.7 and 1.8 not applicable for this review.

** D4 - 4.3-6 not applicable for this review

Supplementary table 2B: RoB2 risk of bias assessment

Author	Tumor type	**D1 Randomization process**	1.1 Allocation sequence random?	1.2 Allocation concealed until assignement?	1.3 Baseline differences suggest a problem with the randomization process?	**D2 Deviation from intended intervention***	2.1 Participants aware of intervention?	2.2 Carers aware of intervention?	2.3 Were there deviations from intervention because trial context?	2.4 Deviations likely affected outcome?	2.5 Deviations balanced between groups?	2.6 Appropriate analysis? (i.e. intention to treat)	2.7 Impact on results due to per-protocol analysis?	**D3 Missing outcome data**	3.1 Outcome data available for nearly all participants?	3.2 Missing data biased results?	3.3 Could missingness depend on true value?	3.4 Likely that missingness of outcome depends on true value?	**D4 Measurement of outcome**	4.1 Method of outcome measurement inappropriate?	4.2 Outcome measurement differed between interventions?	4.3 Were the outcome assessors aware of intervention?	4.4 Assessment of outcome influenced by knowledge of intervention?	4.5 Likely that outcome influenced by knowledge of intervention?	**D5 Selection of reported outcomes**	5.1 Pre-specified analysis plan?	5.2 Multiple eligible outcome measurements?	5.3 Multiple eligible analyses?	**Overall bias assessment**
Augestad et al. (2013)	CRC	Low	Y	Y	N	Low	NA	NA	Y	N		Y		High	N	Y	Y	Y	High	Y	N	NI	N	N	Low	Y	N	N	High
Kjeldsen et al. (1997)	CRC	SC	Y	NI	N	High	NA	NA	NI	NI	NI	NI	NI	Low	Y				High	Y	N	NI	N	N	Low	Y	N	N	High
Mäkelä et al. (1995)	CRC	SC	NI	NI	N	High	NA	NA	NI	NI	NI	NI	NI	Low	PY				High	NI	N	NI	N	N	SC	NI	N	N	High
Ohlsson et al. (1995)	CRC	SC	NI	NI	N	SC	NA	NA	N			NI	N	Low	Y				Low	N	N	NI	N	N	SC	NI	N	N	SC
Pietra et al. (1998)	CRC	High	NI	NI	PY	HIgh	NA	NA	NI	NI	NI	NI	NI	High	NI	NI	NI	NI	High	NI	N	NI	N	N	Low	Y	N	N	High
Primrose et al. (2014)	CRC	Low	Y	Y	N	Low	NA	NA	Y	N	N	Y	N	Low	Y				Low	N	N	N	N	N	Low	Y	N	N	Low
Rodriguez et al. (2006)	CRC	Low	Y	Y	N	Low	NA	NA	N			PY		Low	Y				SC	Y	N	NI	N	N	Low	Y	N	N	SC
Rosati et al. (2016)	CRC	Low	Y	Y	N	Low	NA	NA	N			Y		Low	Y				Low	PN	N	NI	N	N	Low	Y	N	N	Low
Schoemaker et al. (1998)	CRC	High	Y	NI	Y	Low	NA	NA	N			PY		Low	Y				Low	N	N	NI	N	N	Low	Y	N	N	High
Secco et al. (2002)	CRC	High	NI	NI	Y	High	NA	NA	NI	NI	NI	NI	NI	Low	PY				High	Y	N	NI	N	N	Low	PY	N	N	High
Sobhani et al. (2018)	CRC	High	PY	NI	Y	Low	NA	NA	NI			Y		Low	Y				High	Y	N	NI	N	N	Low	Y	N	N	High
Strand et al. (2011)	CRC	Low	Y	Y	N	SC	NA	NA	N			NI	N	Low	PY				High	Y	N	NI	N	N	Low	Y	N	N	High
Treasure et al. (2014)	CRC	Low	Y	Y	PN	Low	PN	PN				Y		Low	Y				Low	SC	N	N	N	N	Low	Y	N	N	SC
Wang et al. (2009)	CRC	SC	Y	NI	N	SC	NA	NA	PY	PN		NI	PN	Low	Y				Low	N	N	NI	N	N	Low	Y	N	N	SC
Wattchow et al. (2006)	CRC	SC	Y	PY	Y	Low	NA	NA	NI	NI	NI	Y		Low	PY				High	Y	N	NI	N	N	Low	Y	N	N	High
Wille-Jorgensen et al. (2018)	CRC	Low	Y	Y	N	Low	NA	NA	N			Y		Low	Y				Low	N	N	N	N	N	Low	Y	N	N	Low
Monteil et al. (2010)	Lung	SC	PY	Y	PY	High	NA	NA	NI	NI	NI	NI	NI	Low	PY				High	Y	N	NI	N	N	Low	Y	N	N	High
Sui et al. (2020)	Lung	Low	Y	Y	N	Low	NA	NA	N			Y		SC	PY	PY	PY	PN	High	NI	Y	NI	N	N	Low	Y	N	N	High
Del Turco et al. (1994) Palli et al. (1999)	Breast	Low	Y	Y	N	Low	NA	NA	N			Y		Low	Y				Low	N	N	N	N	N	Low	PY	N	N	Low
Grunfeld et al. (2006)	Breast	Low	Y	Y	N	SC	NA	NA	NI	NI	NI	Y		Low	Y				High	Y	N	NI	N	N	Low	Y	N	N	High
Koinberg et al.(2004)	Breast	Low	Y	Y	N	High	NA	NA	Y	PY	N	Y		High	NI	NI	NI	NI	High	PY	N	N	N	N	Low	Y	N	N	High
Liberati et al. (1994)	Breast	Low	Y	Y	N	Low	NA	NA	N			Y		Low	Y				Low	N	N	NI	N	N	Low	Y	N	N	Low
Bjerring et al. (2019)	Upper GI	SC	Y	Y	PY	Low	NA	NA	PN			Y		Low	PY				Low	PN	N	NI	N	N	Low	Y	N	N	SC
Verschuur et al. (2009)	Upper GI	Low	Y	Y	PN	SC	NA	NA	NI	PN		Y		Low	PY				High	Y	N	NI	N	N	Low	Y	N	N	High

CRC = colorectal cancer, N = no, NA = not applicable, NI = no information, PN = probably no, PY = probably yes, SC = some concerns, Upper GI = upper gastro-intestinal, Y = yes
* D2 - 2.1 and 2.2 not applicable for this review.

CHAPTER FOUR

CHAPTER FOUR

POSTTREATMENT SURVEILLANCE IN PATIENTS WITH PROLONGED DISEASE-FREE SURVIVAL AFTER RESECTION OF COLORECTAL LIVER METASTASIS.

Boris Galjart*, Eric P. van der Stok*, Joost Rothbarth,
Dirk J. Grünhagen , Cornelis Verhoef

* *Shared first authorship*

Published in the Annals of Surgical Oncology

Abstract

Introduction: Posttreatment surveillance protocols most often endure for 5 years after resection of colorectal liver metastasis (CRLM). Most recurrences occur within 3 years after surgical removal of the tumour. This study analyses the need of surveillance for patients with at least 3 years of disease-free survival after potentially curative resection of CRLM.

Methods: A single-centre retrospective analysis of all consecutive patients who underwent treatment for CRLM with curative intent between 2000 and 2011.

Results: In total 152 out of 545 patient (28%) remained disease-free for 3 years after successful resection of the CRLM. The estimated recurrence rate after 10 years of follow-up in this group of 152 patients was 27%. More than half of these patients (55%) could be treated with curative intent for their recurrences. Multivariable analysis revealed that the nodal status of the primary tumour is of significant prognostic value for developing recurrences after 3 years of DFS. A disease-free interval (DFI) of less than 12 months between resection of primary tumour and detection of CRLM shows a trend towards significance. Both factors were used to create a risk score, showing that patients with a low-risk profile (node negative status and a DFI <12 months) have an estimated recurrence rate of 5% and might not benefit from intensive surveillance beyond three years of follow up without a recurrence.

Conclusion: The currently developed risk score shows that follow-up can be stopped in a specific subgroup, 3 years after treatment for their CRLM with curative intent.

Introduction

Liver metastases are common in patients with colorectal cancer (CRC), developing in approximately half of patients with colorectal tumours.[1,2] Surgical treatment of colorectal liver metastasis (CRLM) results in 5 years overall survival (OS) of 40-60%.[3,4] Although the treatment of CRLM has improved, disease recurrence is seen in almost 70% of the patients. Most often recurrences develop during the first 3 years after surgery.[5-7] Both hepatic and pulmonary recurrences can be treated with local therapy repeatedly, thereby still offering the potential of cure.[8-13] The opportunity to control recurrent disease as a curable condition, increased interest in the surveillance of patients after hepatectomy. No consensus on the optimal follow-up protocol for curatively treated patients with stage IV CRC has been reached however.

Patients treated with curative intent for CRLM enter a surveillance scheme, enduring for 5 years in most centres. Research on the surveillance and prognosis of patients with CRLM mainly focuses on the first 3 years after surgery, as most recurrences occur in this period. Literature is scarce on the follow-up of patients with a disease-free survival (DFS) of 3 and more years.[14] The current study aims to analyse the need for surveillance in these patients, by determining the recurrence pattern, treatment for recurrences and oncological outcome. This study assesses the possibilities for a risk-based surveillance protocol in this highly selected but growing group of patients.

Patients and methods

Patient data were extracted from a prospectively maintained database in Erasmus MC Cancer Institute. The database consists of perioperative and clinicopathological characteristics of primary CRC, CRLM and recurrent metastatic disease. In this retrospective analysis patients receiving surgical or ablative therapy for CRLM between January 2000 and November 2011 were included. In this group all patients with a DFS of more than 3 years were identified. In case of relapsing disease after liver surgery, data on recurrence location, diagnosis and of treatment were collected.

Follow-up of patients with CRLM
Surveillance consists of physical examination, thoracoabdominal Computed Tomography (CT) and regular serum Carcinoembryonic Antigen (CEA) level measurements. Patient surveillance was carried out for up to 5 years after treatment of CRLM. During this period serum CEA measurements and radiological imaging were performed every 3 to 6 months during the first 3 years after surgery and yearly thereafter.

Recurrent disease
In the present study, recurrences detected within 3 years of CRLM treatment with curative intent were categorized as early recurrences. All recurrences detected after 3 years were considered to be late recurrences. CEA blood levels above 5,00 µg/L were considered elevated. In case of normal CEA levels the absolute difference between baseline post-operative CEA levels and CEA levels at time of recurrence was calculated.

Treatment of recurrent disease was assessed in a multidisciplinary tumour board for all patients. As long-term local control of metastatic CRC is achieved using surgery, radiofrequency ablation (RFA) or stereotactic radiotherapy (SRx), all of these modalities were considered to be potentially curative treatments for recurrent disease.[15,16]

Disease-free and overall survival
DFS was calculated as the time in months between the resection of CRLM and the diagnosis of recurrent disease (either by radiology, physical examination or endoscopy). When an elevated CEA level was the first sign of possible recurrence, this was followed by confirmative imaging or biopsies. The dates of the latter were used for survival calculations.

OS was the time between treatment of CRLM and the date of death or last follow-up. For both patients with a DFS of 3 and 5 years, conditional OS and DFS curves were created, using 36 and 60 months as the starting points (t0). In order to compare oncological outcome after potentially curative treatment for early and late recurrences, the survival estimate DFS2 (from start treatment of recurrence until re-recurrence) was calculated.

Statistical analysis
The categorical data are presented as absolute numbers and percentages. Continuous variables were displayed as means (and standard deviations (SD)) or medians (and interquartile ranges (IQR)). Different proportions between groups were tested using the Chi-squared test. Univariable and multivariable regression models were created to identify factors related to late disease recurrence, for which Hazard Ratios (HR) and 95% confidence intervals (CI) were calculated. Prognostic factors were used to create a risk score. The score was internally validated for discrimination (concordance index) and calibration (calibration plot), using bootstrap resampling. The Kaplan-Meier method was used to estimate (conditional) survival. All (conditional) survival estimates were compared using the Log-Rank test. A p-value of less than 0,05 was considered significant. All analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, Ill., USA) and R version 3.2.5 (http://www.r-project.org).

RESULTS

Of the 607 patients with a minimal potential follow-up of 3 years and potentially curative treatment for CRLM, 545 consecutive patients (90%) were eligible for analysis in this study. Exclusion criteria are presented in figure 1. One hundred fifty-two patients were disease-free after 3 years of follow-up (28%), of which 31 patients (20%) developed recurrences beyond 3 years. Median follow-up time (t0=36 months after first hepatectomy) was 40 months (IQR: 18-63 months) in this group. Twenty-four patients (16%) died during the follow-up period. In patients with 3 years of DFS the Kaplan-Meier analysis showed an estimated recurrence rate of 27% in the following 7 years of follow-up.

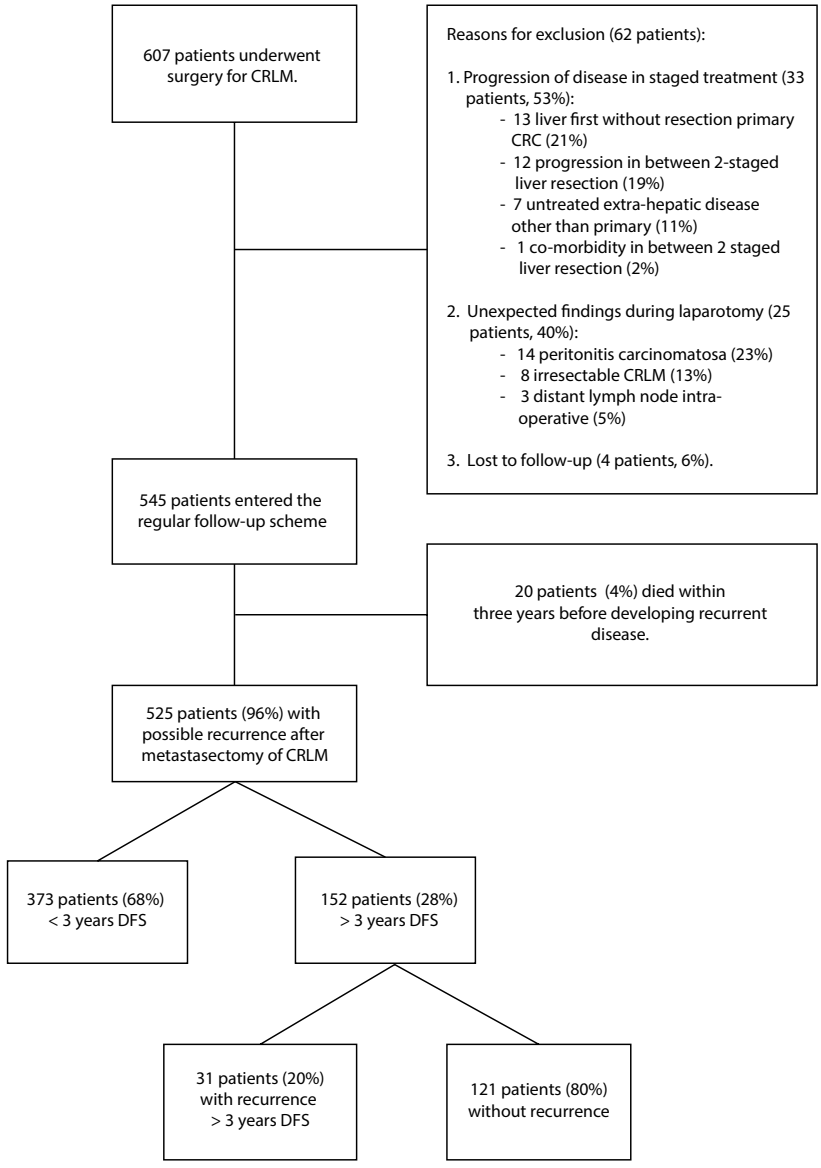


Figure 1: Flowchart of the study.

Eighty-one patients were disease-free for more than 5 years (15%). Median follow-up time in this group of patients (t0=60 months after first hepatectomy) was 31 months (IQR: 20-52 months). Seven recurrences (9%) and 6 deaths (7%) were observed and the estimated (Kaplain-Meier) probabilities of recurrence and mortality in the following 5 years were 11% and 12% respectively. Conditional OS and DFS curves are presented in figure 2, for both patients with 3 and 5 years of DFS. In total 393 patients (72%) had a DFS of less than 3 years. When comparing the recurrence pattern of early (< 3 years DFS) and late recurrences (> 3 years DFS), no significant differences in tumour location were seen (table 1).

After evaluation of the late recurrences, 17 patients (55%) could be treated with curative treatment modalities, compared to 168 (45%) of the early recurrences (p=0,293). In patients with curatively treated early recurrences, re-recurrence occurred earlier than in patients with curatively treated late recurrences. Median time to relapse (DFS2) was 28 months (75th percentile at 12 months, 25th not reached) in patients with late recurrences and 8 months (IQR: 4-30 months) in patients with early recurrences (p=0,041). Table 1 displays treatment and surveillance results of early and late recurrences.

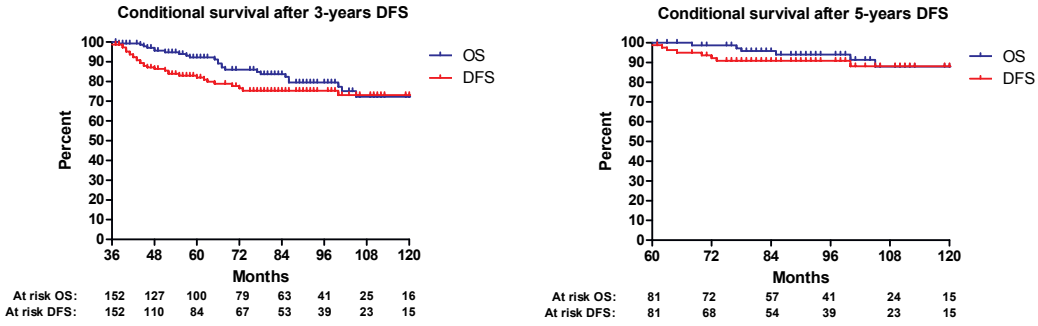


Figure 2: Conditional DFS and OS for patients with 3 and 5-years DFS.

Table 1: Recurrence pattern, surveillance and treatment results.

	Recurrence < 3 years (N=373)	Recurrence > 3 years (N=31)	P-value
Location recurrence			
Intrahepatic only	144 (39%)	9 (29%)	0.291
Extrahepatic location recurrences	229 (61%)	22 (71%)	0.904
Pulmonary recurrence	84 (23%)	11 (36%)	
Local recurrence	15 (4%)	1 (3%)	
Distant lymph nodes	21 (6%)	1 (3%)	
Hepatic and pulmonary	35 (9%)	1 (3%)	
Hepatic and other	28 (8%)	4 (13%)	
Pulmonary and other	15 (4%)	2 (7%)	
Multi-organ metastasis (≥3)	10 (3%)	1 (3%)	
Other locations	21 (6%)	1 (3%)	
Surveillance			
Median CEA (IQR) µg/L	7.0 (2.9-20.0)	7.1 (3.9-12.7)	0.849
Elevated CEA (> 5.0 µg/L)	204 (55%)	22 (71%)	0.087
Non elevated CEA (≤ 5.0 µg/L)	152 (40%)	8 (26%)	
Missing CEA values	17 (5%)	1 (3%)	
Perc. increase (when normal CEA)	152 (40%)	8 (26%)	0.255
>25% compared to baseline	49 (29%)	4 (50%)	
1-25% compared to baseline	25 (15%)	2 (25%)	
Decreased compared to baseline	26 (16%)	2 (25%)	
Not calculated	52 (34%)	0 (0%)	
Treatment			
Curative	168 (45%)	17 (55%)	0.293
Non-curative	205 (55%)	14 (45%)	

In order to define which patients could potentially be excluded from follow-up, the chi-squared test and univariable Cox regression analysis were performed. Factors associated with developing late disease recurrences were the nodal status of the primary tumour, the absence of neo-adjuvant chemotherapy for CRLM and the disease-free interval (DFI) between resection of the primary CRC and the detection of CRLM. The Clinical Risk Score (CRS) described by Fong et al. [17] showed no additional value in assessing the probability of developing late recurrence.

After multivariable analysis, the nodal status remained a statistically significant prognostic factor for late disease recurrence after an initial DFS of 3 years. A DFI of more than 12 months between resection primary and development CRLM) shows a trend towards significance (table 2).

Risk categories for late recurrences were created, in which patients with node negative primary tumours and a DFI of less than 12 months (n=50, 33%) were considered at low-risk. All other patients (with either a N+ status, a DFI of more than 12 months or a combination of both characteristics) were considered at high-risk of late recurrence (n=101, 66%). In 1 patient no risk score could be determined. In the low-risk group 2 patients (4%) developed recurrence during the 2 following years of surveillance (after the initial 3 disease-free years), compared to 22 patients (22%) in the high-risk group. The estimated 10 years recurrence rate in the low risk group was 5% and 25% in the high risk group (p=0,005). The sensitivity of this risk score for prediction of late recurrences during the last 2 years of follow-up is 92%. The estimated difference in recurrence rate between the "high-risk" group and the complete group of patients with 3 years of DFS is 2%. This means that 50 patients with a DFS of 3 years need to remain in follow-up for another 2 years, in order to detect 1 "low-risk" patient with late recurrent disease.

After 5 years of DFS 1 recurrence (3%) was observed in the low-risk group (n=32), compared to 6 recurrences (12%) in the high-risk group (n=49). The estimated 10 years recurrence rate in the following 5 years (after 5 years of DFS) is 3% in the low-risk group versus 15% in the high-risk group (p=0,207). Kaplan-Meier curves after 3 and 5 years of DFS are presented in figure 3.

The created risk model had a moderate capacity to predict late disease recurrence (bootstrap corrected concordance index: 0,71) and acceptable calibration (see supplementary material).

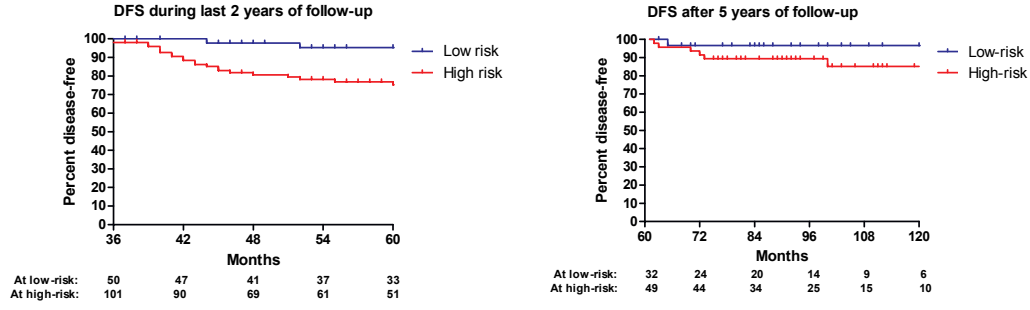


Figure 3: Risk stratification for late recurrences. The graph on the left illustrates the DFS during the last 2 years of follow-up (from 36 to 60 months after hepatectomy). The graph on the right illustrates the DFS after more than 60 months after hepatectomy

Table 2: Baseline characteristics of patients with 3 years of DFS and the results of univariable and multivariable analysis.

Variables	Total (N=152)	Recurrence >3 years (N=31)	χ^2 p-value	Univariable HR (95%CI)	p-value	Multivariable HR (95%CI)	p-value
Gender							
Male	94	19 (20.2%)	0.943	0.942 (0.456-1.943)	0.871		
Female	58	12 (20.7%)					
Age							
Median (range)	64 (30-86)	66 (30-86)	0.326	1.030 (0.994-1.067)	0.106		
Mean ± SD	63.3 ± 11.1	65.9 ± 13.2					
Primary CRC							
Location							
Colon	93	19 (20.4%)	0.989	0.978 (0.475-2.015)	0.952		
Rectum	59	12 (20.3%)					
T-stage							
T3-4	37	3 (8.1%)	0.086	3.250 (0.989-10.682)	0.052		
T1-2	114	28 (24.6%)					
Node status							
Positive	72	20 (27.8%)	0.035	2.316 (1.109-4.837)	0.025	2.279 (1.090 - 4.764)	0.029
Negative	79	11 (13.9%)					
Adjuvant CTx							
Yes	31	9 (29.0%)	0.181	1.890 (0.868-4.116)	0.109		
No	121	22 (18.2%)					
CRLM							
DFI < 12 months							
Yes	93	12 (12.9%)	0.004	0.372 (0.180-0.766)	0.007	0.471 (0.215 - 1.029)	0.059
No	59	19 (32.2%)					
Number of CRLM							
>1	75	14 (18.7%)	0.602	1.002 (4.94-2.033)	0.996		
1	77	17 (22.1%)					
Size of tumours							
≥ 5,00 cm	26	7 (26.9%)	0.386	1.382 (0.595-3.210)	0.451		
≤ 4,99 cm	124	24 (19.4%)					
CEA preoperative							
≥200 µg/L	8	1 (12.5%)	0.305	0.045 (0.00- 46.585)	0.381		
≤ 199 µg/L	120	22 (18.3%)					
Bilobar metastases							
Yes	43	9 (20.9%)	0.918	1.218 (0.560-2.647)	0.691		
No	109	22 (20.2%)					
Neoadjuvant CTx							
Yes	70	8 (11.4%)	0.011	0.411 (0.184-0.920)	0.03	0.577 (0.241-1.380)	0.216
No	82	23 (28.0%)					
Margin < 1mm							
Yes	22	4 (18.2%)	0.743	0.985 (0.344-2.815)	0.977		
No	127	27 (21.3%)					
EHD							
Yes	3	0 (0.0%)	0.376	0.048 (0.00-8158.217)	0.621		
No	149	31 (20.8%)					
Clinical Risk Score							
HR (3-5)	39	7 (17.9%)	0.55	0.809 (0.347-1.886)	0.624		
LR (0-2)	102	23 (22.5%)					

Missing values were observed for T-stage (1), nodal status (1), tumour size (2), preoperative CEA (24), margin status (3 patients with RFA only) and the Clinical Risk Score (11)

CTx = chemotherapy, EHD = extra-hepatic disease, CEA = carcinoembryonic antigen, LR = low-risk, HR = high-risk

Discussion

The current study demonstrates that still a considerable proportion of patients with a DFS of more than 3 years develops recurrences, with an estimated 10 years recurrence rate of 27%. Patients with late recurrences received potentially curative treatment as often as patients with early recurrences did. This may justify surveillance in patients with CRLM, even after a DFS of 3 years.

