Towards more individualized treatment of patients with colorectal cancer liver metastases

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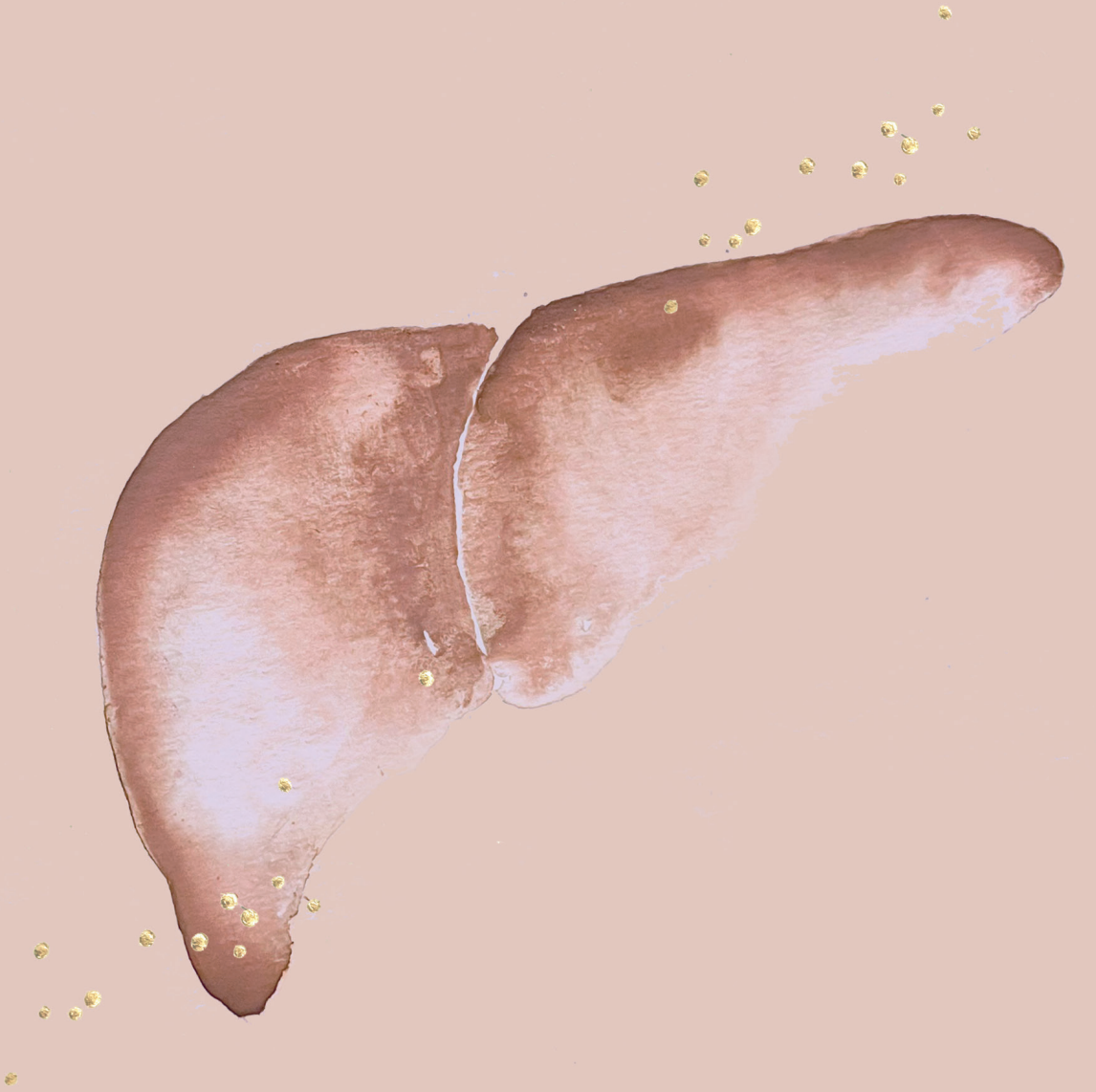

KAREN BOLHUIS

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Towards more individualized treatment of patients with colorectal cancer liver metastases

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CHAPTER 1

General introduction and thesis outline

General introduction and thesis outline

Globally, colorectal cancer (CRC) is the third most common cancer and its incidence is rising.[1,2] CRC predominantly disseminates to the liver, and as many as one-third of CRC patients develop colorectal liver metastases (CRLM). In the majority of these patients the metastases are confined to the liver.[3,4] Although the last decades, outcomes of patients with metastatic CRC have improved due to increasingly effective systemic therapies, unravelling of predictive and prognostic markers by molecular analysis of tumorgenetics, and improved multimodal approaches, CRLM remains the major cause of CRC-related death.[5,6] Unlike in other malignancies, resection of CRLM can offer long-term survival, 5-year-survival rates of 45-60%, or even be curative.[7] However, upfront only 20% of patients with CRLM are considered resectable.[8] Systemic treatment with the combination of chemotherapy and targeted therapy improves these rates with 20-40% by converting patients with initially unresectable CRLM to secondary resectable CRLM.[8-11] Long-term survival rates of patients with so called 'secondary resectable CRLM' are similar to patients considered to have 'primary resectable CRLM'. Furthermore, long-term survival rates of secondary resectable CRLM is significantly better than for patients receiving palliative systemic treatment only (5-year-survival rates 32% vs <10%, respectively).[8,12] Thus treatment should be directed towards achieving complete CRLM resection[8]. Unfortunately, CRLM recurrence rates up to 80% are reported, and over half of CRLM patients die within five years following resection due to their aggressive tumor biology.[13] To further improve long-term survival outcomes in CRLM patients, treatments should be directed towards a more individualized approach by optimizing conversion therapies, risk stratification, and use of predictive and prognostic markers in combination with other novel diagnostic strategies to closely monitor disease status and early detection of recurrences.

Part I Outcomes of induction systemic treatment and resection of CRLM

The effectiveness of systemic treatment for patients with metastatic CRC has improved with response rates of 60-80% and median overall survival (OS) of 30 months in clinical trials.[6] However, interpretation and comparison of data from systemic treatment trials in patients with initially unresectable CRLM is challenging. First, the field is constantly and rapidly evolving with acknowledgement of new prognostic and predictive markers such as *RAS/BRAF*^{V600E} tumor mutations and sidedness of the primary tumor. These developments complicate interpretation of outcomes of prior studies in which these markers were not available or, when available, concern retrospective analyses in small subgroups of patients.[14,15] Second, a lack of clear criteria to determine resectability induces practice variation and selection bias leading to heterogeneity of study populations which complicates translation of study outcomes to clinical practice guidelines.[9,16] Hence, there is no consensus regarding the optimal systemic conversion treatment in the undefined group of patients with initially unresectable CRLM.[6,17]

Although doublet chemotherapy (fluoropyrimidine with oxaliplatin or irinotecan) is preferred over fluoropyrimidine mono-chemotherapy, guidelines offer no clear preference for either doublet or triplet chemotherapy, nor do they offer clear preference regarding the type of targeted therapy when considering systemic conversion treatment, with the only exception that anti-EGFR therapy is restricted to patients with *RAS/BRAF*^{V600E} wildtype and left-sided primary tumors.[6]

The CAIRO5 study was designed to provide answers to the abovementioned issues. CAIRO5 is a phase 3 randomized controlled trial (RCT) executed by the Dutch Colorectal Cancer Group (DCCG) investigating the optimal systemic conversion therapy by randomizing CRLM patients with left-sided and *RAS/ BRAF*^{V600E} wildtype primary tumors between FOLFOX/FOLFIRI (patient preference) plus either bevacizumab or panitumumab and CRLM patients with right-sided and/or *RAS/ BRAF*^{V600E} mutated primary tumors between either FOLFOX/FOLFIRI bevacizumab or FOLFOXIRI bevacizumab.[18] Resectability of CRLM was judged by an expert liver panel based on predefined criteria. In **Chapter 2** we present a systematic review of RCTs comparing first line systemic conversion treatment regimens in (subgroups of) patients with initially unresectable CRLM. We focus on patient characteristics, basic methodology including clinical endpoints, criteria for (un)resectability, and long-term survival outcomes after systemic conversion treatments. In **Chapter 3** we hypothesized that the use of a liver expert panel to perform resectability assessments in CRLM patients would decrease practice variation by reducing individual subjectivity and subsequently would improve consensus on criteria for resection of CRLM. As such, the national DCCG Liver Expert Panel was created and incorporated in the CAIRO5 study assessing all patients for resectability at baseline and during induction treatment. In this chapter we analyzed the feasibility and outcomes of this expert panel.

Novel intensified systemic conversion treatments in combination with rapid evolution of (complex) surgical techniques such as two-staged hepatectomies including Associating Liver Partition and Portal vein Ligation for Staged hepatectomy (ALPPS), minimally invasive laparoscopic or robotic techniques, parenchymal sparing procedures, and multimodal approaches with local ablative therapy, have increased the numbers of patients to be assessed technically resectable.[19-21] Safety data in this patient group is essential. In **Chapter 4** we describe short-term postoperative morbidity and mortality after modern systemic conversion therapy followed by local treatment of the liver and determine risk factors for severe postoperative morbidity in patients participating in the phase 3 CAIRO5 study.

Part II Risk stratification of patients with CRLM

In the absence of resection criteria which are defined by oncological outcomes, all patients with technically resectable CRLM should currently be considered for surgery.[6]

However, high recurrence rates and early extrahepatic recurrences associated with poor survival underline the urgent clinical need to better risk-stratify patients prior to surgery.[22] Although multiple clinical risk scores (CRSs) predicting outcomes after CRLM resection have been proposed[23-26], clinical usefulness is limited since prediction models have mostly been developed in single institutions within selected populations. While the peak incidence of CRC patients is beyond 70 years of age, elderly patients are underrepresented in these CRS development cohorts.[27] In addition, the rate of perioperative systemic treatment in these studies varies according to local guidelines.[6,17,28] As such, generalizability is hampered by a lack of external validation in the general population and in underrepresented subgroups. Moreover, the majority of these studies did not adhere to recommended guidelines for appropriate statistical methodology.[25,29] With extended molecular profiling, possibilities arise to combine patient characteristics, technical-anatomical factors and molecular features to predict outcomes of treatments.[30] Finally, recurrence-free survival (RFS) is often used as predicted outcome for prediction models in CRLM patients, while RFS is an inadequate surrogate endpoint for OS after local treatment of CRLM[31]. In **Chapter 5** we evaluated whether tumor-biological factors could support the technical-anatomical resectability assessment. Therefore, we analyzed whether these factors were predictive of conversion to secondary resectable CRLM, early recurrence and early recurrence not amenable for local treatment with curative intent in patients participating in CAIRO5. In **Chapter 6** the generalizability and clinical validity of two established CRSs[23,24] were evaluated in a nationwide population-based cohort of the Netherlands Cancer Registry (NCR). The cohort comprised patients after local treatment of CRLM divided into pre-specified subgroups: with/without perioperative systemic therapy and age below/above 70 years. In **Chapter 7,** early extrahepatic recurrence within six months after local liver treatment (EHR) is proposed as a novel and clinically relevant endpoint in patients with CRLM. The prognostic relevance of this endpoint is analyzed by landmark analysis and in addition, a prediction model for EHR after local treatment of CRLM is developed and internally validated. After further external validation this prediction model can be used to guide therapeutic decision-making.

Part III Novel diagnostic strategies in patients with CRLM

According to the Response Evaluation Criteria in Solid Tumors (RECIST1.1), computed tomography (CT) is the most used imaging modality to evaluate systemic therapy efficacy and to detect disease recurrences after resection of CRLM.[6,32] Recurrences are caused by minimal residual disease (MRD) left *in situ* after CRLM resection. However, CT-scans have limited accuracy for detecting MRD due to low sensitivity and specificity[33] and the validity of RECIST1.1 has been questioned since it is hampered by high inter- and intraobserver variability, and its use of unidimensional size changes of only two target lesions per organ instead of total tumor volume (TTV).[34-36] Circulating tumor DNA (ctDNA) may offer an alternative diagnostic approach to determine MRD or monitor

treatment response. In **Chapter 8** we determined the prognostic value of postoperative ctDNA for detection of MRD and RFS in patients with CRLM after induction systemic therapy and we evaluated the associations between postoperative ctDNA detection and pathologic tumour response in liver metastases. In **Chapter 9** total tumor volume (TTV) response was compared to systemic treatment to RECIST 1.1. In addition, the prognostic value of TTV and RECIST 1.1 for RFS was assessed in patients with initially unresectable CRLM.

References

1. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16:713-732.
2. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. Lancet. 2019;394:1467-1480.
3. Manfredi S, Lepage C, Hatem C, et al. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. 2006;244:254-259.
4. Engstrand J, Nilsson H, Stromberg C, et al. Colorectal cancer liver metastases - a population-based study on incidence, management and survival. BMC Cancer. 2018;18:78.
5. Zhou H, Liu Z, Wang Y, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther.2022;7:70.
6. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386-1422.
7. Morris EJ, Forman D, Thomas JD, et al. Surgical management and outcomes of colorectal cancer liver metastases. Br J Surg. 2010;97:1110-1118.
8. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3:50-56.
9. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11:38-47.
10. Tomasello G, Petrelli F, Ghidini M, et al. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol. 2017;3:e170278.
11. Cremolini C, Antoniotti C, Stein A, et al. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. J Clin Oncol. 2020;2001225.
12. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer. 2018;88:77-86.
13. 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. 2012;99:477-486.
14. Tsilimigras DI, Ntanasis-Stathopoulos I, Bagante F, et al. Clinical significance and prognostic relevance of KRAS, BRAF, PI3K and TP53 genetic mutation analysis for resectable and unresectable colorectal liver metastases: A systematic review of the current evidence. Surg Oncol. 2018;27:280-288.
15. Holch JW, Ricard I, Stintzing S, et al. The relevance of primary tumour location in patients with metastatic colorectal cancer: A meta-analysis of first-line clinical trials. Eur J Cancer. 2017;70:87-98.
16. Lam VW, Spiro C, Laurence JM, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol. 2012;19:1292-1301.
17. National Coprehensive Cancer Nerwork. Colon Cancer (version 2.2021).
18. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
19. Wakabayashi G, Cherqui D, Geller DA, et al. Recommendations for laparoscopic liver resection: a report from the second international consensus conference held in Morioka. Ann Surg. 2015;261: 619-629.
20. Karanicolas PJ, Jarnagin WR, Gonen M, et al. Long-term outcomes following tumor ablation for treatment of bilateral colorectal liver metastases. JAMA Surg. 2013;148:597-601.
21. Lang H, de Santibanes E, Schlitt HJ, et al. 10th Anniversary of ALPPS-Lessons Learned and quo Vadis. Ann Surg. 2019;269:114-119.
22. 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. 2009;250:440-448.

23. Fong Y, Fortner J, Sun RL, et al. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. 1999;230:309-318; discussion 318-321.
24. Margonis GA, Sasaki K, Gholami S, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105:1210-1220.
25. Mahar AL, Compton C, Halabi S, et al. Personalizing prognosis in colorectal cancer: A systematic review of the quality and nature of clinical prognostic tools for survival outcomes. J Surg Oncol. 2017;116: 969-982.
26. Paredes AZ, Hyer JM, Tsilimigras DI, et al. A Novel Machine-Learning Approach to Predict Recurrence After Resection of Colorectal Liver Metastases. Ann Surg Oncol. 2020;27;5139-5147.
27. Papamichael D, Audisio RA, Glimelius B, et al. Treatment of colorectal cancer in older patients: International Society of Geriatric Oncology (SIOG) consensus recommendations 2013. Ann Oncol. 2015; 26:463-476.
28. Tanis PJ, Intven M. Modulaire revisie richtlijn colorectaal carcinoom. Ned Tijdschr Oncol. 2020; 100-102.
29. Moons KG, Altman DG, Reitsma JB, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015;162: W1-73.
30. Lang H, Baumgart J, Heinrich S, et al. Extended Molecular Profiling Improves Stratification and Prediction of Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;270:799-805.
31. Ecker BL, Lee J, Saadat LV, et al. Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. Lancet Oncol. 2022;23:1332-1342.
32. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-247.
33. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. J Clin Oncol. 2009;27:e279-280; author reply e281.
34. Beaumont H, Evans TL, Klifa C, et al. Discrepancies of assessments in a RECIST 1.1 phase II clinical trial - association between adjudication rate and variability in images and tumors selection. Cancer Imaging. 2018;18:50.
35. Yoon SH, Kim KW, Goo JM, et al. Observer variability in RECIST-based tumour burden measurements: a meta-analysis. Eur J Cancer. 2016;53:5-15.
36. Kuhl CK, Alparslan Y, Schmoee J, et al. Validity of RECIST Version 1.1 for Response Assessment in Metastatic Cancer: A Prospective, Multireader Study. Radiology. 2019;290:349-356.

PART I

OUTCOMES OF INDUCTION SYSTEMIC TREATMENT AND LOCAL TREATMENT OF COLORECTAL CANCER LIVER METASTASES

CHAPTER 2

Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review

Karen Bolhuis, Milan Kos, Martijn GH van Oijen,
Rutger-Jan Swijnenburg, Cornelis JA Punt

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Abstract

Background

There is no consensus on the optimal systemic conversion therapy in patients with unresectable colorectal cancer liver-only metastases (CRLM) to achieve a complete resection. Interpretation of trials is complicated by heterogeneity of patients caused by emerging prognostic and predictive characteristics, such as *RAS*/*BRAF* mutation status, lack of consensus on unresectability criteria and lack of data on clinical outcome of secondary resections. A systematic review was performed of characteristics of study populations and methodology of trials regarding patients with initially unresectable colorectal cancer liver-only metastases.

Methods

Phase II/III randomised trials, published after 2008, regarding first line systemic conversion therapy in patients or subgroups of patients with CRLM were included. Data on secondary resection outcomes were collected.

Results

Overall, 20 trials were included for analysis: seven prospective trials in patients with unresectable CRLM and 13 trials in the overall population of unresectable metastatic CRC (mCRC) with retrospective subgroup analysis of CRLM patients. Fourteen trials did not provide unresectability criteria at baseline, and criteria differed among the remaining studies. Trials and study populations were heterogeneous in prognostic/predictive factors, use of primary end-points, and reporting on long-term clinical outcomes. R0-RRs in CRLM patients varied between CRLM studies and mCRC studies, with rates of 22-57% and 11-38%, respectively.

Conclusions

Cross-study comparison of (subgroups of) studies regarding first-line systemic treatment in patients with unresectable CRLM is hampered by heterogeneity in study populations, trial designs, use of *(K)RAS*/*BRAF* mutational tumour status, and differences/absence of unresectability criteria. No optimal conversion systemic regimen can be selected from available data. Prospective studies with well-defined criteria of these issues are warranted.

Highlights

- Cross-study comparison of systemic conversion therapies in CRLM patients is complicated
- Study populations and resection rates vary considerably between CRLM studies
- No preferential systemic regimen for conversion therapy can be given
- Transparent baseline (un)resectability criteria for CRLM are needed
- Long-term survival outcomes after resection were not reported in the majority of studies.

Introduction

The prognosis of unresectable metastatic colorectal cancer (mCRC) is poor, with 5-year survival rates of less than 15%.[1] Patients with CRC metastases confined to the liver (colorectal cancer liver only metastases [CRLM]) who are candidates for resection/ablative treatment of metastases may have 5-year-survival rates of 45-60%.[2-4] Initially unresectable CRLM patients may convert to resectable disease upon systemic therapy[5], with superior 5-year survival outcomes compared with systemic therapy only (32% versus 9%, respectively).[3]

There is no consensus regarding the optimal systemic conversion therapy.[6,7] However, doublet chemotherapy (fluoropyrimidine with oxaliplatin or irinotecan) is preferred over fluoropyrimidine monochemotherapy because increased response rates have been correlated with higher resection rates (RRs).[8-10] The addition of a targeted agent (anti-vascular endothelial growth factor (VEGF) or anti-endothelial growth factor receptor (EGFR) antibody) to doublet chemotherapy has further increased response rates to around 60%.[11-13] These combination-regimens are currently recommended as conversion systemic therapy for CRLM patients.[6] No regimen is preferred, except that anti-EGFR therapy is limited to patients with left-sided primary tumours that are *RAS*/*BRAF*^{V600E} wildtype.[13,14] More recent data show higher response rates for triplet chemotherapy (FOLFOXIRI) plus bevacizumab than cytotoxic doublet chemotherapy with bevacizumab.[15] However, outcomes in terms of R0-RRs and overall survival in the subgroup of CRLM patients were not consistently improved.[15-17] FOLFOXIRI in combination with anti-EGFR therapy, mainly studied in single-arm phase II trials, is also effective (response rates 70-86%) but at the cost of significantly more toxicity.[18-21] To further investigate this combination, a randomised phase III study comparing FOLFOXIRI-panitumumab with FOLFOX-panitumumab, is ongoing.[22]

Translating outcomes of trials with aforementioned systemic conversion therapies to individual CRLM patients is challenging. Firstly, only few randomised controlled studies (RCTs) have been performed in patients with initially unresectable CRLM, and many of these trials do not report on long-term survival outcomes.[10] Secondly, because of the constantly evolving field with the acknowledgement of prognostic and predictive factors such as *RAS* and *BRAF* mutation status and sidedness of the primary tumour, most data on CRLM patients are retrieved from (often unplanned) small and retrospective subgroup analyses without upfront selection.[14,23] Thirdly, lack of consensus on criteria for surgical unresectability causes variation in patient selection among studies and even between centres participating to the same study.[10,14,24] Lastly, most studies with bevacizumab-containing regimens have not assessed morphological responses on the computed tomography (CT) scan which are associated with favourable outcomes[25] and correlate poorly with Response Evaluation Criteria in Solid Tumours (RECIST) criteria.[14,26]

We present a systematic review of randomised studies in (subgroups of) patients with initially unresectable CRLM, with focus on patient characteristics and basic methodology including clinical endpoints, criteria for unresectability, and long-term survival outcomes.

Methods

This systematic review was performed as per the PRISMA guidelines for systemic reviews.[27]

Search strategy

PubMed, EMBASE and Web of Science databases were searched up to *July 2019* to identify relevant studies. In august 2020, a final search was done for any updated literature of included trials. Because bevacizumab as the targeted agent for mCRC was FDA approved in 2004 and to minimise cohort effects, studies published before 2008 were excluded. Relevant keywords included terms related to 'Colorectal Cancer' AND 'Combined chemotherapeutic regimens'. In addition, we manually searched for relevant European Society for Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO) meeting abstracts. The complete search strategy is presented in **Supplementary Table S1**. Two reviewers (KB and MK) reviewed the literature independently, and discrepancies were resolved by discussion until consensus was reached.

Eligibility criteria

Studies had to meet all of the following eligibility criteria: (1) phase II/III/IV RCTs, (2) study populations with CRLM or mCRC considered initially unresectable, (3) reporting on outcomes of CRLM patients, and (4) first-line systemic fluoropyrimidine-based (doublet or triplet) therapy combined with either an anti-EGFR antibody or bevacizumab. Anti-EGFR studies were included if they reported relevant outcomes in patients at least selected for *KRAS* wildtype (wt) tumours, because of the recognised predictive value for anti-EGFR therapy. When multiple publications from the same study population were identified, the most relevant publication was included. Definitions of CRLM (subgroups) of studies were screened to ensure patients with extrahepatic metastases were excluded.

Data extraction and statistical analysis

Patient selection data, such as baseline unresectability criteria and *(K)RAS*/ *BRAF*^{V600E} mutational status (wildtype (wt) and mutation (mut), were collected. Response rates (ORR) defined as partial and complete response, R0-RRs, any resection rate (RR) defined

as combined R0/R1/R2 resections, and survival outcomes (OS and PFS) were extracted independently by two authors (KB and MK). We calculated weighted pooled means for efficacy outcomes (ORR, (R0-)RR and median OS) for doublet chemotherapy with bevacizumab, doublet chemotherapy with anti-EGFR targeted therapy and triplet chemotherapy with bevacizumab, based on the number of evaluable CRLM patients per treatment arm. To analyse the association between ORR and R0-RR, we performed a Spearman's rank correlation test. The quality of the included trials and subgroup analysis and potential bias is described.

Results

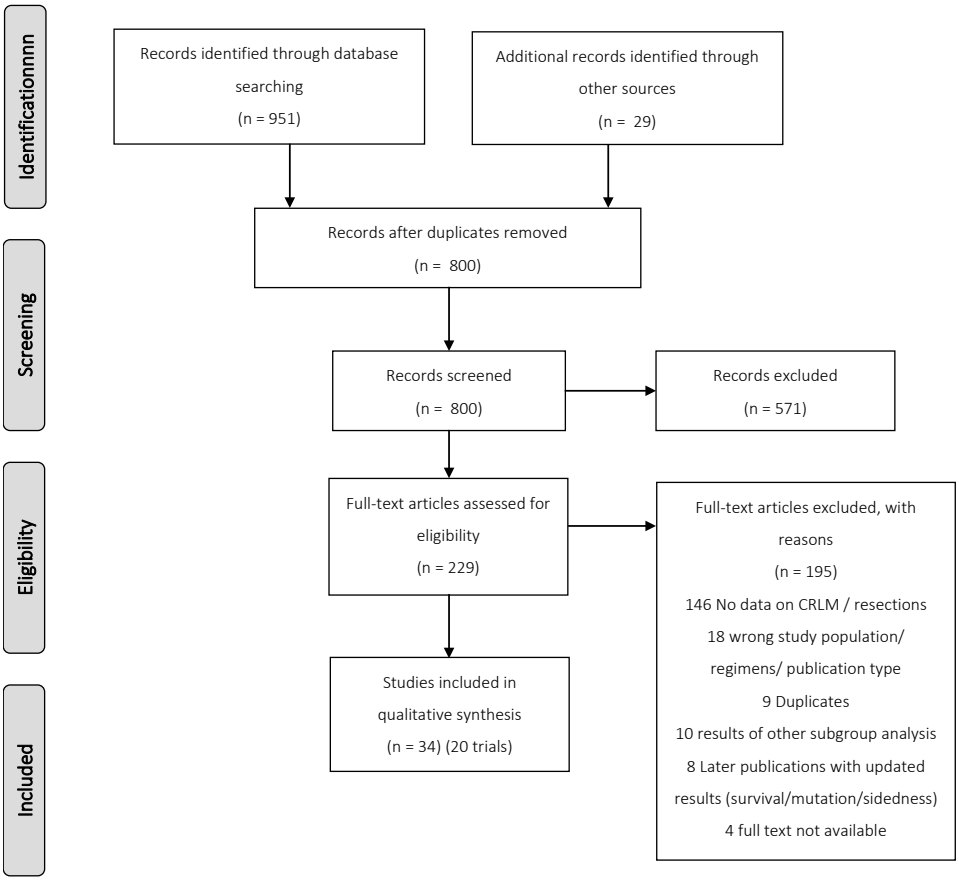
Literature search

Using the search strategy reported, a total of 800 unique records were retrieved and screened on title and abstract. The majority of studies (571) were excluded because they did not comprise first-line RCTs. The full text of the remaining 229 articles was read to select studies reporting on outcomes of patients with initially unresectable CRLM. A total of 195 articles were excluded, mainly because they did not report data on resection outcomes in patients with CRLM (n=146 studies). The flow chart of search and in- and excluded trials is presented in **Figure 1**.

Included studies

After reading the full text, a total of 34 articles, with published results of nine phase II[17,28-39], ten phase III[11,12,15,23,34,35,38,40-51] and one phase IV[52,53] RCTs, were included. Three studies concerned predominantly Asian populations[40,52,54], all other studies concerned western populations. Eleven studies randomised between anti-EGFR (cetuximab or panitumumab) or anti-EGFR and bevacizumab-containing regimens. The other seven studies regarded bevacizumab-containing regimens only. Five of 19 selected studies reported on outcomes of triplet chemotherapy with bevacizumab or cetuximab. In general, there was a high variability with regard to patient characteristics: firstly, some studies included only initially unresectable CRLM patients, while others included the overall population of mCRC patients with unresectable disease; and secondly, study populations differed in selection based on mutational status (*KRAS*, *RAS*, *BRAF* or no selection), synchronous or metachronous disease and sidedness of primary tumour. An overview of differences in basic patient selection of included studies is displayed in **Figure 2 and Tables 1, 2 and 3**.

Figure 1 PRISMA flow diagram outlining search results and study selection.



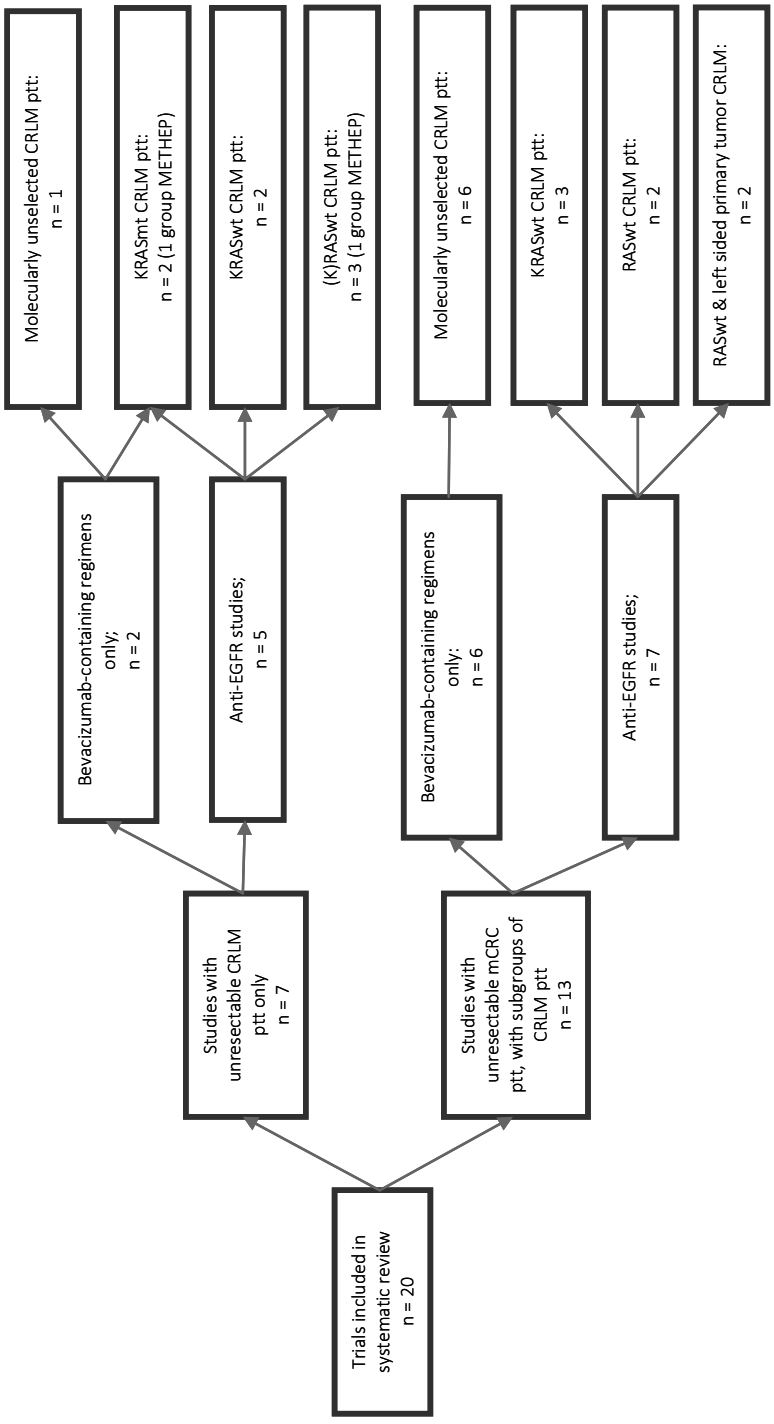


Figure 2 Flow chart showing differences in patient characteristics in selected studies with regard to extend of metastatic disease (unresectable CRLM or unresectable metastatic CRC patients) and selection on prognostic and predictive molecular biomarkers ((K)RAS/BRAF mutation) and sidedness of primary tumor. Abbreviations: mCRC, metastatic colorectal cancer; CRLM, colorectal cancer liver-only metastases; ptt, patients.

Prospective studies limited to unresectable CRLM patients (Table 1)

Study design

We included seven trials with upfront selection of patients with initially unresectable CRLM. Two of these trials randomised between bevacizumab-containing regimens[17,52], four trials used anti-EGFR-containing regimens in one or both treatment arms[28,30,39,40] and one trial selected patients based on *(K)RAS* mutational status (*(K)RAS* wt versus *(K)RAS* mutation (mut)) and randomised the two groups between either systemic combinations with bevacizumab (*(K)RAS* mut) or with cetuximab *(K)RAS* wt).[31] Two studies compared targeted therapy with doublet versus triplet chemotherapy[17,31] and one trial compared a bevacizumab-containing regimen with an anti-EGFR containing regimen.[54] Regimens used in the remaining trials are presented in **Table 1**.

Five primary end-points were used: objective response rates (n=2), progression free survival (n=1), conversion rate (n=2), R0/R1 RRs (n=1) and overall RR (n=1) (**Table 1**).

Patient selection

Two trials recruited only patients with synchronous metastases after resection of the primary tumour.[40,53] Although all CRLM studies described baseline unresectability criteria, each study used a different set of criteria. A total of nine criteria were formulated: 1) inadequate future liver remnant (FLR) (n=6), 2) metastases in contact with major vessels of the FLR (n=2), 3) no upfront R0/R1 resection possible (n=4), 4) more than 4 metastases (n=3), 5) diameter of metastases ≥5 (n=1) or ≥10 cm two contiguous hepatic segments (n=2). Although inadequate FLR was used as criterion in six of seven studies, it should be noted that different cut-off points, ranging between 20-30%, were used to define this criterion (**Table1**). Included patients per trial ranged from 77 to 256 patients. After selection for *(K)RAS* wt status in studies with anti-EGFR regimens, the number of patients ranged from 53 to 147 patients. Data on *RAS* and *KRAS* mutational status were presented in three[28,31,39] and two studies[29,40] with anti-EGFR regimens, respectively. Two trials presented data on patients with *(K)RAS* mut tumours.[31,52,53]

Outcomes

Overall response rates (ORRs) ranged from 55 to 85%. The highest ORR was reached for FOLFOX-cetuximab (85%) in *(K)RAS* wt patients[39] and FOLFOXIRI-bevacizumab (81%) in *(K)RAS* unselected patients.[17] R0-RRs ranged from 22 to 57% and median OS from 25.7 to 49 months, and was not reached in experimental arm of the OLIVIA and ATOM trial. The clinical outcomes for each individual study are provided in **Table 1**.

Table 1 Resection and survival outcomes in trials limited to patients with initially unresectable CRLM.

Study / author	Regimens	CRLM (sub)group[a-g] (n, % of total)	Synchr metastases (%)	Primary endpoint	ORR (%)	RR (%)	R0-resection (n, %)	PFS (mo)	HR PFS (95% CI)	OS (mo)	HR OS (95% CI)	Definition baseline criteria for unresectability
Non-anti EGFR targeted therapy												
OLIVIA[17]				Overall								At least 1 of following criteria as assessed by local MDT:
Phase II	FOLFOXIRI-Bev	41 (100)[a]	73	resection	81	61	54	18.6▫	0.43	NR▫	0.35	• Metastases in contact with major vessels of remnant liver
2015	FOLFOX-Bev	39 (100)	82	rate	62	49	31	11.5	(0.26-0.72)	32.2	(0.15-0.80)	• No upfront R0/R1 resection of all hepatic lesions possible
Europe				(R0/R1/R2)								• < 30% estimated residual liver volume after resection
BECOME*[52,53]												At least 1 of following criteria as assessed by 3 liver surgeons
												• < 30% estimated residual liver volume after resection
Phase IV	FOLFOX-Bev	121 (100)[g]	100	Conversion	55▫▫	23	22▫▫	9.5▫▫	0.49	25.7▫	0.71	• Complete resection (negative margins) not possible
2019	*FOLFOX*[¥]	*120 (100)*	100	rate	*37*	*7*	*6*	*5.6*	(0.38-0.65)	*20.5*	(0.52-0.97)	• Inability to preserve 2 contiguous hepatic segments
Asia												• Inadequate vascular inflow and outflow and/or biliary drainage
Anti-EGFR targeted therapy in one or both treatment arms												
CELIM[∞][29,30]												Technically non-resectable by local surgeon and radiologist
Phase II	FOLFOX-Cet	56[a] / 35 (63)[b]	75	ORR	68[a]	53[a]	38[a]	12.1[b]	1.13	36.1[b]	0.86	• ≥ 5 liver metastases
	FOLFIRI-Cet	55 / 35 (64)	71		57	49	30	11.5	(0.69-1.85)	41.6	(0.48-1.53)	• Inadequate FLR
2010												• Infiltration of all hepatic liver veins, both hepatic arteries or both
Europe												• portal vein branches
Ye et al.**[40]												Defined as non-resectable by MDT (>3 liver surgeons and radiologist)
Phase III	FOLFOX/FOLFIRI+Cet	70 (100)[b]	100	Conversion	57▫▫	27▫▫	26▫▫	10.2▫▫	0.60	30.9▫	0.54	• No adequate FLR (>20%)
	FOLFOX/FOLFIRI[¥]	*68 (100)*	100	rate	*29*	*10*	*7*	*5.8*	(0.41-0.87)	*21.0*	(0.33-0.89)	• Complete resection (negative margins) not possible
2013												• Inability to preserve 2 contiguous hepatic segments
Asia												• Inadequate vascular inflow and outflow and/or biliary drainage
PLANET-TTD[†28]												Unresectable CRLM considered by the local hepatic surgeons' criteria
Phase II	FOLFOX-Pan	27 (71)[c]	85	ORR	78	37▫	26▫	13	0.7	39	0.9	• Or >4 liver metastasis
2017	FOLFIRI-Pan	26 (67)	85		73	69	54	15	(0.4-1.3)	49	(0.5-1.9)	• Or any metastasis > 10 cm
Europe												
METHEP-2[††31]												Defined as non-resectable by surgeon and radiologist at MDT:
Phase II	FOLFOX/FOLFIRI	126 (100)[a]		R0/R1	-	-	48▫	-	-	37.6	0.80	• Impossibility to resect all metastases in a single operation
	+Bev/Cet		88	Resection							(0.56-1.16)	• While preserving at least 30% of healthy liver tissue
2018	FOLFIRINOX	130 (100)		rate	-	-	57	-	-	42.9		• While preserving portal vein and hepatic artery homolateral
Europe	+Bev/Cet											to the remnant liver or a portal pedicle
ATOM[39,54]												At least one of the following criteria:
Phase II	FOLFOX-Cet	59 (100)[d]	92	PFS	85▫	49	37	14.8	0.80	NR	0.83	• ≥5 metastases
	FOLFOX-Bev	57 (100)	91		68	56	44	11.5	(0.51-1.26)	30.4	(0.44-1.56)	• diameter of metastases ≥ 5 cm
												• Inadequate FLR (function)
2019												• Invasion in all hepatic veins or inferior vena cava
Asia												• Invasion into both right and left hepatic arteries or portal veins

Abbreviations table 1: CRLM, colorectal liver-only metastases; Synchr, synchronous; ORR, objective response rate; RR, resection rate (R0+R1+R2); PFS, progression free survival; HR, hazard ratio; OS, overall survival; NR, not reached; wt, wildtype; mt, mutated; MDT, multidisciplinary team; Bev, bevacizumab; Cet, cetuximab; FLR, future liver remnant. ▫p-value < 0.05; ▫▫p-value <0.01.

Results of study concerns (sub)group of patients which are: molecularly unselected[a], KRASwt[b], RASwt[c] or (K)RASwt[d], (K)RASmt[g]

Legend table 1: * Data subtracted from abstract presented at ESMO 2019; ** Study population concerns patients with synchronous metastases only and resection of primary tumour at baseline. Cetuximab was not approved for reimbursement; ǂ Surgical resection rates used for RR, not further specified. R0 and R1 resections were not reported separately and described as R0-RR in this table; ∞ PFS and OS outcomes of KRASwt subgroup. Other outcomes for patients not selected for (K)RAS mutation status; ǂǂ Patients were randomized between doublet or triplet therapy with either bevacizumab for (K)RASmt and cetuximab for (k)RASwt tumours. Outcomes given for both (K)RASmt and wt together. Synchronous disease was not reported separately. R0 and R1 resections were not reported separately and described as R0-RR in this table. Data subtracted from abstract presented at ASCO 2018; ¥ Treatment arm not included in analysis for this systematic review because not (standard) targeted therapy.

Studies in unresectable mCRC patients, with retrospective subgroup analysis of CRLM patients

Thirteen trials concerned patients with unresectable mCRC with subgroup analysis of patients with CRLM. Six trials used bevacizumab-containing regimens and the other seven trials anti-EGFR targeted therapy in at least one treatment arm (**Table 2 and 3**). Trials reporting on outcomes in CRLM subgroups did not present unresectability criteria at baseline.

Studies with bevacizumab-containing regimens (Table 2)

Study design

A total of six studies comprised a bevacizumab-containing regimen.[12,15,32,33,49,55] Three studies compared triplet versus doublet chemotherapy with bevacizumab.[15,49,56]

Patient characteristics

The subgroups of CRLM patients ranged from 80 to 418 patients per study, which comprised 20-30% of the total study population. Patients in these subgroups were not selected based on *(K)RAS* mutational status.

Outcomes

R0-RRs were reported in five out of six studies and ranged between 11-38%. The FOLFOX-Bevacizumab regimen was used in both the HORIZON III and MAVERICC trial, but R0-RRs in CRLM patients varied with 14% versus 39%, respectively.[32,55] None of the included studies reported on median PFS or median OS in CRLM patients. RRs in CRLM patients per study are presented in **Table 2**.

Studies with anti-EGFR-containing regimens (Table 3)

Study design

We identified seven studies regarding patients with unresectable mCRC, with reported outcomes of anti-EGFR therapy in CRLM subgroups (**Table 3**). Four trials compared chemotherapy with or without anti-EGFR therapy[47,48,57,58] and two trials compared chemotherapy with either anti-EGFR- or bevacizumab.[36,45] One trial compared chemotherapy with bevacizumab with chemotherapy, bevacizumab and anti-EGFR therapy.[50] Except for the PEAK and the FIRE-3 trial, the subgroup analyses of CRLM in other trials were of unplanned and retrospective nature.

Table 2 Resection and survival outcomes in studies for patients with initially unresectable mCRC with other than anti-EGFR targeted therapy.

Study	Regimens	Total (n)	Primary endpoint	ORR (%)	PFS (mo)	HR PFS (95% CI)	OS (mo)	HR OS (95% CI)	CRLM pts (n, % of total)	RR CRLM (%)	R0-resection CRLM (n, %)
NO16966[12,42]	XELOX/FOLFOX-Bev	699	PFS	38	9.4▫▫	0.83 (0.72-0.95)	21.3	0.89 (0.76-1.03)	211 (30)	-	12
Phase III	*XELOX/FOLFOX*[¥]	*701*		*38*	*8.0*		*19.9*		*207(30)*	-	*12*
2008											
USA											
HORIZON III[55]	FOLFOX-Bev	713	PFS	47	10.3	1.10 (0.97-1.25)	21.4	0.95 (0.82-1.10)	158 (22)	14	-
Phase III	*FOLFOX-Cediranib*[¥]	*709*		*46*	*9.3*		*22.8*		*132 (19)*	*11*	-
2012											
Europe											
TRIBE[Ω15,41,51)]	FOLFOXIRI-Bev	252	PFS	65▫	12.3▫▫	0.77 (0.65-0.93)	29.8▫	0.80 (0.65-0.98)	59 (23)	41	32
Phase III	FOLFIRI-Bev	256		54	9.7		25.8		46 (18)	39	28
2015											
Europe											
TRIBE-2[H49,67]	FOLFOXIRI-Bev	339	PFS2	62▫▫	19.1▫▫	0.74 (0.62-0.88)	27.6▫	0.81 (0.67-0.98)	108 (32)	-	38
Phase III	Seq doublet-Bev	340		50	16.4		22.6		97 (29)	-	28
2018											
Europe											
STEAM*[33]	cFOLFOXIRI-Bev	93	ORR	72	11.9▫▫	0.7 (0.5-0.9)	34.0	0.8 (0.5-1.2)	28 (30)	29	25
Phase II	Seq doublet-Bev	92	PFS	73	11.4▫	0.7(0.5-1.0)	28.3	1.0 (0.7-1.5)	28 (30)	21	18
2018	FOLFOX-Bev	95		62	9.5	REF	30.7		27 (28)	19	11
USA											
MAVERICC[32]	FOLFOX-Bev	188	PFS	65	10.1	0.79 (0.61-1.01)	23.9	0.76 (0.56-1.04)	44 (23)	39	34
Phase II	FOLFIRI-Bev	188		61	12.6		27.5		36 (19)	31	17
2018											
USA											

▫p-value <0.05 and ▫▫p-value <0.01.

[Ω] Data subtracted from abstract presented at ASCO 2013, RR reported in abstract as: surgical RR with curative intent.

Not further defined; ǂ Data subtracted from abstract presented at ASCO 2019, outcomes of PFS concerns PFS2;

* Liver RR not further specified as R0+R1+R2 in publication;

[¥] Treatment arm not included in analysis for this systematic review because not (standard) targeted therapy.

Abbreviations: mCRC, metastatic colorectal cancer ORR, objective response rate; PFS, progression free survival; mo, months; HR, hazard ratio; OS, overall survival; RR resection rate (R0+R1+R2); CRLM, colorectal cancer liver-only metastases; Bev, Bevacizumab; seq, sequential; c, concurrent.

Table 3 Resection and survival outcomes in studies for patients with initially unresectable mCRC with anti-EGFR targeted therapy

Study	Regimens	mCRC subgroup[a-f] (n, % of total)	Primary endpoint	ORR (%)	PFS (mo)	HR PFS (95% CI)	OS (mo)	HR OS (95% CI)	CRLM subgroup[a-f] (n, % of total)	RR CRLM (%)	R0-resection CRLM (%)	OS CRLM (mo)	HR OS CRLM (95% CI)
CRYSTAL[†11,34,59]													
Phase III	FOLFIRI-Cet	142 (24)[f]	PFS	73▫▫	12.0▫▫▫	0.50	28.7▫▫	0.65	68[b] / 43 (7)[c]	23[b]	16[b]	29.8[c]	0.65
2009	*FOLFIRI*[¥]	*138 (23)*		*41*	*8.9*	(0.34 - 0.72)	*21.7*	(0.50-0.86)	*72 / 46 (8)*	*11*	*7*	*29.5*	(0.38 – 1.10)
Europe													
CAIRO2[50]													
Phase III	*CAPOX-Bev-Cet*[¥]	*158 (42)*[b]	*PFS*	*50*	*10.5*	-	*21.8*	-	*40 (11)*[b]	23	-	*27.6*	0.92
2009	CAPOX-Bev	156 (41)			10.6		22.4		45 (12)	9	-	26.1	(0.58 - 1.46)
Europe													
OPUS[23,37]													
Phase II	FOLFOX-Cet	38 (23)[c]	ORR	58▫▫	12.0	0.53	19.8	0.94	25 (15)[b]	-	16	26.3	0.93
2009	*FOLFOX*[¥]	*49 (29)*		*29*	*5.8*	(0.27-1.04)	*17.8*	(0.56-1.56)	*23 (14)*	-	*4*	*23.9*	(0.44 - 2.00)
Europe													
PRIME[†34,35,46]													
Phase III	FOLFOX-Pan	169 (28)[f]	PFS	68▫▫▫	12.9▫▫	0.72	30.3▫	0.73	48[c] / 33 (6)[f]	30[f]	27[f]	40.7[c]	0.71
2010	*FOLFOX*[¥]	*159 (27)*		*53*	*9.2*	(0.57 - 0.90)	*23.6*	(0.57-0.93)	*41 / 31 (5)*	*26*	*19*	*33.4*	(0.43-1.16)
Europe													
COIN[23,48]	CAPOX/FOLFOX-												
Phase III	Cet	367[b]/292(34)[e]	OS	64▫[b]	8.6[b]	0.96	19.9[e]	1.02	87(11)[b]	-	15	-	-
2011	*CAPOX/FOLFOX*[¥]	*362/289(35)*		*57*	*8.6*	(0.82 - 1.12)	*20.1*	(0.83-1.24)	*91 (11)*	-	*13*	-	
Europe													
FIRE-3[Ω 34,43-45]													
Phase III	FOLFIRI-Cet	157 (53)[f]		69	10.7	0.90	38.3▫▫	0.63	71 (24)[c]	22[Ω]	-	40.0	0.74
2014	FOLFIRI-Bev	149 (51)	ORR	62	10.7	(0.71 - 1.14)	28.0	(0.48-0.85)	62 (21)		-	33.3	(0.47 - 1.15)
Europe													
PEAK[†34,36]													
Phase II	FOLFOX-Pan	53 (37)[f]	PFS	64	14.6	0.68	43.3	0.77	18 (13)[f]	28[f]	22[f]	-	-
2014	FOLFOX-Bev	54 (38)		57	11.5	(0.45 - 1.04)	32.0	(0.46-1.28)	15 (10)	40	33	-	
Europe													

▫ p-value <0.05; ▫▫p-value <0.01; ▫▫▫p-value <0.001.

Results of study concern (sub)group of patients which are: molecularly unselected[a], *KRAS*wt[b], *RAS*wt[c] or *(K)RAS*wt[d], *RAS*wt and *BRAF*wt[e], *RAS*wt and left primary[f].