To date no prospective trials have been performed investigating the efficacy of long-term follow-up of patients with CRLM, nor curatively treated stage IV CRC in general. It is still unclear to what extend surveillance is useful. The primary target of this study was to objectify the necessity of surveillance in patients without evidence of disease 3 years after the first liver metastasectomy. Several groups have shown that repeat resections of recurrences offer survival benefit [18-20] and although the efficacy of RFA and SRx has been studied less intensively, results indicate that long-term disease control can be reached using these treatments.[15,16,21,22] As more than half of the patients with late recurrences were treated with either one or a combination of local treatments, surveillance seems legitimate in this particular group of patients.

Follow-up in the centre of the current study is carried out during 5 years for all patients after resection of CRLM, as is advised in the ASCRS and NCCN guidelines.[23,24] Preferably cancer surveillance should only be performed in those patients benefiting from it. In order to decide in which patients follow-up is desirable, accurate prediction of outcome after metastasectomy is needed. Many efforts to determine prognosis of patients with CRLM have been made [4,17,25-27], of which the CRS is mostly practised.[17] Less evidence is available to predict the likelihood of late disease recurrence, which is demonstrated by the fact that patients with initially poor prognostic factors can still be cured from CRLM.[28] A study by Tan et al. shows that the currently used risk scores for CRLM have little predictive value in 3-years survivors of CRLM with regards to the disease-specific survival and are therefore not suitable to decide whether long-term follow-up is appropriate.[29] In the current study the nodal status of the primary CRC showed to be the only significant prognostic factor with respect to developing late disease recurrence. The DFI was non-significant in multivariable analysis, but showed a trend towards significance. The interval between resection of the primary tumour and occurrence of CRLM has been used in most CRS, as a DFI of less than a year increases the chance of developing recurrent disease shortly after hepatectomy.[4,17,25-27] The results in this study indicate an opposite effect in patients with 3 years of DFS, as patients with a short interval (<12 months) between the primary CRC and the occurrence of CRLM had a favourable outcome in this particular group of patients. Although counterintuitive, this finding might not be illogical. In many studies a short DFI is described as a risk factor for early recurrence and a surrogate for aggressive tumour behaviour, inherently.[17] Moreover, this means that if patients with a short DFI develop recurrences, it is more likely that these will occur in the period shortly after partial hepatectomy rather than after a period of 3 years. This study shows that in the period thereafter, patients with a short DFI have therefore a lower risk of developing recurrence, as it is unlikely that patients with initially aggressive tumour behaviour will still develop recurrences after remaining disease-free for such a significant period of time. Consequently, patients with a prolonged DFI have a decreased risk in the period shortly after surgery, but remain at higher risk of recurrence for an expanded period of time. Considering the more latent tumour behaviour in patients with a prolonged DFI, this might not be implausible. Although patient selection, rather than tumour biology, could also explain the observed effect, this finding might be of interest when considering long-term surveillance in patients with CRLM and should therefore be validated in an external cohort of patients.

To identify patients that could potentially be discharged from (intensive) surveillance, a stratification system was created using both the DFI and nodal status as variables. Patients with optimal prognostic factors (pN0-status and a DFI < 12 months) were considered to be at low-risk, resulting in an estimated recurrence probability of 5%. The results display that this is lower than the estimated 12% recurrence probability after 5 years of DFS, when it is generally accepted to discharge patients from follow-up. The risk score showed moderately good discrimination and acceptable calibration. Although this scoring system needs external validation and could potentially be extended with other variables, this study indicates that there may be patients with a low-risk profile that do not benefit from a surveillance protocol consisting of 5 years and can either be discharged from follow-up after 3 years or undergo less intensive surveillance by the general practitioner.

During the past decade several research groups have retrospectively evaluated the different aspects of follow-up after metastectomy, in order to define an optimal surveillance protocol.[30-37] Jones et al. [14] highlight the lack of evidence surrounding surveillance of patients with CRLM after reviewing all available literature on early intensive follow-up after metastasectomy and therefore remain inconclusive on how to perform optimal follow-up. In a review by Metcalfe et al. [38] 5 years of follow-up is proposed. As was shown in this and other studies, patients with a DFS of 5 years still have a probability of approximately 10% to develop recurrences after being discharged from surveillance. Recent literature states that cure after resection of CRLM might only be achieved after 10 years of survival.[28,39] This suggests that an extended follow-up protocol of more than 5 years could be worthwhile for some patients, again addressing the need for tailor-made follow-up schedules.

The current study has several limitations and its conclusions should therefore be interpreted with care. As a result of the retrospective nature of this study the obtained results might be biased. Due to the limited number of events after 3 years of DFS, only 3 factors could be evaluated in the multivariable analysis. It is likely that other factors are influential, although non-significant in this particular univariable analysis. Also, the identified risk score has not been externally validated, which impairs generalizability.

Nevertheless, this study provides valuable insights regarding the follow-up of patients with 3 years of DFS after surgery for CRLM. The data suggests that follow-up in patients surviving 3 years without evidence of disease is useful and necessary in most patients. Patients with the currently developed low-risk profile might not benefit from the additional 2 years of surveillance, and patients with a high risk profile should be followed beyond 5 years, which emphasizes the importance of a tailor-made long-term follow-up protocol after treatment of CRLM with curative intent.

References

1 Bengmark S, Hafström L. The natural history of primary and secondary malignant tumors of the liver I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy. Cancer. 1969;23(1):198-202.

2 van der Pool AEM, Damhuis RA, Ijzermans JNM, et al. Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series. Colorectal Disease. 2012;14(1):56-61.

3 Choti MA, Sitzmann JV, Tiburi MF, et al. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg. Jun 2002;235(6):759-766.

4 Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. Ann Surg. Jan 2008;247(1):125-135.

5 Butte JM, Gonen M, Allen PJ, et al. Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection. Ann Surg Oncol. Aug 2015;22(8):2761-2771.

6 de Jong MC, Pulitano C, Ribero D, et al. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. Ann Surg. Sep 2009;250(3):440-448.

7 Lam VW, Pang T, Laurence JM, et al. A systematic review of repeat hepatectomy for recurrent colorectal liver metastases. J Gastrointest Surg. Jul 2013;17(7):1312-1321.

8 de Jong MC, Mayo SC, Pulitano C, et al. Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis. J Gastrointest Surg. Dec 2009;13(12):2141-2151.

9 Adam R, Bismuth H, Castaing D, et al. Repeat hepatectomy for colorectal liver metastases. Ann Surg. Jan 1997;225(1):51-60; discussion 60-52.

10 Petrowsky H, Gonen M, Jarnagin W, et al. Second liver resections are safe and effective treatment for recurrent hepatic metastases from colorectal cancer: a bi-institutional analysis. Ann Surg. Jun 2002;235(6):863-871.

11 Suzuki H, Kiyoshima M, Kitahara M, Asato Y, Amemiya R. Long-term outcomes after surgical resection of pulmonary metastases from colorectal cancer. Ann Thorac Surg. Feb 2015;99(2):435-440.

12 Warwick R, Page R. Resection of pulmonary metastases from colorectal carcinoma. Eur J Surg Oncol. Dec 2007;33 Suppl 2:S59-63.

13 Pfannschmidt J, Dienemann H, Hoffmann H. Surgical Resection of Pulmonary Metastases From Colorectal Cancer: A Systematic Review of Published Series. The Annals of Thoracic Surgery.84(1):324-338.

14 Jones RP, Jackson R, Dunne DF, et al. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. Br J Surg. Apr 2012;99(4):477-486.

15 Tree AC, Khoo VS, Eeles RA, et al. Stereotactic body radiotherapy for oligometastases. The Lancet. Oncology. Jan 2013;14(1):e28-37.

16 Abdalla EK, Vauthey JN, Ellis LM, et al. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. Ann Surg. Jun 2004;239(6):818-825; discussion 825-817.

17 Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: Analysis of 1001 consecutive cases. Ann Surg. 1999;230(3):309-321.

18 Oba M, Hasegawa K, Shindoh J, et al. Survival benefit of repeat resection of successive recurrences after the initial hepatic resection for colorectal liver metastases. Surgery. Feb 2016;159(2):632-640.

19 Kulik U, Bektas H, Klempnauer J, Lehner F. Repeat liver resection for colorectal metastases. Br J Surg. Jun 2013;100(7):926-932.

20 Wicherts DA, de Haas RJ, Salloum C, et al. Repeat hepatectomy for recurrent colorectal metastases. Br J Surg. May 2013;100(6):808-818.

21 Rusthoven KE, Kavanagh BD, Cardenes H, et al. Multi-institutional phase I/II trial of stereotactic body radiation therapy for liver metastases. J Clin Oncol. Apr 1 2009;27(10):1572-1578.

22 Van Tilborg AA, Meijerink MR, Sietses C, et al. Long-term results of radiofrequency ablation for unresectable colorectal liver metastases: a potentially curative intervention. Br J Radiol. Jun 2011;84(1002):556-565.

23 Steele SR, Chang GJ, Hendren S, et al. Practice Guideline for the Surveillance of Patients After Curative Treatment of Colon and Rectal Cancer. Dis Colon Rectum. Aug 2015;58(8):713-725.

24 Benson AB, 3rd, Venook AP, Bekaii-Saab T, et al. Colon cancer, version 3.2014. J Natl Compr Canc Netw. Jul 2014;12(7):1028-1059.

25 Kattan MW, Gönen M, Jarnagin WR, et al. A Nomogram for Predicting Disease-specific Survival After Hepatic Resection for Metastatic Colorectal Cancer. Annals of Surgery. 2008;247(2):282-287.

26 Iwatsuki S, Dvorchik I, Madariaga JR, et al. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. Journal of the American College of Surgeons. Sep 1999;189(3):291-299.

27 Nordlinger B, Guiguet M, Vaillant JC, et al. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. Cancer. Apr 1 1996;77(7):1254-1262.

28 Tomlinson JS, Jarnagin WR, DeMatteo RP, et al. Actual 10-Year Survival After Resection of Colorectal Liver Metastases Defines Cure. Journal of Clinical Oncology. October 10, 2007 2007;25(29):4575-4580.

29 Tan MC, Butte JM, Gonen M, et al. Prognostic significance of early recurrence: a conditional survival analysis in patients with resected colorectal liver metastasis. HPB (Oxford). Oct 2013;15(10):803-813.

30 Bakalakos EA, Burak WE, Jr., Young DC, Martin EW, Jr. Is carcino-embryonic antigen useful in the follow-up management of patients with colorectal liver metastases? American journal of surgery. Jan 1999;177(1):2-6.

31 Gomez D, Sangha VK, Morris-Stiff G, et al. Outcomes of intensive surveillance after resection of hepatic colorectal metastases. Br J Surg. Oct 2010;97(10):1552-1560.

32 Metcalfe M, Mann C, Mullin E, Maddern G. Detecting curable disease following hepatectomy for colorectal metastases. ANZ journal of surgery. Jul 2005;75(7):524-527.

33 Bhattacharjya S, Aggarwal R, Davidson BR. Intensive follow-up after liver resection for colorectal liver metastases: results of combined serial tumour marker estimations and computed tomography of the chest and abdomen - a prospective study. British journal of cancer. Jul 3 2006;95(1):21-26.

34 Connor S, Hart MG, Redhead DN, et al. Follow-up and outcomes for resection of colorectal liver metastases in Edinburgh. Eur J Surg Oncol. Feb 2007;33(1):55-60.

35 Hyder O, Dodson RM, Mayo SC, et al. Post-treatment surveillance of patients with colorectal cancer with surgically treated liver metastases. Surgery. Aug 2013;154(2):256-265.

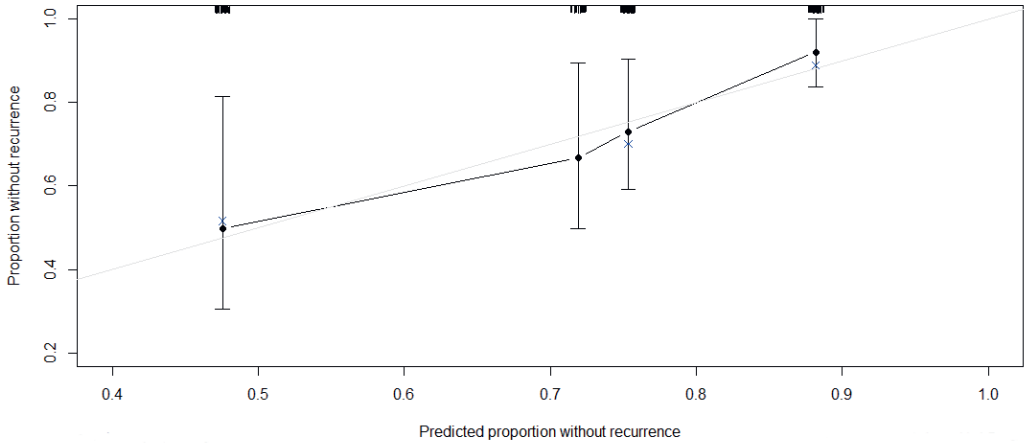
36 Verberne CJ, Wiggers T, Vermeulen KM, de Jong KP. Detection of recurrences during follow-up after liver surgery for colorectal metastases: both carcinoembryonic antigen (CEA) and imaging are important. Ann Surg Oncol. Feb 2013;20(2):457-463.

37 Langenhoff BS, Krabbe PF, Ruers TJ. Efficacy of follow-up after surgical treatment of colorectal liver metastases. Eur J Surg Oncol. Feb 2009;35(2):180-186.

38 Metcalfe MS, Mullin EJ, Maddern GJ. Choice of surveillance after hepatectomy for colorectal metastases. Arch Surg. Jul 2004;139(7):749-754.

39 Viganò L, Ferrero A, Lo Tesoriere R, Capussotti L. Liver Surgery for Colorectal Metastases: Results after 10 Years of Follow-Up. Long-Term Survivors, Late Recurrences, and Prognostic Role of Morbidity. Annals of Surgical Oncology. 2008/09/01 2008;15(9):2458-2464.

Supplementary figure 1: Calibration plot.



PART II

Histopathological growth patterns of colorectal liver metastases

CHAPTER SIX

CHAPTER SIX

ANGIOGENIC DESMOPLASTIC HISTOPATHOLOGICAL GROWTH PATTERN AS A PROGNOSTIC MARKER OF GOOD OUTCOME IN PATIENTS WITH COLORECTAL LIVER METASTASES.

Boris Galjart*, Pieter M.H. Nierop*, Eric P. van der Stok,
Robert R.J. Coebergh van den Braak, Diederik J. Höppener,
Sophie Daelemans, Luc Y. Dirix, Cornelis Verhoef,
Peter B. Vermeulen, Dirk J. Grünhagen

* *Shared first authorship*

Published in Angiogenesis

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. 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 blinded for patient outcome.[2] 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.

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 (CIs).

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).

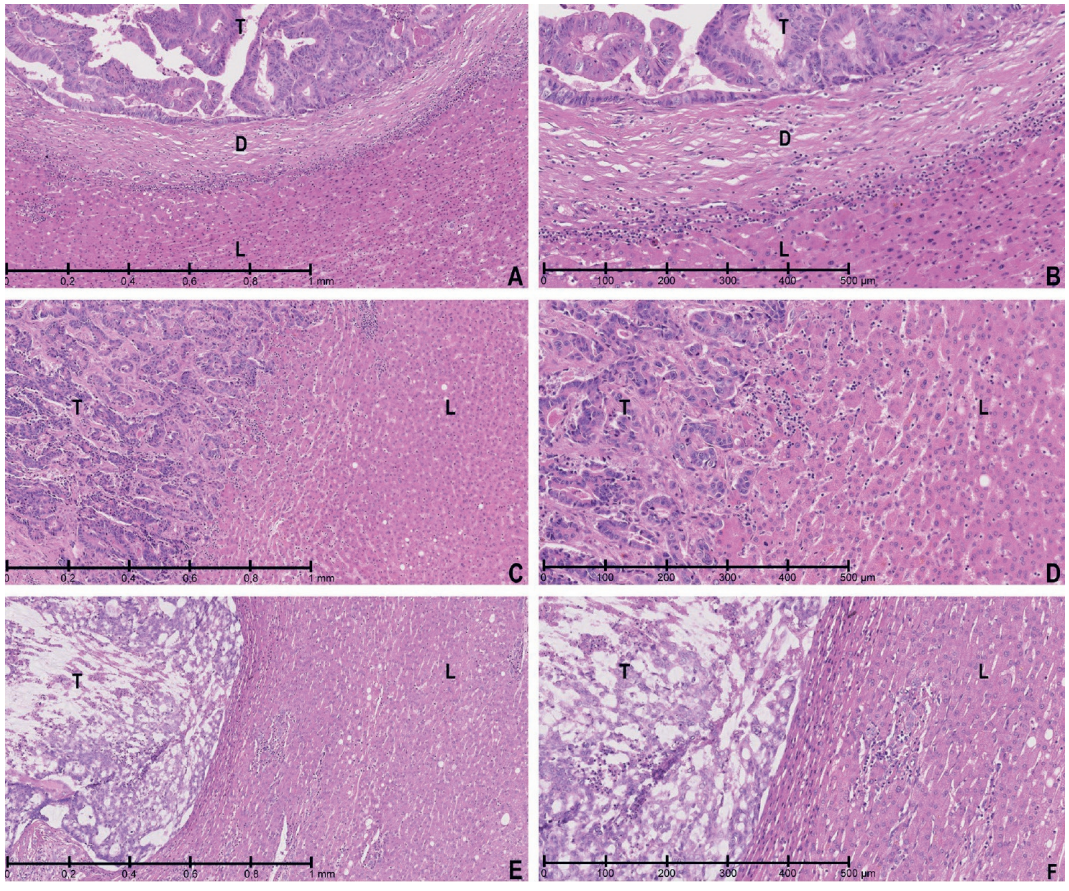


Figure 1A-F: Collated HE tissue sections. 1A-B: dHGP low and high magnification; 1C-D: rHGP low and high magnification; 1E-F: pHGP low and high magnification. T: tumour; D: desmoplastic stroma; L: liver parenchyma.

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.

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.

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).

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).

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.

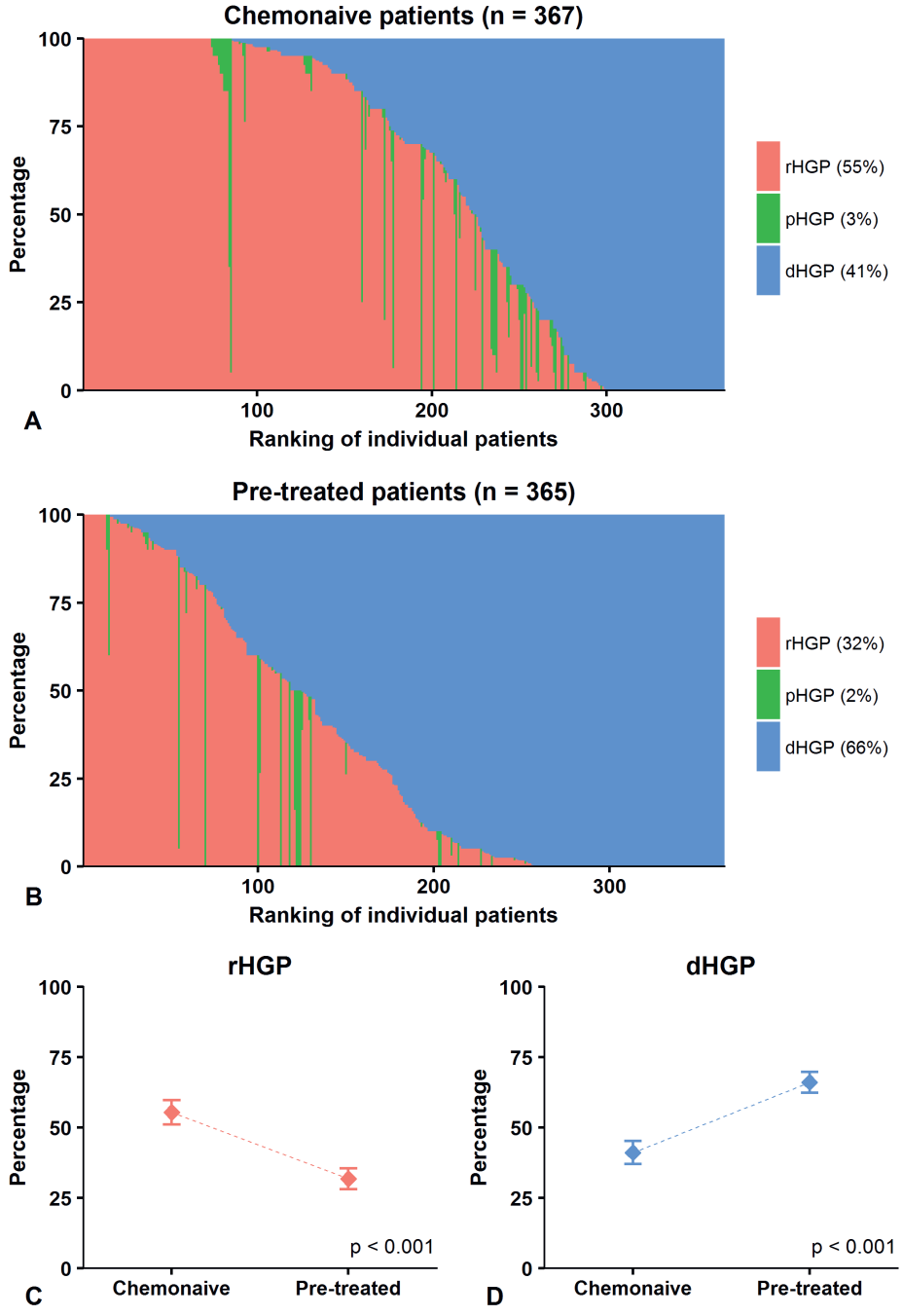


Figure 2A-D: 2A: Distribution of HGPs. Ranking based on percentage dHGP. 2A: distribution of HGPs in the chemo-naive cohort. 3B: distribution of HGPs in the pre-treated cohort. 2C-D: total proportion rHGP (C) and dHGP (D) in chemo-naive patients compared to pre-treated patients. (percentages do not always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

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

Overall survival	Univariable Hazard ratio [95% CI]	p-value	Multivariable 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]	0.03
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 Hazard ratio [95% CI]**	**p-value**	**Multivariable 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

ASA = American Society of Anaestesiologists, CEA = carcinoembryonic antige, cont. = continuous, CRLM = colorectal liver metastases, dHGP = desmoplastic histopathological growth patterns, R1 = irradical resection margin.

Table 2: Overall survival cox regression cut-off analysis in the chemo-naive cohort.

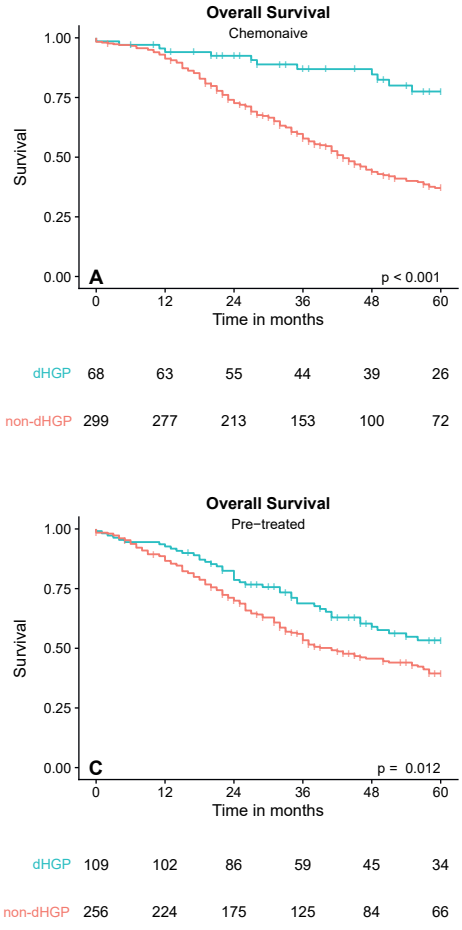
Overall survival	Univariable Hazard ratio [95% CI]	p-value	Multivariable Hazard ratio [95% CI]	p-value
Age at resection CRLM (cont.)	1.011 [0.997-1.025]	0.126	1.017 [1.002-1.032]	0.031
ASA > II	1.018 [0.648-1.600]	0.939	0.968 [0.600-1.564]	0.896
Right-sided primary	1.477 [1.053-2.072]	0.024	1.563 [1.088-2.247]	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]	0.01
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

ASA = American Society of Anaestesiologists, CEA = carcinoembryonic antige, cont. = continuous, CRLM = colorectal liver metastases, dHGP = desmoplastic histopathological growth patterns, R1 = irradical resection margin.