[†] Resection rate defined as any resection, not further specified. RR in CRYSTAL study defined as R0+R1, R2 not given;

[Ω] Resection rates not given for separate treatment arms and defined as any hepatic resection;

[¥]Treatment arm not included in analysis for this systematic review because not (standard) targeted therapy.

Abbreviations: mCRC, metastatic colorectal cancer; ORR, objective response rate; PFS, progression free survival; HR, hazard ratio; OS, overall survival; CRLM, colorectal cancer liver-only metastases; RR resection rates (R0+R1+R2); Cet, cetuximab, Pan, panitumumab; Bev, bevacizumab.

Patient characteristics

The population of the CRLM subgroups differed between the studies in regard to *RAS*/*BRAF* mutational status. Data on *(K)RAS* mutation status and sidedness of primary tumour in CRLM patients was available for patients with *KRAS* wt tumours in three studies[48,50,59], patients with *RAS* wt tumours in two studies[44,59], and *RAS* wt and left sided primary tumours in two studies.[35] The number of patients in the subgroups of patients with CRLM and *(K)RAS* wt patients ranged from 33 to 178 patients, comprising 5-22% of total study populations (Table 3).

Outcomes

R0-RRs ranged from 15-33%. Five out of seven trials presented data on median OS in the anti-EGFR treatment arms in CRLM patients, which varied from 26.3 to 40.7 months. Response rates, RRs and survival outcomes for each individual study are presented in Table 3.

Pooled efficacy outcomes of systemic therapy for (subgroups of) CRLM patients (Table 4)

Doublet chemotherapy with anti-EGFR therapy in patients with (K)RAS wt tumours

Eight and nine studies reported on response and RRs and median overall survival. In CRLM studies compared to non-CRLM studies the weighted pooled mean for ORR was 68% and 81%, for R0-RR 34% and 18% and median OS 39 compared to 36 months, respectively.

Doublet chemotherapy with bevacizumab in (K)RAS unselected patients

Five and six studies reported on (R0)-RR. In CRLM studies compared to non-CRLM studies a weighted pooled mean for RR of 49% vs. 23% and R0-RR of 31% vs. 19% was reported, respectively. Response and survival outcomes in CRLM patients were reported in one study only, with an ORR of 62% and median OS of 32.2 months.

Doublet chemotherapy with bevacizumab in patients with (K)RAS wt tumours

Two to four selected studies reported on efficacy outcomes in CRLM patients. In CRLM studies compared to non-CRLM studies the weighted pooled mean ORR was 68% and 72%, R0-RR 44 and 33% and median OS was 30.4 and 30.3 months, respectively.

Triplet chemotherapy with bevacizumab in (K)RAS unselected patients

Three and four studies reported on (R0)-RR. In CRLM studies compared to non-CRLM studies the weighted pooled mean RR was 61% and 37% and R0-RR of 44% and 33%, respectively. Response and survival outcomes in CRLM patients were reported in one study only, with an ORR of 81% and median OS was not reached. Results are presented in Table 4.

Table 4 Summary of efficacy results of first line systemic therapy in (subgroups of) CRLM patients according to selection for *(K)RAS* mutational status and stratified for studies with or without upfront selection of CRLM patients.

Characteristics	Studies recruiting CRLM patients				Studies recruiting mCRC and evaluating CRLM subgroups			
	Studies reporting on outcome* (n)	Sample size (n)	Weighted pooled mean	Range [†]	Studies reporting on outcome* (n)	Sample size (n)	Weighted pooled mean	Range [†]
Doublet-anti EGFR therapy, *(K)RAS wt* [a]								
ORR, %	4	293	68	57-85	4	197	81	76 – 84
RR, %	4	293	45	27-69	4	190	24	22-30
R0-RR, %	4	293	34	26-54	5	231	18	15-27
Median OS, mo[¥]	4	252	39	30.9-49	4	187	36	26.3-40.7
Doublet-bevacizumab, *(K)RAS unselected* [b]								
ORR, %	1	39	62	NA	0	-	-	-
RR, %	1	39	49	NA	4	339	23	14-39
R0-RR, %	1	39	31	NA	5	489	19	11-34
Median OS, mo	1	39	32.2	NA	0	-	-	-
Doublet-bevacizumab, *KRAS wt* [c]								
ORR, %	1	59	68	NA	1	62	72	NA
RR, %	1	59	56	NA	3	122	19	9-40
R0-RR, %	1	59	44	NA	1	15	33	NA
Median OS, mo	1	59	30.4	NA	2	107	30.3	26.1 – 33.3
Triplet-bevacizumab, *(K)RAS unselected* [d]								
ORR, %	1	41	81	NA	0	-	-	-
RR, %	1	41	61	NA	2	87	37	29-41
R0-RR, %	1	41	54	NA	3	195	34	25-38
Median OS, mo	1	41	NR	NA	0	-	-	-

[†] No range is given if outcomes were reported in < 2 studies; [¥] Median OS was not reached in ATOM trial. Median OS was estimated based on a Kaplan Meier OS curve.

*The number of studies reporting on outcome does not always count up to the total number of studies selected for this pooled analysis because some studies did not report on a specific outcome.

[a] Studies selected for this analysis: CRLM Studies: CELIM[29,30,35], Ye et al.[40], PLANET-TTD[28], ATOM[39]. mCRC studies with CRLM subgroup analysis: CRYSTAL[59], OPUS[68], PRIME[46], COIN[48], FIRE-3[44] and PEAK[36],

[b] Studies selected for this analysis: CRLM Study: OLIVIA[17].

mCRC studies with CRLM subgroup analysis: NO16966[42], TRIBE[51], TRIBE-2[49], STEAM[33], MAVERICC[32] and HORIZON III[55].

[c] Studies selected for this analysis: CRLM Study: ATOM[39].

mCRC studies with CRLM subgroup analysis: CAIRO2[50], FIRE-3[44] and PEAK[44].

[d] Studies selected for this analysis: CRLM Study: OLIVIA[17].

mCRC studies with CRLM subgroup analysis: TRIBE[51], TRIBE-2[49] and STEAM[33].

Abbreviations: CRLM, colorectal cancer liver-only metastases; wt, wildtype; ORR, overall response rate; RR, resection rate; R0-RR, R0-resection rate; OS, overall survival; Doublet, fluorouracil based chemotherapy with irinotecan or oxaliplatin; Triplet, fluorouracil based chemotherapy with irinotecan and oxaliplatin.

Correlation of response and R0-resection rate

No significant correlations were observed between RECIST response rate and R0-RR either for doublet chemotherapy (r=0.19, p=0.41), triplet chemotherapy (r=0.32, p=0.68), chemotherapy plus bevacizumab (r=0.55, p=0.12), or chemotherapy plus anti-EGFR therapy (r=0.057, p=0.87). This correlation was also absent in pooled data from prospective studies with upfront selection of CRLM patients (r=0.57, p=0.084) and retrospective studies without upfront selection of CRLM patients (r=-0.015, p=0.96). (Data not shown)

Discussion

Cross-study comparison of outcomes of studies regarding systemic conversion therapy in patients with initially unresectable CRLM is complicated by heterogeneity in study populations, surgical decision making, and trial designs. Currently available data do not allow to select a preferential systemic regimen for conversion therapy. Colorectal cancer is increasingly recognized as a heterogeneous disease with varying treatment effects based on different prognostic (bio)markers such as *(K)RAS* and *BRAF* mutation status.[60] In the selected CRLM (subgroup) analyses we noticed a large variation among trials in distribution of synchronous/metachronous disease, and the prognostic and predictive value of *(K)RAS* mutation status and sidedness of primary tumour were not yet established at the time most studies were designed.[34] Data from studies in which these factors were not included are difficult to interpret, and, when available in retrospective subgroup analysis, concerned only small subgroups ranging from 5-22% of the original mCRC study population resulting in insufficient power to detect any differences in outcomes.

Criteria for unresectability at baseline differed significantly in trials limited to CRLM, and none of the trials in the overall population of unresectable mCRC patients presented such criteria for its CRLM subgroups. This implies a selection bias and heterogeneity of study populations and leads to high variation in resectability assessment.[30,45,54,61] Also, compared to prospective trials in CRLM patients, patients in trials in the population of unresectable mCRC reporting on the CRLM subgroup may not have been screened as rigorously on extrahepatic metastases at diagnosis or on resectability of disease recurrence by an expert multidisciplinary team (MDT), and more often may have presented with permanently unresectable CRLM. These factors may explain the higher rates of liver resection in the prospective studies compared to the studies with retrospective subgroup analyses.

Furthermore, lack of consensus on criteria for (un)resectability has been demonstrated[30], and influences the outcomes of trials as was shown by a retrospective surgical review of the FIRE-3 trial. In this trial more than 70% of centrally reviewed patients with CRLM were in retrospect considered resectable at the time of best response during systemic treatment, but only 36% of these patients actually underwent resection.[45] Additionally, more patients in this study underwent secondary resections when treated in university hospitals compared to other treatment settings while resections were comparable in terms of technical difficulty and anticipated clinical benefit. Patients with centrally reviewed resectable CRLM who actually underwent resection had a significantly better median OS compared to patients without resection.[45] This suggests that surgical treatments with chance of long-term survival is denied to a significant number of patients with initially unresectable mCRC. This is supported by the large difference in R0-RRs among the studies in this review, which range from 22-57% in prospective studies in CRLM only patients and from 11-38% in retrospective subgroup analyses of CRLM patients in the overall mCRC population. A striking example are the results of the HORIZON III and the MAVERICC studies which concern seemingly comparable study populations and identical treatment arms (FOLFOX-bevacizumab), but present divergent CRLM RRs of 14% versus 39%, respectively.[32,55] Efforts should be made to ensure that CRLM patients are being discussed in an MDT which includes a liver surgeon at initial diagnosis as well as during follow-up of systemic treatment.

Long-term survival outcomes for CRLM patients were not reported in the majority of CRLM subgroup analyses in trials in the overall population of mCRC patients. Only one of seven CRLM studies considered PFS as primary endpoint[54], with the remaining trials using short-term outcomes like conversion, (overall) resection or response rate as primary endpoint. However, emerging multimodal procedures like 2-stage resections, using portal vein embolization or Associating Liver Partition and Portal vein Ligation for Staged hepatectomy (ALPPS)-procedure or combined resection and thermal ablation render more patients technically resectable, but evidence that these extensive procedures are clinically meaningful in terms of improved long-term survival are lacking.

Caution is warranted in using response rate as a surrogate marker for effectivity of systemic conversion therapies. The absence of a correlation between ORR and R0-RRs in our correlation analysis refutes the prevailing paradigm of high response rates translating in high CRLM RRs.[10,14] For example, the ATOM trial showed the highest ORR of 85% for FOLFOX-cetuximab, compared to 68% in the bevacizumab arm, but the liver RR was numerically lower in the cetuximab compared to the bevacizumab treatment arm[31]. This phenomenon may be due to a decreased validity of response rate as assessed by RECIST-criteria for targeted therapy and especially for bevacizumab-containing regimens, since favorable morphological responses on CT scans are not being taken into account.[14,25,26]

FOLFOXIRI-bevacizumab is considered an option as conversion therapy in CRLM patients.[17] This is supported by two studies performing a pooled analysis of CRLM patients receiving FOLFOXIRI-bevacizumab with a reported pooled R0-RR of 30.7%[62] and 53.7%.[63] When evaluating these studies, it should be noted that the reported pooled R0-RRs vary considerably, most of included studies in these pooled analyses were not RCT's, and no comparison with doublet chemotherapy was performed.[62,63] In addition, a meta-analyses comparing RCT's with first-line FOLFOXIRI-bevacizumab to doublet-bevacizumab as first-line therapy for patients with unresectable mCRC showed a significant benefit in favor of FOLFOXIRI-bevacizumab in terms of PFS, ORR and secondary R0-RR not limited to CRLM.[64] However, there was no significant interaction between treatment arm and the achievement of R0 resection in terms of OS.[64] Based on these data no preference for a triplet chemotherapeutic backbone can be given as systemic conversion therapy in CRLM patients. Results of FOLFOXIRI combined with panitumumab are promising with a high response rate of 87%, however outcomes in CRLM subgroups were not published.[21]

Lastly, the role of induction immunotherapy in patients with known mismatch repair deficiency (dMMR) and initially unresectable CRLM is not clear yet. The KEYNOTE-177 randomized trial compared first line immunotherapy with standard chemo- and targeted therapy in patients with dMMR mCRC. Preliminary data showed a benefit in terms of PFS for immunotherapy. However, immunotherapy only performed better until after six months when the curves crossed. Furthermore, there was a higher risk of early progression for immunotherapy (30% vs. 12%). Based on these data the use of immunotherapy as systemic conversion therapy for CRLM patients cannot be recommended.[65]

In conclusion, currently available data do not allow to select an optimal systemic conversion regimen in patients with initially unresectable CRLM. For future trials in (subgroups of) patients with CRLM, we recommend the inclusion of relevant prognostic/predictive factors (i.e. *RAS*/*BRAF*^{V600E} mutational status and sidedness of primary tumour), presentation of transparent (un)resectability criteria, and reporting on long-term clinical outcome of patients. In the ongoing phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group patients with initially unresectable CRLM are randomized according to stratification by *RAS/BRAFV600E* mutation status and primary tumour sidedness between either doublet versus triplet chemotherapy both plus bevacizumab, or doublet chemotherapy plus bevacizumab versus panitumumab[66] (Un)resectability of CRLM at baseline and during follow-up is evaluated by a panel of experienced liver surgeons according to predefined and transparent criteria.[61] Lastly, we recommend consecutive resectability assessments at baseline and during systemic therapy by an experienced MDT including a liver surgeon. Future research should improve the

selection of patients in whom local treatment of CRLM not only is feasible but also results in a meaningful clinical outcome.

Conclusion

Current data do not allow to select an optimal systemic conversion regimen in patients with initially unresectable CRLM. The constantly evolving field of the treatment of metastatic CRC and emerging biomarkers make comparison of outcomes in trials with CRLM patients both challenging and crucial. Interpretation of results in CRLM patients is complicated by heterogeneity in study populations and trial designs, differences/absence of unresectability criteria, and often lack of data on long-term clinical outcome. These issues should be addressed in future trials in this group of patients.

Author contributions

Study concept and design: KB, MK, MvO, RJS, CJAP
Financial support: not applicable
Data collection: KB, MK
Statistical analyses: KB, MK, MvO
Data interpretation: KB, MK, MvO, RJS, CJAP
Manuscript writing: KB, MK, MvO, RJS, CJAP
Critical revision of the manuscript: KB, MK, MvO, RJS, CJAP

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References

1. Surveillance E, and End Results (SEER) Program. https://seer.cancer.gov/statfacts/html/colorect.html [
2. de Ridder JAM, van der Stok EP, Mekenkamp LJ, et al. Management of liver metastases in colorectal cancer patients: A retrospective case-control study of systemic therapy versus liver resection. Eur J Cancer. 2016;59:13-21.
3. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3(1):50-56.
4. Adam R, De Gramont A, Figueras J, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17(10):1225-1239.
5. Bartlett EK, Simmons KD, Wachtel H, et al. The rise in metastasectomy across cancer types over the past decade. Cancer. 2015;121(5):747-757.
6. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27(8):1386-1422.
7. Benson AB, 3rd, Venook AP, Cederquist L, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15(3):370-398.
8. Golfinopoulos V, Salanti G, Pavlidis N, et al. Survival and disease-progression benefits with treatment regimens for advanced colorectal cancer: a meta-analysis. Lancet Oncol. 2007;8(10):898-911.
9. Pozzo C, Basso M, Cassano A, et al. Neoadjuvant treatment of unresectable liver disease with irinotecan and 5-fluorouracil plus folinic acid in colorectal cancer patients. Ann Oncol. 2004;15(6):933-939.
10. Folprecht G, Grothey A, Alberts S, et al. Neoadjuvant treatment of unresectable colorectal liver metastases: correlation between tumour response and resection rates. Ann Oncol. 2005;16(8): 1311-1319.
11. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011;29(15):2011-2019.
12. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008;26(12):2013-2019.
13. Venook AP, Niedzwiecki D, Lenz HJ, et al. Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. JAMA. 2017;317(23):2392-2401.
14. Okuno M, Hatano E, Nishino H, et al. Does response rate of chemotherapy with molecular target agents correlate with the conversion rate and survival in patients with unresectable colorectal liver metastases?: A systematic review. Eur J Surg Oncol. 2017;43(6):1003-1012.
15. Loupakis F, Cremolini C, Masi G, et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med. 2014;371(17):1609-1618.
16. Hurwitz H, Tan BR, Reeves JA, et al. Updated efficacy, safety, and biomarker analyses of STEAM, a randomized, open-label, phase II trial of sequential (s) and concurrent (c) FOLFOXIRI-bevacizumab (BV) vs FOLFOX-BV for first-line (1L) treatment (tx) of patients with metastatic colorectal cancer (mCRC). Journal of Clinical Oncology Conference. 2017;35(4 Supplement 1).
17. Gruenberger T, Bridgewater J, Chau I, et al. Bevacizumab plus mFOLFOX-6 or FOLFOXIRI in patients with initially unresectable liver metastases from colorectal cancer: the OLIVIA multinational randomised phase II trial. Ann Oncol. 2015;26(4):702-708.
18. Cremolini C, Antoniotti C, Lonardi S, et al. Activity and safety of cetuximab plus modified folfoxiri followed by maintenance with cetuximab or bevacizumab for RAS and BRAF wild-type metastatic colorectal cancer a randomized phase 2 clinical trial. JAMA Oncology. 2018;4(4):529-536.
19. Folprecht G, Hamann S, Schutte K, et al. Dose escalating study of cetuximab and 5-FU/folinic acid (FA)/oxaliplatin/irinotecan (FOLFOXIRI) in first line therapy of patients with metastatic colorectal cancer. BMC Cancer. 2014;14:521.
20. Garufi C, Torsello A, Tumolo S, et al. Cetuximab plus chronomodulated irinotecan, 5-fluorouracil, leucovorin and oxaliplatin as neoadjuvant chemotherapy in colorectal liver metastases: POCHER trial. Br J Cancer. 2010;103(10):1542-1547.

21. Geissler M, Klingler T, Riera-Knorrenschield J, et al. MFOLFOXIRI 1 Panitumumab versus FOLFOXIRI as first-line treatment in patients with RAS wild- type metastatic colorectal cancer m(CRC): A randomized phase II VOLFI trial of the AIO (AIOKRK0109). Ann Oncol. 2018;29 (Supplement 5):v108.
22. Borelli B, Moretto R, Lonardi S, et al. TRIPLETE: A randomised phase III study of modified FOLFOXIRI plus panitumumab versus mFOLFOX6 plus panitumumab as initial therapy for patients with unresectable RAS and BRAF wild-type metastatic colorectal cancer. ESMO Open. 2018;3 (4) (no pagination)(e000403).
23. Petrelli F, Barni S, Anti Eaflm. Resectability and outcome with anti-EGFR agents in patients with KRAS wild-type colorectal liver-limited metastases: a meta-analysis. Int J Colorectal Dis. 2012;27(8):997-1004.
24. Jones RP, Hamann S, Malik HZ, et al. Defined criteria for resectability improves rates of secondary resection after systemic therapy for liver limited metastatic colorectal cancer. Eur J Cancer. 2014; 50(9):1590-1601.
25. Chun YS, Vauthey JN, Boonsirikamchai P, et al. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. JAMA. 2009;302(21):2338-2344.
26. Punt CJA, Huiskens J, van Gulik T, et al. Pseudoprogression on bevacizumab treatment: tumor-dynamics in the modern era of systemic treatment for metastatic colorectal cancer. Acta Oncol. 2018;57(5): 681-682.
27. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. 2010;8(5):336-341.
28. Carrato A, Abad A, Massuti B, et al. First-line panitumumab plus FOLFOX4 or FOLFIRI in colorectal cancer with multiple or unresectable liver metastases: A randomised, phase II trial (PLANET-TTD). Eur J Cancer. 2017;81:191-202.
29. Folprecht G, Gruenberger T, Bechstein W, et al. Survival of patients with initially unresectable colorectal liver metastases treated with FOLFOX/cetuximab or FOLFIRI/cetuximab in a multidisciplinary concept (CELIM study). Ann Oncol. 2014;25(5):1018-1025.
30. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11(1):38-47.
31. Ychou M, Rivoire M, Thezenas S, et al. Induction chemotherapy (CT) with FOLFIRINOX or FOLFOX/FOLFIRI, plus cetuximab (CET) or bevacizumab (BEV) (by RAS status), in patients (pts) with primarily unresectable colorectal liver metastases (CRLM): Results of the randomized UNICANCER PRODIGE 14-ACCORD 21 (METHEP-2) trial. J Clin Oncol. 2018;36(15_suppl):3535-3535.
32. Parikh AR, Lee FC, Yau L, et al. MAVERICC, a Randomized, Biomarker-stratified, Phase II Study of mFOLFOX6-Bevacizumab versus FOLFIRI-Bevacizumab as First-line Chemotherapy in Metastatic Colorectal Cancer. Clin Cancer Res. 2019;25(10):2988-2995.
33. Hurwitz HI, Tan BR, Reeves JA, et al. Phase II Randomized Trial of Sequential or Concurrent FOLFOXIRI-Bevacizumab Versus FOLFOX-Bevacizumab for Metastatic Colorectal Cancer (STEAM). Oncologist. 2018.
34. Arnold D, Lueza B, Douillard JY, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol. 2017;28(8):1713-1729.
35. Peeters M, Price T, Taieb J, et al. Relationships between tumour response and primary tumour location, and predictors of long-term survival, in patients with RAS wild-type metastatic colorectal cancer receiving first-line panitumumab therapy: retrospective analyses of the PRIME and PEAK clinical trials. Br J Cancer. 2018;119(3):303-312.
36. Rivera F, Karthaus M, Hecht JR, et al. Final analysis of the randomised PEAK trial: overall survival and tumour responses during first-line treatment with mFOLFOX6 plus either panitumumab or bevacizumab in patients with metastatic colorectal carcinoma. Int J Colorectal Dis. 2017;32(8):1179-1190.
37. Bokemeyer C, Kohne CH, Ciardiello F, et al. FOLFOX4 plus cetuximab treatment and RAS mutations in colorectal cancer. Eur J Cancer. 2015;51(10):1243-1252.
38. Van Cutsem E, Folprecht G, Bokemeyer C, et al. Chemotherapy plus cetuximab as conversion therapy for patients with initially unresectable colorectal cancer liver metastases. Eur J Cancer. 2013;49:S490-S490.
39. Oki E, Emi Y, Yamanaka T, et al. Randomised phase II trial of mFOLFOX6 plus bevacizumab versus mFOLFOX6 plus cetuximab as first-line treatment for colorectal liver metastasis (ATOM trial). Br J Cancer. 2019;121(3):222-229.

40. Ye LC, Liu TS, Ren L, et al. Randomized controlled trial of cetuximab plus chemotherapy for patients with KRAS wild-type unresectable colorectal liver-limited metastases. J Clin Oncol. 2013;31(16):1931-1938.
41. Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol. 2015;16(13):1306-1315.
42. Okines A, Puerto OD, Cunningham D, et al. Surgery with curative-intent in patients treated with first-line chemotherapy plus bevacizumab for metastatic colorectal cancer First BEAT and the randomised phase-III NO16966 trial. Br J Cancer. 2009;101(7):1033-1038.
43. Heinemann V, von Weikersthal LF, Decker T, et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. Lancet Oncol. 2014;15(10):1065-1075.
44. Holch JW, Ricard I, Stintzing S, et al. Relevance of liver-limited disease in metastatic colorectal cancer: Subgroup findings of the FIRE-3/AIO KRK0306 trial. Int J Cancer. 2018;142(5):1047-1055.
45. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer. 2018;88:77-86.
46. Douillard JY, Siena S, Peeters M, et al. Impact of early tumour shrinkage and resection on outcomes in patients with wild-type RAS metastatic colorectal cancer. Eur J Cancer. 2015;51(10):1231-1242.
47. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009;360(14):1408-1417.
48. Maughan TS, Adams RA, Smith CG, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet. 2011;377(9783):2103-2114.
49. Cremolini C. Updated results of TRIBE2, a phase III, randomized strategy study by GONO in the first and second-line treatment of unresectable mCRC. J Clin Oncol 37, 2019 (suppl; abstr 3508). 2019.
50. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med. 2009;360(6):563-572.
51. Falcone A, Cremolini C, Masi G, et al. FOLFOXIRI/bevacizumab (bev) versus FOLFIRI/bev as first-line treatment in unresectable metastatic colorectal cancer (mCRC) patients (pts): Results of the phase III TRIBE trial by GONO group. J Clin Oncol. 2013;31(15_suppl):3505-3505.
52. Xu J, Liu T, Tang W, et al. LBA31Bevacizumab plus chemotherapy versus chemotherapy alone as first-line treatment for patients with RAS mutant unresectable colorectal liver-limited metastases: A single center randomized control trial. Ann Oncol. 2019;30(Supplement_5).
53. Tang W, Ren L, Ye Q, et al. Bevacizumab Plus mFOLFOX6 Versus mFOLFOX6 Alone as First-Line Treatment for RAS Mutant Unresectable Colorectal Liver-Limited Metastases: The BECOME Randomized Controlled Trial. J Clin Oncol. 2020:JCO2000174.
54. Uetake H, Emi Y, Yamanaka T, et al. A randomized phase II study of mFOLFOX6 plus bevacizumab versus mFOLFOX6 plus cetuximab for previously untreated, liverlimited metastatic colorectal cancer that is unsuitable for resection (ATOM trial). Journal of Clinical Oncology Conference. 2018;36(4 Supplement 1).
55. Schmoll HJ, Cunningham D, Sobrero A, et al. Cediranib with mFOLFOX6 versus bevacizumab with mFOLFOX6 as first-line treatment for patients with advanced colorectal cancer: a double-blind, randomized phase III study (HORIZON III). J Clin Oncol. 2012;30(29):3588-3595.
56. Hurwitz HI, Tan BR, Reeves JA, et al. Phase II Randomized Trial of Sequential or Concurrent FOLFOXIRI-Bevacizumab Versus FOLFOX-Bevacizumab for Metastatic Colorectal Cancer (STEAM). Oncologist. 2019;24(7):921-932.
57. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol. 2009;27(5):663-671.
58. Douillard JY, Siena S, Cassidy J, et al. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. Ann Oncol. 2014;25(7):1346-1355.
59. Kohne CH, Poston G, Folprecht G, et al. FOLFIRI plus cetuximab in patients with liver-limited or non-liver-limited RAS wild-type metastatic colorectal cancer: A retrospective subgroup analysis of the CRYSTAL study. Eur J Surg Oncol. 2016;42(10):1540-1547.

60. Punt CJ, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol. 2017;14(4):235-246.
61. Huiskens J, Bolhuis K, Engelbrecht MR, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229(6):523-532 e522.
62. Cremolini C, Casagrande M, Loupakis F, et al. Efficacy of FOLFOXIRI plus bevacizumab in liver-limited metastatic colorectal cancer: A pooled analysis of clinical studies by Gruppo Oncologico del Nord Ovest. Eur J Cancer. 2017;73:74-84.
63. Tomasello G, Petrelli F, Ghidini M, et al. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol. 2017;3(7):e170278.
64. Cremolini C, Antoniotti C, Stein A, et al. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. J Clin Oncol. 2020:JCO2001225.
65. Andre T, Shiu K-K, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high/mismatch repair deficient metastatic colorectal cancer: The phase 3 KEYNOTE-177 Study. J Clin Oncol. 2020;38(18_suppl):LBA4-LBA4.
66. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
67. Cremolini C, Marmorino F, Loupakis F, et al. TRIBE-2: a phase III, randomized, open-label, strategy trial in unresectable metastatic colorectal cancer patients by the GONO group. BMC Cancer. 2017;17(1):408.
68. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol. 2011;22(7):1535-1546.

Supplemental material

Table S1 Literature search strategy

Search strategy PUBMED		
#	**Search**	**Items found**
#7	Search ("Antibodies, Monoclonal/therapeutic use*" [Mesh] OR "Antineoplastic Agents/therapeutic use*" [Mesh] OR "Antibodies, Monoclonal, Humanized"[Mesh] OR "Molecular Targeted Therapy" [Mesh] OR Molecular Targeted Therap* [tiab] OR Targeted Molecular Therap* [tiab] OR Target therap* [tiab] OR targeted therapy* [tiab] OR EGFR [tiab] OR bevacizumab [tiab] OR anti EGFR [tiab] OR EGFR treatment* [tiab] OR anti-EGFR [tiab] OR avastin [tiab])	383399
#6	Search ("Antineoplastic Combined Chemotherapy Protocols/therapeutic use*" [Mesh] OR "Neoadjuvant Therapy" [Mesh] OR (Chemotherap* [tiab] OR systemic therap* [tiab] OR systemic treatment* [tiab] OR induction therap* [tiab] OR induction treatment* [tiab] OR first line [tiab] OR First-line [tiab] OR neoadjuvant therap* [tiab] OR neoadjuvant treatment* [tiab])	514239
#20	Search ("Neoplasm Metastasis"[Mesh] OR Neoplasm Metastases [tiab] OR Metastasis [tiab] OR Metastases [tiab] OR Neoplasm Metastasis [tiab] OR metastatic colo* [tiab])	429693
#19	Search ("Colorectal Neoplasms/drug therapy"[Mesh] OR Colorectal Neoplasm* [tiab] OR Colorectal Tumor* [tiab] OR Colorectal Carcinoma* [tiab] OR Colorectal Cancer* [tiab] OR Colonic Neoplasm* [tiab] OR Colon Neoplasm* [tiab] OR Cancer of Colon [tiab] OR Colon Cancer* [tiab] OR Cancer of the Colon [tiab] OR Colonic Cancer* [tiab] OR Colon tumor* [tiab] OR colon tumour* [tiab] OR colorectal tumor* [tiab] OR colorectal tumour [tiab])	155877
#22	Search (#19 AND #20 AND #6 AND #7 AND #8)	453
#23	Search (#19 AND #20 AND #6 AND #7 AND #8) Filters: Publication date from 2008/01/01 to 2019/05/22	338
Search strategy EMBASE		
#1	exp colon cancer/dm, dt, su, th or (Colorectal Neoplasm* or Colorectal Tumor* or Colorectal Carcinoma* or Colorectal Cancer* or Colonic Neoplasm* or Colon Neoplasm* or Cancer of Colon or Colon Cancer* or Cancer of the Colon or Colonic Cancer* or Colon tumor* or colon tumour* or colorectal tumor* or colorectal tumour*).ti,ab,kw.	241614
#2	metastasis/ or (Neoplasm Metastases or Metastasis or Metastases or Neoplasm Metastasis or metastatic colo*).ti,ab,kw.	314137
#3	chemotherapy/ or (Neoadjuvant Therapy or Chemotherap* or systemic therap* or systemic treatment* or induction therap* or induction treatment* or first line or First-line or neoadjuvant therap* or neoadjuvant treatment* or cytotoxic therap* or cytotoxic treatment*).ti,ab,kw.	769152
#4	monoclonal antibody/ or (Antibodies, Monoclonal, Humanized or Molecular Targeted Therap or Targeted Molecular Therap* or Target therap* or targeted therapy* or EGFR or bevacizumab or anti EGFR or EGFR treatment* or anti-EGFR or avastin or monoclonal antibod* or cetuximab or panitumumab).ti,ab,kw.	443983
#5	1 and 2 and 3 and 4	5730
#6	limit 5 to (english language and (randomized controlled trial or controlled clinical trial) and (article or article in press or conference abstract or conference paper))	613

CHAPTER 3

Outcomes of resectability assessment of the Dutch colorectal cancer group liver metastases expert panel

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Abstract

Introduction

Decision making on optimal treatment strategy in patients with initially unresectable colorectal cancer liver metastases (CRLM) remains complex because uniform criteria for (un)resectability are lacking. This study reports on the feasibility and short-term outcomes of The Dutch Colorectal Cancer Group Liver Expert Panel.

Methods

The Expert Panel consists of thirteen hepatobiliary surgeons and four radiologists. Resectability assessment is performed independently by three randomly assigned surgeons. CRLM are scored as resectable, potentially resectable or permanently unresectable. In absence of consensus, two additional surgeons are invited for a majority consensus. Patients with potentially resectable or unresectable CRLM at baseline are evaluated every two months of systemic therapy. Once CRLM are considered resectable, a treatment strategy is proposed.

Results

Overall, 398 panel evaluations in 183 patients were analyzed. The median time to panel conclusion was 7 days (IQR 5-11). Inter-surgeon disagreement was observed in 205 (52%) evaluations, with major disagreement (resectable vs permanently unresectable) in 42 (11%) evaluations. After systemic treatment, 106 patients were considered to have resectable CRLM, out of which 84 (79%) patients underwent a curative procedure. R0 resection (n=41) or R0 resection in combination with ablative treatment (n=26) or ablative treatment only (n=4) was achieved in 67/84 (80%) patients.

Conclusion

This study analyzed prospective resectability evaluation of patients with CRLM by a panel of radiologists and liver surgeons. The high rate of disagreement among experienced liver surgeons reflects the complexity in defining treatment strategies for CRLM and supports the use of a panel rather than a single-surgeon decision.

Introduction

Survival rates in patients with metastatic colorectal carcinoma (CRC) have increased over the past decades owing to the increased resection rate of metastases and the development of effective systemic drugs. In 30-40% of patients, CRC metastases are limited to the liver.[1,2] Resection of colorectal liver metastases (CRLM) offers the chance of long-term disease-free survival or cure, with 5-year survival rates ranging between 25% and 58%.[3-5] In addition to standard, one-stage resections, several other options are currently available to achieve clearance of the liver from all tumors. The combination of resection with local ablative techniques enables sparing of parenchyma, and preoperative portal vein embolization can be used to induce hypertrophy of the future liver remnant rendering patients with upfront too small liver remnant amenable to resection.[6] Two-stage hepatectomy and Associating Liver Partition and Portal vein Ligation for Staged hepatectomy (ALPPS) are strategies to allow extensive resections in patients with bilobar metastases. Despite these novel techniques, only a minority of patients with CRLM (20%) present with metastases that are considered upfront resectable.[7,8] In patients with upfront unresectable CRLM, a number of studies have shown that downsizing of CRLM by induction systemic treatment may allow secondary resections with survival rates comparable to primary resections.[9-11]

The lack of criteria for (un)resectability in most studies induces selection bias and thereby complicates the interpretation of patient outcomes. Historically, the number and size of CRLM and 1 cm resection margins were the dominant criteria that were used to define (un)resectability. These criteria have gradually been abandoned, because multiple studies have shown significant survival benefits of liver resection even in patients with very advanced CRLM.[12,13] Currently, the main issue is whether a complete resection with tumor-free margins is feasible while preserving at least 20-30% of total liver volume, with adequate vascular in- and outflow and biliary drainage.[14] To enable adequate assessment of resectability, the presence of at least one experienced liver surgeon in a dedicated multidisciplinary team conference is considered mandatory.[15,16] However, the criteria for resectability are subject to individual interpretation.[17,18] Although there may exist consensus on the extremes of upfront resectable versus permanently unresectable CRLM, large interobserver variability concerning resectability has been observed even among experienced liver surgeons.[19-22]

The ongoing CAIRO5, multicenter, randomized, phase 3 trial of the Dutch Colorectal Cancer Group (DCCG) investigates the optimal systemic induction regimen in patients with initially unresectable, colorectal liver-only metastases.[23] An innovative aspect of the study design is that all patients are prospectively evaluated for resectability by an expert panel consisting of experienced hepatobiliary surgeons and radiologists according to predefined criteria. We hypothesized that the use of such a panel may decrease

individual subjectivity in defining (un)resectability and subsequently may improve consensus on criteria for resection of CRLM.

This study analyses the feasibility and outcomes of the CAIRO5 national DCCG Liver Expert Panel in resectability assessment for patients with CRLM at baseline and during induction systemic treatment.

Methods

Patients

All patients registered between November 2014 and August 2017 in the ongoing CAIRO5 study; a multicenter, randomized, phase 3 trial of the DCCG (EudraCT 2013-005435-24, ClinicalTrials.gov: NCT02162563) were selected for this analysis.[23] The CAIRO5 study randomizes patients with unresectable or potentially resectable CRLM and no extrahepatic metastases in 1) doublet chemotherapy plus either bevacizumab or panitumumab for left sided primary, *RAS* and *BRAF* wild-type tumors, or 2) doublet or triplet chemotherapy, both with bevacizumab for *RAS* or *BRAF* mutated tumors or right sided primary tumors. Patients were evaluated for resectability by the panel at baseline and during systemic treatment. The following outcome parameters were recorded: time required by the expert panel to reach a panel conclusion, inter-surgeon variation on resectability assessment, and adherence to the panel recommendation for local treatment by the collaborating center.

Patient imaging

Tumor staging and response analysis were assessed using contrast enhanced, abdomen-pelvic CT scan and thoracic helical CT scan or a conventional thoracic radiograph at baseline and every 8-9 weeks after baseline imaging. Use of MRI of the liver or PET scan was left to the discretion of the local treatment team, since these imaging modalities were not mandatory according to the Dutch colorectal cancer guideline. If results of these studies were available and showed additional information concerning the metastases, these scans were reviewed by the expert panel as well.

Predefined resectability criteria

For the purpose of transparency and homogeneity of the trial population and to reduce selection bias, consensus among liver surgeons was achieved on criteria for initial (un)resectability during a meeting of the Dutch Liver Surgery working group.[24] Resectability at baseline was defined as the ability to obtain a complete (R0) resection of all lesions in one single surgical procedure (i.e. excluding 2-stage resections and/or use

of portal vein embolization) by resection only (i.e. excluding the use of additional ablative treatments or other local methods), leaving an estimated minimum remnant liver volume of 25-30% in uncompromised livers, or 35-40% in compromised livers prior to treatment (fibrosis, cirrhosis or steatosis). Options for local treatment during induction systemic therapy included 2-stage resections, use of preoperative PVE, ALPPS, and combinations with local ablative treatments.

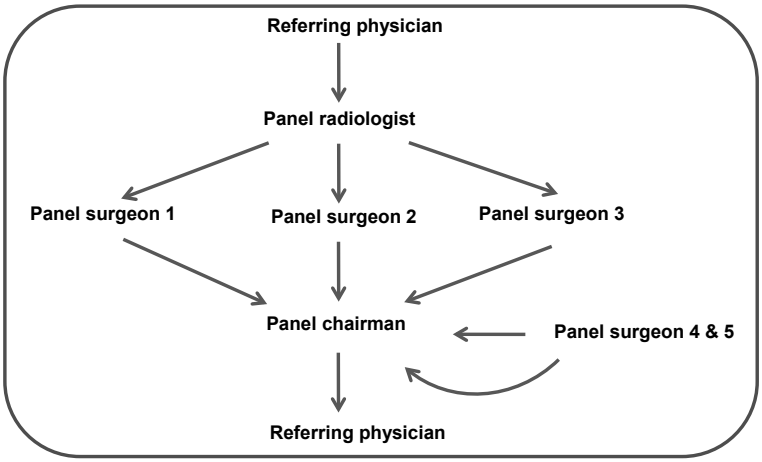
Design of the DCCG Liver Metastases Expert Panel

The DCCG Liver Expert Panel consists of 13 liver surgeons and 4 radiologists from 12 hospitals. The liver surgeons are all member of the Dutch Study Group for Liver Surgery, have extensive experience in treating patients with CRLM. All liver surgeons are part of a local surgical team that performs more than 20 liver resections per year.[25]

A digital online platform was designed that allowed uploading of the images by the local hospital and the independent assessment of resectability by each panel member (ALEA®, FormsVision, Abcoude, The Netherlands).

CT Scans were digitally anonymized and reviewed by a panel radiologist. The radiologist evaluated metastases according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1. When the panel radiologist confirmed that no extrahepatic metastases were present, three liver surgeons were randomly selected. Based on the available imaging studies and the accompanying radiology report, all three panel surgeons individually voted on resectability by choosing one of the following three categories: 1) resectable 2) potentially resectable, or 3) permanently unresectable. In case CRLM were considered to be potentially resectable, the panel surgeons were requested to differentiate between a) technically unresectable but potentially resectable after (further) downsizing and b) technically resectable but start/continuation of systemic treatment is preferred. If no consensus was reached among the three panel surgeons, two additional panel surgeons were randomly selected to evaluate resectability. Minor disagreement is defined as: a panel evaluation in which one of the panel surgeons assessed the CRLM as potentially resectable and one other surgeon in the same panel assessed the CRLM as resectable or permanently unresectable. Major disagreement is defined as: a panel evaluation in which at least one of the panel surgeons assessed the CRLM as resectable and another surgeon in the same panel voted for permanently unresectable CRLM. The final decision on resectability was made according to the majority of votes among the selected panel members. The chairman of the panel, who is not one of the voting members, coordinated the voting process, confirmed the final decision of the panel and strived for a panel conclusion within 14 days. One central study coordinator (JH, KB) monitored the progress of the evaluation and solved problems such as questions from the participating hospitals or technical problems experienced by the panel members. The logistics of the panel is schematically represented in **Figure 1**.

Figure 1 Logistics of resectability assessment by Dutch Colorectal Cancer Group Liver Expert Panel.



To confirm unresectability of CRLM, panel evaluation was performed at baseline prior to randomization and after every eight weeks, equal to four treatment cycles. At baseline, patients with CRLM assessed as resectable, did not qualify for inclusion in the CAIRO5 study. At follow-up evaluations, further resectability assessments were discontinued when CRLM were assessed as permanently unresectable or resectable. In case CRLM were considered resectable, the local treatment team was notified and a surgical plan was proposed. All patients assessed as having potentially resectable CRLM at first follow-up evaluation, corresponding to four treatment cycles, are re-evaluated after eight treatment cycles at 16 weeks and, if still considered potentially resectable, for a final assessment after 12 treatment cycles at 24 weeks.

To evaluate the feasibility and predictive accuracy of the panel conclusions, clinical outcomes in terms of resection rate, type of resection with adjunctive use of local additional modalities (ablative treatments, PVE, 2-stage resection, ALPPS) and R0 resection rate were analyzed. Reasons for deviation from panel conclusions were documented.

Outcomes of resections were evaluated by type of resection as well as the R0 resection rate. R0 resection was defined as microscopically margin-negative resection, in which no microscopic tumor cells have remained in the resection margins of surgically removed metastases. R1 resection indicates the removal of all macroscopic disease, but microscopic margins are positive for tumor cells. In case only local ablative treatment was performed, no R status could be defined. The local physician judged the local ablative procedure to be complete or incomplete. Discrepancies between the local

treatment plan proposed by the DCCG Liver Expert Panel and the actual treatment procedures were also documented.

The design of the DCCG Liver Expert Panel including the procedure of assessment was part of the study protocol and approved by the Institutional Review Board of the Amsterdam UMC.

The proposed surgical plan for patients with CRLM assessed as resectable at follow-up, should attempt to include all lesions as demonstrated at baseline imaging. However, pretreatment lesions in complete radiological remission and not detectable during surgery were left *in situ*. The decision to perform resection by laparoscopic or by open procedure was left to the discretion of the performing surgeon.[26]

Patients with synchronous metastatic disease were eligible for study participation, provided that the primary tumor was deemed resectable by the local MDT in case of few or absent symptoms of this primary tumor or in case patient had recovered from immediate surgery necessitated by symptoms of primary tumor. Patients with a primary tumor in situ, whose liver metastases became resectable upon induction systemic treatment are to undergo subsequent surgical treatment for the primary tumor, usually at the end of protocol treatment.

Statistical analysis

Continuous variables were displayed as median with inter-quartile-range (IQR) and categorical variables by number with percentages. Categorical variables were analyzed using chi-square test. Statistical analyses were performed using SPSS 25.0 (IBM, Chicago, IL). Chi-square tests were two-tailed and $p<0.05$ was considered significant.

Results

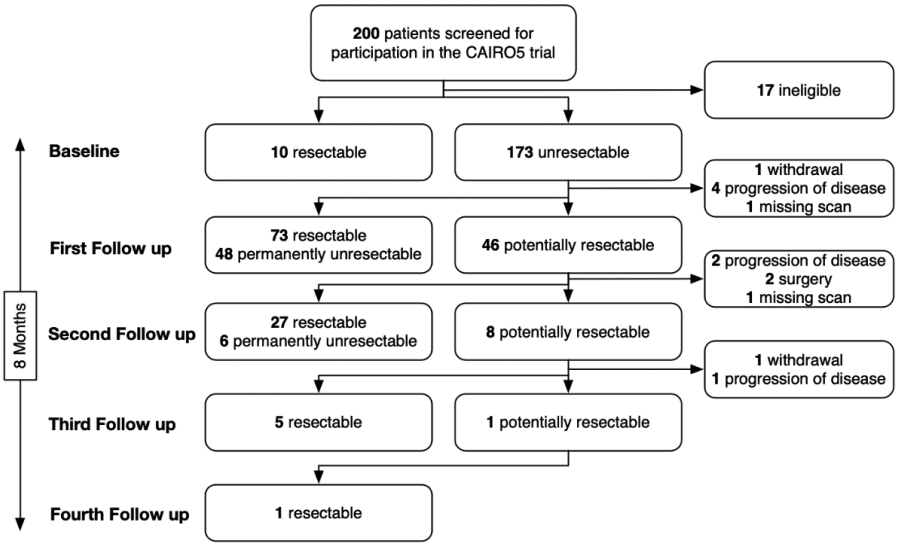
Between June 2014 and August 2017, 200 patients with CRLM from 41 Dutch hospitals were registered and screened for eligibility for the CAIRO5 trial (**Figure 2**). Of these patients, 17 were found to be ineligible for study participation at registration prior to panel evaluation: 13 patients did not meet the inclusion criteria of whom seven had extrahepatic metastases, and four patients withdrew from participation before panel conclusion at baseline was reached.

Evaluation of resectability

Overall, 398 panel evaluations in 183 patients were analyzed (183 baseline, 215 follow up evaluations). At baseline, 10 patients were assessed to have initially resectable CRLM.

These patients were considered ineligible for participation in the CAIRO5 study. The panel conclusion along with the proposed surgical plan was forwarded to the referring treatment team. The remaining 173 patients were assessed as having initially unresectable CRLM, of which 127 potentially resectable and 46 permanently unresectable CRLM. Of the 173 patients with initially unresectable CRLM, six patients were not re-evaluated at first follow-up. Reasons for which patients were not re-evaluated are presented in Figure 2.

Figure 2 Flowchart with numbers of patients assessed to be resectable, potentially resectable or permanently unresectable at baseline, and follow up evaluations and reasons for patients not to be re-evaluated.

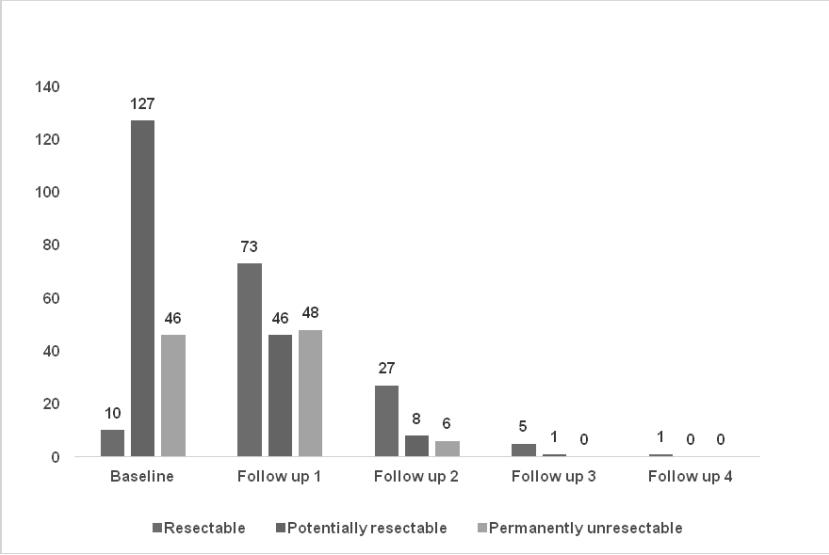


At first follow-up evaluation (FU1), 73 of 167 (45%) patients were considered to have resectable CRLM, 48 (29%) permanently unresectable and 46 (28%) potentially resectable CRLM. In patients with potentially resectable CRLM, systemic treatment was continued with a panel evaluation after four more cycles of systemic therapy. Five of 46 (11%) patients with CRLM considered as potentially resectable at FU1 were not re-evaluated by the panel at second follow-up evaluation (FU2), leaving 41 patients for second follow-up evaluation (FU2).

At FU2, 27 of 41 (66%) patients were considered to have resectable, 6 (15%) permanently unresectable and 8 (20%) potentially resectable CRLM. At FU3, 5 of 6 patients (83%) were considered to have resectable CRLM while in one patient the scan showed ongoing response with still extensive CRLM. In this case the panel preferred to

continue systemic treatment for another four cycles, i.e. for a total of eight months. At fourth follow-up evaluation (FU4), the CRLM of this patient were considered resectable. (**Figure 2 and 3**)

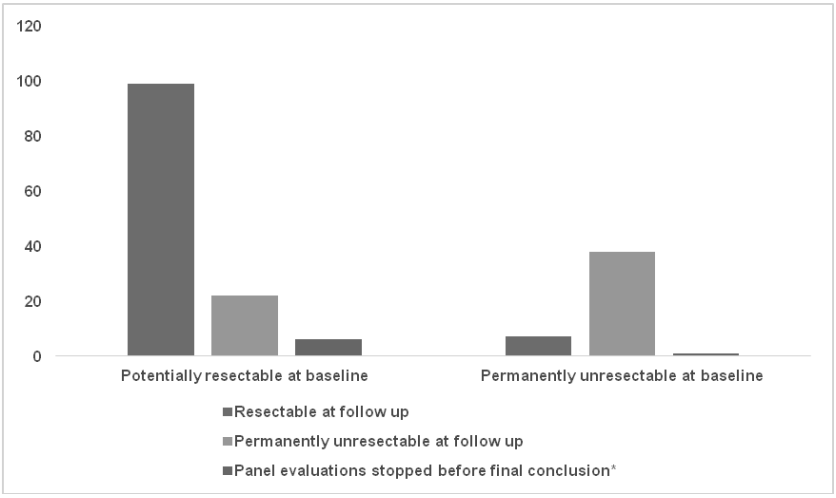
Figure 3 Distributions of panel conclusions at baseline and during follow up.