Table 3: Cox regression analysis for OS and PFS of pre-treated patients.

Overall survival	**Univariable Hazard ratio [95% CI]**	**p-value**	**Multivariable 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]	0.34	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 Hazard ratio [95% CI]**	**p-value**	**Multivariable 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]	0.84
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

ASA = American Society of Anaestesiologists, CEA = carcinoembryonic antige, cont. = continuous, CRLM = colorectal liver metastases, dHGP = desmoplastic histopathological growth patterns, R1 = irradical resection margin.



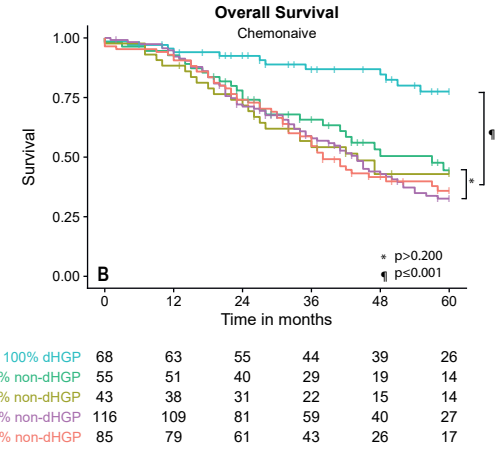


Figure 3A-C: 3A OS chemo-naive patients; 3B Cut-off analysis with OS for percentage dHGP amongst chemo-naive patients; 3C pre-treated patients.

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).

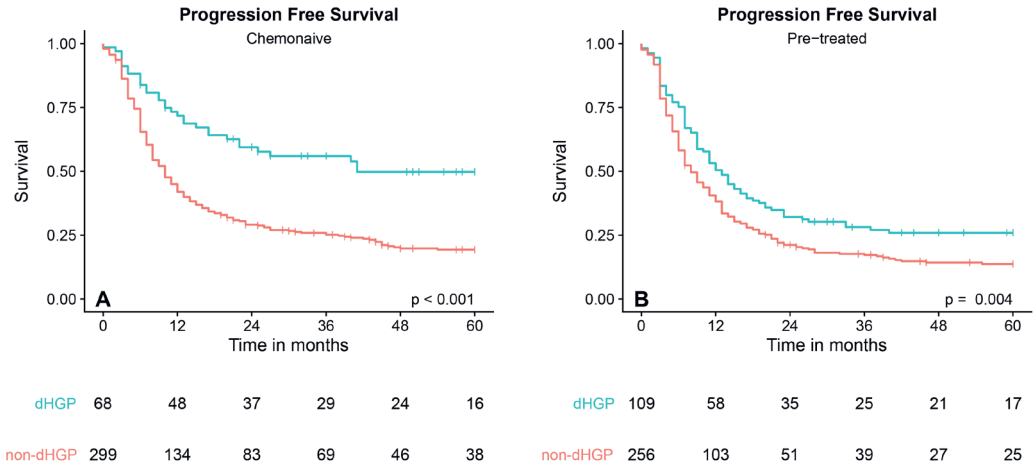


Figure 4A-B PFS: 4A: PFS of chemo-naive patients. 4B: PFS of pre-treated patients.

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).

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).

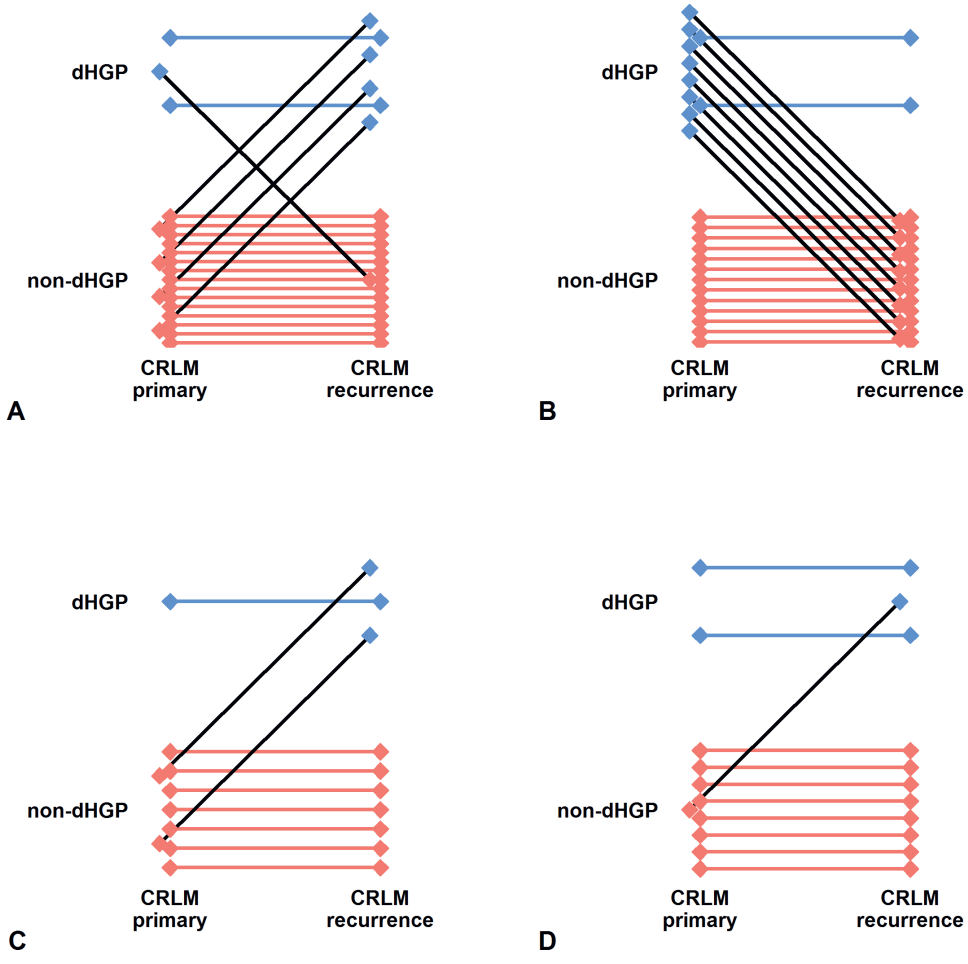


Figure 5A-D: Graphical display of the changes in HGPs between 1st and 2nd surgery for CRLM found per group: 5A: -/-; 5B:+/-; 5C: -/+; 5D: +/+.

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.

References

1 Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. Oct 2001;195(3):336-342.

2 van Dam PJ, van der Stok EP, Teuwen LA, et al. International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer. Nov 7 2017;117(10):1427-1441.

3 Stessels F, Van den Eynden G, Van der Auwera I, et al. Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. Br J Cancer. Apr 05 2004;90(7):1429-1436.

4 Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med. Nov 2016;22(11):1294-1302.

5 Brunner SM, Kesselring R, Rubner C, et al. Prognosis according to histochemical analysis of liver metastases removed at liver resection. Br J Surg. Dec 2014;101(13):1681-1691.

6 Mlecnik B, Tosolini M, Kirilovsky A, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol. Feb 20 2011;29(6):610-618.

7 Eefsen RL, Vermeulen PB, Christensen IJ, et al. Growth pattern of colorectal liver metastasis as a marker of recurrence risk. Clin Exp Metastasis. Apr 2015;32(4):369-381.

8 Nielsen K, Rolff HC, Eefsen RL, Vainer B. The morphological growth patterns of colorectal liver metastases are prognostic for overall survival. Mod Pathol. Dec 2014;27(12):1641-1648.

9 Siriwardana PN, Luong TV, Watkins J, et al. Biological and Prognostic Significance of the Morphological Types and Vascular Patterns in Colorectal Liver Metastases (CRLM): Looking Beyond the Tumor Margin. Medicine (Baltimore). Feb 2016;95(8):e2924.

10 Van den Eynden GG, Bird NC, Majeed AW, Van Laere S, Dirix LY, Vermeulen PB. The histological growth pattern of colorectal cancer liver metastases has prognostic value. Clin Exp Metastasis. Aug 2012;29(6):541-549.

11 Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration. J Natl Cancer Inst. Jun 4 2018.

12 Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. Sep 1999;230(3):309-318; discussion 318-321.

13 Passiglia F, Bronte G, Bazan V, Galvano A, Vincenzi B, Russo A. Can KRAS and BRAF mutations limit the benefit of liver resection in metastatic colorectal cancer patients? A systematic review and meta-analysis. Crit Rev Oncol Hematol. Mar 2016;99:150-157.

14 Loupakis F, Schirripa M, Caparello C, et al. Histopathologic evaluation of liver metastases from colorectal cancer in patients treated with FOLFOXIRI plus bevacizumab. Br J Cancer. Jun 25 2013;108(12):2549-2556.

15 Rubbia-Brandt L, Giostra E, Brezault C, et al. Importance of histological tumor response assessment in predicting the outcome in patients with colorectal liver metastases treated with neo-adjuvant chemotherapy followed by liver surgery. Ann Oncol. Feb 2007;18(2):299-304.

16 Rahbari NN, Kedrin D, Incio J, et al. Anti-VEGF therapy induces ECM remodeling and mechanical barriers to therapy in colorectal cancer liver metastases. Sci Transl Med. Oct 12 2016;8(360):360ra135.

17 Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. Dec 2014;15(12):1243-1253.

18 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. Lancet Oncol. Nov 2013;14(12):1208-1215.

19 Nowak-Sliwinska P, Alitalo K, Allen E, et al. Consensus guidelines for the use and interpretation of angiogenesis assays. Angiogenesis. May 15 2018.

20 Donnem T, Reynolds AR, Kuczynski EA, et al. Non-angiogenic tumours and their influence on cancer biology. Nat Rev Cancer. May 2018;18(5):323-336.

21 Kuczynski EA, Yin M, Bar-Zion A, et al. Co-option of Liver Vessels and Not Sprouting Angiogenesis Drives Acquired Sorafenib Resistance in Hepatocellular Carcinoma. J Natl Cancer Inst. Aug 2016;108(8).

22 Bentolila LA, Prakash R, Mihic-Probst D, et al. Imaging of Angiotropism/Vascular Co-Option in a Murine Model of Brain Melanoma: Implications for Melanoma Progression along Extravascular Pathways. Sci Rep. Apr 6 2016;6:23834.

23 Lugassy C, Wadehra M, Li X, et al. Pilot study on "pericytic mimicry" and potential embryonic/stem cell properties of angiotropic melanoma cells interacting with the abluminal vascular surface. Cancer Microenviron. Apr 2013;6(1):19-29.

24 Lu J, Ye X, Fan F, et al. Endothelial cells promote the colorectal cancer stem cell phenotype through a soluble form of Jagged-1. Cancer Cell. Feb 11 2013;23(2):171-185.

25 van Dam PJ, Daelemans S, Ross E, et al. Histopathological growth patterns as a candidate biomarker for immunomodulatory therapy. Semin Cancer Biol. Jan 26 2018.

26 Karagkounis G, Torbenson MS, Daniel HD, et al. Incidence and prognostic impact of KRAS and BRAF mutation in patients undergoing liver surgery for colorectal metastases. Cancer. Dec 1 2013;119(23):4137-4144.

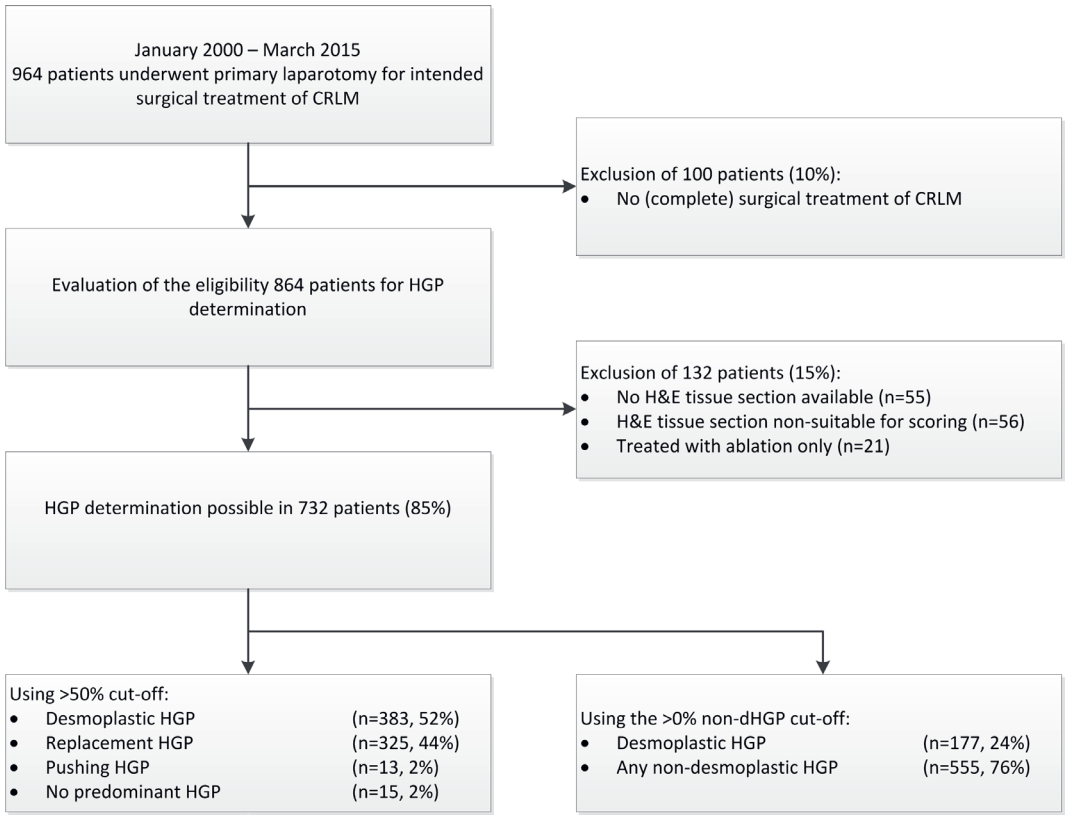
27 Vauthey JN, Zimmitti G, Kopetz SE, et al. RAS mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. Ann Surg. Oct 2013;258(4):619-626; discussion 626-617.

28 Brudvik KW, Jones RP, Giuliante F, et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. May 25 2017.

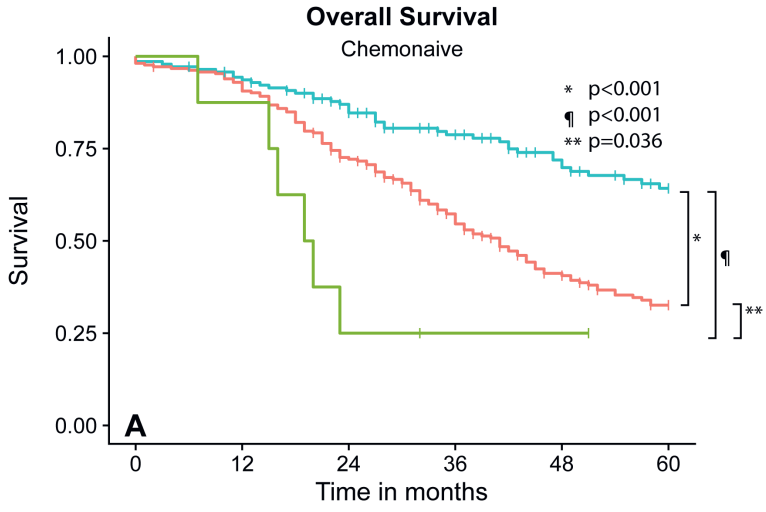
29 Gonsalves WI, Mahoney MR, Sargent DJ, et al. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. J Natl Cancer Inst. Jul 2014;106(7).

30 Smith CG, Fisher D, Claes B, et al. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/- cetuximab. Clin Cancer Res. Aug 01 2013;19(15):4104-4113.

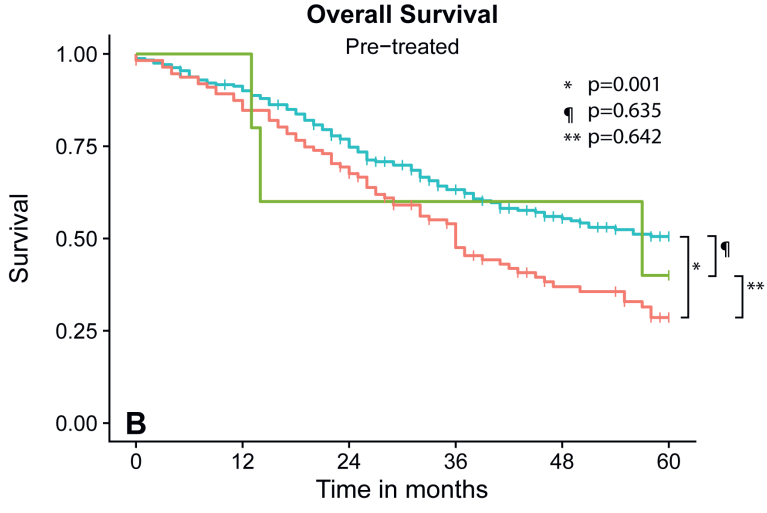
31 Yaeger R, Cercek A, Chou JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. Aug 01 2014;120(15):2316-2324.



Supplementary figure 1: A flowchart of the patient inclusion.



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.

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

		dHGP N=68 (19%)	non-dHGP N=299 (81%)	P-value	Missing N
General characteristics					
Age at resection (median [IQR])		68.0 [59.0, 75.2]	66.0 [59.0, 73.0]	0.18	
Gender (%)	Female	25 (37)	109 (36)	0.962	
	Male	43 (63)	190 (64)		
ASA classification(%)	ASA I-II	57 (85)	265 (91)	0.142	9
	ASA >II	10 (15)	26 (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	3 (4)	9 (3)		
Pathological T-stage (%)	pT 0-2	19 (28)	61 (21)	0.188	3
	pT 3-4	49 (72)	235 (79)		
Pathological N-stage (%)	N0	35 (52)	118 (40)	0.07	6
	N+	32 (48)	176 (60)		
Adjuvant chemotherapy (%)	No	59 (87)	231 (77)	0.082	
	Yes	9 (13)	68 (23)		
CRLM characteristics					
Synchronous CRLM (%)	No	43 (63)	212 (71)	0.215	
	Yes	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])		2.0 [1.5, 3.6]	3.2 [2.2, 4.1]	<0.001*	1
Preoperative CEA (median [IQR])		6.0 [3.0, 12.0]	13.6 [4.7, 44.2]	<0.001*	14
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*	2
	R1	3 (4)	38 (13)		
CRS (%)	Low (0-2)	53 (80)	230 (78)	0.746	8
	High (3-5)	13 (20)	63 (22)		
Major resection (≥3 segments) (%)	No	59 (87)	226 (76)	0.046*	
	Yes	9 (13)	73 (24)		
Major complications (Clavien-Dindo ≥3)	No	61 (90)	277 (93)	0.363	1
	Yes	7 (10)	21 (7)		
Postoperative death (%)	No	67 (99)	293 (98)	0.77	
	Yes	1 (1)	6 (2)		

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

		dHGP N=109 (30%)	non-dHGP N=256 (70%)	P-value	Missing N
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 I-II	100 (92)	234 (92)	0.995	1
	ASA >II	9 (8)	21 (8)		
Primary tumour characteristics					
Location (%)	Right-sided	20 (18)	38 (15)	0.657	
	Left-sided	45 (41)	115 (45)		
	Rectum	42 (39)	101 (39)		
	Double	2 (2)	2 (1)		
Pathological T-stage (%)	pT 0-2	20 (19)	36 (16)	0.483	31
	pT 3-4	86 (81)	192 (84)		
Pathological N-stage (%)	N0	44 (42)	77 (34)	0.152	32
	N+	61 (58)	151 (66)		
Adjuvant chemotherapy (%)	No	105 (96)	228 (90)	0.056	4
	Yes	4 (4)	24 (10)		
CRLM characteristics					
Synchronous CRLM (%)	No	22 (20)	60 (23)	0.495	
	Yes	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])		2.9 [2.1, 4.7]	3.4 [2.4, 5.3]	0.047	1
Preoperative CEA (median [IQR])		12.2 [3.6, 51.2]	21.0 [7.0, 93.0]	0.008	18
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	1
	R1	11 (10)	56 (22)		
CRS (%)	Low (0-2)	48 (48)	89 (37)	0.073	24
	High (3-5)	53 (52)	151 (63)		
Major resection (≥3 segments) (%)	No	67 (61)	129 (50)	0.052	
	Yes	42 (39)	127 (50)		
Major complications (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	Missing N
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	10
	ASA > II	66 (9%)	36 (10 %)	30 (8%)		
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	34
	pT3-4	562 (81%)	284 (78.0%)	278 (83.2%)		
Nodal status	N0	274 (40%)	153 (42%)	121 (36%)	0.104	38
	N+	420 (61%)	208 (58%)	212 (64%)		
Adjuvant chemotherapy	No	623 (86%)	290 (79%)	333 (92%)	<0.001*	4
	Yes	105 (14%)	77 (21%)	28 (8%)		
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*	2
Preoperative CEA	Median (IQR)	14.7 (4.8-51.8)	11.0 (4.2-29.8)	19.7 (5.3-74.0)	<0.001*	32
Fong CRS	Low	420 (60%)	283 (79%)	137 (40%)	<0.001*	32
	High	280 (40%)	76 (21%)	204 (60%)		
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*	3
	R1	108 (15%)	41 (11%)	67 (18%)		
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 segments	481 (66%)	285 (78%)	196 (54%)	<0.001*	
	≥3 segments	251 (34%)	82 (22%)	169 (46%)		
Major complications	No	665 (91%)	338 (92%)	327 (90%)	0.193	1
	Yes	66 (9%)	28 (8%)	38 (10%)		
Postoperative death	No	718 (98%)	360 (98%)	358 (98%)	0.992	
	Yes	14 (2%)	7 (2%)	7 (2%)		

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

	Univariable Odds Ratio [95% CI]	P-value	Multivariable Odds Ratio [95% CI]	P-value
Right-sided primary	1.112 [0.710-1.742]	0.644	1.264 [0.789-2.026]	0.33
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]	0.53	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]	0.8	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*

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

	Univariable Odds Ratio [95% CI]	P-value	Multivariable 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]	0.03
Diameter largest CRLM (cont.)	0.933 [0.850-1.024]	0.145	0.936 [0.847-1.035]	0.2
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: Overall Survival Cox regression analysis all neoadjuvantly treated patients +/- Bevacizumab.

Overall Survival	Univariable Odds Ratio [95% CI]	P-value	Multivariable Odds 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]	0.14
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]	0.34	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: Progression-Free Survival Cox regression all neoadjuvantly treated patients +/- Bevacizumab.

Progression free survival	Univariable Odds Ratio [95% CI]	P-value	Multivariable Odds 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]	0.83
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]	0.54

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

		>50% dHGP 142 (39%)	>50% rHGP 213 (58%)	>50% pHGP 8 (2%)	P-value	Missing N
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]	0.32	
Gender	Male	46 (32)	80 (38)	4 (50)	0.426	
	Female	96 (68)	133 (62)	4 (50)		
ASA	ASA I-II	122 (87)	188 (91)	8 (100)	0.29	9
	ASA > II	18 (13)	18 (9)	0 (0)		
Primary tumour characteristics						
Location	Right-sided	23 (16)	37 (17)	1 (12)	0.7	
	Left-sided	62 (44)	82 (38)	2 (25)		
	Rectum	53 (37)	87 (41)	4 (50)		
	Double tumour	4 (3)	7 (3)	1 (12)		
pTumour stage	pT0-2	36 (25)	40 (19)	3 (38)	0.21	3
	pT3-4	106 (75)	170 (81)	5 (62)		
Nodal status	N0	75 (53)	75 (36)	2 (25)	0.004*	6
	N+	66 (47)	133 (64)	6 (75)		
Adjuvant chemotherapy	No	120 (85)	161 (76)	7 (88)	0.107	
	Yes	22 (15)	52 (24)	1 (12)		
CRLM characteristics						
Synchronous CRLM	No	95 (67)	152 (71)	4 (50)	0.333	
	Yes	47 (33)	61 (29)	4 (50)		
Disease-free interval	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	
Size of largest CRLM	Median (IQR)	2.8 [1.9, 4.0]	3.0 [2.0, 4.0]	4.0 [2.9, 4.9]	0.066	1
Preoperative CEA	Median (IQR)	7.8 [3.4, 17.5]	13.7 [5.0, 51.5]	20.6 [2.9, 23.3]	0.002*	14
Bilobar metastases	No	110 (77)	167 (78)	7 (88)	0.796	
	Yes	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*	2
	R1	9 (6)	30 (14)	0 (0)		
Fong CRS	Low	112 (81)	162 (78)	6 (75)	0.692	8
	High	26 (19)	47 (22)	2 (25)		
Major liver resection	No	118 (83)	158 (74)	7 (88)	0.112	
	Yes	24 (17)	55 (26)	1 (12)		
Major complications (i.e. Clavien-Dindo ≥3)	No	127 (89)	199 (94)	8 (100)	0.22	1
	Yes	15 (11)	13 (6)	0 (0)		
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, using 50% cut-off.

Overall Survival	Univariable Hazard Ratio [95% CI]	P-value	Multivariable Hazard Ratio [95% CI]	P-value
Age at resection CRLM (cont.)	1.012 [0.998-1.026]	0.1	1.016 [1.001-1.032]	0.038
ASA > II	1.021 [0.650-1.606]	0.927	1.047 [0.650-1.685]	0.85
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, using 50% cut-off.