Conversion rate

Of 127 patients with CRLM assessed as potentially resectable at baseline evaluation, 71 (56%), 24 (19%) and 4 (3%) patients were converted to resectable disease after systemic induction therapy at FU1, FU2 and FU3, respectively. CRLM were considered permanently unresectable in 13 (10%) and 5 (4%) patients at FU1 and FU2, respectively. Thirty-six (78%) of 46 patients considered to have permanently unresectable CRLM at baseline, remained permanently unresectable during follow-up assessment, whereas 2 (4%), 3 (7%), 1 (2%) and 1 (2%) patients converted from permanently unresectable disease to resectable disease at FU1, FU2, FU3 and FU4, respectively. (**eFigure 1**)

eFigure 1 Distributions of panel conclusions at follow-up according to panel conclusion at baseline. *Patients with missing panel evaluations because of progression were scored as permanently unresectable



Time to panel conclusion

Overall, the median time to panel conclusion was 7 days (IQR 5-11). At baseline and follow-up evaluations, the median time to panel conclusion was 6 days (IQR 4-9 days) and 9 days (IQR 6-13 days), respectively.

Inter-surgeon variation in panel evaluations

Overall, any form of inter-surgeon disagreement was observed in 206 (52%) baseline and follow up evaluations, with major disagreement (resectable vs. permanently unresectable) in 42 (11%) evaluations. Any inter-surgeon disagreement was lower at baseline compared to follow-up panel evaluations; 80 (43.7%) versus 126 (58.6%), respectively, p=0.003. Major inter-surgeon disagreement was lower at baseline compared to follow-up panel evaluations; 3 (1.6%) vs. 39 (18.1%), respectively, p<0.001.

For all panel evaluations at FU1, FU2 and FU3 a vast majority of panel evaluations resulted in any form of disagreement among the selected panel surgeons. The rates of panel disagreement per time of evaluation are presented in **Table 1**.

Over time the number of evaluations with panel disagreement increased. In the first 199 panel evaluations, panel disagreement existed in 91 (46%) of evaluations compared to 115 (58%) in the second group, defined by the last 199 panel evaluations (p=0.021).

Table 1 Inter-Surgeon Variation in Panel Evaluations.

Time of evaluation	Panel agreement		Minor disagreement*		Major disagreement†	
	n	%	n	%	n	%
Overall	193	48	163	41	42	11
Baseline	103	56	77	42	3	2
Follow-up 1	69	41	74	45	24	14
Follow-up 2	19	47	10	24	12	29
Follow-up 3	1	17	2	33	3	50
Follow-up 4	1	100	0	0	0	0

*In one panel evaluation at least one panel surgeon judged "potentially resectable" whereas at least one other surgeon judged "permanently unresectable or "resectable"; †In one panel evaluation at least one panel surgeon judged "resectable" whereas at least one other surgeon judged "permanently unresectable"

Adherence to panel conclusion

Of 10 patients with CRLM considered resectable at baseline, who did not receive systemic therapy in the CAIRO5 study, 2 patients underwent R0 resection while 5 patients first started systemic treatment upon decision of the local surgeon or MDT and in 3 patients extrahepatic metastases were found on additional imaging.

A total of 106 (61%) patients with initially unresectable CRLM (73 patients at FU1, 27 at FU2, 5 at FU3 and 1 patient at FU4) were assessed as having resectable CRLM at follow up evaluation. In 93 (88%) of these patients, resection of CRLM was attempted. Complete local treatment of CRLM by resection (n=51) or resection in combination with ablative therapy (n=29) or ablative therapy only (n=4) was performed in a total of 84 (79%) patients. Reasons for non-adherence to the panel decision are presented in Table 2. In 38% of resections and/or local ablative treatments, the final procedure was carried out exactly similar to the treatment plan suggested by the panel.

Characteristics of the intervention in patients that underwent resection and/or local ablative treatment

Out of 84 patients that underwent a procedure with curative intent, 21 (25%) patients required preoperative portal vein embolization. Twenty-seven (32%) patients underwent a right hemihepatectomy, 21 (25%) a segmentectomy or local resection plus ablative treatment, 17 (21%) a segmentectomy or local resection, 7 (8%) a left hemihepatectomy, another 7 (8%) an extended right hemihepatectomy, 4 (5%) patients were treated with a local ablative procedure only and 1 patient underwent an extended left hemihepatectomy. Twenty-two (26%) two-stage procedures and 11 (13%) laparoscopic procedures were performed (Table 3).

In patients who underwent resection (n=51), R0, R1 and R2 resections were achieved in 41 (80%), 9 (18%) and 1 (2%) cases, respectively. In patients who underwent resection in combination with local ablative treatment (n=29), R0, R1 and R2 resections were achieved in 22 (76%), 6 (21%) and 1 (3%) cases, respectively.

Table 2 Adherence to panel conclusion and panel treatment plan in resectable patients at follow-up evaluation.

Panel conclusion adherence	Total n=106
Resection and/or local ablative treatment, n (%)	
Yes	84 (79)
No	22 (21)
Reason no resection and/or local ablative treatment, n	
Perioperatively unresectable (open-close)	6
Patient condition	4
Decision local surgeon/MDT	4
New intra- and/or extrahepatic metastases	4
2nd stage not executed due to insufficient liver remnant	3
Patient decision	1
Final resection similar to panel treatment plan, n (%)	
Yes	32 (38)
No	52 (62)
Reason resection not similar to panel conclusion, n	
Final resection more extensive	12
Final resection less extensive	16
1-stage converted to 2-stage resection	8
2-stage converted to 1-stage resection	7
Local ablative treatment instead of wedge/segment resection	8
Wedge/segment resection instead of local ablative treatment	1

MDT, multidisciplinary team

Table 3 Procedure characteristics.

Procedure	Total (n=84)	
	n	%
Portal vein embolization		
Yes	21	25
No	63	75
Surgical and/or ablative treatment		
Surgical procedure	51	60
Surgical + local ablative treatment	29	35
Local ablative treatment	4	5
Procedure type		
Left hemihepatectomy	7	8
Extended left hemihepatectomy	1	1
Right hemihepatectomy	27	32
Extended right hemihepatectomy	7	8
Segmentectomy/local resection	17	21
Segmentectomy/local resection + local ablative treatment	21	25
Only local ablative treatment	4	5
Two-stage procedure		
Yes	17	20
ALPPS	5	6
No	62	74

Table 3 (continued)

Procedure	Total (n=84)	
	n	%
Laparoscopic procedure		
Yes	11	13
No	70	83
Unknown	3	4
Radicality		
R0	63	75
R1	15	18
R2	2	2
Local ablative treatment only	4	5

ALPPS, associating liver partition and portal vein ligation for staged hepatectomy

Outcome in relation to panel agreement

Out of 106 patients evaluated to have resectable CRLM, 52 (49%) patients had assessments with panel agreement whereas in 54 (51%) patients panel disagreement occurred. In patients with panel agreement and resectable CRLM, resections (26) and/or ablative treatment (19) was undertaken in 45 of 52 (87%) patients, whereas 39 of 54 (72%) patients received resections (25) and/or ablative treatments (14) when there was panel disagreement at evaluation (p = 0.069). In patients with panel agreement, R0 resection (22) and/or ablative treatment (17) was achieved in 39 out of 52 (75%) patients, compared to 28 (19 resections and 9 resections with ablative therapy) out 54 (52%) patients when disagreement occurred (p=0.013).

In panel evaluations with major panel disagreement and resectable outcome, 9 of 18 (50%) patients did not receive a liver resection. The reasons for non-resection in this group included: intraoperative unresectability in 3 patients, insufficient future liver remnant in 2, decision overruled by the local MDT or surgeon in 3 and decision of the patient in 1.

Further analysis was done excluding 13 patients in whom no surgery was initiated for reasons of extrahepatic disease, decision of local MDT or condition of the patient. Of the remaining 93 patients, 47 (51%) had an assessment with panel agreement. R0 resection (22) and/or local ablative treatment (17) was achieved in 39 out of 47 (83%) patients with panel agreement, compared to 28 (19 resections and 9 resections with ablative therapy) out 46 (61%) patients with panel disagreement (p=0.018). (**Table 4**)

Table 4 Outcomes in Relation to Panel Agreement in Patients with Colorectal Cancer Liver Metastases Considered Resectable by the Panel after Systemic Therapy.

Outcomes	Total patients with resectable CRLM n=106		Panel agreement n=52		Panel disagreement n=54		p Value
	n	%	n	%	n	%	
Resection without ablative treatment							
R0	41	39	22	42	19	35	-
R1	9	8	3	6	6	11	
R2	1	1	1	2	0	0	
Resection with ablative treatment							
R0	22	21	13	25	9	17	-
R1	6	6	1	2	5	9	
R2	1	1	1	2	0	0	
Local ablative treatment only	4	4	4	8	0	0	-
Perioperatively unresectable	6	6	2	4	4	7	-
2nd stage not done, insufficient liver remnant	3	3	0	0	3	6	-
No operation	13	12	5	10	8	15	-
Resection and/or ablative treatment	84	79	45	86	39	72	0.069
No resection and/or ablative treatment	22	21	7	14	15	28	
R0 resection and/or ablative treatment	67	63	39	75	28	52	0.013
R1 or R2 or incomplete or no resection	39	37	13	25	26	48	

CRLM, colorectal cancer liver metastases

Discussion

This study demonstrates successful implementation and feasibility of the CAIRO5 national DCCG Liver Expert Panel in clinical practice. The median time to panel conclusion of 7 days was considerably faster than the preconceived maximum of 14 days allowing efficient assessment by multiple experienced liver surgeons in these very complex patients.

Despite resectability assessments by a panel of experienced liver surgeons, a high level of inter-surgeon disagreement per assessment was observed, as shown in earlier studies.[17-19,21] This underlines the complexity of defining (un)resectability. However, with consensus on baseline criteria for (un)resectability, we noted significantly less inter-surgeon variation compared to follow-up evaluations, which underscores the value of well-defined resection criteria. Our data, supports the evaluation of CRLM patients by a panel of liver surgeons rather than by an individual surgeon or MDT in order to achieve a more reproducible and more balanced decision per patient. The true value of the panel can be assessed when further clinical, translational and outcome data are available. The high R0 resection rate and / or ablative treatment rate after patients were considered

resectable, confirms the feasibility of resectability assessment by the panel. The difference in successful local treatment (R0 resection and/or ablative treatment) between patients with evaluations with panel agreement versus panel disagreement shows resectability is more difficult to predict in this subgroup of patients and calls for the definition of more stringent resectability criteria. The design of this panel enables further prospective analysis of these subgroups, incorporating follow-up data on clinical outcomes and translational research data on clinical characteristics and biomarkers, in order to provide improved selection criteria for (un)resectability. The increase over time of panel evaluations with disagreement, confirms lack of resection criteria remains an important issue today and that the outcomes of these future analyses are as vital as when the CAIRO5 study started in 2014.

In 62% of patients, the final resection carried out was different from the surgical plan proposed by the panel. We assign this high rate to the fact that the surgical plan itself was not mandatory. Other explanations could be that most hospitals perform an additional preoperative MRI because of better diagnostic performance and the finding of new lesions.[27-29] Furthermore, intraoperative adjustment of the surgical plan is a well-known phenomenon since intraoperative ultrasonography is still known to be the golden standard in revealing the total extent of the disease.[30-32] We did not note much resistance from local MDTs or surgeons regarding the proposed treatment plans by the panel. Two patients with potentially resectable CRLM as assessed by the panel, did not finish further panel evaluations because the local surgeon or MDT decided to proceed with surgical resection. In four patients considered resectable by the panel, the resection was not executed due to disagreement of the local surgeon or MDT.

In the absence of formal international consensus on resectability criteria for CRLM, baseline criteria for unresectability were defined by consensus among Dutch liver surgeons for the purpose of more uniform selection of CRLM patients for multimodality treatment according to state-of-the-art management of CRLM in a governed, auditable and reproducible manner, allowing improved reproducibility and minimal selection bias in the CAIRO5 study, as well as a better interpretation of patient outcomes.[23]

Our criteria imply that more patients are exposed to perioperative systemic treatment in the CAIRO5 study than in routine Dutch practice, since (neo)adjuvant systemic therapy is not recommended in the Dutch treatment guidelines for patients with resectable CRLM due to the lack of survival benefit in the EPOC trial.[33] This will have contributed to the high conversion rate of 61%. However, there was general consensus that the administration of induction systemic treatment is ethical and appropriate in the relatively high-risk patient group qualifying for the CAIRO5 study, moreover because some studies suggest a survival benefit of systemic therapy in patients with high risk CRLM.[34-36]

This study has some limitations. Evaluation of the panel is by observational design, which may introduce bias. However, a randomized selection for evaluation by the panel was considered unethical. Furthermore, the panel evaluation of patients was performed only by radiological imaging, without considering patient's clinical condition and possible comorbidity. Notwithstanding these facts, the CAIRO5 eligibility criteria included the most relevant assessments of performance status and organ functions to allow the safe administration of systemic treatment and surgery, while the final decision for implementation of the panel decision remained with the treating physician.

Conclusion

This study analyzed prospective evaluation of patients with unresectable CRLM as defined by uniform criteria using an online expert panel of radiologists and liver surgeons. The high inter-surgeon variation reflects the complexity in defining treatment strategies for CRLM and supports the use of a panel rather than a single-surgeon decision. Our results demonstrate that the DCCG CAIRO5 Liver Expert Panel is feasible and provides a platform for prospective initial and follow-up assessments on resectability in patients with advanced CRLM on a national level.

Author contributions

Study conception and design: CJAP, TvG
Acquisition of data: KB, JH, MA, CD, ME, MG, DG, LH, JH, KdJ, GK, AK, JK, ML, KvL, IQM, GP, AR, TR, RJS, HvT, CV, JdW, CJAP, TvG
Analysis and interpretation of data: KB, JH, CJAP, TvG
Drafting of manuscript: KB, JH, CJAP, TvG
Critical revision: KB, JH, MA, CD, ME, MG, DG, LH, JH, KdJ, GK, AK, JK, ML, KvL, IQM, GP, AR, TR, RJS, HvT, CV, JdW, CJAP, TvG

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References

1. Borner MM. Neoadjuvant chemotherapy for unresectable liver metastases of colorectal cancer--too good to be true? Ann Oncol. 1999;10:623–626.
2. van der Geest LGM, Lam-Boer J, et al. Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis. 2015;32: 457–465.
3. Noren A, Eriksson HG, Olsson LI. Selection for surgery and survival of synchronous colorectal liver metastases; a nationwide study. Eur J Cancer. 2016;53:105–114.
4. Angelsen J-H, Horn A, Sorbye H, et al. Population-based study on resection rates and survival in patients with colorectal liver metastasis in Norway. Br J Surg. 2017;104:580–589.
5. de Ridder JAM, van der Stok EP, Mekenkamp LJ, et al. Management of liver metastases in colorectal cancer patients: A retrospective case-control study of systemic therapy versus liver resection. Eur J Cancer. 2016;59:13–21.
6. Huiskens J, Olthof PB, van der Stok EP, et al. Does portal vein embolization prior to liver resection influence the oncological outcomes - A propensity score matched comparison. Eur J Surg Oncol. 2018;44:108–114.
7. Adam R. Chemotherapy and surgery: new perspectives on the treatment of unresectable liver metastases. Ann Oncol. 2003;14 Suppl 2:ii13-6.
8. Wicherts DA, de Haas RJ, Adam R. Bringing unresectable liver disease to resection with curative intent. Eur J Surg Oncol. 2007;33 Suppl 2:S42-51.
9. Adam R, Delvart V, Pascal G, et al. Rescue Surgery for Unresectable Colorectal Liver Metastases Downstaged by Chemotherapy. Ann Surg. 2004;240:644–658.
10. Lam VWT, Spiro C, Laurence JM, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol. 2012;19:1292–1301.
11. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3:50–56.
12. Adam R, de Gramont A, Figuerras J, et al. The Oncosurgery Approach to Managing Liver Metastases from Colorectal Cancer: A Multidisciplinary International Consensus. Oncologist. 2012:1225–1239.
13. Brouquet A, Abdalla EK, Kopetz S, et al. High survival rate after two-stage resection of advanced colorectal liver metastases: response-based selection and complete resection define outcome. J Clin Oncol. 2011;29:1083–1090.
14. Voorthuizen T Van, Gulik TM Van, Punt CJA. Defining resectability of colorectal liver metastases : how and why ? Colorectal Cancer. 2013;2:61–72.
15. Lordan JT, Karanjia ND, Quiney N, et al. A 10-year study of outcome following hepatic resection for colorectal liver metastases - The effect of evaluation in a multidisciplinary team setting. Eur J Surg Oncol. 2009;35:302–306.
16. Jones RP, Vauthey J-N, Adam R, et al. Effect of specialist decision-making on treatment strategies for colorectal liver metastases. Br J Surg. 2012;99:1263–1269.
17. Homayounfar K, Bleckmann A, Helms H-J, et al. Discrepancies between medical oncologists and surgeons in assessment of resectability and indication for chemotherapy in patients with colorectal liver metastases. Br J Surg. 2014;101:550–557.
18. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer. 2018;88:77–86.
19. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11:38–47.
20. Choti MA, Thomas M, Wong SL, et al. Surgical Resection Preferences and Perceptions among Medical Oncologists Treating Liver Metastases from Colorectal Cancer. Ann Surg Oncol. 2016;23(2):375-381
21. Mohammad WM, Martel G, Mimeault R, et al. Evaluating agreement regarding the resectability of colorectal liver metastases: a national case-based survey of hepatic surgeons. HPB (Oxford). 2012;14: 291–297.

22. Aubin J-M, Bressan AK, Grondin SC, et al. Assessing resectability of colorectal liver metastases: How do different subspecialties interpret the same data? Can J Surg. 2018;61:251–256.
23. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
24. Anon. Available at: http://www.werkgroep-leverchirurgie.nl.
25. Couinaud C, Delmas A, Patel J. Le foie etudes anatomiques et chirurgicales. In: Paris: Masson & Cie, 1957.
26. Van Vledder MG, Boctor EM, Assumpcao LR, et al. Intra-operative ultrasound elasticity imaging for monitoring of hepatic tumour thermal ablation. HPB (Oxford). 2010;12:717–723.
27. Zech CJ, Korpraphong P, Huppertz A, et al. Randomized multicentre trial of gadoxetic acid-enhanced MRI versus conventional MRI or CT in the staging of colorectal cancer liver metastases. Br J Surg. 2014;101: 613–621.
28. Jhaveri KS, Fischer SE, Hosseini-Nik H, et al. Prospective comparison of gadoxetic acid-enhanced liver MRI and contrast-enhanced CT with histopathological correlation for preoperative detection of colorectal liver metastases following chemotherapy and potential impact on surgical plan. HPB (Oxford). 2017;19: 992–1000.
29. Schulz A, Viktil E, Godt JC, et al. Diagnostic performance of CT, MRI and PET/CT in patients with suspected colorectal liver metastases: the superiority of MRI. Acta Radiol. 2016;57:1040–1048.
30. D'Hondt M, Vandenbroucke-Menu F, Preville-Ratelle S, et al. Is intra-operative ultrasound still useful for the detection of a hepatic tumour in the era of modern pre-operative imaging? HPB (Oxford). 2011;13: 665–669.
31. Arita J, Ono Y, Takahashi M, et al. Routine Preoperative Liver-specific Magnetic Resonance Imaging Does Not Exclude the Necessity of Contrast-enhanced Intraoperative Ultrasound in Hepatic Resection for Colorectal Liver Metastasis. Ann Surg. 2015;262:1086–1091.
32. Hoareau J, Venara A, Lebigot J, et al. Intraoperative Contrast-Enhanced Ultrasound in Colorectal Liver Metastasis Surgery Improves the Identification and Characterization of Nodules. World J Surg. 2016; 40:190–197.
33. 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. 2013;14:1208–15.
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. 2014;14:174.
35. Ayez N, Lalmahomed ZS, van der Pool AEM, et al. Is the Clinical Risk Score for Patients with Colorectal Liver Metastases Still Useable in the Era of Effective Neoadjuvant Chemotherapy? Ann Surg Oncol. 2011;18:2757–2763.
36. Liu W, Zhou J-G, Sun Y, et al. The role of neoadjuvant chemotherapy for resectable colorectal liver metastases: a systematic review and meta-analysis. Oncotarget. 2016;7(24): 37277-37287.

CHAPTER 4

Short-term outcomes of secondary liver surgery for initially unresectable colorectal liver metastases following modern induction systemic therapy in the Dutch CAIRO5 trial

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Abstract

Objective

To present short-term outcomes of liver surgery in patients with initially unresectable colorectal liver metastases (CRLM) downsized by chemotherapy plus targeted agents.

Summary of background data

The increase of complex hepatic resections of CRLM, technical innovations pushing boundaries of resectability and use of intensified induction systemic regimens warrants for safety data in a homogeneous multicenter prospective cohort.

Methods

Patients with initially unresectable CRLM, who underwent complete resection after induction systemic regimens with doublet or triplet chemotherapy, both plus targeted therapy, were selected from the ongoing phase III CAIRO5 study (NCT02162563). Short-term outcomes and risk factors for severe postoperative morbidity (Clavien-Dindo grade≥3) were analyzed using logistic regression analysis.

Results

A total of 173 patients underwent resection of CRLM after induction systemic therapy. The median number of metastases was 9 and 161 (93%) patients had bilobar disease. Thirty-six (20.8%) 2-stage resections and 88 (51%) major resections (>three liver segments) were performed. Severe postoperative morbidity and 90-day mortality was 15.6% and 2.9%, respectively. After multivariable analysis, blood transfusion (OR 2.9 [95%CI 1.1-6.4] p=0.03), major resection (OR 2.9 [95%CI 1.1-7.5] p=0.03) and triplet chemotherapy (OR 2.6 [95%CI 1.1-7.5] p=0.03) were independently correlated with severe postoperative complications. No association was found between number of cycles of systemic therapy and severe complications (r=-0.038, p=0.31).

Conclusion

In patients with initially unresectable CRLM undergoing modern induction systemic therapy and extensive liver surgery, severe postoperative morbidity and 90-day mortality was 15.6% and 2.7%, respectively. Triplet chemotherapy, blood transfusion and major resections were associated with severe postoperative morbidity.

Introduction

Over the last decennium, the number of complex hepatic resections in patients with colorectal liver metastases (CRLM) have increased gradually, with improved long-term survival outcomes.[1-4] Short-term postoperative morbidity and mortality rates of major liver resections are reported up to 45% and 7%, respectively.[4-8] Perioperative blood transfusion and major liver resections are independent prognostic factors for severe short-term complications[9] and occurrence of postoperative complications after liver resection has been associated with deprived long-term oncological outcomes.[10] Innovations in treatment strategies in patients with advanced CRLM have led to an increased number of patients deemed technically eligible for resection. This is mainly attributable to technical improvements with advances in liver augmentation and parenchymal-sparing techniques like treatment combinations with local ablative therapy, portal vein embolization, two-stage resections and Associating Liver Partition and Portal vein ligation for Staged hepatectomy (ALPPS).[11,12] Secondly, novel systemic regimens consisting of chemotherapy including targeted agents with high efficacy have become available with response rates of up to 80% and allowing secondary resections in 40% of patients with CRLM initially deemed unresectable.[13-15] After induction therapy, patients often require complex resections to clear the liver from tumor. Still, five-year survival rates of up to 40% have been described with this regimen.[4,16]

Systemic treatment may compromise the liver, with histologic changes such as portal and parenchymal inflammation, sinusoidal obstruction syndrome associated with oxaliplatin-based chemotherapy and steatohepatitis associated with irinotecan-based chemotherapy with a risk of progressing to fibrosis, cirrhosis and liver failure.[17,18] Parenchymal necrosis and sinusoidal dilatation was found to be increased in patients with triplet chemotherapy as compared to controls without systemic therapy.[19] The presence of hepatic parenchymal toxicity is correlated with an increased risk of postoperative morbidity and mortality after secondary resection as a result of impaired liver function, bleeding or infection.[17,18,20-23] In contrast, bevacizumab has been reported to reduce the risk of oxaliplatin-based sinusoidal injury.[20]

Reported postoperative morbidity and mortality rates after induction systemic therapy and extensive hepatic resections of CRLM range widely[9,21,24-26] and data is derived mostly from single center retrospective studies.[5] These studies concern heterogeneous patient populations with varying numbers of metastases, types of resections and number of cycles, type and intention of systemic therapy (neo-adjuvant or induction).[21,22] With increasing use of modern intensified induction systemic therapy in combination with ongoing innovations leading to increased surgical possibilities, safety data in this specific patient group in a multicenter prospective cohort on a national level is warranted.

The aim of this study was to describe short-term postoperative morbidity and mortality after modern induction systemic therapy followed by hepatic resection and to determine risk factors for severe postoperative morbidity in patients participating in the ongoing phase 3 CAIRO5 study.

Methods

Patient selection

Patients were selected from the ongoing CAIRO5 study, a phase three clinical trial of the Dutch Colorectal Cancer Group (DCCG), investigating the currently most effective first-line systemic regimens of chemotherapy (5-fluorouracil, oxaliplatin and/or irinotecan) plus targeted therapy in patients with initially unresectable, liver-only CRLM.[27] Patients are randomized between FOLFOX/FOLFIRI-Bevacizumab and FOLFOX/FOLFIRI-Panitumumab, or FOLFOX/FOLFIRI-Bevacizumab and FOLFOXIRI-Bevacizumab according to *RAS/BRAF* tumor mutation status and sidedness (right-sided or left-sided hemicolon) of primary tumor. A central expert panel of liver surgeons and abdominal radiologists evaluates patients at baseline for eligibility based on predefined baseline resectability criteria. Given the lack of (inter)national consensus on criteria for (un)resectability, these criteria were selected to allow a homogeneous study population. Following these baseline criteria, CRLM are deemed unresectable if an R0 resection cannot be achieved in one procedure with one surgical intervention based on computed tomography (CT) and/or magnetic resonance imaging (MRI) scan. Thereafter, patients are evaluated by the panel every two months during systemic treatment to assess resectability of CRLM according to current and more liberal guidelines[28,29], and thus abandoning baseline resectability criteria. If CRLM are deemed resectable, a surgical plan is provided and forwarded with the resectability assessment to the local multidisciplinary team (MDT).[30] According to CAIRO5 study protocol, adjuvant therapy after surgery of CRLM is initiated until a total length of induction and adjuvant systemic therapy of 12 cycles. Accrual of patients started in July 2014. All patients from the start of the study until April 2019 who underwent complete resection with or without local ablative treatment of CRLM were included for this study. Patients with planned two-stage resections and who underwent only the first (minor) stage of surgery, for reasons other than postoperative complications, were excluded. All patients signed a written consent form and the study was conducted according to the ethical standards of the Helsinki Declaration of 1975.

Data selection

All data in the CAIRO5 study were prospectively collected by certified local data managers and checked by central data managers of the Netherlands Comprehensive Cancer Institute. Baseline characteristics were collected such as: age, sex, site of primary

tumor, time to metastases, *RAS/BRAF* mutational status and Fong clinical risk score categorized in low risk (0 or 1 point), moderate risk (2 or 3 points) and high risk (4 or 5 pionts)[31]. Synchronous disease was defined as a disease-free interval (DFI) of <6 months after initial diagnosis of CRC.[32] Furthermore, oncological characteristics were scored such as: number and largest diameter of metastases, hepatic location (unilobar/bilobar and segments as described by Couinaud[33]) and involvement of diaphragm, hepatic arteries and veins and inferior vena cava. Information about induction systemic therapy, such as type of systemic therapy (triplet versus doublet and anti-EGFR versus anti-VEGF therapy), response to therapy according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1)[34], number of cycles and days between last chemotherapy and surgery, were collected. Surgical-technical details were collected such as: type of surgery (one-stage versus two-stage), combination with local ablative treatment, portal vein embolization (PVE) yes/no, and R0/R1-resection status (R1 was defined as microscopic tumor involvement in the resection margin[35]), blood loss and number of days of hospitalization. Major resections were defined as resections of ≥4 segments.[36] Postoperative morbidity was scored according to Clavien Dindo grading system.[37] Severe complications were defined as Clavien Dindo 3a and higher. Mortality was scored at 30 and 90 days.

Histopathological analysis

The assessment of hepatic resection specimens available at time of analysis was centrally performed by reviewing the original hematoxylin and eosin- stained slides of liver metastases by a dedicated pathologist (CM). Pathologic response was scored based on the Tumor Regression Grading[38] and correlation analysis was performed for number of cycles of induction systemic therapy and pathologic response. Furthermore, liver parenchymal inflammation surrounding the liver metastases based on lymphocyte infiltration (peritumoral, portal and combined inflammation) was assessed and the association of parenchymal inflammation and occurrence of severe postoperative complications was assessed.[23]

Statistical analysis

Patient and tumor characteristics were displayed with counts and percentages or medians with inter-quartile range (IQR). Differences between groups were analyzed using chi-square tests and Fisher's exact tests, as appropriate. Values of $p<0.05$ were considered statistically significant. Potential predictive factors were analyzed following logistic regression analyses. After univariable analyses was performed, multicollinearity was tested. Variables with significant interaction were not tested in the multivariable model. Because of the limited number of events a maximum of three factors were selected for multivariable analysis. Based on literature and variable of interest, blood transfusion and chemotherapy (triplet versus doublet) were selected in advance for

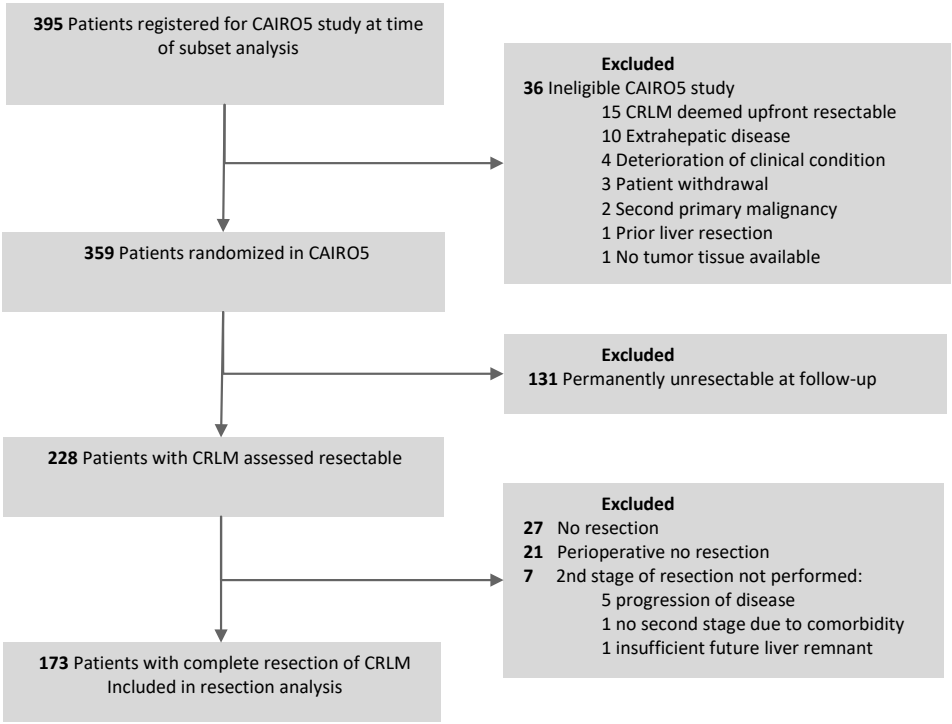
multivariable testing. The third factor was selected with backward selection. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) were calculated. Correlation was tested by the Spearman's correlation coefficient. Analyses were performed using SPSS software version 26 (IBM, New York, USA).

Results

Patient cohort

A total of 395 patients were registered for the CAIRO5 trial between start of the study and April 2019. Thirty-six patients were excluded due to ineligibility for the study. The remaining 359 patients were enrolled and randomized to systemic treatment. After systemic treatment, 131 patients with permanently unresectable CRLM as assessed by central expert panel evaluation, were excluded for this analysis. Of the remaining 228 patients deemed to have resectable disease after induction systemic therapy, 55 (24.1%) were excluded because resection was not successfully completed. In 27 of these 55 patients, surgery was abandoned due to new intrahepatic (n=6) or extrahepatic (n=5) metastases as shown by additional imaging, decision of the local MDT (n=8), condition (n=4) or request (n=2) of the patients, complete radiological response (n=1) and in one patient the reason was missing. Twenty-one patients were considered unresectable perioperatively. Lastly, seven of 43 patients (16.3%) scheduled for a two-stage resection, did not undergo the second resection due to progression of disease (n=5), comorbidity (n=1), or insufficient future liver remnant (FLR) (n=1). After applying inclusion and exclusion criteria, 173 of 359 (48.2%) randomized patients, who underwent liver surgery with curative intent in 19 centers, were analyzed. Resections were performed in a total of eight university and 14 non-university hospitals. The flow-diagram with reasons of in- and exclusion of patients is shown in **Figure 1**.

Figure 1 Flowchart patients.



Baseline characteristics

Patient characteristics are provided in **Table 1** and showed predominantly synchronous disease in 146 (84.4%) patients, bilobar distribution of liver metastases in 161 (93%) patients, with a median of 5 (IQR 4-6) liver segments involved and a median of 9 (IQR 5-14) CRLM per patient. No patients had a low Fong clinical risk score, whereas 97 patients (56.1%) had a medium and 73 patients (42.2%) had a high Fong clinical risk score[31]. The tumor carried a *RAS* or *BRAF* mutation in 83 patients (48.0%). All patients (100%) were treated with chemotherapy with targeted therapy before resection. FOLFOX/FOLFIRI-Bevacizumab, FOLFOX/FOLFIRI-Panitumumab and FOLFOXIRI-Bevacizumab was administered in 92 (53.2%), 37 (21.4%) and 44 (25.4%) of patients, respectively. Patients received a median of six cycles of induction systemic therapy prior to resection and had a median interval between last administration of systemic therapy and liver resection of 41 days in patients without PVE and 47 days in patients with PVE prior to resection. At baseline, diaphragm, portal vein or inferior vena cava were clinically involved in 52 (30.1%), 60 (34.7%) and 35 (20.2%) patients, respectively, as assessed by a central radiologic assessment by the panel radiologist.

Table 1 Baseline patient characteristics total cohort.

Clinical characteristics	All patients (N=173)
Age - yr	
Median (IQR)	62 (55-70)
Sex – no (%)	
Male	112 (64.7)
Female	61 (35.3)
Site of primary tumor – no (%)	
Right colon	35 (20.2)
Left colon or rectum	138 (79.8)
Time to metastases – no (%)	
Synchronous	146 (84.4)
Metachronous	27 (15.6)
Mutational status – no (%)	
RAS / BRAF wildtype	90 (52.0)
RAS / BRAF mutation	83 (48.0)
Fong risk score – no (%)	
Low	0
Medium	97 (56.1)
High	73 (42.2)
Unknown	3 (1.7)
Number of liver metastases – no	
Median (IQR)	9 (5-14)
Diameter of largest metastases – mm	
Median (IQR)	34 (24-58)
Diaphragm involved – no (%)	
Yes	52 (30.1)
No	113 (65.3)
Unknown	8 (4.6)
Hepatic vein involved – no (%)	
Yes	109 (63.0)
No	59 (34.1)
Unknown	5 (2.9)
Portal vein involved – no (%)	
Yes	60 (34.7)
No	109 (63.0)
Unknown	4 (2.3)
Vena cava involved – no (%)	
Yes	35 (20.2)
No	137 (79.2)
Unknown	1 (0.6)
Number of liver segments involved – no	
Median (IQR)	5 (4-6)
Distribution of liver metastases – no (%)	
Unilobar	13 (7.5)
Bilobar	161 (92.5)
Induction systemic therapy – no (%)	
Doublet + bevacizumab	92 (53.2)
Doublet + panitumumab	37 (21.4)
Triplet + bevacizumab	44 (25.4)

Table 1 (continued)

Clinical characteristics	All patients (N=173)
Number of cycles induction systemic therapy	
Median (IQR)	6 (5-9)
Days between last systemic therapy and surgery	
Median (IQR)	42 (32-42)
Best radiological response – no (%)	
Partial response	110 (63.6)
Stable disease	61 (35.3)
Progressive disease	2 (1.2)

Abbreviations: IQR, interquartile range.

Surgical techniques

Thirty-six patients (20.8%) underwent a 2-stage resection including ten (5.7%) ALPPS procedures. PVE was performed in 40 patients (23.1%) and 88 (50.8%) resections were classified as major resections. In 67 patients (38.5%) liver resection was combined with local tumor ablation. Surgeries had a median duration of 241 minutes and in 136 patients (78.6%) an R0 resection was achieved. In patients with synchronous CRLM (n=97), 88 patients (82.1%) underwent a liver-first procedure, six patients (6.2%) underwent combined liver and primary tumor resection and three (3.1%) patients had their primary tumor resected first. Approximately half of the surgeries (54.3%) were performed in a university referral hospital. Blood transfusions were transmitted to 29 (16.8%) patients. **Table 2** summarizes all the surgical-technical specifications.

Table 2 Surgical specifications total cohort.

Surgical specifications	Total cohort (N=173)
Surgical and/or ablative treatment – no (%)	
Surgery only	98 (56.3)
Surgery + local ablative treatment	67 (38.5)
Local ablative treatment only	9 (5.2)
Two stage procedure – no (%)	
Yes	36 (20.8)
Conventional two stage	26
ALPPS	10
No	137 (79.2)
Portal vein embolization – no (%)	
Yes	40 (23.1)
Right	39
Left	1
No	133 (76.9)

Table 2 (continued)

Surgical specifications	Total cohort (N=173)
Type of procedure – no (%)	
Right HHT only	13 (7.5)
Right HHT with local resection and/or local ablative treatment	46 (26.6)
Left HHT only	2 (1.2)
Left HHT with local resection and/or local ablative treatment	6 (3.5)
Extended HHT only	6 (3.6)
Extended HHT with local resection and/or local ablative treatment	8 (4.6)
Local resection with/without local ablative treatment	83 (48.0)
Local ablative treatment only	9 (5.2)
Major resection	
Yes	88 (51)
No	85 (49)
Margin status – no (%)	
R0	136 (78.6)
R1	27 (15.6)
Local ablative treatment only	9 (5.2)
Unknown	1 (0.6)
Primary tumor resection – no (%)	
Resection at baseline	76 (43.9)
Combined primary and liver resection	6 (3.5)
Liver-first procedure	69 (39.9)
Primary-first procedure	3 (1.7)
Primary tumor not resected	17 (9.8)
Primary tumor resection unknown	2 (1.2)
Duration of surgery – min	
Median (IQR)	237 (176 – 335)
Hospital setting of resections	
University hospital	95 (54.3)
Non-university hospital	79 (45.7)
Length of hospital stay (LOH)	
One stage procedure	
Median (IQR)	8 (6 – 10)
Two stage procedure – total LOH of both procedures	
Median (IQR)	18 (15-23)
Blood transfusion	
Yes	29 (16.8)
Median amounts of units RBC, (IQR)	2 (2 – 4)
No	140 (80.9)
Unknown	4 (2.3)

Abbreviations: IQR, interquartile range; LOH, length of hospital stay; ALPPS, Associating Liver Partition and Portal vein ligation for Staged hepatectomy; HHT, hemihepatectomy; RBC, red blood cells

Overall, 119 postoperative complications were documented in 66 (38.2%) patients. A total of 41 severe complications were reported in 27 (15.6%) patients. The number and type of severe complications are shown in **Table 3.** Thirty-day and 90-day mortality rates were 1.7% and 2.9%, respectively.

Table 3 Type of postoperative morbidity according to Clavien Dindo and type of severe complications in total cohort. Patients may have had more than one complication.

Total complications (n=119)	
Clavien Dindo Grade	
1	29 (24.0)
2	48 (39.7)
3a	23 (19.0)
3b	6 (5.0)
4a	5 (4.1)
4b	4 (3.3)
5	3 (2.5)
Type of severe complications (Clavien Dindo grade ≥3a only, n=41)	
Aspiration	1
Sepsis	4
Biliary leakage	3
Wound infection	3
Shock	1
Ascites	2
Thrombo-embolic event	3
(Anastomotic) leakage	1
Urine retention	1
Gastroparesis	4
Inadequate nerve block	1
Fluid collection at resection site	1
Pneumothorax	1
Saturation decrease	1
Renal insufficiency and hypotension	1
Intra-abdominal infection/abcess	4
Fever	1
Ileus	1
Supraventricular tachycardia	1
Pleural effusion	2
Liver failure	1
Diabetic ketoacidosis	1
Fever, biloma abdomen & pneumonia	1
Breath depression & delirium	1

Predictive factors associated with severe complications

After univariable analyses, four factors were significantly correlated with severe postoperative complications: perioperative blood transfusion, two-stage resection including ALPSS, major surgery and triplet chemotherapy. No multicollinearity was found. After backward selection based on highest p-value of three factors, intraoperative blood transfusion, major surgery and triplet chemotherapy were analyzed in a multivariable model. All three factors remained independently correlated with severe postoperative complications. This resulted for blood transfusion in an OR of 2.9 (95% CI 1.1-6.4, *p*=0.03), for triplet chemotherapy in an OR of 2.6 (95% CI 1.1-7.5, *p*=0.03) and for major resections in an OR of 2.9 (95% CI 1.1-7.5, *p*=0.03). See **Table 4.**

Table 4 Univariable and multivariable analysis factors predicting severe postoperative complications.

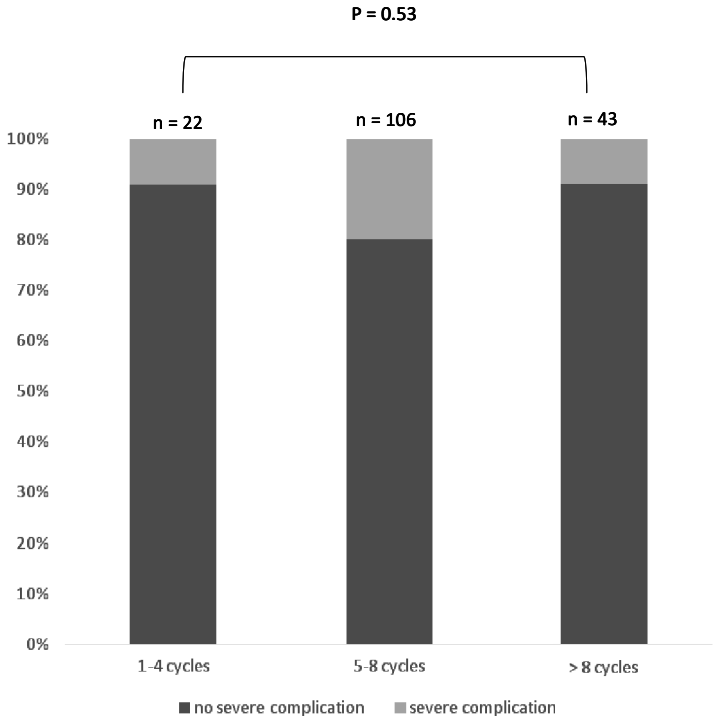
Parameter	Univariable analysis			Multivariable analysis		
	OR	95% CI	*p*	OR	95% CI	*p*
Age > 67	1.6	0.70-3.8	0.26	-		
RAS/BRAF mutation vs wildtype	1.0	0.44-2.3	0.99	-		
Bilobar disease	0.6	0.17-2.5	0.52	-		
Blood transfusion yes vs no	3.1	1.2-7.7	0.019	2.9	1.1-6.4	0.03
Triplet vs doublet chemotherapy	2.4	1.0-5.4	0.041	2.6	1.1-7.5	0.03
Targeted therapy; Anti-EGFR vs anti-VEGF therapy	0.9	0.34-2.4	0.81	-		
2-stage vs 1-stage	2.7	1.1-6.6	0.028	-		
PVE yes vs no	1.0	0.37-2.6	0.98	-		
ALPPS	2.3	0.57-9.8	0.23	-		
Major vs Minor resection	3.0	1.1-7.9	0.028	2.9	1.1-7.5	0.03
Primary tumor first vs liver first resection	3.5	0.76-16.1	0.11	-		
University vs non-university hospitals	1.5	0.65-3.6	0.33	-		
Number of cycles chemotherapy before resection	0.92	0.78-1.1	0.32	-		
Number of days between last chemotherapy and resection	0.99	0.97-1.0	0.41	-		

Importantly, no correlation was found in number of cycles of induction systemic therapy and occurrence of both overall postoperative morbidity and severe postoperative morbidity (OR 0.92, 95% CI 0.82-1.04, *p*=0.18 and OR 0.92, 95% CI 0.78-1.09, *p*=0.32, respectively), neither in the subgroup of patients who underwent major resections (OR 0.96, 95% CI 0.80-1.15, *p*=0.69 and OR 0.96, 95% CI 0.78-1.19, *p*=0.72, respectively). Furthermore, after categorizing the number of cycles of preoperative systemic therapy in 1-4 cycles, 5-8 cycles and >8 cycles, corresponding to 0-2 months, 2-4 months and >4 months, no difference was found between the groups and severe complications (**Figure 2).** In patients with an indication for further adjuvant systemic therapy after resection (until total length of 12 cycles), less patients with severe complications received adjuvant systemic therapy, as compared to patients without severe complications, 5 (18.5%) versus 52 (41.9%) patients, *p*=0.023.

Major resections and severe postoperative complications

Severe complications were more common after major resections compared to minor resections, 23.9% vs. 8.2%, *p*=0.005. The 90 day mortality after major vs. minor resections was 4.5% vs. 1.2%, *p*=0.186. Ten (6%) patients underwent an ALPPS procedure. Among patients who underwent an ALPPS procedure compared to patients with other liver procedures, the severe complication rate was 30% versus 15.3 % and the 90-day mortality rate was 10% compared to 2.5%, respectively. However, this difference was not statistically significant. Nine of ten ALPPS procedures were performed in a university hospital.

Figure 2 Number of cycles induction systemic therapy and severe complication rate following resection of colorectal liver metastases.



Pathologic response, parenchymal inflammation and number of cycles

Central histopathological analysis was performed on resection specimens of 84 patients. A minor or no pathological response was found in 28 (33%) patients, and partial and major pathological response was found in 28 (33%) and 28 (33%) patients, respectively. The number of cycles of induction systemic therapy were comparable across the pathological response groups, with a median of 6 cycles and no correlation was found between number of cycles of systemic therapy and pathologic response (r=-0.108, p=0.336). Peritumoral lymphocyte infiltration was present in 69 (81%) patients and portal lymphocyte infiltration in 23 (27%) patients. Combined peritumoral and portal lymphocyte infiltration was present in 23 (22%) patients. The number of severe complications was the same among patients with and without peritumoral, portal or combined lymphocyte infiltration (data not shown).

Discussion

In this prospective multicenter study a well-defined group of patients with initially unresectable CRLM underwent extensive liver resections after modern induction systemic therapy. We report acceptable short-term postoperative morbidity and 90-day mortality. Risk factors independently correlated with severe postoperative complications were intraoperative blood transfusions, triplet chemotherapy and major liver resection. The number of cycles of preoperative systemic therapy was not related to severe postoperative complications.

We observed an overall postoperative severe complication rate of 15.6% in the total cohort and 23.9% after major resections. The overall 90-day mortality rate was 2.9%. The cohort comprised mostly patients with very advanced disease based on number of liver metastases, the bilobar distribution of metastases and radiological involvement of diaphragm, portal vein or inferior vena cava. This subsequently resulted in extensive and complex hepatic resections with a high PVE rate and high major resection rate of 51%.

Previously published data in the last decade on postoperative morbidity and mortality of resections of CRLM after preoperative systemic therapy comprise predominantly retrospective, single center studies and are depicted in **Table 5**.[4,6-8,21,23-25,39-51] Severe postoperative morbidity and 90-day mortality in these studies ranged from 0 to 39.0% and 0 - 10.3%, respectively. The studies vary widely in median number of metastases (2 to 8 metastases), number of cycles of preoperative systemic therapy (6 to 12 cycles) and major resection rates (25% to 100%). This probably contributes to the variation in reported short-term postoperative outcomes. Furthermore, in eight studies the preoperative systemic therapy was specified as induction therapy for initially unresectable CRLM[4,7,24,39-42], one study comprised neo-adjuvant therapy[48], while the other studies did not specify the intention of preoperative therapy (neo-adjuvant/induction). Percentage of patients receiving targeted therapy ranged from 23% to 100% and was missing in five studies. Three studies[23,45,51] described patients who had received FOLFOXIRI induction systemic therapy and only one study reported on postoperative morbidity or mortality outcomes in these patients.[51] Five prospective RCTs published data on short-term postoperative outcomes after 2010, although this data was minimal for resections details, postoperative complications, and 90-day mortality rate.[40,41,48,51-54] Although these factors hamper inter-study comparison, the severe morbidity and mortality rate reported in the present study compare favorably with the majority of previously reported data after preoperative systemic therapy and support the increase of complex liver surgeries, whereas the long-term survival benefits need to be taken into account as well. These acceptable short-term outcomes might reflect the overall improvement of surgical (parenchymal-sparing) techniques[55] and careful selection of patients based on FLR volume and function.[1,8]

Table 5 Overview of characteristics and short-term postoperative outcomes of studies or subgroups of studies published after 2010 regarding patients with resection of colorectal liver metastases after preoperative systemic therapy and compared to this study.