	Univariable Odds Ratio [95% CI]	P-value	Multivariable 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]	0.39
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*

Supplementary table 11: Baseline characteristics pre-treated patients, using 50% cut-off.

		>50% dHGP N=241 (67%)	>50% rHGP N=112 (31%)	>50% pHGP N=5 (1%)	P-value	Missing N
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	Male	84 (35)	42 (38)	1 (20)	0.682	
	Female	157 (65)	70 (62)	4 (80)		
ASA	ASA I-II	219 (91)	103 (92)	5 (100)	0.773	1
	ASA > II	21 (9)	9 (8)	0 (0)		
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)		
pTumour stage	pT0-2	44 (20)	11 (11)	1 (20)	0.174	31
	pT3-4	181 (80)	88 (89)	4 (80)		
Nodal status	N0	93 (41)	24 (24)	2 (40)	0.015	32
	N+	132 (59)	74 (76)	3 (60)		
Adjuvant chemotherapy	No	224 (94)	100 (90)	4 (80)	0.282	4
	Yes	15 (6)	11 (10)	1 (20)		
CRLM characteristics						
Synchronous CRLM	No	58 (24)	22 (20)	1 (20)	0.646	
	Yes	183 (76)	90 (80)	4 (80)		
Disease-free interval	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	
Size of largest CRLM	Median (IQR)	3.3 [2.3, 5.1]	3.1 [2.0, 5.2]	5.1 [2.5, 5.4]	0.619	1
Preoperative CEA	Median (IQR)	19.7 [5.2, 65.2]	21.0 [6.8, 112.5]	3.0 [2.4, 21.0]	0.17	18
Bilobar metastases	No	101 (42)	44 (39)	2 (40)	0.896	
	Yes	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	1
	R1	41 (17)	25 (22)	0 (0)		
Fong CRS	Low	102 (45)	32 (31)	1 (20)	0.047	24
	High	126 (55)	70 (69)	4 (80)		
Major liver resection	No	139 (58)	52 (46)	2 (40)	0.117	
	Yes	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, using 50% cut-off.

Overall Survival	**Univariable**	**P-value**	**Multivariable**	**P-value**
	Hazard Ratio [95% CI]		**Hazard Ratio [95% CI]**	
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]	0.64	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]	0.14
pHGP	1.020 [0.324-3.209]	0.973	0.829 [0.245-2.801]	0.763

CHAPTER SEVEN

CHAPTER SEVEN

HISTOPATHOLOGICAL GROWTH PATTERNS AND SURVIVAL AFTER RESECTION OF COLORECTAL LIVER METASTASIS: AN EXTERNAL VALIDATION STUDY.

Diederik J. Höppener, Boris Galjart, Pieter M.H. Nierop, Florian E. Buisman, Eric P. van der Stok, Robert R.J. Coebergh van den Braak, Martin J. van Amerongen, Vinod P. Balachandran, William R. Jarnagin, T. Peter Kingham, Michail Doukas, Jinru Shia, Iris D. Nagtegaal, Peter B. Vermeulen, Bas Groot Koerkamp, Dirk J. Grünhagen, Johannes H.W. de Wilt, Michael I. D'Angelica, Cornelis Verhoef

Published in JNCI Cancer Spectrum

Abstract

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

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

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

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

Introduction

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

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

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

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

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

Methods

Patient selection and data

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

Patient demographics, clinicopathological disease characteristics and survival data were extracted from the respective center's prospectively maintained databases. The study adheres to the REMARK guidelines for tumor marker prognostic studies.[10] Institutional ethical review and approval was obtained from the medical ethics committee of the Erasmus University Medical Center Rotterdam (MEC-2018-1743), which granted a waiver for informed consent.

Treatment strategy and postoperative course

The Erasmus MC Cancer Institute, Memorial Sloan Kettering Cancer Center, and the Radboud University Medical Center are tertiary referral centers for liver surgery. All patients with suspected CRLM were discussed by a multidisciplinary team of surgical oncologists, medical oncologists, radiation oncologists, and radiologists. Presence of limited extrahepatic disease amenable to local treatment did not preclude complete surgical treatment. Noticeable practice differences between centers exist in use of perioperative chemotherapeutic therapies. HAIP chemotherapy is commonly used at the Memorial Sloan Kettering Cancer Center and is administered frequently in selected patients[11], whereas in the Netherlands HAIP chemotherapy is only administered within the context of randomized controlled clinical trials.[12,13] Moreover, perioperative systemic chemotherapy is considered standard of care throughout the United States. In the Netherlands, guidelines advocate to only administer preoperative chemotherapy to increase resectability in patients with unresectable disease, or to facilitate a parenchymal sparing approach. Postoperative systemic chemotherapy is not advocated. Practice variation regarding perioperative systemic chemotherapy does however exist in the Netherlands.[14]

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

Pathological assessment

Pathological assessment of HGP was performed retrospectively on H&E sections by at least two trained observers simultaneously and blinded for patient characteristics and outcome. Dedicated liver pathologists were consulted when necessary. All available H&E tissue sections of all resected CRLM of each individual patient were assessed for HGP phenotype by light microscopy or digital evaluation of digitalized sections.

In accordance with international consensus guidelines, the tumor-liver interface was evaluated for pathological phenotype. The three previously described HGP phenotypes are discussed in depth in these guidelines.[15] In summation, the desmoplastic phenotype is characterized by separation of tumor and liver parenchyma by a band of desmoplastic stroma (Figure 1A). This band of desmoplastic stroma separating cancer cells from the liver parenchyma is absent in the non-desmoplastic phenotypes (Figure 1B). As multiple phenotypes can appear in conjunction, the relative proportion of each phenotype is estimated on each H&E section and expressed as percentage. The final patient-level score is the average of each metastasis with equal weights assigned to discrete metastases and to individual slides within metastases. There is no minimum section requirement for HGP assessment. Sections are considered unsuitable if only a small fraction of the tumor-liver interface (less than 20%) is assessable, if tissue preservation quality is deemed unsuitable (e.g. tear of tissue at the transition zone) or when viable tumor tissue is absent (i.e. complete pathological response). Patients were classified as desmoplastic if all slides of all resected CRLM uniformly displayed a desmoplastic phenotype (i.e. 100% desmoplastic, Figure 1A), and as non-desmoplastic if any non-desmoplastic phenotype was observed in any slide of any resected CRLM (i.e. <100%

desmoplastic, Figure 1B).[7] For cut-off analyses patients were classified in subgroups according to the extent of non-desmoplastic phenotypes observed: 100% desmoplastic versus 0.1%-33%, 33.1%-67% and 67.1%-100% non-desmoplastic, respectively.

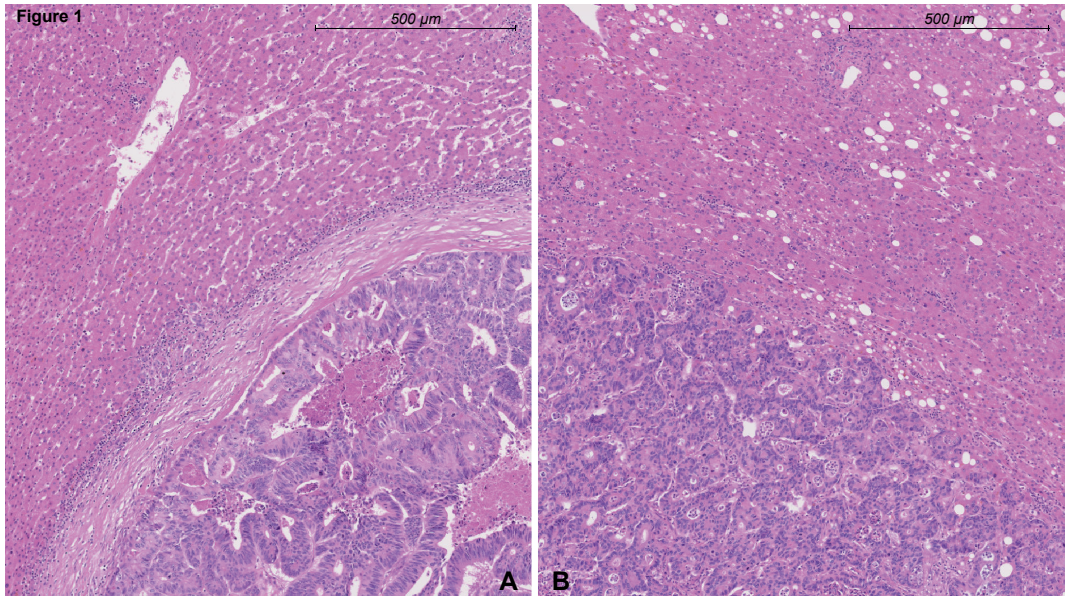


Figure 1. Hematoxylin & eosin stained tissue sections of resected CRLM viewed at 5 times magnification are shown with corresponding scale bars in the upper-right. A) Hematoxylin & eosin stained tissue section of a resected colorectal liver metastasis displaying a desmoplastic phenotype. Note the rim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

Outcomes

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

Statistical analyses

Categorical data are reported as absolute count with corresponding percentage. Non-parametric continuous data are reported as median with corresponding interquartile range (IQR). Differences in proportions were evaluated by means of the Chi-squared test. Medians were compared by the Kruskall-Wallis test. Survival curves were estimated according to Kaplan-Meier analysis and compared by means of the log-rank test. Five-year survival estimates with corresponding 95% confidence intervals (CIs) are reported. Median follow-up for survivors was determined using the reverse Kaplan-Meier method. Uni- and multivariable Cox proportional hazards regression survival analyses were performed and reported as hazard ratios (HRs) with corresponding 95% CIs. All known clinicopathological risk factors were added to the regression models. With regards to missing data,

full case analyses were performed. The proportional hazards assumption was visually assessed by plotting Schoenfeld residuals and Kaplan-Meier curves. Since data on KRAS and BRAF mutational status was only available for less than half of the patients, separate Cox regression models were computed with additional correction for these genetic risk factors. Cox regression models with interaction terms were created to evaluate effect modification of HGP by preoperative chemotherapy.[7] All log-rank tests and Cox regression analyses were performed with center as stratification factor. The statistical significance level was set at an α of .05. All statistical tests were 2-sided and were performed using the R Project for Statistical Computing version 4.0.3 (https://www.r-project.org/) with the packages ggplot2 (v3.3.2), rms (6.0-1), survival (v3.2-7), survminer (v0.4.8) and tableone (v0.12.0).

Results

Between 2000 and 2019 a total of 2708 consecutive patients underwent resection of CRLM at either the Erasmus MC Cancer Institute (n=1044), Memorial Sloan Kettering Cancer Center (n=1352) or Radboud University Medical Center (n=312) and had resection specimens suitable for pathological HGP assessment. Of these, 732 patients treated at the Erasmus MC Cancer Institute are described in our previous paper[7], 582 received perioperative HAIP chemotherapy, 446 were treated with postoperative systemic chemotherapy, and 168 did not undergo complete surgical treatment, resulting in a total of 780 patients included in the current external validation study. Baseline characteristics stratified by center are reported in Supplementary Table 1. A total of 213 patients were treated at the Erasmus MC Cancer Institute, 338 at the Memorial Sloan Kettering Cancer Center, and 229 at the Radboud University Medical Center. Of the 213 newly described patients treated at the Erasmus MC Cancer Institute, 163 (76.5%) underwent surgery outside (i.e. after march 2015) the inclusion period of the previous study, 10 (4.7%) were additionally identified through data requests at the IT department, and for the remaining 40 (18.7%) H&E resection specimens were previously missing but have since been recovered.[7] Primary tumor and CRLM clinicopathological characteristics were comparable between centers, with the exception of the number of CRLM, presence of extrahepatic disease, and the disease-free interval between resection of primary tumor and detection of liver metastasis, all being more favorable in patients treated at the Radboud University Medical Center (Supplementary Table 1).

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

Table 1. Baseline characteristics stratified by histopathological phenotype

Characteristic	Missing, No. (%)	Desmoplastic (n = 149)	Non-desmoplastic (n = 631)	*P-value* [a]
Treatment center, No. (%)				
Erasmus MC	--	45 (30.2)	168 (26.6)	0.66
MSKCC		63 (42.3)	275 (43.6)	
Radboud UMC		41 (27.5)	188 (29.8)	
Median age at resection CRLM (IQR), y	--	65.0 (52.0, 72.0)	65.0 (56.0, 72.0)	0.31
Sex, No. (%)				
Male	--	92 (61.7)	374 (59.3)	0.58
Female		57 (38.3)	257 (40.7)	
ASA classification, No. (%)				
ASA I-II	4 (0.5)	87 (59.2)	377 (59.9)	0.87
ASA >II		60 (40.8)	252 (40.1)	
Primary tumor location, No. (%)				
Left-sided	24 (3.1)	49 (34.8)	254 (41.3)	0.35
Right-sided		41 (29.1)	166 (27.0)	
Rectal		51 (36.2)	195 (31.7)	
T stage, No. (%)				
pT 0-2	56 (7.2)	21 (15.7)	76 (12.9)	0.39
pT 3-4		113 (84.3)	514 (87.1)	
N stage, No. (%)				
N0	10 (1.3)	64 (43.5)	220 (35.3)	0.06
N+		83 (56.5)	403 (64.7)	
Median No. of CRLM (IQR)	2 (0.3)	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	0.12
Median diameter of largest CRLM (IQR), cm	3 (0.4)	2.2 (1.3, 3.3)	3.0 (2.0, 4.6)	<0.001
Median disease-free interval[b] (IQR), months	11 (1.4)	0.0 (0.0, 11.8)	2.0 (0.0, 16.0)	0.03
Median Preoperative CEA (IQR), µg/L	65 (8.3)	5.3 (2.7, 16.4)	11.2 (4.2, 32.5)	<0.001
Preoperative systemic chemotherapy, No. (%)				
No	--	64 (43.0)	343 (54.4)	0.01
Yes		85 (57.0)	288 (45.6)	
Resection margin involved, No. (%)				
No	1 (0.1)	136 (91.3)	541 (85.9)	0.08
Yes		13 (8.7)	89 (14.1)	
Extrahepatic disease, No. (%)				
No	--	140 (94.0)	556 (88.1)	0.04
Yes		9 (6.0)	75 (11.9)	
KRAS mutational status, No. (%)				
Wildtype	450 (57.7)	29 (50.0)	155 (57.0)	0.33
Mutant		29 (50.0)	117 (43.0)	
BRAF mutational status, No. (%)				
Wildtype	491 (62.9)	48 (96.0)	231 (96.7)	0.82
Mutant		2 (4.0)	8 (3.3)	
Microsatellite stability status, No. (%)				
MSS	600 (76.9)	35 (85.4)	134 (96.4)	0.01
MSI		6 (14.6)	5 (3.6)	

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

b Between resection of primary tumor and detection of CRLM

Overall and disease-free survival

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

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

Effect of preoperative chemotherapy

No statistically significant interaction between preoperative chemotherapy and HGP was observed (OS p=0.61, DFS p=0.64). OS and DFS differed statistically significantly between desmoplastic and non-desmoplastic HGP patients in both the chemo-naive and pre-treated subpopulations. In chemo-naive patients the 5-year (95%CI) OS estimate for a desmoplastic phenotype was 81.5% (95%CI = 68.9-96.5%) compared to 51.8% (95%CI = 44.4-60.5%) for a non-desmoplastic phenotype (Figure 3A, p<0.001). Again, similar differences were observed for DFS, with 5-year DFS estimates of 36.4% (95%CI = 22.6%-58.6%) for desmoplastic versus 19.9% (95%CI = 15.0%-26.2%) for non-desmoplastic (Figure 3B, p<0.001).

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

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

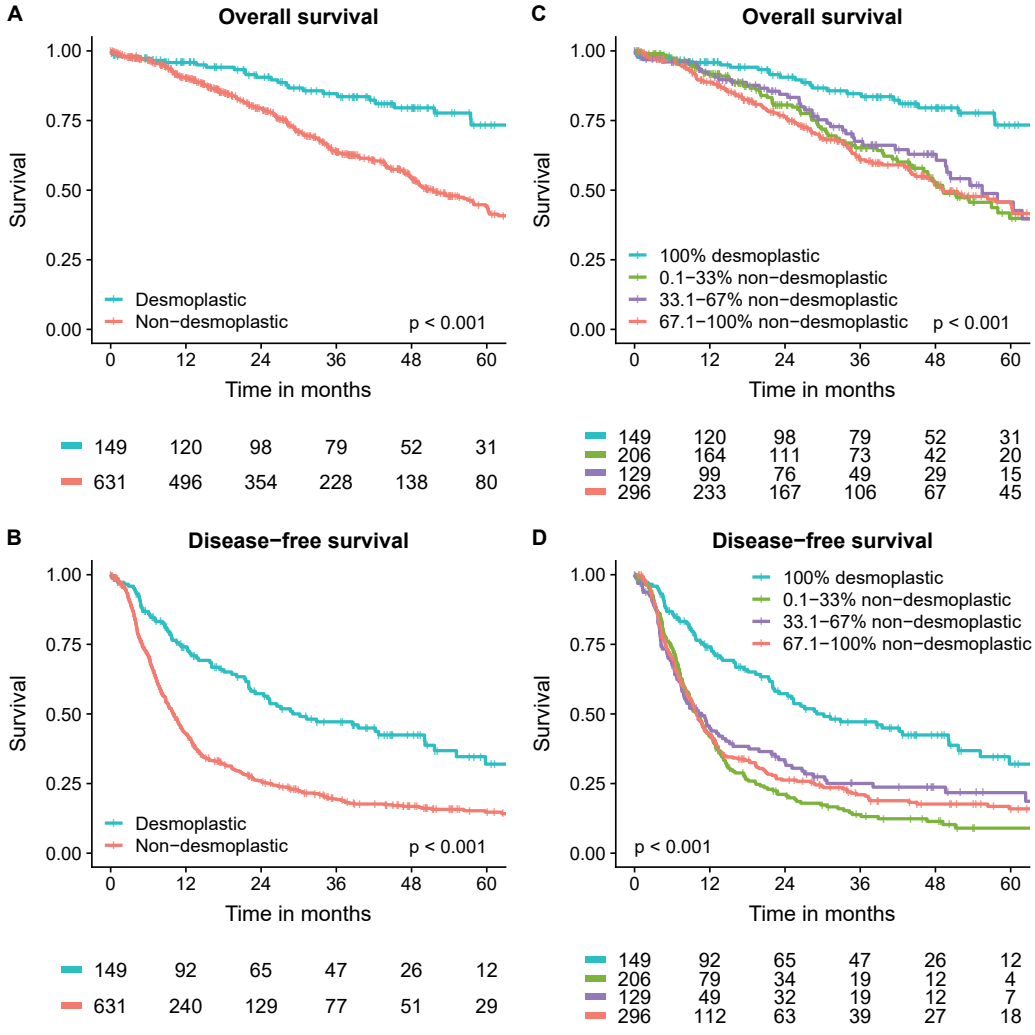


Figure 2. Kaplan-Meier overall and disease-free survival estimates are shown. Figures A and B display the overall (A) and disease-free (B) survival estimates of patients with a desmoplastic versus a non-desmoplastic phenotype. Figures C and D display the overall (C) and disease-free (D) survival estimates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

Table 2. Uni- and multivariable Cox regression analyses for overall and disease-free survival

Characteristic	Overall survival (n=625)				Disease-free survival (n=625)			
	Univariable		Multivariable		Univariable		Multivariable	
	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Age at resection CRLM - years	1.01 (1.00-1.02)	0.01	1.01 (1.00-1.02)	0.13	1.00 (0.99-1.00)	0.34	1.00 (0.99-1.01)	0.95
ASA classification - >II vs I-II	1.26 (0.94-1.71)	0.13	1.29 (0.90-1.87)	0.17	1.14 (0.91-1.41)	0.25	1.22 (0.95-1.57)	0.12
Right-sided primary - yes vs no	1.46 (1.13-1.88)	0.004	1.36 (1.00-1.86)	0.05	1.05 (0.86-1.27)	0.65	1.03 (0.82-1.29)	0.81
T-stage - pT3-4 vs pT0-2	1.36 (0.92-2.00)	0.12	1.28 (0.82-2.01)	0.28	1.24 (0.95-1.61)	0.11	1.09 (0.81-1.46)	0.57
N-stage - N+ vs N0	1.18 (0.93-1.51)	0.18	1.23 (0.91-1.66)	0.18	1.29 (1.08-1.55)	0.005	1.24 (1.01-1.53)	0.04
Disease-free interval[a] (cont.) - months	1.00 (0.99-1.01)	0.65	1.00 (0.99-1.01)	0.67	0.99 (0.99-1.00)	0.01	0.99 (0.98-1.00)	0.01
Number of CRLM (cont.)	1.10 (1.06-1.15)	<0.001	1.09 (1.04-1.14)	<0.001	1.11 (1.08-1.15)	<0.001	1.08 (1.04-1.12)	<0.001
Diameter of largest CRLM (cont.) - cm	1.06 (1.03-1.10)	<0.001	1.06 (1.02-1.11)	0.006	1.06 (1.03-1.09)	<0.001	1.05 (1.01-1.09)	0.009
Preoperative CEA (cont.) - 100 µg/L	1.01 (1.00-1.02)	0.006	1.01 (1.00-1.02)	0.03	1.01 (1.00-1.02)	0.09	1.01 (1.00-1.02)	0.24
Resection margin involved - yes vs no	1.83 (1.36-2.47)	<0.001	1.22 (0.84-1.76)	0.3	1.84 (1.47-2.31)	<0.001	1.46 (1.11-1.92)	0.007
Extrahepatic disease - yes vs no	1.63 (1.15-2.29)	0.005	1.59 (1.05-2.41)	0.03	1.85 (1.44-2.38)	<0.001	2.21 (1.64-2.98)	<0.001
Preoperative chemotherapy - yes vs no	1.25 (0.96-1.62)	0.1	1.26 (0.93-1.71)	0.13	1.45 (1.20-1.74)	<0.001	1.26 (1.01-1.56)	0.04
Desmoplastic phenotype - yes vs no	0.39 (0.27-0.56)	<0.001	0.36 (0.23-0.58)	<0.001	0.44 (0.35-0.56)	<0.001	0.50 (0.37-0.66)	<0.001

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

Table 3. Uni- and multivariable Cox regression analyses for overall and disease-free survival including KRAS and BRAF status

Characteristic	Overall survival				Disease-free survival			
	Univariable		Multivariable (n=227)		Univariable		Multivariable (n=227)	
	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value	HR (95%CI)	P-value
Age at resection CRLM - years	1.01 (1.00-1.02)	0.01	1.02 (1.00-1.04)	0.05	1.00 (0.99-1.00)	0.34	1.00 (0.99-1.01)	0.99
ASA classification - >II vs I-II	1.26 (0.94-1.71)	0.13	0.91 (0.52-1.61)	0.75	1.14 (0.91-1.41)	0.25	1.02 (0.71-1.48)	0.91
Right-sided primary - yes vs no	1.46 (1.13-1.88)	0.004	1.01 (0.59-1.71)	0.98	1.05 (0.86-1.27)	0.65	0.83 (0.58-1.19)	0.32
T-stage - pT3-4 vs pT0-2	1.36 (0.92-2.00)	0.12	1.74 (0.73-4.11)	0.21	1.24 (0.95-1.61)	0.11	1.48 (0.86-2.56)	0.16
N-stage - N+ vs N0	1.18 (0.93-1.51)	0.18	0.98 (0.58-1.66)	0.95	1.29 (1.08-1.55)	0.005	1.15 (0.80-1.67)	0.45
Disease-free interval[a] (cont.) - months	1.00 (0.99-1.01)	0.65	0.97 (0.95-0.99)	0.003	0.99 (0.99-1.00)	0.01	0.99 (0.97-1.00)	0.01
Number of CRLM (cont.)	1.10 (1.06-1.15)	<0.001	1.03 (0.95-1.11)	0.46	1.11 (1.08-1.15)	<0.001	1.06 (1.00-1.12)	0.04
Diameter of largest CRLM (cont.) - cm	1.06 (1.03-1.10)	<0.001	1.02 (0.94-1.11)	0.56	1.06 (1.03-1.09)	<0.001	0.99 (0.93-1.06)	0.81
Preoperative CEA (cont.) - 100 µg/L	1.01 (1.00-1.02)	0.006	0.95 (0.83-1.10)	0.53	1.01 (1.00-1.02)	0.09	1.02 (0.91-1.15)	0.71
Resection margin involved - yes vs no	1.83 (1.36-2.47)	<0.001	1.87 (1.01-3.47)	0.05	1.84 (1.47-2.31)	<0.001	1.63 (1.07-2.46)	0.02
Extrahepatic disease - yes vs no	1.63 (1.15-2.29)	0.005	1.49 (0.81-2.76)	0.2	1.85 (1.44-2.38)	<0.001	2.16 (1.41-3.29)	<0.001
Preoperative chemotherapy - yes vs no	1.25 (0.96-1.62)	0.1	1.44 (0.82-2.51)	0.2	1.45 (1.20-1.74)	<0.001	0.98 (0.68-1.41)	0.91
KRAS status - mutant vs wildtype	1.55 (1.11-2.18)	0.01	2.21 (1.33-3.65)	0.002	1.33 (1.04-1.70)	0.03	1.43 (1.03-1.98)	0.03
BRAF status - mutant vs wildtype	1.59 (0.58-4.37)	0.37	3.42 (1.00-11.71)	0.05	1.08 (0.53-2.23)	0.83	1.03 (0.39-2.72)	0.95
Desmoplastic phenotype - yes vs no	0.39 (0.27-0.56)	<0.001	0.43 (0.20-0.92)	0.03	0.44 (0.35-0.56)	<0.001	0.42 (0.25-0.70)	<0.001