Study	Type study	Total patients	Study period	Preoperative systemic Rx %	Intention syst Rx %	Targeted Rx %	Triplet Rx %	Median number cycles	Median number CRLM	Major resection %	Morbidity %	Severe morbidity %	90-day mortality %	Remark
CAIRO5 trial 2021	*RCT*	*173*	*2014 - 2019*	*100*	*Induction*	*100*	*25*	*6*	*9*	*51*	*38*	*16*	*2.9*	*Present study*
BECOME trial 2020 - JCO	RCT	34	2013 - 2017	100	Induction	79	0	-	-	-	-	15	-	No data reported on surgical specifications
PLANET-TTD 2017 - EJC	RCT	28	2009 - 2012	100	Induction	100	0	-	-	-	32	-	-	No data reported on surgical specifications
OLIVIA 2015 – Ann Oncol	RCT	44	2008 - 2011	100	Induction	100	51	6	-	-	68	36	4.5	No data reported on surgical specifications
EPOC-2 2014 - Lancet	RCT	198	2007-2012	100	Neo-adjuvant	50	0	-	<3	-	-	23	-	Resectable CRLM only
Ye et al. 2013 - JCO	RCT	22	2008 -2011	100	Induction	51	0	-	-	-	0	0	0	No data published on surgical specifications. No explanation morbidity and mortality rates
Tsim et al. 2011 Ann Surg Oncol	Prosp.	38	2003 - 2006	100	Induction	-	-	8-10	4	100	33	12	0	All patients underwent 2-stage resections
Brouquet et al. 2011 - JCO	Prosp	47	2002 - 2010	100	Induction	74	0	6	7	85	49	26	6.4	All patients underwent 2-stage resections Reported rates of completed 2-stage resection
Elfrink et al. 2020 - EJSO	Retrosp	1314	2014-2018	100	n.r.	-	-	-	-	33	15	10	1.9	Major resection defined as segmentectomy of 3 or more.
Wiseman et al. 2019 - JACS	Retrosp.	1416	2014 - 2016	100	n.r.	-	-	-	< 3	25	34	-	0.8	Type of systemic therapy unknown
Fukuoka et al. 2017 - WJS	Retrosp	439	2005 - 2014	19	n.r.	38	0	187^	2	25	29	2	0	Systemic therapy comprised: systemic therapy for CRLM including adjuvant systemic therapy primary tumor
Ubink et al. 2016 Clin Colorect Canc	Retrosp.	270	2000 - 2015	29	n.r.	-	-	-	2	43	-	27	4	Type of systemic therapy unknown

Table 5 (continued)

Study	Type study	Total patients	Study period	Preoperative systemic Rx %	Intention syst Rx %	Targeted Rx %	Triplet Rx %	Median number cycles	Median number CRLM	Major resection %	Morbidity %	Severe morbidity %	90-day mortality %	Remark
Lock et al. 2017 - JACS	Retrosp.	204	2006 - 2012	62	n.r.	59	14	11	-	53	-	14	3	Any Chemotherapy < 12 months prior to liver resection
Passot et al. 2016 - JACS	Retrosp.	89	2003 - 2014	100	n.r.	-	-	6	6	82	-	26	7	All patients underwent 2-stage resections Reported rates of patients with completed 2-stage resection
Giakoustidis et al. 2014 Hepat Oncol	Retrosp	236	2005 - 2012	100	n.r.	44	0	6	2	57	37	17	3	Only 2-stage >80% VPE 12 % extrahepatic disease
Reissfelder et al 2014 - Surgery	Retrosp.	119	2002 - 2010	100	n.r.	47	20	-	-	50	30	-	1.6	No effect of FOLFOXIRI described
Wolf et al. 2013 - JACS	Retrosp	506	2003 - 2007	65	n.r.	29	0	168^	-	61	32	10	0	Any Chemotherapy < 6 months prior to liver resection
Shindoh et al. 2013 Ann Surg Oncol	Retrosp.	194	1993 - 2011	66	n.r.	63	0	-	-	100	48	24	4	All extended right hemihepatectomy
Turrini et al. 2012 - EJSO	Retrosp.	48	2000 - 2010	100	Induction	42	0	8	8	71	20	12	6	All patients underwent 2-stage resections
Cauchy et al. 2012 - Ann Surg	Retrosp.	257	2000- 2011	100	Induction	55	-	12	6	94	84	39	10.3	All patients underwent VPO as this was the definition of initially unresectable disease
Spelt et al. 2012 - WJS	Retrosp.	97	2000-2009	100	n.r.	23	-	7	2	63	63	4	0	-

Abbreviations**:** RCT, randomized clinical trial; Prosp, prospective; Retrosp, retrospective; Rx, therapy; CRLM, colorectal liver metastases; n.r., not reported. ^ value presents the number of days of systemic therapy.

Varying results have been published concerning the association between number of cycles of systemic therapy and severe postoperative outcomes. In patients with more than 12 cycles induction systemic therapy, Cauchy et al[24] reported remarkably high postoperative mortality (19%) and major morbidity (55%) rates strongly correlated with parenchymal liver injury and Aloia et al.[56] reported a higher re-operation rate in these patients. Other studies deny a correlation of postoperative severe morbidity and number of cycles of preoperative systemic therapy.[1,23,24] However, the cohort of Cauchy et al. concerned a heavily pretreated population with a median number of cycles of 12, 30% of patients had received more than one line systemic therapy prior to liver surgery and all patients underwent portal vein occlusion as this was their definition of initially unresectable CRLM. In the present study no correlation was found between both number of cycles of induction systemic therapy and liver parenchymal inflammation and severe postoperative complications, neither in the subgroup with major resections, but it should be noted that the median number of cycles in our cohort was six and comprised first-line systemic therapy in all patients. Furthermore, bevacizumab has shown to prevent liver parenchymal injury caused by cytotoxic agents and this might have played a role in our results since 75% of patients in our cohort received bevacizumab.[43,57,58]

To our knowledge this study is the first to analyze the prognostic impact of triplet and doublet chemotherapy in combination with targeted agents in regard to postoperative morbidity and mortality after liver resection. A surprising outcome was the strong association between severe postoperative complications and triplet chemotherapy as compared to doublet chemotherapy. This is in line with the results of the OLIVIA trial, which reported a severe postoperative morbidity rate of 40% in patients receiving triplet induction chemotherapy with bevacizumab[51]. Since oxaliplatin is known to contribute to occurrence of a “blue liver” based on sinusoidal injury[59] and irinotecan may cause a “yellow liver” by steatohepatitis[16,60], the combination of both oxaliplatin and irinotecan in triplet chemotherapy may have an extra detrimental effect on the liver. Further histopathological analysis of livers of patients receiving triplet chemotherapy might help to further analyze the association between postoperative complications and triplet chemotherapy. For clinicians treating patients with CRLM, this study shows that intensification of systemic therapy in an attempt to increase resection rates and long-term outcomes comes at a cost of increased short-term postoperative outcomes. The postoperative mortality did not differ between doublet and triplet chemotherapy backbone.

This study has some limitations. Histopathologic assessment was performed on liver metastases only and to better assess the liver injury caused by cytotoxic agents and association with postoperative morbidity rate, assessment of liver parenchyma for the presence of sinusoidal obstruction syndrome, steatohepatitis and parenchymal necrosis

would be informative. Furthermore, underlying liver disease is an important risk factor for postoperative complications, but remains difficult to predict without the availability of a preoperative tissue biopsy or liver function tests.

Conclusion

In this large prospective multicenter randomized controlled trial in patients with advanced initially unresectable CRLM undergoing liver resections after modern induction systemic therapy, we report acceptable postoperative morbidity and mortality rates. Number of cycles of preoperative systemic therapy was not related to severe postoperative complications. These results support the increase of complex liver surgery and number of cycles of first-line induction systemic therapy should not be a contraindication to liver resection. Risk factors that independently correlated with severe postoperative complications were major resection, intraoperative blood transfusions and triplet induction chemotherapy. Careful patient selection considering the type of preoperative systemic treatment as well as efforts to perform parenchymal-sparing resections might help to further reduce the severe complication and mortality rate.

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Author contributions

K.B., L.G., C.J.A.P., and R.J.S. participated in research design. K.B., L.G., N.J.W., C.J.A.P., R.J.S., C.H.C.D., D.J.G., T.M.v.G., G.K., J.M.K., I.Q.M., A.M.R., C.V., and J.H.W.W. were involved in manuscript writing. K.B., L.G., N.J.W., A.K., T.C., C.H.C.D., M.F.G., D.J.G., T.M.G., J.H., K.P.J., G.K., J.M.K., M.S.L., I.Q.M., G.A.P., A.M.R., T.M.R., C.V., J.H.W.W., C.J.A.P., and R.J.S. participated in the performance of the research. K.B., L.G., and A.K. were involved in data collection. K.B., A.K., L.G., C.J.A.P., and R.J.S. participated in data analysis. All authors participated in critical revision of the manuscript.

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References

1. Cloyd JM, Mizuno T, Kawaguchi Y, et al. Comprehensive Complication Index Validates Improved Outcomes Over Time Despite Increased Complexity in 3707 Consecutive Hepatectomies. Ann Surg. 2020;271(4):724-731.
2. Hamers PAH, Elferink MAG, Stellato RK, et al. Informing metastatic colorectal cancer patients by quantifying multiple scenarios for survival time based on real-life data. Int J Cancer. 2020.
3. Kopetz S, Chang GJ, Overman MJ, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol. 2009;27(22):3677-3683.
4. Brouquet A, Abdalla EK, Kopetz S, et al. High survival rate after two-stage resection of advanced colorectal liver metastases: response-based selection and complete resection define outcome. J Clin Oncol. 2011;29(8):1083-1090.
5. Ironside N, Bell R, Bartlett A, McCall J, Powell J, Pandanaboyana S. Systematic review of perioperative and survival outcomes of liver resections with and without preoperative portal vein embolization for colorectal metastases. HPB (Oxford). 2017;19(7):559-566.
6. Passot G, Chun YS, Kopetz SE, et al. Predictors of Safety and Efficacy of 2-Stage Hepatectomy for Bilateral Colorectal Liver Metastases. J Am Coll Surg. 2016;223(1):99-108.
7. Turrini O, Ewald J, Viret F, Sarran A, Goncalves A, Delpero JR. Two-stage hepatectomy: who will not jump over the second hurdle? Eur J Surg Oncol. 2012;38(3):266-273.
8. Shindoh J, Tzeng CW, Aloia TA, et al. Optimal future liver remnant in patients treated with extensive preoperative chemotherapy for colorectal liver metastases. Ann Surg Oncol. 2013;20(8):2493-2500.
9. Kingham TP, Correa-Gallego C, D'Angelica MI, et al. Hepatic parenchymal preservation surgery: decreasing morbidity and mortality rates in 4,152 resections for malignancy. J Am Coll Surg. 2015;220(4):471-479.
10. Dorcaratto D, Mazzinari G, Fernandez M, et al. Impact of Postoperative Complications on Survival and Recurrence After Resection of Colorectal Liver Metastases: Systematic Review and Meta-analysis. Ann Surg. 2019;270(6):1018-1027.
11. Farges O, Belghiti J, Kianmanesh R, et al. Portal vein embolization before right hepatectomy: prospective clinical trial. Ann Surg. 2003;237(2):208-217.
12. Sandstrom P, Rosok BI, Sparrelid E, et al. ALPPS Improves Resectability Compared With Conventional Two-stage Hepatectomy in Patients With Advanced Colorectal Liver Metastasis: Results From a Scandinavian Multicenter Randomized Controlled Trial (LIGRO Trial). Ann Surg. 2018;267(5):833-840.
13. Tomasello G, Petrelli F, Ghidini M, Russo A, Passalacqua R, Barni S. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol. 2017;3(7):e170278.
14. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer. 2018;88:77-86.
15. Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020;141:225-238.
16. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3(1):50-56.
17. Robinson SM, Wilson CH, Burt AD, Manas DM, White SA. Chemotherapy-associated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis. Ann Surg Oncol. 2012;19(13):4287-4299.
18. Vauthey JN, Pawlik TM, Ribero D, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol. 2006;24(13):2065-2072.
19. 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. 2013;108(12):2549-2556.
20. Zhao J, van Mierlo KMC, Gomez-Ramirez J, et al. Systematic review of the influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases. Br J Surg. 2017;104(8):990-1002.

21. Ubink I, Jongen JMJ, Nijkamp MW, et al. Surgical and Oncologic Outcomes After Major Liver Surgery and Extended Hemihepatectomy for Colorectal Liver Metastases. Clin Colorectal Cancer. 2016;15(4): e193-e198.
22. Konopke R, Kersting S, Bunk A, et al. Colorectal liver metastasis surgery: analysis of risk factors predicting postoperative complications in relation to the extent of resection. Int J Colorectal Dis. 2009;24(6): 687-697.
23. Reissfelder C, Brand K, Sobiegalla J, et al. Chemotherapy-associated liver injury and its influence on outcome after resection of colorectal liver metastases. Surgery. 2014;155(2):245-254.
24. Cauchy F, Aussilhou B, Dokmak S, et al. Reappraisal of the risks and benefits of major liver resection in patients with initially unresectable colorectal liver metastases. Ann Surg. 2012;256(5):746-752; discussion 752-744.
25. Wiseman JT, Guzman-Pruneda F, Xourafas D, et al. Impact of Neoadjuvant Chemotherapy on the Postoperative Outcomes of Patients Undergoing Liver Resection for Colorectal Liver Metastases: A Population-Based Propensity-Matched Analysis. J Am Coll Surg. 2019;229(1):69-77 e62.
26. Lam VW, Laurence JM, Johnston E, Hollands MJ, Pleass HC, Richardson AJ. A systematic review of two-stage hepatectomy in patients with initially unresectable colorectal liver metastases. HPB (Oxford). 2013;15(7):483-491.
27. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
28. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27(8):1386-1422.
29. Adam R, De Gramont A, Figueras J, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17(10):1225-1239.
30. Huiskens J, Bolhuis K, Engelbrecht MR, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229(6):523-532 e522.
31. 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):309-318; discussion 318-321.
32. Mekenkamp LJ, Koopman M, Teerenstra S, et al. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010;103(2): 159-164.
33. Couinaud. Le Foie: Études anatomiques et chirurgicales. Paris: Masson. In:1957:400-409.
34. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247.
35. College of American Pathologists. https://www.cap.org/. Accessed March 20, 2021.
36. Reddy SK, Barbas AS, Turley RS, et al. A standard definition of major hepatectomy: resection of four or more liver segments. HPB (Oxford). 2011;13(7):494-502.
37. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2):205-213.
38. 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. 2007;18(2):299-304.
39. Tang W, Ren L, Liu T, et al. Bevacizumab Plus mFOLFOX6 Versus mFOLFOX6 Alone as First-Line Treatment for RAS Mutant Unresectable Colorectal Liver-Limited Metastases: The BECOME Randomized Controlled Trial. J Clin Oncol. 2020;38(27):3175-3184.
40. Carrato A, Abad A, Massuti B, et al. First-line panitumumab plus FOLFOX4 or FOLFIRI in colorectal cancer with multiple or unresectable liver metastases: A randomised, phase II trial (PLANET-TTD). Eur J Cancer. 2017;81:191-202.
41. Ye LC, Liu TS, Ren L, et al. Randomized controlled trial of cetuximab plus chemotherapy for patients with KRAS wild-type unresectable colorectal liver-limited metastases. J Clin Oncol. 2013;31(16):1931-1938.
42. Tsim N, Healey AJ, Frampton AE, et al. Two-stage resection for bilobar colorectal liver metastases: R0 resection is the key. Ann Surg Oncol. 2011;18(7):1939-1946.

43. Kishi Y, Zorzi D, Contreras CM, et al. Extended preoperative chemotherapy does not improve pathologic response and increases postoperative liver insufficiency after hepatic resection for colorectal liver metastases. Ann Surg Oncol. 2010;17(11):2870-2876.
44. Fukuoka K, Nara S, Honma Y, Kishi Y, Esaki M, Shimada K. Hepatectomy for Colorectal Cancer Liver Metastases in the Era of Modern Preoperative Chemotherapy: Evaluation of Postoperative Complications. World J Surg. 2017;41(4):1073-1081.
45. Lock JF, Westphal T, Rubin T, et al. LiMAx Test Improves Diagnosis of Chemotherapy-Associated Liver Injury Before Resection of Colorectal Liver Metastases. Ann Surg Oncol. 2017;24(9):2447-2455.
46. Wolf PS, Park JO, Bao F, et al. Preoperative chemotherapy and the risk of hepatotoxicity and morbidity after liver resection for metastatic colorectal cancer: a single institution experience. J Am Coll Surg. 2013;216(1):41-49.
47. Giakoustidis A, Neofytou K, Khan A, Mudan S. Addition of bevacizumab to preoperative chemotherapy for colorectal liver metastases does not increase perioperative morbidity and mortality. Hepat Oncol. 2014;1(4):363-375.
48. Primrose J, Falk S, Finch-Jones M, et al. Systemic chemotherapy with or without cetuximab in patients with resectable colorectal liver metastasis: the New EPOC randomised controlled trial. Lancet Oncol. 2014;15(6):601-611.
49. Spelt L, Hermansson L, Tingstedt B, Andersson R. Influence of preoperative chemotherapy on the intraoperative and postoperative course of liver resection for colorectal cancer metastases. World J Surg. 2012;36(1):157-163.
50. 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;46(9):1742-1755.
51. Gruenberger T, Bridgewater J, Chau I, et al. Bevacizumab plus mFOLFOX-6 or FOLFOXIRI in patients with initially unresectable liver metastases from colorectal cancer: the OLIVIA multinational randomised phase II trial. Ann Oncol. 2015;26(4):702-708.
52. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11(1):38-47.
53. Ychou M, Rivoire M, Thezenas S, et al. Induction chemotherapy (CT) with FOLFIRINOX or FOLFOX/FOLFIRI, plus cetuximab (CET) or bevacizumab (BEV) (by RAS status), in patients (pts) with primarily unresectable colorectal liver metastases (CRLM): Results of the randomized UNICANCER PRODIGE 14-ACCORD 21 (METHEP-2) trial. J Clin Oncol. 2018;36(15_suppl):3535-3535.
54. Oki E, Emi Y, Yamanaka T, et al. Randomised phase II trial of mFOLFOX6 plus bevacizumab versus mFOLFOX6 plus cetuximab as first-line treatment for colorectal liver metastasis (ATOM trial). Br J Cancer. 2019;121(3):222-229.
55. Elfrink AKE, Nieuwenhuizen S, van den Tol MP, et al. Hospital variation in combined liver resection and thermal ablation for colorectal liver metastases and impact on short-term postoperative outcomes: a nationwide population-based study. HPB (Oxford). 2021;23(6):827-839.
56. Aloia T, Sebagh M, Plasse M, et al. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. J Clin Oncol. 2006;24(31):4983-4990.
57. Rubbia-Brandt L, Lauwers GY, Wang H, et al. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. Histopathology. 2010;56(4):430-439.
58. Hisaka T, Ishikawa H, Sakai H, et al. Sinusoidal Obstruction Syndrome and Postoperative Complications Resulting from Preoperative Chemotherapy for Colorectal Cancer Liver Metastasis. Anticancer Res. 2019;39(8):4549-4554.
59. Soubrane O, Brouquet A, Zalinski S, et al. Predicting high grade lesions of sinusoidal obstruction syndrome related to oxaliplatin-based chemotherapy for colorectal liver metastases: correlation with post-hepatectomy outcome. Ann Surg. 2010;251(3):454-460.
60. Fernandez FG, Ritter J, Goodwin JW, Linehan DC, Hawkins WG, Strasberg SM. Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases. J Am Coll Surg. 2005;200(6):845-853.

PART II

RISK STRATIFICATION OF PATIENTS WITH COLORECTAL CANCER LIVER METASTASES

CHAPTER 5

The role of tumor-biological factors in technical-anatomical resectability assessment of colorectal liver metastases following induction systemic treatment: An analysis of the Dutch CAIRO5 trial

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Submitted

Abstract

Background

Large inter-surgeon variability exists in resectability assessments of colorectal cancer liver-only metastases (CRLM), which is primarily based on technical-anatomical factors. We evaluated the predictive value of tumor-biological factors for secondary resectability and outcomes after local treatment for CRLM.

Methods

482 patients with initially unresectable CRLM from the phase 3 CAIRO5 trial were selected, with two-monthly resectability assessments by a liver expert panel. If no consensus existed among panel surgeons (i.e. same vote for (un)resectability of CRLM), conclusion was based on majority vote. The association of tumor-biological (sidedness, synchronous CRLM, CEA and RAS/BRAF^{V600E} mutation status) and technical-anatomical factors with consensus among panel surgeons, secondary resectability, early recurrence (<6 months) without curative-intent repeat local treatment was analyzed by uni- and prespecified multivariable logistic regression.

Results

After systemic treatment, 240 (50%) patients received complete local treatment of CRLM of which 75 (31%) experienced early recurrence without repeat local treatment. Higher number of CRLM (OR 1.09 [95% CI 1.03-1.15]) and age (OR 1.03 [95% CI 1.00-1.07]) were independently associated with early recurrence without repeat local treatment. In 138 (52%) patients no consensus among panel surgeons was present prior to local treatment. Postoperative outcomes in patients with and without consensus were comparable.

Conclusion

Almost a third of patients selected by an expert panel for secondary CRLM surgery following induction systemic treatment, experienced an early recurrence only amenable to palliative treatment. Number of CRLM and age but no tumor-biological factors were predictive, suggesting that until there are better biomarkers, resectability assessment remains a technical-anatomical decision.

Introduction

Resectability assessments of CRLM are primarily based on technical-anatomical features and is defined as the ability to perform a complete resection, while preserving a sufficient future liver remnant.[1,2] Improved surgical, liver augmentation, and ablation techniques paralleled with optimization of induction systemic therapies have increased the number of patients with technically resectable disease.[3-5] However, up to 45% of patients have early disease recurrence within 6-8 months after local treatment of CRLM, which is often not amenable to repeat local treatment and is negatively associated with overall survival (OS).[6-12] Additionally, resectability assessment is subject to large inter-surgeon variability due to lack of consensus on (un)resectability criteria.[13-17] These issues warrant studies on the potential added predictive value of currently clinically available tumor-biological factors on clinically relevant outcomes after local treatment of CRLM.

Varying factors have been reported to be associated with survival outcomes after CRLM resection such as node-positive primary tumor, number and size of CRLM, serum carcinoembryonic antigen (CEA), disease-free interval between primary tumor and CRLM[18-21], sidedness of primary tumor and *RAS* / *BRAF*^{V600E} mutation status.[22-25] Combining these factors into prediction models are of limited clinical utility caused by the relatively low discriminative power and limitations such as the lack of entry resectability criteria and retrospective nature of the data.[26]

This analysis of patients who received local treatment of CRLM after induction systemic therapy in the CAIRO5 study[27] overcomes the limitations due to the prospective design, the clear entry resectability criteria and repeat resectability assessments by a liver expert panel. We evaluated the predictive value of technical-anatomical and tumor-biological factors on conversion to resectable CRLM, early recurrence and early recurrence without curative-intent repeat local treatment. Outcomes after local treatment of CRLM were compared according to the degree of consensus among panel surgeons in resectability assessments of CRLM.

Methods

Patient selection

Patients were selected from the phase 3 randomized controlled CAIRO5 trial of the DCCG (NCT02162563), investigating first-line systemic regimens of chemotherapy (5-fluorouracil, oxaliplatin and/or irinotecan) plus targeted therapy (bevacizumab or panitumumab) in patients with initially unresectable CRLM. The design of the study has

been published.[27] To allow a meaningful follow-up period, patients randomized between start of the study until April 2021 were selected for this analysis.