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

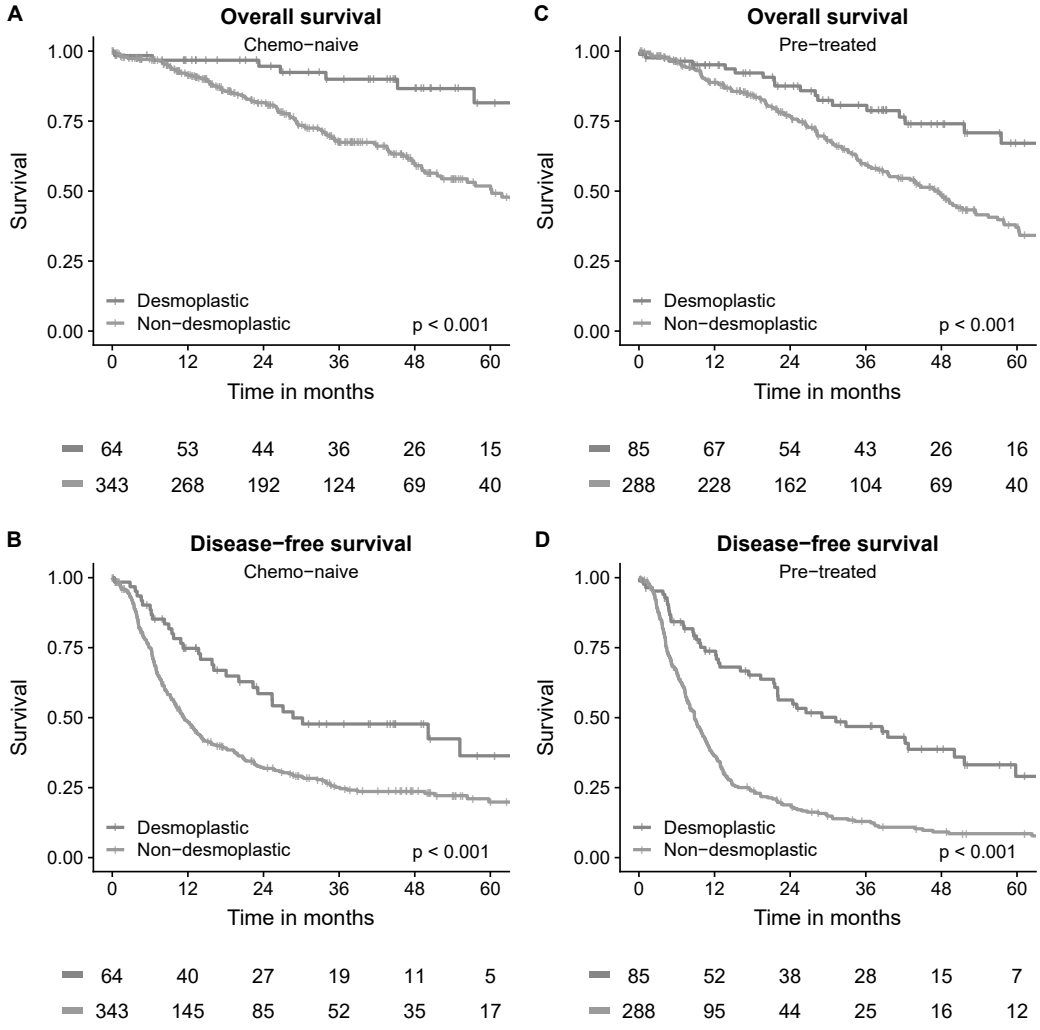


Figure 3. Kaplan-Meier overall and disease-free survival estimates stratified by preoperative chemotherapy are shown. Figures A and B display the overall (A) and disease-free (B) survival estimates for chemo-naive patients with a desmoplastic versus a non-desmoplastic phenotype. Figures C and D display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

Discussion

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

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

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

Besides implications for HGP assessment and postoperative prognosis, this observation is also interesting from a cancer biology perspective as it suggests that HGPs can be regarded as a binary biological switch. While this paper does not provide a clear indication for the actual underlying process, in the 23% of patients with available data we did observe a statistically significant association between MSI and a desmoplastic phenotype. Because of their genetic hypermutability MSI tumors express more mutational neoantigens which can become targets for T cells.[18,19] The more potential immune targets are present, the more likely an effective antitumor response can be elicited.[19] This is why MSI tumors are thought to form metastases less often and why MSI represents the only indication for systemic immunotherapy in metastatic CRC so far.[20,21] Since MSI tumors only accounted for 15% of patients with a desmoplastic phenotype in our study, a desmoplastic HGP could reflect more a state of (hepatic) anticancer immunity. This is supported by several other studies which demonstrated that a desmoplastic phenotype was associated with an enrichment of immune cells in the tumor microenvironment, specifically CD8+ T cells.[5,6] One

could therefore hypothesize that a non-desmoplastic histopathological phenotype, observed in however small a quantity, may be a reflection of the tumor's intrinsic or obtained ability to evade the anticancer immune response. Our study is however at serious risk of selection bias regarding availability of MSI status and validation should therefore be pursued, as well as research into the other biological and immunological aspects of these histopathological phenotypes.

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

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

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

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

References

1 Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. 2006;244(2):254-259.

2 Kanas GP, Taylor A, Primrose JN, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. Clin Epidemiol. 2012;4:283-301.

3 Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. 2001;195(3):336-342.

4 Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med. 2016;22(11):1294-1302.

5 Höppener DJ, Nierop PMH, Hof J, et al. Enrichment of the tumour immune microenvironment in patients with desmoplastic colorectal liver metastasis. Br J Cancer. 2020.

6 Brunner SM, Kesselring R, Rubner C, et al. Prognosis according to histochemical analysis of liver metastases removed at liver resection. Br J Surg. 2014;101(13):1681-1691.

7 Galjart B, Nierop PMH, van der Stok EP, et al. Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases. Angiogenesis. 2019;22(2):355-368.

8 Buisman FE, Van der Stok EP, Galjart B, et al. Histopathological growth patterns as biomarker for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases. Clin Exp Metastasis. 2020.

9 Hoppener DJ, Nierop PMH, Herpel E, et al. Histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy. Clin Exp Metastasis. 2019.

10 Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration. J Natl Cancer Inst. 2018;110(8):803-811.

11 Groot Koerkamp B, Sadot E, Kemeny NE, et al. Perioperative Hepatic Arterial Infusion Pump Chemotherapy Is Associated With Longer Survival After Resection of Colorectal Liver Metastases: A Propensity Score Analysis. J Clin Oncol. 2017;35(17):1938-1944.

12 Buisman FE, Homs MYV, Grünhagen DJ, et al. Adjuvant hepatic arterial infusion pump chemotherapy and resection versus resection alone in patients with low-risk resectable colorectal liver metastases - the multicenter randomized controlled PUMP trial. BMC Cancer. 2019;19(1):327.

13 Buisman FE, Grünhagen DJ, Homs MYV, et al. Adjuvant Hepatic Arterial Infusion Pump Chemotherapy After Resection of Colorectal Liver Metastases: Results of a Safety and Feasibility Study in The Netherlands. Ann Surg Oncol. 2019;26(13):4599-4607.

14 Elfrink AKE, Kok NFM, van der Werf LR, et al. Population-based study on practice variation regarding preoperative systemic chemotherapy in patients with colorectal liver metastases and impact on short-term outcomes. Eur J Surg Oncol. 2020.

15 van Dam PJ, van der Stok EP, Teuwen LA, et al. International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer. 2017;117(10):1427-1441.

16 Morino T, Tanaka J, Tobe T. Clinico-pathological features of liver metastases from colorectal cancer in relation to prognosis. Nihon Geka Hokan. 1991;60(3):154-164.

17 Fernández Moro C, Bozóky B, Gerling M. Growth patterns of colorectal cancer liver metastases and their impact on prognosis: a systematic review. BMJ Open Gastroenterol. 2018;5(1):e000217.

18 Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138(6):2073-2087 e2073.

19 Chen DS, Mellman I. Elements of cancer immunity and the cancer–immune set point. Nature. 2017;541(7637):321-330.

20 Buckowitz A, Knaebel HP, Benner A, et al. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. Br J Cancer. 2005;92(9):1746-1753.

21 André T, Shiu K-K, Kim TW, et al. Pembrolizumab in Microsatellite-Instability–High Advanced Colorectal Cancer. New England Journal of Medicine. 2020;383(23):2207-2218.

22 Eefsen RL, Van den Eynden GG, Hoyer-Hansen G, et al. Histopathological growth pattern, proteolysis and angiogenesis in chemonaive patients resected for multiple colorectal liver metastases. J Oncol. 2012;2012:907971.

23 Eefsen RL, Vermeulen PB, Christensen IJ, et al. Growth pattern of colorectal liver metastasis as a marker of recurrence risk. Clin Exp Metastasis. 2015;32(4):369-381.

24 Nielsen K, Rolff HC, Eefsen RL, Vainer B. The morphological growth patterns of colorectal liver metastases are prognostic for overall survival. Mod Pathol. 2014;27(12):1641-1648.

25 Nierop PMH, Galjart B, Hoppener DJ, et al. Salvage treatment for recurrences after first resection of colorectal liver metastases: the impact of histopathological growth patterns. Clin Exp Metastasis. 2019;36(2):109-118.

26 Pinheiro RS, Herman P, Lupinacci RM, et al. Tumor growth pattern as predictor of colorectal liver metastasis recurrence. Am J Surg. 2014;207(4):493-498.

27 van Dam PJ, Daelemans S, Ross E, et al. Histopathological growth patterns as a candidate biomarker for immunomodulatory therapy. Semin Cancer Biol. 2018;52(Pt 2):86-93.

28 Van den Eynden GG, Bird NC, Majeed AW, Van Laere S, Dirix LY, Vermeulen PB. The histological growth pattern of colorectal cancer liver metastases has prognostic value. Clin Exp Metastasis. 2012;29(6):541-549.

29 Nierop PMH, Höppener DJ, van der Stok EP, et al. Histopathological growth patterns and positive margins after resection of colorectal liver metastases. HPB (Oxford). 2019.

30 Hof J, Visser L, Höppener DJ, et al. B Cells as Prognostic Biomarker After Surgery for Colorectal Liver Metastases. Front Oncol. 2020;10:249.

31 Cheng J, Wei J, Tong T, et al. Prediction of Histopathologic Growth Patterns of Colorectal Liver Metastases with a Noninvasive Imaging Method. Ann Surg Oncol. 2019;26(13):4587-4598.

32 Kawaguchi Y, Kopetz S, Newhook TE, et al. Mutation Status of RAS, TP53, and SMAD4 is Superior to Mutation Status of RAS Alone for Predicting Prognosis after Resection of Colorectal Liver Metastases. Clin Cancer Res. 2019;25(19):5843-5851.

33 Gonsalves WI, Mahoney MR, Sargent DJ, et al. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. J Natl Cancer Inst. 2014;106(7).

34 Margonis GA, Buettner S, Andreatos N, et al. Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer. JAMA Surg. 2018;153(7):e180996.

35 Smith CG, Fisher D, Claes B, et al. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy +/- cetuximab. Clin Cancer Res. 2013;19(15):4104-4113.

36 Yaeger R, Cercek A, Chou JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. 2014;120(15):2316-2324.

37 Buisman FE, Galjart B, Buettner S, Groot Koerkamp B, Grünhagen DJ, Verhoef C. Primary tumor location and the prognosis of patients after local treatment of colorectal liver metastases: a systematic review and meta-analysis. HPB (Oxford). 2020;22(3):351-357.

Supplementary Table 1. Baseline characteristics stratified by treatment center

		Missing (%)	Erasmus MC n = 213 (%)	MSKCC n = 338 (%)	Radboud UMC n = 229 (%)	P-value
Age at resection CRLM (median [IQR])			65.0 [58.0, 71.0]	61.0 [52.0, 72.0]	67.0 [60.0, 73.0]	<0.001
Gender	Male		146 (69)	175 (52)	145 (63)	<0.001
	Female		67 (31)	163 (48)	84 (37)	
ASA classification	ASA I-II	4 (1)	187 (88)	93 (28)	184 (81)	<0.001
	ASA >II		25 (12)	244 (72)	43 (19)	
Primary tumor location	Left-sided	24 (3)	87 (42)	132 (41)	84 (38)	0.1
	Right-sided		48 (23)	101 (31)	58 (26)	
	Rectal		74 (35)	90 (28)	82 (37)	
T-stage	pT 0-2	56 (7)	28 (13)	31 (11)	38 (17)	0.15
	pT 3-4		182 (87)	256 (89)	189 (83)	
N-stage	N0	10 (1)	86 (41)	118 (35)	80 (35)	0.36
	N+		124 (59)	215 (65)	147 (65)	
Number of CRLM (median [IQR])		2 (0)	2.0 [1.0, 4.0]	2.0 [1.0, 3.0]	1.0 [1.0, 3.0]	<0.001
Diameter of largest CRLM in cm (median [IQR])		3 (0)	2.8 [1.9, 4.5]	2.8 [2.0, 4.5]	2.8 [1.9, 4.3]	0.67
Disease-free interval in months* (median [IQR])		11 (1)	0.0 [0.0, 11.0]	0.0 [0.0, 19.0]	6.0 [0.0, 18.0]	0.004
Preoperative CEA in µg/L (median [IQR])		65 (8)	11.3 [4.5, 33.5]	8.6 [3.4, 25.9]	10.0 [3.8, 30.0]	0.28
Preoperative systemic chemotherapy	No		135 (63)	103 (30)	169 (74)	<0.001
	Yes		78 (37)	235 (70)	60 (26)	
Resection margin involved	No	1 (0)	179 (84)	294 (87)	204 (89)	0.35
	Yes		33 (16)	44 (13)	25 (11)	
Extrahepatic disease	No		190 (89)	283 (84)	223 (97)	<0.001
	Yes		23 (11)	55 (16)	6 (3)	
KRAS mutational status	Wildtype	450 (58)	24 (50)	131 (56)	29 (60)	0.59
	Mutant		24 (50)	103 (44)	19 (40)	
BRAF mutational status	Wildtype	491 (63)	43 (96)	198 (97)	38 (95)	0.75
	Mutant		2 (4)	6 (3)	2 (5)	
Microsatellite stability status	MSS	600 (77)	54 (96)	60 (91)	55 (95)	0.42
	MSI		2 (4)	6 (9)	3 (5)	
Histopathological phenotype	Desmoplastic		45 (21)	63 (19)	41 (18)	0.66
	Non-desmoplastic		168 (79)	275 (81)	188 (82)	

* Between resection of primary tumor and detection of CRLM

Abbreviations in alphabetical order: ASA: American Society of Anesthesiologists; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis; Erasmus MC: Erasmus MC Cancer Institute; IQR: interquartile range; MSI: microsatellite instable; MSKCC: Memorial Sloan Kettering Cancer Center; MSS: microsatellite stable; Radboud UMC: Radboud University Medical Center.

Supplementary Table 2. Uni- and multivariable Cox regression models of cut-off analyses for overall and disease-free survival

	Overall survival				Disease-free survival			
	Univariable		Multivariable (n=625)		Univariable		Multivariable (n=625)	
	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value
Age at resection CRLM - years	1.01 [1.00-1.02]	0.01	1.01 [1.00-1.02]	0.11	1.00 [0.99-1.00]	0.34	1.00 [0.99-1.01]	0.94
ASA classification - >II vs I-II	1.26 [0.94-1.71]	0.13	1.27 [0.88-1.84]	0.2	1.14 [0.91-1.41]	0.25	1.21 [0.94-1.56]	0.13
Right-sided primary - yes vs no	1.46 [1.13-1.88]	0.004	1.37 [1.00-1.88]	0.05	1.05 [0.86-1.27]	0.65	1.04 [0.82-1.31]	0.75
T-stage - pT3-4 vs pT0-2	1.36 [0.92-2.00]	0.12	1.28 [0.82-2.00]	0.28	1.24 [0.95-1.61]	0.11	1.09 [0.81-1.46]	0.57
N-stage - N+ vs N0	1.18 [0.93-1.51]	0.18	1.23 [0.91-1.66]	0.19	1.29 [1.08-1.55]	0.005	1.25 [1.01-1.54]	0.04
Disease-free interval* (cont.) - months	1.00 [0.99-1.01]	0.65	1.00 [0.99-1.01]	0.71	0.99 [0.99-1.00]	0.01	0.99 [0.98-1.00]	0.01
Number of CRLM (cont.)	1.10 [1.06-1.15]	<0.001	1.09 [1.04-1.14]	<0.001	1.11 [1.08-1.15]	<0.001	1.08 [1.04-1.12]	<0.001
Diameter of largest CRLM (cont.) - cm	1.06 [1.03-1.10]	<0.001	1.07 [1.02-1.11]	0.005	1.06 [1.03-1.09]	<0.001	1.05 [1.01-1.09]	0.008
Preoperative CEA (cont.) - 100 µg/L	1.01 [1.00-1.02]	0.006	1.01 [1.00-1.02]	0.03	1.01 [1.00-1.02]	0.09	1.01 [1.00-1.02]	0.24
Resection margin involved - yes vs no	1.83 [1.36-2.47]	<0.001	1.23 [0.85-1.78]	0.27	1.84 [1.47-2.31]	<0.001	1.45 [1.10-1.91]	0.008
Extrahepatic disease - yes vs no	1.63 [1.15-2.29]	0.005	1.62 [1.07-2.45]	0.02	1.85 [1.44-2.38]	<0.001	2.19 [1.62-2.95]	<0.001
Preoperative chemotherapy - yes vs no	1.25 [0.96-1.62]	0.1	1.27 [0.93-1.73]	0.13	1.45 [1.20-1.74]	<0.001	1.25 [1.00-1.55]	0.05
Desmoplastic phenotype	Reference		Reference		Reference		Reference	
0.1-33% non-desmoplastic	2.53 [1.68-3.82]	<0.001	2.90 [1.75-4.82]	<0.001	2.49 [1.89-3.27]	<0.001	2.07 [1.51-2.85]	<0.001
33.1-67% non-desmoplastic	2.15 [1.37-3.36]	<0.001	2.30 [1.33-3.97]	0.003	2.02 [1.48-2.74]	<0.001	1.82 [1.27-2.60]	0.001
67.1-100% non-desmoplastic	2.80 [1.91-4.11]	<0.001	2.89 [1.77-4.73]	<0.001	2.24 [1.72-2.91]	<0.001	2.07 [1.51-2.82]	<0.001

* Between resection of primary tumor and detection of CRLM

Abbreviations in alphabetical order: ASA: American Society of Anesthesiologists; Cont.: entered as continuous variable; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis.

Supplementary Table 3. Uni- and multivariable Cox regression analyses for overall and disease-free survival within the chemo-naive subpopulations

	Overall survival				Disease-free survival			
	Univariable		Multivariable (n=352)		Univariable		Multivariable (n=352)	
	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value
Age at resection CRLM - years	1.02 [1.00-1.04]	0.02	1.02 [1.00-1.05]	0.05	1.00 [0.99-1.01]	0.78	1.00 [0.99-1.02]	0.47
ASA classification - >II vs I-II	1.57 [0.97-2.55]	0.07	1.47 [0.87-2.48]	0.15	1.27 [0.92-1.76]	0.14	1.24 [0.88-1.75]	0.22
Right-sided primary - yes vs no	1.65 [1.12-2.44]	0.01	1.36 [0.86-2.15]	0.19	1.22 [0.93-1.59]	0.16	1.12 [0.82-1.53]	0.47
T-stage - pT3-4 vs pT0-2	2.07 [1.11-3.86]	0.02	1.75 [0.89-3.43]	0.11	1.58 [1.07-2.33]	0.02	1.41 [0.93-2.13]	0.1
N-stage - N+ vs N0	1.32 [0.92-1.89]	0.14	1.41 [0.92-2.16]	0.11	1.27 [0.99-1.63]	0.06	1.32 [1.00-1.76]	0.05
Disease-free interval* (cont.) - months	1.00 [0.99-1.01]	0.43	1.00 [0.99-1.02]	0.51	0.99 [0.99-1.00]	0.15	0.99 [0.98-1.00]	0.1
Number of CRLM (cont.)	1.08 [1.00-1.17]	0.06	1.08 [0.99-1.17]	0.09	1.16 [1.09-1.22]	<0.001	1.14 [1.07-1.22]	<0.001
Diameter of largest CRLM (cont.) - cm	1.07 [1.01-1.13]	0.02	1.08 [1.01-1.16]	0.03	1.04 [0.99-1.09]	0.11	1.06 [1.00-1.13]	0.05
Preoperative CEA (cont.) - 100 µg/L	1.05 [1.01-1.11]	0.03	1.04 [0.98-1.10]	0.24	1.02 [0.97-1.07]	0.43	0.99 [0.93-1.06]	0.88
Resection margin involved - yes vs no	1.32 [0.76-2.27]	0.32	1.25 [0.67-2.34]	0.49	1.63 [1.12-2.38]	0.01	1.63 [1.07-2.48]	0.02
Extrahepatic disease - yes vs no	1.61 [0.85-3.04]	0.15	1.63 [0.76-3.49]	0.21	2.01 [1.34-3.03]	<0.001	1.69 [1.07-2.69]	0.03
Desmoplastic phenotype - yes vs no	0.34 [0.18-0.64]	<0.001	0.29 [0.13-0.65]	0.003	0.49 [0.33-0.72]	<0.001	0.53 [0.34-0.82]	0.005

* Between resection of primary tumor and detection of CRLM

Abbreviations in alphabetical order: ASA: American Society of Anesthesiologists; Cont.: entered as continuous variable; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis.

Supplementary Table 4. Uni- and multivariable Cox regression analyses for overall and disease-free survival within the pre-treated subpopulations

	Overall survival				Disease-free survival			
	Univariable		Multivariable (n=273)		Univariable		Multivariable (n=273)	
	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value
Age at resection CRLM - years	1.01 [1.00-1.02]	0.06	1.01 [0.99-1.03]	0.22	1.00 [0.99-1.01]	0.49	1.00 [0.98-1.01]	0.7
ASA classification - >II vs I-II	1.17 [0.80-1.72]	0.42	1.01 [0.60-1.71]	0.97	1.05 [0.79-1.41]	0.73	1.16 [0.79-1.70]	0.45
Right-sided primary - yes vs no	1.54 [1.09-2.18]	0.01	1.46 [0.93-2.30]	0.1	0.95 [0.72-1.25]	0.69	0.92 [0.65-1.31]	0.65
T-stage - pT3-4 vs pT0-2	1.01 [0.60-1.71]	0.96	1.01 [0.52-1.95]	0.98	0.88 [0.61-1.27]	0.5	0.76 [0.48-1.19]	0.23
N-stage - N+ vs N0	1.06 [0.76-1.49]	0.73	1.14 [0.72-1.80]	0.59	1.19 [0.92-1.55]	0.18	1.18 [0.84-1.65]	0.34
Disease-free interval* (cont.) - months	1.00 [0.98-1.01]	0.38	0.98 [0.97-1.00]	0.07	1.00 [0.99-1.00]	0.23	0.99 [0.98-1.00]	0.09
Number of CRLM (cont.)	1.11 [1.06-1.16]	<0.001	1.09 [1.03-1.16]	0.004	1.09 [1.05-1.13]	<0.001	1.05 [1.00-1.10]	0.05
Diameter of largest CRLM (cont.) - cm	1.07 [1.02-1.12]	0.006	1.06 [0.99-1.13]	0.1	1.06 [1.03-1.10]	<0.001	1.05 [1.00-1.11]	0.05
Preoperative CEA (cont.) - 100 µg/L	1.01 [1.00-1.02]	0.03	1.01 [1.00-1.02]	0.05	1.01 [1.00-1.01]	0.18	1.00 [0.99-1.01]	0.53
Resection margin involved - yes vs no	2.11 [1.45-3.09]	<0.001	1.31 [0.79-2.18]	0.29	1.89 [1.41-2.53]	<0.001	1.73 [1.17-2.55]	0.006
Extrahepatic disease - yes vs no	1.67 [1.11-2.52]	0.01	2.23 [1.31-3.81]	0.003	1.60 [1.16-2.20]	0.004	2.62 [1.73-3.98]	<0.001
Desmoplastic phenotype - yes vs no	0.41 [0.26-0.65]	<0.001	0.43 [0.23-0.79]	0.007	0.37 [0.27-0.52]	<0.001	0.43 [0.29-0.64]	<0.001

* Between resection of primary tumor and detection of CRLM

Abbreviations in alphabetical order: ASA: American Society of Anesthesiologists; Cont.: entered as continuous variable; CEA: carcinoembryonic antigen; CRLM: colorectal liver metastasis.

CHAPTER EIGHT

CHAPTER EIGHT

SALVAGE TREATMENT FOR RECURRENCES AFTER FIRST RESECTION OF COLORECTAL LIVER METASTASES: THE IMPACT OF HISTOPATHOLOGICAL GROWTH PATTERNS

Boris Galjart*, Pieter M. H. Nierop*, Diederik J. Höppener, Eric P. van der Stok, Robert R. J. Coebergh van den Braak, Peter B. Vermeulen, Dirk J. Grünhagen, Cornelis Verhoef

* *Shared first authorship*

Published in Clinical and Experimental Metastasis

Abstract

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. 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. 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. 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 vs. 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 5 years after resection of CLRM. The follow-up consists of carcinoembryonic antigen (CEA) monitoring every 3 months for the entire follow-up duration and imaging every 6 months in the first 3 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 6 months again undergoing local treatment for the recurrence have poor survival outcomes.[25] Therefore, when patients present with recurrent disease within 6 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 6 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 20% 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).

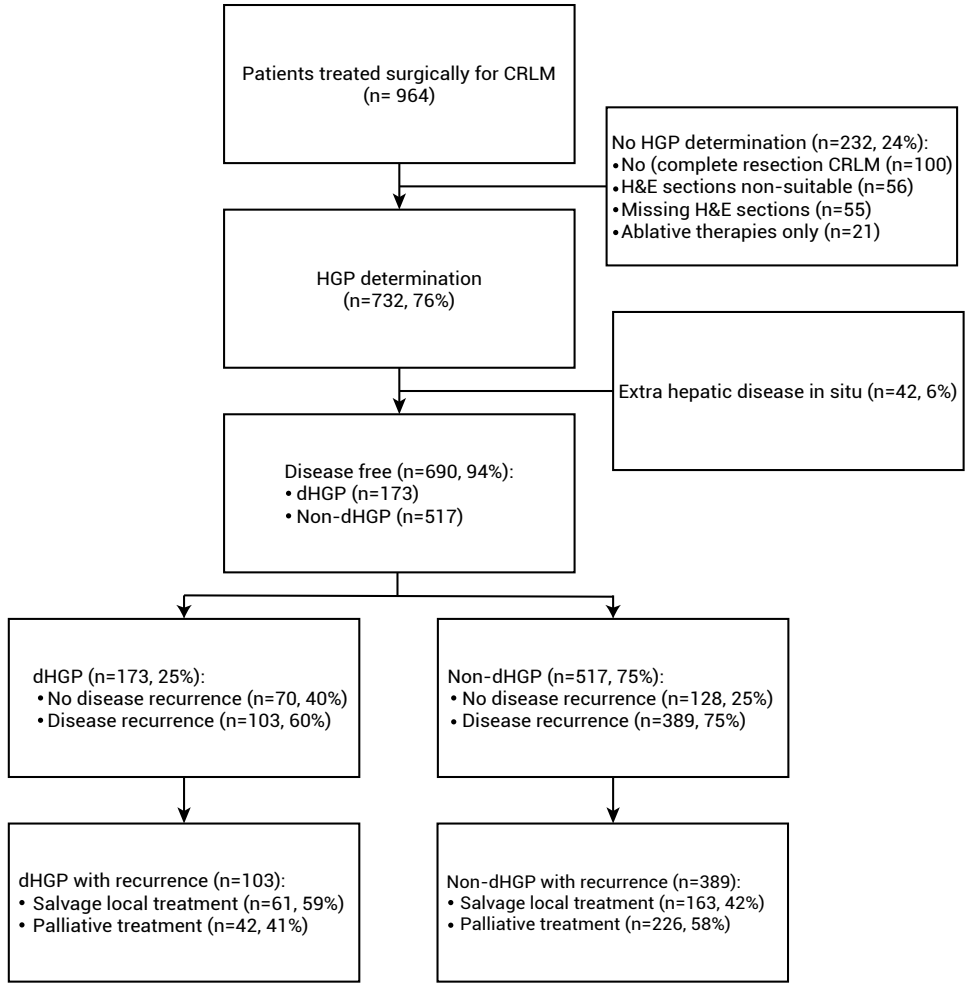


Figure 1: Flowchart of patient selection.

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 Fig. 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).

Table 1: Baseline characteristics of all patients stratified for HGP.

		Total N=690	dHGP N=173	Non-dHGP N=517	P-value	Missing N
Male gender		445 (65%)	109 (63%)	336 (65%)	0.637	
Age (median (IQR))		65 (58–71)	65 (56–72)	64 (58–71)	0.984	
ASA > II		63 (9%)	19 (11%)	44 (9%)	0.351	10
Primary tumour characteristics						
Location	Right-sided	84 (17%)	20 (19%)	64 (17%)	0.313	
	Left-sided	205 (42%)	44 (43%)	161 (41%)		
	Rectum	193 (39%)	35 (34%)	158 (41%)		
	Double	10 (2%)	4 (4%)	6 (2%)		
pT3-4 stage		546 (80%)	132 (77%)	414 (81%)	0.239	10
pN+ stage		407 (60%)	90 (53%)	317 (62%)	0.035*	13
Adjuvant chemotherapy		103 (15%)	13 (8%)	90 (17%)	0.002*	
CRLM characteristics						
Synchronous CRLM		361 (52%)	253 (49%)	108 (62%)	0.002*	
Disease-free interval (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 (median (IQR))		3.1 (2.0-4.5)	2.5 (1.8–4.2)	3.3 (2.3–4.8)	<0.001*	2
Preoperative CEA (median (IQR))		14.0 (4.7–50.0)	7.6 (3.2–30.0)	16.2 (5.1–53.0)	<0.001*	28
Fong CRS high (3-5)		262 (39%)	64 (39%)	198 (39%)	0.924	20
Bilobar metastases		272 (39%)	67 (39%)	205 (40%)	0.83	
Preoperative CTx		325 (47%)	105 (61%)	220 (43%)	<0.001*	
Resection margin		102 (15%)	14 (8%)	88 (17%)	0.004*	3
Extra Hepatic Disease		61 (9%)	16 (9%)	45 (9%)	0.827	
Major liver resection		235 (34%)	51 (30%)	184 (36%)	0.142	
Disease recurrence		492 (71%)	103 (60%)	389 (75%)	<0.001*	

* Indicates significant P-value

ASA = American Society of Anaesthesiologists, CEA = carcinoembryonic antigen, CRLM = colorectal liver metastasis, CRS = clinical risk score, CTx = chemotherapy, HGP = histopathological growth patterns, 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 ($p < 0.001$, Fig. 2).

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 6 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).

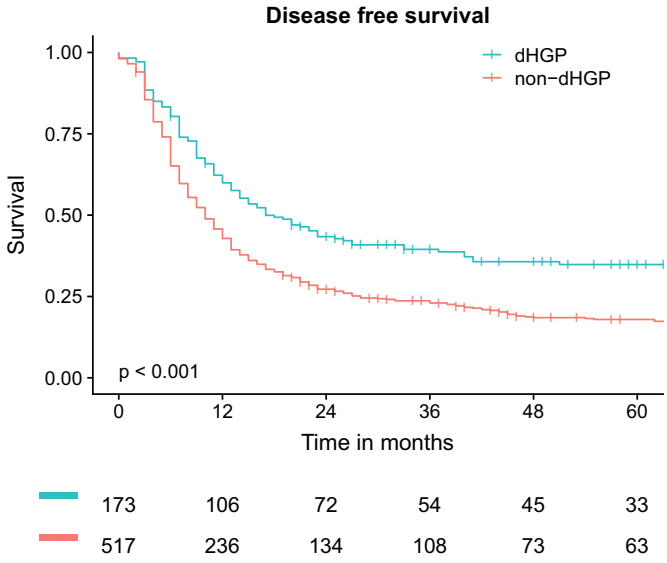


Figure 2: DFS after first hepatic resection for CRLM compared for HGP.

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, $p < 0.001$).

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).

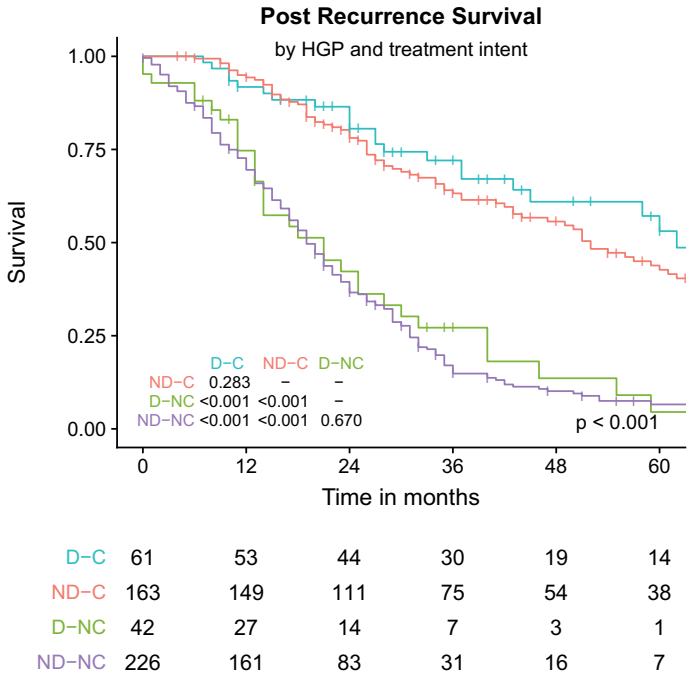


Figure 3: PRS compared for HGP and treatment intent of the recurrence.

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 noncurative intent

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%)	
PC 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%)	
PC 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*

* Indicates significant P-value

dHGP = desmoplastic histopathological growth pattern, PC = peritoneal carcinomatosis

Table 3: Logistic regression for salvageable recurrence.

	Univariable Odds Ratio [95% CI]	P-value	Multivariable 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]	0.27		
Preoperative chemotherapy	1.210 [0.849-1.727]	0.292		
R1 resection CRLM	0.988 [0.616-1.584]	0.959		
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*

* Indicates significant P-value

ASA= American Society of Anaesthesiologists, CEA = carcinoembryonic antigen, cont. = continuous, CRLM = colorectal liver metastases, R1 = irradical resection margin

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 hypothesize 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 reported on the recurrence pattern in the context of HGPs but did not find an association.[18] 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 and BRAF mutations.[37-40] 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.

References

1 de Jong MC, Pulitano C, Ribero D, et al. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. Ann Surg. Sep 2009;250(3):440-448.

2 Galjart B, van der Stok EP, Rothbarth J, Grünhagen DJ, Verhoef C. Posttreatment Surveillance in Patients with Prolonged Disease-Free Survival After Resection of Colorectal Liver Metastasis. Ann Surg Oncol. Nov 2016;23(12):3999-4007.

3 Hamady ZZ, Lodge JP, Welsh FK, et al. One-millimeter cancer-free margin is curative for colorectal liver metastases: a propensity score case-match approach. Ann Surg. Mar 2014;259(3):543-548.

4 Okuno M, Goumard C, Kopetz S, et al. RAS Mutation is Associated with Unsalvageable Recurrence Following Hepatectomy for Colorectal Cancer Liver Metastases. Ann Surg Oncol. Aug 2018;25(8):2457-2466.

5 Tomlinson JS, Jarnagin WR, DeMatteo RP, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol. Oct 10 2007;25(29):4575-4580.

6 Hof J, Wertenbroek MW, Peeters PM, Widder J, Sieders E, de Jong KP. Outcomes after resection and/or radiofrequency ablation for recurrence after treatment of colorectal liver metastases. Br J Surg. Jul 2016;103(8):1055-1062.

7 Oba M, Hasegawa K, Shindoh J, et al. Survival benefit of repeat resection of successive recurrences after the initial hepatic resection for colorectal liver metastases. Surgery. Feb 2016;159(2):632-640.

8 Butte JM, Gönen M, Allen PJ, et al. Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection. Ann Surg Oncol. Aug 2015;22(8):2761-2771.

9 Andreou A, Brouquet A, Abdalla EK, Aloia TA, Curley SA, Vauthey JN. Repeat hepatectomy for recurrent colorectal liver metastases is associated with a high survival rate. HPB (Oxford). Nov 2011;13(11):774-782.

10 Mise Y, Imamura H, Hashimoto T, et al. Cohort study of the survival benefit of resection for recurrent hepatic and/or pulmonary metastases after primary hepatectomy for colorectal metastases. Ann Surg. May 2010;251(5):902-909.

11 Shaw IM, Rees M, Welsh FK, Bygrave S, John TG. Repeat hepatic resection for recurrent colorectal liver metastases is associated with favourable long-term survival. Br J Surg. Apr 2006;93(4):457-464.

12 Wicherts DA, de Haas RJ, Salloum C, et al. Repeat hepatectomy for recurrent colorectal metastases. Br J Surg. May 2013;100(6):808-818.

13 Viganò L, Capussotti L, Lapointe R, et al. Early recurrence after liver resection for colorectal metastases: risk factors, prognosis, and treatment. A LiverMetSurvey-based study of 6,025 patients. Ann Surg Oncol. Apr 2014;21(4):1276-1286.

14 Schmidt T, Nienhüser H, Kuna C, et al. Prognostic indicators lose their value with repeated resection of colorectal liver metastases. Eur J Surg Oncol. Oct 2018;44(10):1610-1618.

15 Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. Oct 2001;195(3):336-342.

16 van Dam PJ, van der Stok EP, Teuwen LA, et al. International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer. Nov 7 2017;117(10):1427-1441.

17 Brunner SM, Kesselring R, Rubner C, et al. Prognosis according to histochemical analysis of liver metastases removed at liver resection. Br J Surg. Dec 2014;101(13):1681-1691.

18 Eefsen RL, Vermeulen PB, Christensen IJ, et al. Growth pattern of colorectal liver metastasis as a marker of recurrence risk. Clin Exp Metastasis. Apr 2015;32(4):369-381.

19 Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med. Nov 2016;22(11):1294-1302.

20 Nielsen K, Rolff HC, Eefsen RL, Vainer B. The morphological growth patterns of colorectal liver metastases are prognostic for overall survival. Mod Pathol. Dec 2014;27(12):1641-1648.

21 Siriwardana PN, Luong TV, Watkins J, et al. Biological and Prognostic Significance of the Morphological Types and Vascular Patterns in Colorectal Liver Metastases (CRLM): Looking Beyond the Tumor Margin. Medicine (Baltimore). Feb 2016;95(8):e2924.

22 Van den Eynden GG, Bird NC, Majeed AW, Van Laere S, Dirix LY, Vermeulen PB. The histological growth pattern of colorectal cancer liver metastases has prognostic value. Clin Exp Metastasis. Aug 2012;29(6):541-549.

23 Galjart B, Nierop PMH, van der Stok EP, et al. Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases. Angiogenesis. May 2019;22(2):355-368.

24 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. Lancet Oncol. Nov 2013;14(12):1208-1215.

25 van der Pool AE, Lalmahomed ZS, de Wilt JH, Eggermont AM, Ijzermans JM, Verhoef C. Local treatment for recurrent colorectal hepatic metastases after partial hepatectomy. J Gastrointest Surg. May 2009;13(5):890-895.

26 Hill CR, Chagpar RB, Callender GG, et al. recurrence following hepatectomy for metastatic colorectal cancer: development of a model that predicts patterns of recurrence and survival. Ann Surg Oncol. Jan 2012;19(1):139-144.

27 Hadden WJ, de Reuver PR, Brown K, Mittal A, Samra JS, Hugh TJ. Resection of colorectal liver metastases and extra-hepatic disease: a systematic review and proportional meta-analysis of survival outcomes. HPB (Oxford). Mar 2016;18(3):209-220.

28 Hwang M, Jayakrishnan TT, Green DE, et al. Systematic review of outcomes of patients undergoing resection for colorectal liver metastases in the setting of extra hepatic disease. Eur J Cancer. Jul 2014;50(10):1747-1757.

29 Leung U, Gönen M, Allen PJ, et al. Colorectal Cancer Liver Metastases and Concurrent Extrahepatic Disease Treated With Resection. Ann Surg. Jan 2017;265(1):158-165.

30 Chua TC, Saxena A, Liauw W, Chu F, Morris DL. Hepatectomy and resection of concomitant extrahepatic disease for colorectal liver metastases--a systematic review. Eur J Cancer. Aug 2012;48(12):1757-1765.

31 Adam R, de Haas RJ, Wicherts DA, et al. Concomitant extrahepatic disease in patients with colorectal liver metastases: when is there a place for surgery? Ann Surg. Feb 2011;253(2):349-359.

32 Pulitanò C, Bodingbauer M, Aldrighetti L, et al. Liver resection for colorectal metastases in presence of extrahepatic disease: results from an international multi-institutional analysis. Ann Surg Oncol. May 2011;18(5):1380-1388.

33 Ayez N, van der Stok EP, Grünhagen DJ, et al. The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator. Eur J Surg Oncol. Jul 2015;41(7):859-867.

34 Rahbari NN, Reissfelder C, Schulze-Bergkamen H, et al. Adjuvant therapy after resection of colorectal liver metastases: the predictive value of the MSKCC clinical risk score in the era of modern chemotherapy. BMC Cancer. Mar 11 2014;14:174.

35 Groot Koerkamp B, Sadot E, Kemeny NE, et al. Perioperative Hepatic Arterial Infusion Pump Chemotherapy Is Associated With Longer Survival After Resection of Colorectal Liver Metastases: A Propensity Score Analysis. J Clin Oncol. Jun 10 2017;35(17):1938-1944.

36 Höppener DJ, Nierop PMH, Herpel E, et al. Histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy. Clin Exp Metastasis. Aug 2019;36(4):311-319.

37 Gonsalves WI, Mahoney MR, Sargent DJ, et al. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. J Natl Cancer Inst. Jul 2014;106(7).

38 Smith CG, Fisher D, Claes B, et al. Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy ± cetuximab. Clin Cancer Res. Aug 1 2013;19(15):4104-4113.

39 Margonis GA, Buettner S, Andreatos N, et al. Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer. JAMA Surg. Jul 18 2018;153(7):e180996.

40 Yaeger R, Cercek A, Chou JF, et al. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. Cancer. Aug 1 2014;120(15):2316-2324.

CHAPTER NINE

CHAPTER NINE

GENERAL DISCUSSION, SUMMARY AND FUTURE PERSPECTIVES

General discussion, summary and future perspectives

Within the two parts of this thesis, detection, treatment and prognostication of CRC recurrences were studied. The overarching targets of this thesis were to individualize follow-up and treatment strategies (Part I), and to evaluate histopathological growth patterns as a novel risk factor for CRLM (Part II).

Part I: Individualize follow-up and treatment after surgery for (metastatic) colorectal cancer

Non-metastatic colorectal cancer

Surveillance after resection of CRC has been studied extensively, with several high quality randomized controlled trials being available.[1-4] There has been a longstanding interest in follow-up for CRC, for several reasons. In contrast to most cancer types, local treatments (e.g. resection, ablation, radiotherapy) for isolated recurrences are frequently performed and considered potentially curative, making early detection appealing.[5,6] In addition, a relatively large proportion of CRC patients will develop recurrent disease.[7-9] Despite the clear rationale behind CRC follow-up, 14 out of the 16 performed randomized controlled trials failed to demonstrate a significant survival benefit after intensified surveillance, including the largest and most recent trials.[1-4] This is in line with the results presented in **chapter 2**. Although it seems as if intensive follow-up does not improve survival outcomes, intensive surveillance does increase curative intent treatment rates for recurrences after resection of CRC.

Follow-up schemes are more or less identical for all patients with non-metastatic CRC, despite considerable variability in terms of prognosis and probability of relapses.[10-12] Efforts to individualize follow-up schedules have failed for now. Intensive follow-up did not prove to be more effective in patients with advanced tumour stages, despite an increased risk of recurrences.[1,13] And although the site of the primary tumour (i.e. rectum or colon) results in differential patterns of recurrences, this has little impact on the effectiveness of intensive follow-up. Preoperative CEA has been another factor of interest with regards to follow-up. Thirty to fifty percent of the CRC population presents with low CEA values upon diagnosis, and it is often believed that sequential CEA measurements are less sensitive in this subgroup of patients.[14,15] Nevertheless, frequent imaging during follow-up has no impact on overall and cancer-specific survival outcomes in CEA negative patients, as shown in **chapter 3**. As of now, postoperative follow-up intensity for CRC cannot be tailored based on current risk factors.

European and United States guidelines recommend intensive follow-up protocols. According to the *American Society of Clinical Oncology* and the *European Society for Medical Oncology*, imaging (i.e. CT scans of the thorax and abdomen) should be performed every six months during the initial years after resection. Clinical evaluations and CEA level measurements should be performed even more frequent.[10-12] These guidelines appear counterintuitive in light of the available literature, and highlight the ongoing debate surrounding follow-up. This debate can be explained by the complexity of the data and study limitations. The latter mainly concerns a lack of statistical power to actually

detect survival differences. Twenty percent of CRCs relapse after surgery, of which only 30 percent are suitable for curative treatment.[7,16] Five-year overall survival rates of patients treated with curative intent range from 20 to 50 percent.[5,17] Considering the outcomes of **chapter 3** (relative risk 1.25), intensive follow-up would increase curative treatment rates to approximately 40 percent. On a population basis, the impact of such an increase in curative treatment rates is largely diluted, as CRC in most patients does not recur at all. One could take the position that local treatment rate, rather than survival, is the most important and reliable outcome when evaluating follow-up. This would imply that intensive follow-up is effective. On the other hand, a hazard ratio of 0.99 suggests hardly any impact of intensive follow-up on survival, even when considering the potential issue of statistical power. This would imply that both low and high intensity follow-up schedules identify those patients that actually benefit from local treatments. Some individual countries are now taking the latter position and are reducing frequency of imaging,[1,3] shifting towards less intensive, CEA based follow-up, with one or two CT scans performed in total.

Colorectal liver metastasis

Follow-up for patients with resected CRLM is conducted in a similar fashion. A considerable proportion of patients with recurrent disease after resection of CRLM remains amenable for repeat salvage treatment, which again is the main reason for surveillance.[18] Follow-up for CRLM has been studied far less than for non-metastatic CRC. Jones et al. performed a meta-analysis, comparing intensive to less intensive surveillance and found no survival benefit of intensified strategies. [19] In contrast to non-metastatic CRC, these results are fully based on observational studies, underscoring the lack of high-quality evidence. Follow-up is carried out for a period of at least 5 years, which seems reasonable given the results in **chapter 4**.

In order to reduce recurrence rates during CRLM follow-up, additional (neo)adjuvant treatments are often administered. Although Dutch guidelines do not recommend systemic chemotherapy for patients with resectable CRLM, treatment with oxaliplatin- or irinotecan-based regimens remains the golden standard in many countries worldwide. Systemic chemotherapy improves disease-free survival, but has not been associated with longer overall survival in three randomized studies.[20-22] Hepatic arterial infusion chemotherapy is currently only provided in highly specialized centres, and may provide an overall survival benefit over systemic chemotherapy or no perioperative treatment.[23]

In **chapter 5** we demonstrate that extrahepatic disease recurrence risk after resection of CRLM predicts the effectiveness of (neo)adjuvant therapies. Systemic chemotherapy is most effective in patients at high risk of recurring outside the liver, while hepatic arterial infusion chemotherapy is least effective in this subgroup of patients. These observations are in line with several other studies, showing a significant overall survival benefit after systemic chemotherapy in patients with a high clinical risk score.[24,25] Groot Koerkamp et al. showed that the expected survival gain from hepatic arterial infusion chemotherapy is largest in patients with favourable oncological characteristics (e.g. node negative primary tumour).[26] All of this suggests that risk stratification for (neo)adjuvant therapy is possible in patients with resectable CRLM. Priorly, adequate validation of the findings in **chapter 5** is needed, but such validation remains complex. Retrospective patient cohorts with all modern-day risk factors incorporated are available, but convincing high level evidence from these cohorts will not be obtained, due to the risk of selection bias. In the Netherlands mostly patients with initially irresectable disease receive chemotherapy, while in other countries, patients with severe co-morbidities will be treated with resection alone. In each case, confounding by indication will considerably influence outcomes. Large multicentre and international databases will be needed. Another option would be to validate within randomized studies, but many of the new biomarkers are not available in the older studies. Analysis in ongoing randomized controlled trials therefore needs to be awaited.

Part II: Histopathological growth patterns of colorectal liver metastases

Disease recurrence rates after resection of CRLM greatly vary between patient subgroups.[5] In order to predict the risk of recurrence and death, several general and tumour characteristics are available. Many of these risk factors relate to the tumour load prior to surgery (i.e. size and number of CRLM, preoperative CEA level) or the primary tumour (i.e. lymph node status, time between surgery and detection of CRLM). These factors are often combined into risk scores, such as Fong's Clinical Risk Score or the Genetic and Morphological Evaluation score.[27,28] Despite being associated with survival, the predictive capacity of these models upon external validation is poor. As such, these scores have little to no impact on the clinical management of CRLM. A need for accurate biomarkers for patients with resectable CRLM therefore remains.[29,30]

Histopathological growth patterns (HGPs) have emerged as a new potential risk factor, and classify CRLM in three histological phenotypes (i.e. desmoplastic, replacement, pushing).[31] Several biological differences in vascularization and tumour immunology have been described, resulting in an impact on prognosis (**chapter 6 and 7**). Patients with desmoplastic CRLM seem to have a superior prognosis over the patients with non-desmoplastic CRLM. Five-year overall survival rates between 73 and 78 percent were observed among patients with desmoplastic CRLM. In line with these results, recurrence rates considerably differ between the both subgroups, with 50 percent of patients with desmoplastic CRLM remaining disease-free for five years or longer. Multiple studies have reported similar outcomes, also when applying different cut-offs.[32] These outcomes are remarkable, as they compare to patients with stage III CRC.[33] Besides an impact on survival, others have shown a potential relationship between HGPs and the effectiveness of chemotherapy. Adjuvant chemotherapy seems to be more effective in patients with non-desmoplastic HGP, which is why HGPs were incorporated in the risk score presented in chapter 5.[34] A differential pattern of recurrences between desmoplastic (more often liver-limited recurrence) and non-desmoplastic CRLM (more often multi-organ recurrences) seems to partially explain the differences in prognosis and treatment effectiveness (**chapter 8**).

In order to standardize HGP scoring, international consensus guidelines have been published in 2017.[35] As the three different HGPs can co-exist within one tumour and patient, a scoring system based on pre-dominancy was advocated. However, **chapter 6 and 7** showed that presence of any non-desmoplastic growth impairs prognosis, while the extent of non-desmoplastic growth does not. A novel classification system, in which patients are classified into desmoplastic (i.e. in all CRLM only desmoplastic type HGP was observed) or non-desmoplastic HGP (i.e. in any of the CRLM more than 1% non-desmoplastic type HGP was observed) has recently been adopted by the updated consensus guidelines.[36]

Alongside HGPs, several new risk factors for patients with resected CRLM have emerged over recent years. Genetic alterations have shown important impact on recurrence rates and survival outcomes. BRAF V600E mutations have been associated with poor prognosis,[37] and the same was observed for patients with various mutations in KRAS.[38] In addition, so-called liquid biopsies were introduced over recent years. The presence of both circulating tumour cells or circulating DNA is highly associated with disease recurrence and poor prognosis in CRC patients.[39] In comparison to the analysis of genetic alterations or liquid biopsies, the use of HGPs provides several advantages. These mainly relate to costs, reproducibility and accessibility. HGPs can be assessed on standard hematoxylin eosin slides within several minutes, and little heterogeneity between tumours and within a patient exists. Inter-observer variability is low, especially regarding the distinction between desmoplastic and non-desmoplastic tumours, and adequate scoring of HGPs can be reached after a short learning curve.[35,40] In contrast to other new biomarkers, this enables swift and broad

application of HGPs within clinical practice, with little to no additional expenses.

Despite being easily applicable and available, there are some barriers that limit the use of HGPs in the clinic for now. First and foremost, resection remains a pre-requisite for determination of HGPs. This largely limits the utility of HGPs, especially in countries in which adjuvant chemotherapy is not administered, such as the Netherlands. Second, we observed a potential impact of preoperative chemotherapy on HGPs, with a diminished prognostic impact in these patients. As approximately 30 percent of patients receives preoperative chemotherapy in the Netherlands,[41] often to convert CRLM to a resectable state, HGP assessment prior to the start of chemotherapy may be warranted. Chinese studies have shown promising results of a radiomics approach, using analysis of CT or MRI scans.[42,43] Both reached an area under the curve of over 0.9 in predicting the predominant HGP type, but have not yet been validated using the novel cut-off. In addition, studies are ongoing that relate primary tumour characteristics with the HGP type. Eventually, combining all sorts of approaches may need to be required for optimal prediction of HGP type prior to surgery.

Future perspectives

Follow-up and treatment of (metastatic) CRC
Important changes in the way patients are followed-up after resection of CRC may be expected over the coming years. First, as many CRC patients with recurrences still present with symptoms, patient reported outcomes measures (PROMs) may become increasingly important.[3] In stage II-IV lung cancer, PROMs have shown to facilitate earlier detection of disease progression, suggesting that these measures may be useful during CRC follow-up as well.[44] PROMs can be obtained digitally on regular time points and deterioration of PROMS over time could become a selection tool for further clinical evaluation. The use of PROMs would enable efficient, economical, comprehensive assessment of all aspects related to patient welfare and could easily be implemented in today's technological society. Second, CRC surveillance could (partly) be conducted out of hospital, with the general practitioner in a central role. General practitioner follow-up does not seem to impair survival in patients with CRC,[45-47] or other types of cancer.[48] One of the main arguments in favour of general practitioner led, out of hospital follow-up is the simultaneous management of non-cancer related health problems. As shown in several studies, general health dictates prognosis, especially in cancer patients at low risk of recurrence.[49] Adequate design of general practitioner-based follow-up might facilitate increased focus on general health issues, and thereby eventually even improve patient outcome and wellbeing. Third, guidelines should advocate a more patient-centred approach. Despite the lacking effectiveness in terms of survival, there are numerous reasons to retain follow-up, even partially in-hospital. A considerable part of patients has unmet needs during follow-up, but these needs vary widely between patients and may change over time.[50-52] As intensity of follow-up does not importantly impact survival outcomes, the frequency of evaluations should, to a certain point, be dependent on patients wishes. This tailored approach would ensure optimal follow-up care in a more cost-effective way, and is currently evaluated in the ongoing FUTURE trials. Fourth and last, sequential data gathered during follow-up may be used to discharge patients from follow-up earlier on. For now, CEA remains the only factor that is sequentially analysed during follow-up. Factors such as circulating DNA or PROMS may be used to continuously assess a patient's risk on recurrence during follow-up.[53,54] Combining the information from all of these biomarkers using high-end statistical modelling (e.g. joint models) may be used to identify subgroups that can safely be discharged from follow-up.

All in all, many aspects of follow-up remain to be clarified, in spite of all the studies performed. Future studies should incorporate new biomarkers and sequential analysis of available data. In

terms of outcomes, other aspects should also be considered. Many of the studies focusing on quality of life are relatively small and the same applies to cost-effectiveness analyses. As the actual impact on oncological outcomes is likely small, these outcomes should also play a major role in the studies that are going to be conducted.

In line with non-metastatic CRC patients, follow-up of CRLM patients will likely change as well. Many of the non- metastatic CRC practices will eventually be adopted, in lack of high quality studies within this specific population. Considerable changes may be expected in the perioperative treatment of CRLM as well. The results of the PUMP trials will shed a new light on the effectiveness of hepatic arterial infusion chemotherapy. In addition, immunotherapy will likely play an increasing role in patients with CRLM, or CRC in general. Next to new treatments becoming available, other technical improvements might influence treatment strategies in CRLM patients too. Over the last years, promising results from artificial intelligence approaches have been published. Machine learning algorithms may be applied to large datasets, to improve risk predictions and stratification.[55,56] When further developed in very large datasets, such models may well perform better than current standard statistical techniques. In addition, machine learning can be applied to imaging scans (i.e. radiomics) or pathology slides (i.e. pathomics). Pathomics and radiomics models are currently developed to predict the expected benefit from several treatments, and may be add in selecting CRLM patients for optimal (neo)adjuvant treatment as well.

Histopathological growth patterns of colorectal liver metastasis

Although HGPs may not have a large impact on clinical practice yet, additional research may provide valuable insights in the biology of CRLM, and potentially related treatment targets. Several biological mechanisms have been linked to HGPs, especially with regards to differences in tumour vascularization and infiltration of immune cells.[31,57-59] Replacement type CRLM seem to use pre-existing sinusoidal vessels for blood supply (i.e. vessel co-option), by replacing normal epithelial cells without destruction of the surrounding tissue. On the other hand, desmoplastic type CRLM gain vascular access through newly formed vessels (i.e. angiogenesis). This process induces a reaction resembling wound healing, with fibrosis and an inflammatory response. In line with these observations, replacement growth has been associated with increased cell motility, pericytic mimicry and a reduced infiltration of CD8+ immune cells.[57-60] These biological differences suggest there may be potential therapeutic targets related to HGPs. Given the differences in tumour vascularisation, an association with anti-angiogenic therapies has been evaluated. A study by Frentzas et al. suggests that vessel co-option mediates resistance to anti-angiogenic therapy, making bevacizumab less effective in patients with replacement type CRLM.[58] These results could not be replicated in **chapter 6**. In both studies, bevacizumab was administered prior to liver resection and HGP assessment, which is a considerable limitation in view of the histopathological changes induced by chemotherapy. Ideally, the effectiveness of anti-angiogenic therapeutics should be evaluated in an adjuvant setting. In contrast to anti-angiogenic therapy, the effectiveness of monoclonal antibodies that target the epidermal growth factor receptor (e.g. cetuximab, panitumumab) has yet to be determined in relation to HGPs. However, since we found no association between HGPs and RAS/RAF mutations in **chapter 7**, no interaction between HGPs and the effectiveness of these treatments is to be expected. The effectiveness of cetuximab and panitumumab is largely dictated by mutations in these genes.[61]

One of the main advancements in the systemic treatment of CRC has to be the introduction of immunotherapy. Neoadjuvant immunotherapy with combined PD-1/CTLA-4 blockade was highly effective in microsatellite instable early-stage CRC, while palliative therapy with PD-1 blockade led to improved progression-free survival in advanced microsatellite high CRC.[62,63] Impressive results have been reached in other types of tumours as well, especially in immunogenic tumours

such as melanoma and non-small cell lung cancer.[64,65] Among the determinants of response to immunotherapy in CRC are tumour mutation burden, immune cell densities and the expression levels of potential targets (e.g. PD-1, PDL-1, CTLA-4).[66] Efforts to identify novel targets for immunotherapy are ongoing. Factors such as LAG3 and TIMP3 could potentially act as new immunomodulating targets.[66] Several reasons to assume a potential relationship with HGPs are present. First, microsatellite instability was strongly associated with desmoplastic CRLM in **chapter 7**, which often results in a high tumour mutational burden. Second, multiple studies showed increased infiltration of several types of T- cells in desmoplastic CRLM.[57,59] Both observations hint towards HGPs as a potential target for immunotherapy and shed a different light on the biology behind HGPs. The presence of non-desmoplastic HGP could be an expression of immune evasion, in particular since the extent of non-desmoplastic growth does not seem to impact prognosis.

Next to evaluation of therapeutic targets, translational and clinical studies should be initiated to gain further insights in the biological processes behind these histological phenotypes. The relationship between HGPs and the host-immune status (e.g. neutrophil to lymphocyte ratio) or hepatic homeostasis in general (e.g. steatosis of the liver) could further explain whether this is a tumour based or a host based phenomenon. In addition, novel techniques such as spatial transcriptomics and single-cell RNA sequencing may be used to explore the tumour microenvironment en determine what cell types are present, how they relate to each other, and what genes regulate the interaction between those cells.[67] Together with assessment of expression levels for immune checkpoint inhibition, such an analysis would be of particular interest in the evaluation of HGPs. Different patterns often co-exist within one tumour and patient, which could be caused by local micro environmental processes. All in all, additional research to further explore the biology behind HGPs seems warranted to gain further insights in potential treatment targets and underlying cancer biology.

References

1 Wille-Jørgensen P, Syk I, Smedh K, et al. Effect of More vs Less Frequent Follow-up Testing on Overall and Colorectal Cancer-Specific Mortality in Patients With Stage II or III Colorectal Cancer: The COLOFOL Randomized Clinical Trial. Jama. May 22 2018;319(20):2095-2103.

2 Rosati G, Ambrosini G, Barni S, et al. A randomized trial of intensive versus minimal surveillance of patients with resected Dukes B2-C colorectal carcinoma. Ann Oncol. Feb 2016;27(2):274-280.

3 Primrose JN, Perera R, Gray A, et al. Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. Jama. Jan 15 2014;311(3):263-270.

4 Rodríguez-Moranta F, Saló J, Arcusa A, et al. Postoperative surveillance in patients with colorectal cancer who have undergone curative resection: a prospective, multicenter, randomized, controlled trial. J Clin Oncol. Jan 20 2006;24(3):386-393.

5 Tomlinson JS, Jarnagin WR, DeMatteo RP, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol. Oct 10 2007;25(29):4575-4580.

6 Suzuki H, Kiyoshima M, Kitahara M, Asato Y, Amemiya R. Long-term outcomes after surgical resection of pulmonary metastases from colorectal cancer. Ann Thorac Surg. Feb 2015;99(2):435-440.

7 Qaderi SM, Galjart B, Verhoef C, et al. Disease recurrence after colorectal cancer surgery in the modern era: a population-based study. Int J Colorectal Dis. Nov 2021;36(11):2399-2410.

8 Grimm P, Billiet I, Bostwick D, et al. Comparative analysis of prostate-specific antigen free survival outcomes for patients with low, intermediate and high risk prostate cancer treatment by radical therapy. Results from the Prostate Cancer Results Study Group. BJU Int. Feb 2012;109 Suppl 1:22-29.

9 van Maaren MC, de Munck L, Strobbe LJA, et al. Ten-year recurrence rates for breast cancer subtypes in the Netherlands: A large population-based study. Int J Cancer. Jan 15 2019;144(2):263-272.

10 Meyerhardt JA, Mangu PB, Flynn PJ, et al. Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol. Dec 10 2013;31(35):4465-4470.

11 Argilés G, Tabernero J, Labianca R, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. Oct 2020;31(10):1291-1305.

12 Glynne-Jones R, Wyrwicz L, Tiret E, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. Jul 1 2017;28(suppl_4):iv22-iv40.

13 Pugh SA, Shinkins B, Fuller A, Mellor J, Mant D, Primrose JN. Site and Stage of Colorectal Cancer Influence the Likelihood and Distribution of Disease Recurrence and Postrecurrence Survival: Data From the FACS Randomized Controlled Trial. Ann Surg. Jun 2016;263(6):1143-1147.

14 Konishi T, Shimada Y, Hsu M, et al. Association of Preoperative and Postoperative Serum Carcinoembryonic Antigen and Colon Cancer Outcome. JAMA Oncol. Mar 1 2018;4(3):309-315.

15 Ramphal W, Boeding JRE, van Iwaarden M, et al. Serum carcinoembryonic antigen to predict recurrence in the follow-up of patients with colorectal cancer. Int J Biol Markers. Mar 2019;34(1):60-68.

16 Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. Aug 2006;244(2):254-259.

17 Kanas GP, Taylor A, Primrose JN, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. Clin Epidemiol. 2012;4:283-301.

18 de Jong MC, Mayo SC, Pulitano C, et al. Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis. J Gastrointest Surg. Dec 2009;13(12):2141-2151.

19 Jones RP, Jackson R, Dunne DF, et al. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. Br J Surg. Apr 2012;99(4):477-486.

20 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. Lancet Oncol. Nov 2013;14(12):1208-1215.

21 Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. Lancet. Mar 22 2008;371(9617):1007-1016.

22 Kanemitsu Y, Shimizu Y, Mizusawa J, et al. Hepatectomy Followed by mFOLFOX6 Versus Hepatectomy Alone for Liver-Only Metastatic Colorectal Cancer (JCOG0603): A Phase II or III Randomized Controlled Trial. J Clin Oncol. Dec 1 2021;39(34):3789-3799.

23 Buisman FE, Filipe WF, Galjart B, et al. Adjuvant intra-arterial chemotherapy for patients with resected colorectal liver metastases: a systematic review and meta-analysis. HPB (Oxford). Nov 19 2021.

24 Ayez N, van der Stok EP, Grünhagen DJ, et al. The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator. Eur J Surg Oncol. Jul 2015;41(7):859-867.

25 Rahbari NN, Reissfelder C, Schulze-Bergkamen H, et al. Adjuvant therapy after resection of colorectal liver metastases: the predictive value of the MSKCC clinical risk score in the era of modern chemotherapy. BMC Cancer. Mar 11 2014;14:174.

26 Groot Koerkamp B, Sadot E, Kemeny NE, et al. Perioperative Hepatic Arterial Infusion Pump Chemotherapy Is Associated With Longer Survival After Resection of Colorectal Liver Metastases: A Propensity Score Analysis. J Clin Oncol. Jun 10 2017;35(17):1938-1944.

27 Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. Sep 1999;230(3):309-318; discussion 318-321.

28 Margonis GA, Sasaki K, Gholami S, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. Aug 2018;105(9):1210-1220.

29 Roberts KJ, White A, Cockbain A, et al. Performance of prognostic scores in predicting long-term outcome following resection of colorectal liver metastases. Br J Surg. Jun 2014;101(7):856-866.

30 Zakaria S, Donohue JH, Que FG, et al. Hepatic resection for colorectal metastases: value for risk scoring systems? Ann Surg. Aug 2007;246(2):183-191.

31 Vermeulen PB, Colpaert C, Salgado R, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. Oct 2001;195(3):336-342.

32 Fernández Moro C, Bozóky B, Gerling M. Growth patterns of colorectal cancer liver metastases and their impact on prognosis: a systematic review. BMJ Open Gastroenterol. 2018;5(1):e000217.

33 Dulskas A, Gaizauskas V, Kildusiene I, Samalavicius NE, Smailyte G. Improvement of Survival over Time for Colorectal Cancer Patients: A Population-Based Study. J Clin Med. Dec 14 2020;9(12).

34 Buisman FE, van der Stok EP, Galjart B, et al. Histopathological growth patterns as biomarker for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases. Clin Exp Metastasis. Oct 2020;37(5):593-605.

35 van Dam PJ, van der Stok EP, Teuwen LA, et al. International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer. Nov 7 2017;117(10):1427-1441.

36 Latacz E, Höppener D, Bohlok A, et al. Histopathological growth patterns of liver metastasis: updated consensus guidelines for pattern scoring, perspectives and recent mechanistic insights. Br J Cancer. Jun 1 2022.

37 Margonis GA, Buettner S, Andreatos N, et al. Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer. JAMA Surg. Jul 18 2018;153(7):e180996.

38 Brudvik KW, Jones RP, Giuliante F, et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. Jan 2019;269(1):120-126.

39 Bolhuis K, van 't Erve I, Mijnals C, et al. Postoperative circulating tumour DNA is associated with pathologic response and recurrence-free survival after resection of colorectal cancer liver metastases. EBioMedicine. Aug 2021;70:103498.

40 Höppener DJ, Nierop PMH, Herpel E, et al. Histopathological growth patterns of colorectal liver metastasis exhibit little heterogeneity and can be determined with a high diagnostic accuracy. Clin Exp Metastasis. Aug 2019;36(4):311-319.

41 Elfrink AKE, Kok NFM, van der Werf LR, et al. Population-based study on practice variation regarding preoperative systemic chemotherapy in patients with colorectal liver metastases and impact on short-term outcomes. Eur J Surg Oncol. Sep 2020;46(9):1742-1755.

42 Cheng J, Wei J, Tong T, et al. Prediction of Histopathologic Growth Patterns of Colorectal Liver Metastases with a Noninvasive Imaging Method. Ann Surg Oncol. Dec 2019;26(13):4587-4598.

43 Han Y, Chai F, Wei J, et al. Identification of Predominant Histopathological Growth Patterns of Colorectal Liver Metastasis by Multi-Habitat and Multi-Sequence Based Radiomics Analysis. Front Oncol. 2020;10:1363.

44 Denis F, Lethrosne C, Pourel N, et al. Randomized Trial Comparing a Web-Mediated Follow-up With Routine Surveillance in Lung Cancer Patients. J Natl Cancer Inst. Sep 1 2017;109(9).

45 Augestad KM, Norum J, Dehof S, et al. Cost-effectiveness and quality of life in surgeon versus general practitioner-organised colon cancer surveillance: a randomised controlled trial. BMJ Open. 2013;3(4).

46 Wattchow DA, Weller DP, Esterman A, et al. General practice vs surgical-based follow-up for patients with colon cancer: randomised controlled trial. Br J Cancer. Apr 24 2006;94(8):1116-1121.

47 Grunfeld E, Levine MN, Julian JA, et al. Randomized trial of long-term follow-up for early-stage breast cancer: a comparison of family physician versus specialist care. J Clin Oncol. Feb 20 2006;24(6):848-855.

48 Vos JAM, Wieldraaijer T, van Weert H, van Asselt KM. Survivorship care for cancer patients in primary versus secondary care: a systematic review. J Cancer Surviv. Feb 2021;15(1):66-76.

49 Cho H, Mariotto AB, Mann BS, Klabunde CN, Feuer EJ. Assessing non-cancer-related health status of US cancer patients: other-cause survival and comorbidity prevalence. Am J Epidemiol. Aug 1 2013;178(3):339-349.

50 Wieldraaijer T, Duineveld LA, van Asselt KM, et al. Follow-up of colon cancer patients; causes of distress and need for supportive care: Results from the ICARE Cohort Study. Eur J Surg Oncol. Jan 2017;43(1):118-125.

51 Burg MA, Adorno G, Lopez ED, et al. Current unmet needs of cancer survivors: analysis of open-ended responses to the American Cancer Society Study of Cancer Survivors II. Cancer. Feb 15 2015;121(4):623-630.

52 Molassiotis A, Yates P, Li Q, et al. Mapping unmet supportive care needs, quality-of-life perceptions and current symptoms in cancer survivors across the Asia-Pacific region: results from the International STEP Study. Ann Oncol. Oct 1 2017;28(10):2552-2558.

53 Tie J, Cohen JD, Wang Y, et al. Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. JAMA Oncol. Dec 1 2019;5(12):1710-1717.

54 Waalboer RB, Meyer YM, Galjart B, et al. Sarcopenia and long-term survival outcomes after local therapy for colorectal liver metastasis: a meta-analysis. HPB (Oxford). Sep 9 2021.

55 Skrede OJ, De Raedt S, Kleppe A, et al. Deep learning for prediction of colorectal cancer outcome: a discovery and validation study. Lancet. Feb 1 2020;395(10221):350-360.

56 Huang YQ, Liang CH, He L, et al. Development and Validation of a Radiomics Nomogram for Preoperative Prediction of Lymph Node Metastasis in Colorectal Cancer. J Clin Oncol. Jun 20 2016;34(18):2157-2164.

57 Brunner SM, Kesselring R, Rubner C, et al. Prognosis according to histochemical analysis of liver metastases removed at liver resection. Br J Surg. Dec 2014;101(13):1681-1691.

58 Frentzas S, Simoneau E, Bridgeman VL, et al. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med. Nov 2016;22(11):1294-1302.

59 Höppener DJ, Nierop PMH, Hof J, et al. Enrichment of the tumour immune microenvironment in patients with desmoplastic colorectal liver metastasis. Br J Cancer. Jul 2020;123(2):196-206.

60 Lugassy C, Vermeulen PB, Ribatti D, Pezzella F, Barnhill RL. Vessel co-option and angiotropic extravascular migratory metastasis: a continuum of tumour growth and spread? Br J Cancer. Jan 5 2022.

61 Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. Feb 5 2009;360(6):563-572.

62 André T, Shiu KK, Kim TW, et al. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. N Engl J Med. Dec 3 2020;383(23):2207-2218.

63 Chalabi M, Fanchi LF, Dijkstra KK, et al. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat Med. Apr 2020;26(4):566-576.

64 Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. May 31 2018;378(22):2078-2092.

65 Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. N Engl J Med. Oct 17 2019;381(16):1535-1546.

66 Cui G. The Mechanisms Leading to Distinct Responses to PD-1/PD-L1 Blockades in Colorectal Cancers With Different MSI Statuses. Front Oncol. 2021;11:573547.

67 Wu Y, Yang S, Ma J, et al. Spatiotemporal Immune Landscape of Colorectal Cancer Liver Metastasis at Single-Cell Level. Cancer Discov. Jan 2022;12(1):134-153.

CHAPTER TEN

CHAPTER TEN

NEDERLANDSE SAMENVATTING

Nederlandse samenvatting

Dit proefschrift bestaat uit twee delen, waarin verschillende aspecten van de follow-up, behandeling en prognose van colorectaal carcinoom patiënten worden belicht. Het onderzoek heeft zich gericht op het individualiseren van nacontrole- en behandelingsstrategieën (deel I) en het evalueren van histopathologische groeipatronen als nieuwe prognostische factor in patiënten met colorectale levermetastasen (deel II).

Deel I: Individualiseren van follow-up en behandeling na resectie van het colorectaal carcinoom

Na resectie van een colorectaal carcinoom ondergaat het merendeel van de patiënten zogeheten oncologische follow-up. Patiënten worden normaliter gedurende vijf jaar of langer gecontroleerd. De controle bestaat uit klinische evaluaties in het ziekenhuis, periodieke metingen van de tumormarker CEA en beeldvorming middels CT scans. Oncologische follow-up dient meerdere doelen. Het primaire doel is de vroege detectie van recidief ziekte (zowel lokaal als op afstand gemetastaseerd), om zo hernieuwde locoregionale behandeling mogelijk te maken en de overleving van patiënten te verbeteren. Daarnaast kunnen de controles gebruikt worden om patiënten in te lichten over hun prognose, en om psychologische hulp te bieden waar nodig.

Er zijn verscheidene onderzoeken gedaan naar de invloed van oncologische follow-up op de overleving van patiënten met een colorectaal carcinoom, waaronder multipele gerandomiseerde studies. **Hoofdstuk 2** vat de beschikbare literatuur samen ten aanzien van oncologische nacontrole voor vijf veelvoorkomende tumoren, waaronder het colorectaal carcinoom. In dit hoofdstuk tonen we aan dat intensieve controle voor het colorectaal carcinoom geen invloed heeft op de algehele en kanker-specifieke overleving, maar wel de kans op een in opzet curatieve behandeling voor recidief ziekte verhoogd.

Controle van de tumormarker CEA is een belangrijk onderdeel van de oncologische follow-up bij patiënten met een colorectaal carcinoom. Een stijging van het CEA is indicatief voor recidief ziekte, met name voor lever uitzaaiingen. In een gedeelte van de patiënten is het CEA niet verhoogd voorafgaand aan resectie van het colorectaal carcinoom. Vaak wordt aangenomen dat in deze patiënten CEA een minder sensitieve tumormarker is, en dat beeldvorming mogelijk vaker toegepast zou moeten worden tijdens follow-up. In **hoofdstuk 3** tonen wij aan dat frequente beeldvorming ook in de groep patiënten met een CEA negatief colorectaal carcinoom de overleving niet verbeterd.

De lever is het meest aangedane orgaan in patiënten met uitzaaiingen van het colorectaal carcinoom. Ongeveer 30 procent van de colorectaal carcinoom patiënten wordt uiteindelijk gediagnosticeerd met colorectale levermetastsen, waarvan ongeveer een derde kan worden behandeld met in opzet curatieve behandeling (i.e. resectie en/of ablatie).

Ook patiënten met gereseceerde colorectale levermetastasen worden gedurende 5 jaar gecontroleerd. Dit terwijl veruit de meeste recidieven optreden binnen de eerste drie jaar na chirurgie. In **hoofdstuk 4** hebben wij in een groep patiënten die drie jaar ziektevrij bleven na resectie van colorectale levermetastasen, de noodzaak tot verdere follow-up bekeken. We tonen aan dat in naar schatting 27 procent van deze groep recidieven optreden en dat follow-up daarmee gerechtvaardigd is. Daarnaast werd een risicoscore ontwikkeld, waarmee een groep patiënten kan worden geïdentificeerd die een veel kleinere kans (5%) op terugkeer van ziekte heeft.

Wereldwijd wordt perioperatieve systemisch chemotherapie vaak gegeven aan patiënten die chirurgische behandeling ondergaan van colorectale levermetasten. Deze behandeling verbetert de ziektevrije, maar niet de algehele overleving en wordt daarom niet geadviseerd in de Nederlandse richtlijn. Een andere behandelingsoptie is hepatische intra-arteriële chemotherapie, waarbij hoge doseringen chemotherapie direct worden toegediend aan de lever. Deze behandeling wordt in Nederland alleen in onderzoeksverband gegeven, maar is al jaren beschikbaar in de Verenigde Staten.

In **hoofdstuk 5** presenteren we een risicomodel waarin we proberen te voorspellen welke patiënten binnen twee jaar na resectie van colorectale levermetasen een recidief buiten de lever zullen ontwikkelen. De achterliggende gedachte is dat in patiënten met een hoog risico op extrahepatische recidieven, locoregionale chemotherapie minder effectief is en systemische chemotherapie juist effectiever. Het risicomodel kan met redelijke precisie voorspellen welke patiënten extrahepatische recidieven zullen ontwikkelen. In lijn met de hypothese zagen we dat systemische chemotherapie alleen effectief was in patiënten met een zeer hoog risico op extrahepatische terugkeer van ziekte. Intra-arteriële chemotherapie leek effectief in alle groepen, behalve in de patiënten met een zeer hoog risico op extrahepatische metastasen.

Deel II: Histopathologische groeipatronen van colorectale levermetastasen

Er zijn vele risicomodellen beschikbaar om de prognose na resectie van colorectale levermetasen te voorspellen. Deze modellen worden momenteel nauwelijks gebruikt in de klinische praktijk, omdat ze onvoldoende accuraat zijn. Dit toont aan dat er nog steeds behoefte is aan nieuwe prognostische factoren.

Histopathologische groeipatronen van colorectale levermetasten zijn recentelijk ontdekt als potentiele nieuwe risicofactor na resectie. Deze groeipatronen beschrijven in feite de overgang van tumor naar normale lever. Grofweg wordt er onderscheid gemaakt tussen drie typen groeipatroon. Allereerst is er het desmoplastische groeipatroon, waarbij er een ring van stromaal weefsel tussen de tumorcellen en hepatocyten zit. Dit stromale weefsel verhinderd direct contact tussen beide celtypen. Daarnaast wordt het vervangend groeipatroon beschreven. Hierbij infiltreert het tumorweefsel het normale leverweefsel, en is er dus direct contact tussen beide celtypen. Tot slot wordt het duwend groeipatroon beschreven. Hierbij lijkt er een vorm van compressie van het tumorweefsel op het normale leverweefsel te zijn. Er is geen direct contact tussen tumorcellen en hepatocyten.

Er zijn verscheidene biologische verschillen tussen de verschillende groeipatronen. Deze zijn met name gerelateerd aan de vascularisatie en de immunologische respons van het lichaam. In **hoofdstuk 6 en 7** hebben we in twee patiënt cohorten aangetoond dat patiënten met desmoplastische colorectale levermetastasen een betere prognose hebben dan patiënten met niet-desmoplastische tumoren. De vijf-jaar overleving van patiënten met desmoplastische tumoren was respectievelijk 73 en 78 procent in beide cohorten, hetgeen vergelijkbaar is met stadium III colorectaal carcinoom patiënten. In deze hoofdstukken stellen we een nieuw scoringssysteem voor, waarbij er klinisch onderscheid gemaakt wordt tussen patiënten met een volledig desmoplastisch groeipatroon en patiënten met enige vorm van niet desmoplastische groei. In **hoofdstuk 8** tonen we aan dat patiënten met een desmoplastisch groeipatroon vaker recidieven krijgen die zich alleen in de lever bevinden, en frequenter recidieven krijgen die opnieuw behandeld kunnen worden met chirurgie. Patiënten met niet desmoplastische colorectale levermetastasen ontwikkelen vaak multi-orgaan recidieven die niet meer in aanmerking komen voor in opzet curatieve resectie. Dit zou deels het verschil in overleving kunnen verklaren tussen beide groepen.

APPENDICES

List of publications

Galjart B, Höppener DJ, Aerts JGJV, Bangma CH, Verhoef C, Grünhagen DJ. Follow-up strategy and survival for five common cancers: a meta-analysis. Eur J Cancer. 2022

Hellingman T, Galjart B, Henneman JJ, Görgec B, Bijlstra OD, Meijerink M, et al. Limited Effect of Perioperative Systemic Therapy in Patients Selected for Repeat Local Treatment of Recurrent Colorectal Cancer Liver Metastases. Annals of Surgery Open. 2022

Meyer YM, Galjart B, Waalboer RB, Olthof PB, van Vugt JLA, et al. Absence of association between CT-assessed skeletal muscle mass and long-term oncological outcomes after curative therapy for colorectal liver metastasis. HPB (Oxford). 2022

Höppener DJ, Stook JPL, Galjart B, Nierop PMH, Nagtegaal ID, Vermeulen PB, et al. The relationship between primary colorectal cancer histology and the histopathological growth patterns of corresponding liver metastases. BMC Cancer. 2022

Meyer Y, Bohlok A, Höppener D, Galjart B, Doukas M, et al. Histopathological growth patterns of resected non-colorectal, non-neuroendocrine liver metastases: a retrospective multicenter study. Clin Exp Metastasis. 2022

Vles MJ, Höppener DJ, Galjart B, Moelker A, Vermeulen PB, Grünhagen DJ, Verhoef C. Local tumour control after radiofrequency or microwave ablation for colorectal liver metastases in relation to histopathological growth patterns. HPB (Oxford). 2022

Buisman FE, Giardiello D, Kemeny NE, Steyerberg EW, Höppener DJ, Galjart B, et al. Predicting 10-year survival after resection of colorectal liver metastases; an international study including biomarkers and perioperative treatment. Eur J Cancer. 2022

Nierop PM, Höppener DJ, Buisman FE, van der Stok EP, Galjart B, Balachandran VP, et al. Preoperative systemic chemotherapy alters the histopathological growth patterns of colorectal liver metastases. J Pathol Clin Res. 2022

Galjart B, Büttner S, Beumer BR, van Vugt JLA, van Eijck CHJ, Polak WG, et al. Quality and performance of validated prognostic models for survival after resection of intrahepatic cholangiocarcinoma: a systematic review and meta-analysis. HPB (Oxford). 2021

Qaderi SM, Galjart B, Verhoef C, Slooter GD, Koopman M, Verhoeven RHA, et al. Disease recurrence after colorectal cancer surgery in the modern era: a population-based study. Int J Colorectal Dis. 2021

Höppener DJ, Galjart B, Nierop PMH, Buisman FE, van der Stok EP, Coebergh van den Braak RRJ, et al. Histopathological Growth Patterns and Survival After Resection of Colorectal Liver Metastasis: An External Validation Study. JNCI Cancer Spectr. 2021

Waalboer RB, Meyer YM, Galjart B, Olthof PB, van Vugt JLA, Grünhagen DJ, et al. Sarcopenia and long-term survival outcomes after local therapy for colorectal liver metastasis: a meta-analysis. HPB (Oxford). 2021

Buisman FE, Filipe WF, Galjart B, Grünhagen DJ, Homs MYV, Moelker A, et al. Adjuvant intra-arterial chemotherapy for patients with resected colorectal liver metastases: a systematic review and meta-analysis. HPB (Oxford). 2021

Beumer BR, Buettner S, Galjart B, van Vugt JLA, de Man RA, JNM IJ, et al. Systematic review and meta-analysis of validated prognostic models for resected hepatocellular carcinoma patients. Eur J Surg Oncol. 2021

Amini N, Andreatos N, Margonis GA, Buettner S, Wang J, Galjart B, et al. Mutant KRAS as a prognostic biomarker after hepatectomy for rectal cancer metastases: Does the primary disease site matter? J Hepatobiliary Pancreat Sci. 2021

Buisman FE, Galjart B, van der Stok EP, Kemeny NE, Balachandran VP, Boerner T, et al. The impact of hepatic arterial infusion pump chemotherapy on hepatic recurrences and survival in patients with resected colorectal liver metastases. HPB (Oxford). 2020

Buisman FE, Galjart B, van der Stok EP, Balachandran VP, Boerner T, Drebin JA, et al. Recurrence Patterns After Resection of Colorectal Liver Metastasis are Modified by Perioperative Systemic Chemotherapy. World J Surg. 2020

Buisman FE, Galjart B, Buettner S, Groot Koerkamp B, Grünhagen DJ, Verhoef C. Primary tumor location and the prognosis of patients after local treatment of colorectal liver metastases: a systematic review and meta-analysis. HPB (Oxford). 2020

Buisman FE, van der Stok EP, Galjart B, Vermeulen PB, Balachandran VP, Coebergh van den Braak RRJ, et al. Histopathological growth patterns as biomarker for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases. Clin Exp Metastasis. 2020

Nierop PMH, Höppener DJ, van der Stok EP, Galjart B, Buisman FE, Balachandran VP, et al. Histopathological growth patterns and positive margins after resection of colorectal liver metastases. HPB (Oxford). 2020

Galjart B, Nierop PMH, van der Stok EP, van den Braak R, Höppener DJ, Daelemans S, et al. Angiogenic desmoplastic histopathological growth pattern as a prognostic marker of good outcome in patients with colorectal liver metastases. Angiogenesis. 2019

Galjart B, Nierop PMH, Höppener DJ, van der Stok EP, Coebergh van den Braak RRJ, Vermeulen PB, et al. Salvage treatment for recurrences after first resection of colorectal liver metastases: the impact of histopathological growth patterns. Clin Exp Metastasis. 2019

Nierop PMH, Verseveld M, Galjart B, Rothbarth J, Nuyttens J, van Meerten E, et al. The liver-first approach for locally advanced rectal cancer and synchronous liver metastases. Eur J Surg Oncol. 2019

Höppener DJ, Nierop PMH, van Amerongen MJ, Olthof PB, Galjart B, van Gulik TM, et al. The Disease-Free Interval Between Resection of Primary Colorectal Malignancy and the Detection of Hepatic Metastases Predicts Disease Recurrence But Not Overall Survival. Ann Surg Oncol. 2019

Sideras K, Galjart B, Vasaturo A, Pedroza-Gonzalez A, Biermann K, Mancham S, et al. Prognostic value of intra-tumoral CD8(+) /FoxP3(+) lymphocyte ratio in patients with resected colorectal cancer liver metastasis. J Surg Oncol. 2018

Margonis GA, Buettner S, Andreatos N, Wagner D, Sasaki K, Galjart B, et al. The prognosis of colorectal cancer liver metastases associated with inflammatory bowel disease: An exploratory analysis. J Surg Oncol. 2018

Zhou G, Noordam L, Sprengers D, Doukas M, Boor PPC, van Beek AA, Erkens R, Mancham S, Grünhagen D, Menon AG, Lange JF, Burger PJWA, Brandt A, Galjart B, et al. Blockade of LAG3 enhances responses of tumor-infiltrating T cells in mismatch repair-proficient liver metastases of colorectal cancer. Oncoimmunology. 2018

van Dam PJ, van der Stok EP, Teuwen LA, Van den Eynden GG, Illemann M, Frentzas S, Majeed AW, Eefsen RL, Coebergh van den Braak RRJ, Lazaris A, Fernandez MC, Galjart B, et al. International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer. 2017

Buettner S, Galjart B, van Vugt JLA, Bagante F, Alexandrescu S, Marques HP, et al. Performance of prognostic scores and staging systems in predicting long-term survival outcomes after surgery for intrahepatic cholangiocarcinoma. J Surg Oncol. 2017

Galjart B, van der Stok EP, Rothbarth J, Grünhagen DJ, Verhoef C. Posttreatment Surveillance in Patients with Prolonged Disease-Free Survival After Resection of Colorectal Liver Metastasis. Ann Surg Oncol. 2016

Das AM, Koljenović S, Oude Ophuis CM, van der Klok T, Galjart B, Nigg AL, et al. Association of TIMP3 expression with vessel density, macrophage infiltration and prognosis in human malignant melanoma. Eur J Cancer. 2016

De Lima A, Galjart B, Wisse PH, Bramer WM, van der Woude CJ. Does lower gastrointestinal endoscopy during pregnancy pose a risk for mother and child? - a systematic review. BMC Gastroenterol. 2015

Contributing authors

Prof. dr. J.G.J.V. Aerts
Department of Pulmonology, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. M.J. van Amerongen
Department of Surgery, Radboud University Medical Center, Nijmegen, the Netherlands

Dr. V.P. Balachandran
Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA

Prof. dr. C.H. Bangma
Department of Urology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands

J.L. Boxall
Department of Surgery, University of Southampton, Southampton, United Kingdom

Prof. dr. A. Castells
Gastroenterology Department, Hospital Clinic of Barcelona, IDIBAPS, CIBEREHD, University of Barcelona, Barcelona, Spain

Dr. A. Cercek
Department of Medical Oncology, Memorial Sloan Kettering Cancer Center, New York, USA

Dr. R.R.J. Coebergh van den Braak
Department of Surgery, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. S. Daelemans
Medical Biochemistry, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium

Prof. dr. M.I. D'Angelica
Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA

Prof. dr. L.Y. Dirix
Translational Cancer Research Unit (GZA Hospitals and University of Antwerp), Antwerp, Belgium

Dr. M. Doukas
Department of Pathology, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. B. Groot Koerkamp
Department of Surgery, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. D.J. Grünhagen
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

D.J. Höppener
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

Dr. E. Horváth-Puhó
Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Dr. W.R. Jarnagin
Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA

Dr. N.E. Kemeny
Department of Medical Oncology, Memorial Sloan Kettering Cancer Center, New York, USA

Dr. T.P. Kingham
Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, USA

Dr. D. van Klaveren
Department of Public Health, Erasmus University Medical Center, Rotterdam, the Netherlands

Prof. dr. D. Mant
Department of Primary Care, University of Oxford, Oxford, United Kingdom

Prof. dr. I.D. Nagtegaal
Department of Pathology, Radboud University Medical Center, Nijmegen, the Netherlands

Dr. P.M.H. Nierop
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

Prof. dr. J.N. Primrose
Department of Surgery, University of Southampton, Southampton, United Kingdom

S.A. Pugh
Department of Medical Oncology, Addenbrookes Hospital, Cambridge, United Kingdom

Dr. F. Rodríguez-Moranta
Department of Gastroenterology, Bellvitge University Hospital, Barcelona, Spain

Dr. J. Rothbarth
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

Prof. dr. J. Shia
Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, USA

Prof. dr. H.T. Sørensen
Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Prof. dr. E.W. Steyerberg
Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, the Netherlands
Department of Public Health, Erasmus University Medical Center, Rotterdam, the Netherlands

Dr. E.P. van der Stok
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

Prof. dr. I. Syk
Section of Surgery, Department of Clinical Sciences, Skåne University Hospital, Malmö, Sweden

Prof. dr. C. Verhoef
Department of Surgery, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands

Prof. dr. P.B. Vermeulen
Translational Cancer Research Unit, GZA Hospitals and University of Antwerp, Antwerp, Belgium

Prof. dr. J.H.W. de Wilt
Department of Surgery, Radboud University Medical Center, Nijmegen, The Netherlands

PhD PORTFOLIO

Name PhD student	Boris Galjart
Erasmus MC department	Surgery
PhD period	January 2016 – December 2020
Supervisors	Prof. dr. C. Verhoef Dr. D.J. Grünhagen
Date defense thesis	September 21, 2022

Training	Year	ECTS
Presentations and (inter)national conferences		
ESSO congress, Budapest	2018	1.0
EAHPBA congress, Mainz	2017	1.0
ECCO congress, Amsterdam	2017	1.0
Liver Metastasis Research Network meeting, Rotterdam	2017	1.0
Dutch Colorectal Cancer Group meeting	2017	1.0
Chirurgendagen, Velthoven	2016-2017	2.0
Wetenschapsdag Heelkunde Erasmus MC	2016	1.0
Courses		
Master in Clinical Research (NIHES)	2015-2017	120.0
Basiscursus regelgeving Klinisch Onderzoek, NFU	2021	1.5
34th Annual Graduate Summer Institute of Epidemiology and Biostatistics, Johns Hopkins University, Baltimore	2016	6.0
Teaching		
Supervision master thesis (3)	2017-2021	6.0
Other		
Liver Metastases Research Network meeting organisation	2017	2.0

Acknowledgements

En dan nu het – volgens sommigen – belangrijkste onderdeel van dit boekje. Uiteraard is dit proefschrift tot stand gekomen met hulp en ondersteuning van velen. Ik had me dan ook voorgenomen eenieder uitgebreid te bedanken, maar ben (in lijn met stelling 11) te laat begonnen. Het moet dus (in lijn met stelling 10) kort maar krachtig. Eens kijken of dat gaat lukken!

Geachte promotor, beste Kees, wat begon met een onderzoeksproject als student is uiteindelijk uitgemond in dit proefschrift. Het had ook anders kunnen lopen, maar het barricaderen van je kantoor met de loper uit de Daniel heeft je gelukkig niet op andere gedachten doen brengen... Ik heb genoten van mijn tijd als onderzoeker binnen jouw team, de sfeer die je creëert is ongeëvenaard en maakt hard werken – met soms een potje tafeltennis tussendoor – makkelijk. Dank voor alles!

Geachte co-promotor, beste Dirk, een onmisbare schakel binnen de groep! Ik heb je scherpe commentaar altijd zeer gewaardeerd, en je humoristische toon daarbij evenzo. Naast wat minder succesvolle gezamenlijke projecten (CHARISMA…) staan er inmiddels toch flink wat mooie follow-up projecten in de steigers waar we trots op mogen zijn. Dank voor alle begeleiding en geduld – ook na de 14e indiening van het beruchte review – de afgelopen jaren.

Leden van de promotiecommissie, hartelijk dank voor de tijd die jullie hebben genomen om dit proefschrift te beoordelen. Ik kijk uit naar de verdediging.

Ik wil alle co-auteurs bedanken voor hun bijdrage aan dit proefschrift. In het bijzonder wil ik daarbij noemen Peter Vermeulen en Bas Groot Koerkamp. Peter, als 'godfather' van de groeipatronen is jouw input onmisbaar geweest voor dit proefschrift. Dank voor de mooie samenwerking! Bas, na drie (?) jaar filosoferen en coderen lijkt het EHR predictie model er toch echt te komen. Dank voor je hulp daarbij, ik hoop dat er nog meer projecten mogen volgen.

CRLM onderzoekers, een mooi gezelschap! Eric, dank voor de introductie en begeleiding in het winkeltje. Inmiddels al bijna chirurg, maar mocht het toch nog misgaan hoop ik dat je een dubbel carrière aan de tafeltennistafel overweegt (Parijs 2024 komt eraan!). Tube, een even waardeloze kiter als tafeltennisser (geintje), maar een gouden kerel. De vele avonden en weekenden doorhalen in de Daniel en het EMC hebben tot een mooie vriendschap geleid. Hopelijk betekent het afronden van dit proefschrift ook meer tijd voor bezoeken aan de Brouwersdam! Maarten, nog een 'godfather', maar dan van de baseline tabel, met als magnum opus ons angiogenesis artikel (5 stuks!!). Elkaar begeleiden als master onderzoeker was memorabel, hetzelfde kan gezegd worden van de avonden op het eiland en de congressen. Dank voor de mooie tijd! Wills, bruv, namens Höppener nog excuses voor je TV (en de rest). Veel succes met de laatste loodjes van je boek, ik weet zeker dat het goed komt. We zien je hopelijk snel weer op het water! Kel, succesvol datamanager en uitmuntend padel speelster, er zijn maar weinigen die het je nadoen. Volgende keer pakken wij de schaal (alles geven!!). Yannick, Duitsers en Fransen liggen elkaar over het algemeen niet, maar het onderzoeken is ons toch aardig afgegaan. Eens kijken of we dat in het Maasstad kunnen voortzetten! Hakan en Lissa, jullie hebben het stokje inmiddels alweer een tijdje overgenomen. Ik heb alle vertrouwen in de uitkomst, zet 'm op!

Sandra, misschien wel het belangrijkste onderdeel van bovenstaand team!! Veel dank voor alle ondersteuning en gezelligheid!

Mijn grote dank gaat ook uit naar alle Daniel/OGC onderzoekers. De bbq's op het dak, lunches, congressen en winteruitjes, ik bewaar er vele warme herinneringen aan!

Chirurgen en assistenten uit het Amphia, dank voor de mooie tijd en de support tijdens de laatste loodjes!

Vrienden uit Huize Epidaurus (Yoram, Mits, en ik geloof ook Roy). Na jaren zwoegen is het af en jullie waren er vanaf het begin bij om me uit te lachen na weer een avond in de Daniel. We zien elkaar veel te weinig, maar ik heb me voorgenomen daar vanaf nu verandering in te brengen. Hopen dat Klazen terug appt en Mitta kan.

Moksi peleton (Daniel, Gijs), hopelijk binnenkort weer met elkaar op pad voor wat taaie kilometers langs de Lek. Zo niet, dan is een matige pot Feyenoord kijken – en die zijn er naar verluid regelmatig – natuurlijk een prachtig alternatief om weer eens bij te kletsen.

Buettner, zonder jouw eigenaardige muzieksmaak en gevoel voor humor zouden veel dingen in de Ee kelder maar levenloze dingen zijn. Na het succes van ons review (ik ga zeer binnenkort beginnen met het CRLM deel) en de Bredase nachtdiensten, kan ik alleen maar erg benieuwd zijn naar wat onze toekomstige carrière als glazenwassers ons gaat brengen.

Job, de werkplek is langzaam verschoven van een snijzaal naar het mooie Na-21, en hopelijk binnenkort de OK! Laten we de afsluiting bij Stalles of WP er in ieder geval inhouden. Als ik je nog één advies mag geven: verkoop die fiets, koop een kite!

Sjoerd, als mede-Ajacied en binnenkort bijna buurman mag jij niet ontbreken in het dankwoord. De diners en Champions League avonden zijn een mooie afwisseling op het werk. We gaan weer voor de treeeeble!

Dunweg, zwemvriend van het vroege uur. Inmiddels allebei wat kilootjes zwaarder en behoorlijk wat wijzer. Kaapstad was geniaal, ik kijk uit naar de volgende trip.

En dan de paranimfen, Diederik en Ruben. Höppener, oi mate! Inmiddels 400.000 coupes, meer dumpert filmpjes, een paar congressen, vele statistische problemen en flink wat artikelen verder. Zonder jouw ICT brein – sommige mensen noemen je een tovenaar – was dat laatste in ieder geval nooit gelukt. Straks allebei klaar met de PhD, dan kan de jacht op het Na-21 Woo record echt worden ingezet…. Bogt, ondanks een flink aantal jaren FIFA rivaliteit – no era omhaal – nog steeds maten, voor het leven vrees ik dus. Ik hoop dat de komende twee maanden blijkt dat we, naast muzikaal en sportief, ook academisch behoorlijk onderlegd zijn. Zo niet, dan toch maar trainen voor de Tour...

Mama en papa, het boekje is wat epidemiologischer geworden dan de familietraditie, het mag voor jullie geen verrassing heten dat al het lab werk mislukt is… Aan jullie onvoorwaardelijke liefde en steun heeft het in ieder geval niet gelegen. Dank jullie voor alles!

Claire, lieve zus, het boekje is dankzij jou prachtig geworden! Ik laat het te weinig blijken, maar ben super trots op je. Ik ben benieuwd wat de toekomst je allemaal gaat brengen, maar weet zeker (jaja, het staat zwart op wit) dat het een groot succes gaat worden!!

De slotwoorden zijn natuurlijk voor jou, lieve Tanja. Tja, jou echt genoeg bedanken is nauwelijks mogelijk. Het is eindelijk af, en zoals ik al een aantal jaar roep, vanaf nu wordt alles nog beter (en het was al bijna perfect)! Het mag niet te cheesy – ze lezen allemaal mee…. – maar ik houd van je en kijk uit naar al onze volgende avonturen!

Curriculum vitae

Boris Galjart werd op 11 augustus 1992 geboren te Rotterdam. Hij behaalde zijn middelbare school diploma aan het Erasmiaans Gymnasium in dezelfde stad. Na een jaar Biomedische Wetenschappen te hebben gestudeerd aan de Universiteit van Amsterdam, keerde hij in 2011 terug naar Rotterdam om daar met zijn studie Geneeskunde aan de Erasmus Universiteit te beginnen. Initieel als student, en later in het kader van een master in 'Clinical Research' aan het Netherlands Institute for Health Sciences (NIHES), is hij onderzoek gaan doen bij de afdeling Chirurgische Oncologie van de Daniel den Hoed kliniek. Het onderzoek richtte zich op de behandeling en follow-up van patiënten met colorectale levermetastasen en vormt de basis van het proefschrift dat nu voor u ligt. Na een jaar als ANIOS chirurgie gewerkt te hebben in het Amphia ziekenhuis te Breda (opleider dr. E.J. Veen), is hij per 1 juli 2022 gestart aan zijn opleiding tot chirurg in het Maasstad ziekenhuis te Rotterdam (opleider dr. B. Fioole).