Resectability assessment by the DCCG Liver Expert Panel

Computed tomography (CT) scans of patients were evaluated at baseline for eligibility by a central liver expert panel, consisting of 15 liver surgeons and 3 abdominal radiologists. Given the lack of consensus on (un)resectability criteria, baseline resectability criteria were selected by consensus among Dutch liver surgeons to allow a homogeneous study population. CRLM were deemed unresectable at baseline if an R0 resection could not be achieved in a single procedure by surgical resection. Thereafter, patients were reassessed for resectability by the panel every two months during systemic treatment according to more liberal criteria allowing all established local treatments (i.e. ablation, two-stage surgery, portal vein embolization). CT scans were uploaded in a program specially designed to share patient imaging in a privacy-respecting manner. Each CT scan with panel radiology report (including patient's age, number of treatment cycles, location and resection (yes/no) of primary tumor) was evaluated by three randomly selected panel surgeons, who voted individually on the following categories: resectable, potentially resectable after further induction systemic treatment or permanently unresectable. If no consensus (i.e. same category selected by all three surgeons) was obtained, two additional surgeons were consulted and panel conclusion was accepted by majority vote.[13]

Selection of tumor-biological and technical-anatomical tumor features

The following tumor-biological features were collected: *RAS/BRAF*^{V600E} mutation, metachronous metastases (metastases ≥6 months after diagnosis of primary tumor[28]), histopathological nodal status, sidedness of primary tumor (right-sided was defined as tumors located proximal of the splenic flexure), serum CEA (ng/ml). Patient characteristics and technical-anatomical tumor features were collected: age, gender, and number, size and distribution (unilobar / bilobar) of CRLM, diaphragm involvement, involved liver segments and RECIST 1.1 defined response to induction treatment.[29] Resection margin (R0 was defined by absence of microscopic tumor invasion of the resection margin), type of local CRLM treatment (e.g. resection and/or ablation) and type of curative-intent repeat local treatment (e.g. resection, ablation, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) and/or radiotherapy) were collected. Major liver resections were defined as resection of at least three segments or an (extended) hemihepatectomy. Complete local treatment was defined as R0/R1 resection and/or ablation of all CRLM.

Outcomes

Relapse-free survival (RFS) was calculated from the date of last local liver treatment until progression or death or censored on last clinical visit date. Early recurrence was defined as disease progression or death occurring within six months after complete local treatment of CRLM.[8,9] Death and palliative or no local treatment within six months after recurrence were scored as an event for early recurrence without curative-intent repeat local treatment.

Statistical analysis

Continuous variables were displayed as median with interquartile range (IQR) and categorical variables as counts and percentages and differences were analyzed using Pearson's chi-square test. Univariable and multivariable logistic regression were performed to analyze predictive factors for panel (dis)agreement at first follow-up and prior to local treatment, secondary resectability, early recurrence and early recurrence without curative-intent repeat local treatment. Based on the ten events per variable rule, a maximum of 32, 10 and 7 variables could be introduced in multivariable analyses for secondary resectability, early recurrence and early recurrence without repeat local treatment, respectively. Prespecified variables for the multivariable analyses were: age, sidedness, time to metastases, *RAS*/*BRAF*^{V600E} mutation status, CEA, number and size of largest CRLM. $P \leq 0.05$ was considered statistically significant. Analyses were performed using R (version 4.0.3).

Results

After exclusion of 23 patients, 482 patients were analyzed (**Figure 1**). Baseline patient characteristics show a median age of 62 years, a median number of CRLM of 12 (7-22), synchronous disease in 421 (90%), and *RAS* or *BRAF*^{V600E} mutation in 266 (57%) patients (**Table 1**).

Secondary resectability of CRLM

After induction systemic treatment, the liver panel considered CRLM resectable in 324 (69%) patients (**Figure 1**). At the prespecified multivariable analysis, the probability for resectable CRLM was lower in patients with synchronous versus metachronous CRLM (OR 0.18 [95%CI 0.03-0.68], p=0.029), *RAS* mutation versus *RAS*/*BRAF*^{V600E} wildtype (OR 0.38 [95%CI 0.21-0.69], p=0.002), *BRAF*^{V600E} mutation versus *RAS*/*BRAF*^{V600E} wildtype (OR 0.10 [95%CI 0.03-0.30], p<0.001), larger number of CRLM (OR 0.89 [95%CI 0.86-0.91], p<0.001), and larger size of CRLM (OR 0.97 [95%CI 0.96-0.98], p<0.001) (**Table 1**).

RFS and recurrences after local liver treatment

After a median follow-up of 36.5 months (95%CI 23.8-42.7) of patients who received local treatment, the median RFS was 6.9 months (95%CI 6.2-8.2) with 202 (84%) events (recurrence n=198 and death without recurrence n=4), including 104 (43%; 53% of recurrences) early recurrences or death (n=4). The site of early recurrence was liver-only in 62 (62%), lung-only in 12 (12%), peritoneal-only in 5 (5%), lymph node-only in 2 (2%), colon-only in 1 (1%) and multi-organ recurrence in 18 (18%) patients. Among patients with early recurrence, 29 (28%) patients underwent curative-intent repeat local treatment.

Figure 1 Flowchart eligible patients for analysis.

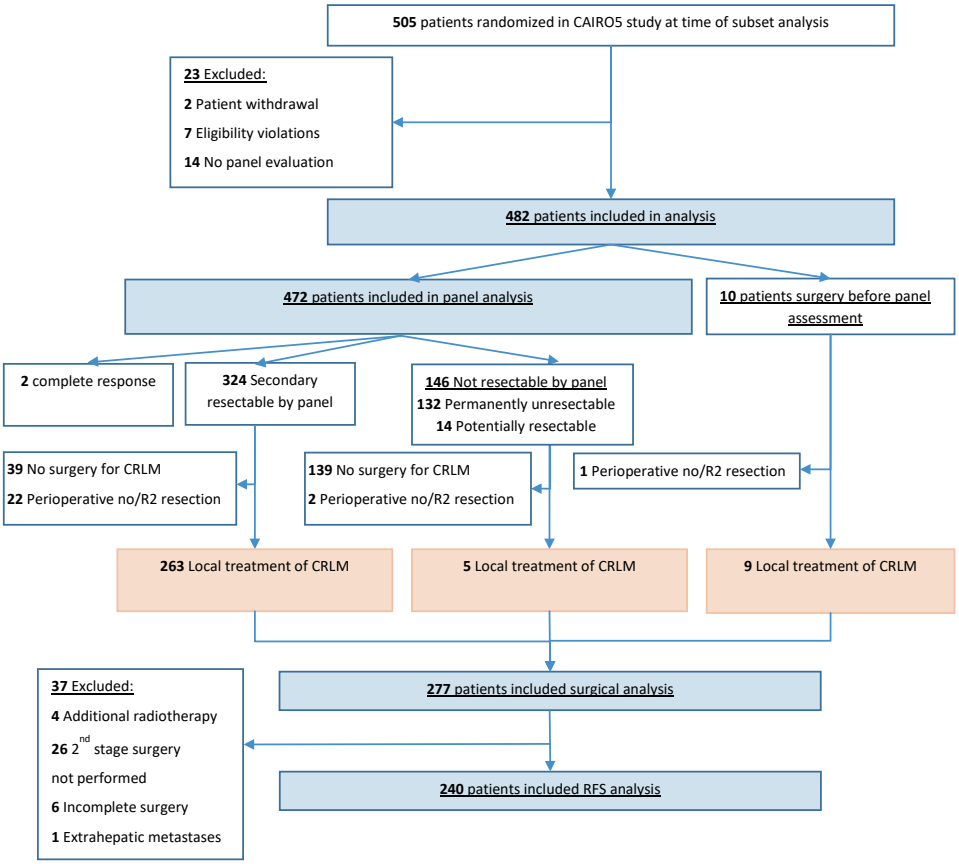


Table 1 Univariable and multivariable logistic regression analysis to evaluate the association of baseline patient and tumor characteristics with probability to be assessed resectable after systemic treatment.

Characteristic	Total cohort[1]	Resectability		Univariable analysis			Multivariable analysis*		
	N=470	Unresectable, N=146	Resectable, N=324	OR	95% CI	p-value	OR	95% CI	p-value
Age patient	62 (54 - 69)	61 (54 - 69)	62 (54 - 70)	1.00	0.98, 1.02	0.829	0.98	0.95, 1.01	0.156
Sex									
Female	182 (39%)	65 (45%)	117 (36%)	—	—				
Male	288 (61%)	81 (55%)	207 (64%)	1.42	0.95, 2.11	0.084			
Site of primary tumor									
Left colon or rectum	344 (73%)	92 (63%)	252 (78%)	—	—		—	—	
Right colon	126 (27%)	54 (37%)	72 (22%)	0.49	0.32, 0.75	<0.001	0.71	0.39, 1.30	0.265
Time to metastases									
Metachronous	49 (10%)	2 (1%)	47 (15%)	—	—		—	—	
Synchronous	421 (90%)	144 (99%)	277 (85%)	0.08	0.01, 0.27	<0.001	0.18	0.03, 0.68	0.029
Tumor nodal status									
Negative	33 (7%)	5 (3%)	28 (9%)	—	—				
Positive	112 (24%)	21 (14%)	91 (28%)	0.77	0.24, 2.11	0.636			
No surgery before registration	325 (69%)	120 (82%)	205 (63%)	0.31	0.10, 0.75	0.017			
Mutational status									
RAS & *BRAF* wildtype	203 (43%)	48 (33%)	155 (48%)	—	—		—	—	
RAS mutation	239 (51%)	80 (55%)	159 (49%)	0.62	0.40, 0.93	0.024	0.38	0.21, 0.69	0.002
BRAF mutation	28 (6%)	18 (12%)	10 (3%)	0.17	0.07, 0.39	<0.001	0.10	0.03, 0.30	<0.001
Serum CEA level, (ng/mL) - baseline	45 (10 - 256)	122 (17 - 409)	28 (8 - 146)	1.00	1.00, 1.00	<0.001	1.00	1.00, 1.00	0.494
Number of liver metastases	12 (7 - 22)	24 (14 - 42)	10 (6 - 15)	0.91	0.89, 0.93	<0.001	0.89	0.86, 0.91	<0.001
Diameter of largest metastasis	41 (27 - 65)	57 (37 - 75)	36 (26 - 57)	0.98	0.98, 0.99	<0.001	0.97	0.96, 0.98	<0.001
Diaphragm involved									
No	257 (55%)	59 (40%)	198 (61%)	—	—				
Yes	178 (38%)	71 (49%)	107 (33%)	0.45	0.29, 0.68	<0.001			
Unknown	35 (7%)	16 (11%)	19 (6%)	0.35	0.17, 0.74	0.005			
Number of liver segments involved	6 (5 - 8)	8 (7 - 9)	5 (4 - 7)	0.51	0.43, 0.58	<0.001			
Distribution of liver metastases									
Unilobar	25 (5%)	3 (2%)	22 (7%)	—	—				
Bilobar	445 (95%)	143 (98%)	302 (93%)	0.29	0.07, 0.85	0.046			

[1] Two patients with complete response were excluded from this analysis. Median (IQR); n (%). OR = Odds Ratio, CI = Confidence Interval. *The variables to include in the multivariable analyses were prespecified.

Predictive factors for early recurrence and early recurrence without repeat local treatment

At the prespecified multivariable analysis, number of CRLM prior to local treatment was the only independent predictive factor for a higher chance of early recurrence (OR 1.10 [95% CI 1.04-1.17], *p*<0.001) (**Table 2**). Number of CRLM was linearly associated with the log-odds of early recurrence which was tested by restricted cubic splines, implying that there is no meaningful cut-off value. To provide more insight on these results, number of CRLM was analyzed on a categorical scale: compared to 1-5 CRLM, 6-10 CRLM are not (OR 1.30 [95% CI 0.70-2.44], *p*=0.411) and >10 CRLM are significantly associated with early recurrence (OR 3.16 [95% CI 1.58-6.51], *p*=0.001). At the prespecified multivariable analysis for early recurrence without repeat local treatment, age (OR 1.03 [95% CI 1.00-1.07], *p*=0.047) and number of CRLM (OR 1.09 [95% CI 1.03-1.15], *p*=0.003) were independent risk factors (**Table 3**). Compared to 1-5 CRLM, 6-10 CRLM are not (OR 1.83 [95% CI 0.92-3.70], *p*=0.089) and >10 CRLM were significantly associated with early recurrence without repeat local treatment (OR 3.20 [95% CI 1.53-6.87], *p*=0.002).

Predictive factors for consensus in resectability assessments

At first follow-up, no consensus among panel surgeons, with panel conclusion based on majority vote, was present in 269 (57%) patients. This included 51 of 269 (19%; 11% of total cohort) evaluations with a totally opposite conclusion among the assigned surgeons (i.e. resectable versus permanently unresectable). No tumor-biological nor technical-anatomical features were predictive for consensus among panel surgeons at first follow-up (data not shown).

In patients receiving local treatment following panel conclusion (n=263), no consensus among panel surgeons was present in 138 (52%) patients at the evaluation prior to local treatment. Factors displaying more advanced disease were associated with no consensus prior to local treatment at univariable analysis: higher CEA (OR 1.00 [95% CI 1.00-1.01], *p*=0.036), larger number of CRLM (OR 1.09 [95% CI 1.04-1.15], *p*=0.001) and larger number of involved liver segments (OR 1.26 [95% CI 1.09-1.45], *p*=0.002) (data not shown). Number of CRLM and involved liver segments were linearly associated with the log-odds of no consensus prior to local treatment which was tested by restricted cubic splines, implying that there is no meaningful cut-off value of these parameters to decide whether patients may benefit from a panel evaluation.

Table 2 Univariable and multivariable logistic regression analysis to evaluate the association of tumor biological and technical-anatomical features at baseline and prior to local treatment, with early recurrence within 6 months following complete local treatment of colorectal liver metastases.

Characteristic	Univariable analysis				Multivariable analysis*		
	Event Rate	OR	95% CI	p-value	OR	95% CI	p-value
Age patient	104 / 240 (43%)	1.01	0.99, 1.04	0.321	1.02	0.99, 1.05	0.203
Sex							
Female	35 / 89 (39%)	—	—				
Male	69 / 151 (46%)	1.30	0.76, 2.22	0.337			
Fong risk score							
Low, <3	48 / 112 (43%)	—	—				
High, ≥3	56 / 128 (44%)	1.04	0.62, 1.73	0.889			
Site of primary tumor							
Left colon or rectum	78 / 185 (42%)	—	—		—	—	
Right colon	26 / 55 (47%)	1.23	0.67, 2.25	0.502	0.99	0.49, 1.99	0.980
Time to metastases							
Metachronous	12 / 34 (35%)	—	—		—	—	
Synchronous	92 / 206 (45%)	1.48	0.71, 3.24	0.309	1.44	0.65, 3.35	0.379
Tumor nodal status							
Negative	9 / 23 (39%)	—	—				
Positive	26 / 63 (41%)	1.09	0.42, 2.98	0.858			
No surgery before registration	69 / 154 (45%)	1.26	0.52, 3.20	0.610			
Mutational status							
RAS & *BRAF* wildtype	50 / 122 (41%)	—	—		—	—	
RAS mutation	50 / 111 (45%)	1.18	0.70, 1.99	0.532	1.03	0.58, 1.84	0.915
BRAF mutation	4 / 7 (57%)	1.92	0.41, 10.1	0.406	1.83	0.35, 10.5	0.472
Serum CEA level, (ng/mL) - PTL	104 / 239 (44%)	1.00	1.00, 1.00	0.139	1.00	1.00, 1.00	0.142
Response - PTL							
Response	63 / 157 (40%)	—	—				
No response (PD/SD)	41 / 83 (49%)	1.46	0.85, 2.49	0.169			
Number of liver metastases - PTL	104 / 239 (44%)	1.10	1.04, 1.16	<0.001	1.10	1.04, 1.17	<0.001
Number of liver metastases - PTL, categorical[1]							
1-5	*33 / 94 (35%)*	—	—		—	—	
6-10	*35 / 87 (40%)*	*1.24*	*0.68, 2.28*	*0.477*	*1.30*	*0.70, 2.44*	*0.411*
>10	*36 / 58 (62%)*	*3.02*	*1.55, 6.04*	*0.001*	*3.16*	*1.58, 6.51*	*0.001*
Number of liver segments involved - PTL	104 / 240 (43%)	1.34	1.15, 1.57	<0.001			
Diameter of largest metastasis - PTL	104 / 239 (44%)	1.00	0.99, 1.01	0.968	1.00	0.99, 1.01	0.732
Distribution of liver metastases - PTL							
Unilobar	9 / 29 (31%)	—	—				
Bilobar	95 / 211 (45%)	1.82	0.81, 4.38	0.158			

OR = Odds Ratio, CI = Confidence Interval, PTL = prior to local treatment.

*The variables to include in the multivariable analyses were prespecified. Number of liver metastases was included in its original continuous form.

[1] A separate multivariable analysis was performed were the continuous number of liver metastases was replaced by the categorical variant.

Table 3 Univariable and multivariable logistic regression analysis to evaluate the association of tumor biological and technical-anatomical features at baseline and prior to local treatment, with early recurrence within 6 months without subsequent local treatment with curative intent.

Characteristic	Univariable analysis				Multivariable analysis		
	Event Rate	OR	95% CI	p-value	OR	95% CI	p-value
Age patient	75 / 240 (31%)	1.03	1.00, 1.06	0.033	1.03	1.00, 1.07	0.047
Sex							
Female	23 / 89 (26%)	—	—				
Male	52 / 151 (34%)	1.51	0.85, 2.73	0.167			
Fong risk score							
Low, <3	37 / 112 (33%)	—	—				
High, ≥3	38 / 128 (30%)	0.86	0.49, 1.48	0.577			
Site of primary tumor							
Left colon or rectum	54 / 185 (29%)	—	—		—	—	
Right colon	21 / 55 (38%)	1.50	0.79, 2.80	0.208	1.12	0.53, 2.29	0.764
Time to metastases							
Metachronous	11 / 34 (32%)	—	—		—	—	
Synchronous	64 / 206 (31%)	0.94	0.44, 2.12	0.881	1.02	0.45, 2.45	0.962
Tumor nodal status							
Negative	6 / 23 (26%)	—	—				
Positive	20 / 63 (32%)	1.32	0.47, 4.10	0.614			
No surgery before registration	49 / 154 (32%)	1.32	0.51, 3.85	0.580			
Mutational status							
RAS & BRAF wildtype	33 / 122 (27%)	—	—		—	—	
RAS mutation	39 / 111 (35%)	1.46	0.84, 2.56	0.183	1.19	0.64, 2.22	0.574
BRAF mutation	3 / 7 (43%)	2.02	0.38, 9.65	0.373	1.67	0.28, 8.86	0.550
Serum CEA level, (ng/mL) - PTL	75 / 239 (31%)	1.00	1.00, 1.00	0.320	1.00	1.00, 1.00	0.340
Response - PTL							
Response	43 / 157 (27%)	—	—				
No response (PD/SD)	32 / 83 (39%)	1.66	0.94, 2.93	0.077			
Number of liver metastases - PTL	75 / 239 (31%)	1.08	1.03, 1.14	0.004	1.09	1.03, 1.15	0.003
Number of liver metastases - PTL, categorical[1]							
1-5	21 / 94 (22%)	—	—		—	—	
6-10	28 / 87 (32%)	1.65	0.85, 3.23	0.138	1.83	0.92, 3.70	0.089
>10	26 / 58 (45%)	2.82	1.40, 5.80	0.004	3.20	1.53, 6.87	0.002
Number of liver segments involved - PTL	75 / 240 (31%)	1.30	1.10, 1.53	0.002			
Diameter of largest metastasis - PTL	75 / 239 (31%)	1.00	0.99, 1.01	0.929	1.00	0.99, 1.01	0.811
Distribution of liver metastases - PTL							
Unilobar	5 / 29 (17%)	—	—				
Bilobar	70 / 211 (33%)	2.38	0.94, 7.31	0.090			

OR = Odds Ratio, CI = Confidence Interval, PTL = prior to local treatment.

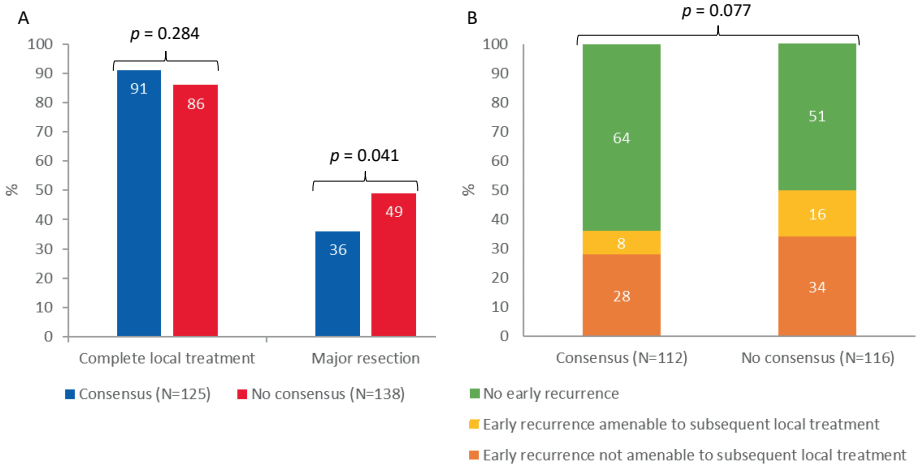
*The variables to include in the multivariable analyses were prespecified. Number of liver metastases was included in its original continuous form.

[1] A separate multivariable analysis was performed were the continuous number of liver metastases was replaced by the categorical variant.

Outcomes of local treatment according to consensus among panel surgeons

At the last panel evaluation prior to local treatment, patients with consensus among panel surgeons compared to no consensus with panel conclusion by majority vote, had a lower rate of major resections (45 [36%] vs. 68 [49%] patients, *p*=0.041) with no difference in complete local treatment rate between these groups (114 [91%] vs. 119 [86%], *p*=0.284). The incidence of no early recurrence, early recurrence with local treatment and early recurrence without local treatment was not statistically different between patients with and without consensus (**Figure 2**). The risk of early recurrence for patients with no panel consensus was increased at univariable analysis (crude OR 1.73 [95% CI 1.03-2.94], *p*=0.040), but not after adjusting for age, primary tumor site, time to metastases, *RAS/BRAF*^{V600E} mutation status, CEA, number and size of CRLM (adjusted OR 1.37 [95% CI 0.78-2.41], *p*=0.274).

Figure 2 Showing outcomes according to degree of consensus among panel surgeons in panel resectability assessments: **A**) short term resection outcomes in patients from 'surgical analysis', **B**) first recurrence outcomes in patients from 'RFS analysis'. All figures include only patients who received local treatment following the panel conclusion. Major liver resections were defined as resection of at least three segments or an (extended) hemihepatectomy.



Benefit of resectability assessments by the panel

In 263 patients who received local treatment following the panel advice, 50 (19%) patients were at least once assessed by an individual panel surgeon as having permanently unresectable CRLM. In 127 patients who were judged as having permanently unresectable CRLM by the panel and without local treatment, 14 (11%) patients were at least once assessed as having resectable CRLM by an individual panel

surgeon. Thus these patients would have potentially received local treatment if resectability was determined by an individual surgeon.

Discussion

In this study with patients with initially unresectable CRLM, age and number of CRLM but not tumor-biological factors were associated with early recurrence without the possibility of repeat local salvage treatment. Consensus among panel surgeons was present in less than half of the resectability assessments. This high inter-surgeon variability has been shown in previous retrospective surgical reviews, with reduced survival outcomes in patients in whom local treatment was considered feasible in retrospect.[16,30,31] Considering the absence of (a meaningful cut-off value of) predictive factors for consensus among panel surgeons and the selection of more patients for curative-intent local treatment, our data support the added value of evaluations by a panel rather than a single surgeon and suggest that panel evaluations should be offered to all patients with initially unresectable CRLM. Patients with consensus or no consensus among panel surgeons prior to local treatment had comparable postoperative outcomes when adjusted for other risk factors at multivariable analysis. This further supports the use of an expert panel.

Patients with CRLM are increasingly offered local treatment due to improved systemic and local treatments. The current study concerned patients with advanced CRLM and showed a rate of early recurrences without curative-intent repeat local treatment of over 30%. This warrants preoperative predictive factors to allow realistic patient expectations and to weigh individualized treatment decisions. As mentioned before, previous studies[8-10,12,22-25,32-34] suggested various factors to affect outcomes after local treatment of CRLM, but these retrospective studies are limited by inter-surgeon variability in assessing CRLM (un)resectability. Strengths of the current study are the prospectively selected cohort based on defined baseline unresectability criteria and that panel surgeons were blinded for tumor-biological factors such as *RAS/BRAF* mutation status, resulting in a predominantly technical-anatomical decision for resectability which reduced bias to a minimum.

RFS was shown to have a weak association with OS after resection of CRLM.[35] As such, an optimal surrogate endpoint for OS after local treatment of CRLM has yet to be defined. In this study we selected early recurrence without curative-intent repeat local treatment as a novel and clinically relevant endpoint in this patient group.

While older patients did not have a higher risk of early recurrence, age was a risk factor for early recurrence without repeat local treatment in this study. This could either be

caused by limitations in terms of technical abilities and/or patient physical condition or preference.

RAS/*BRAF*^{V600E} mutations were reported to be correlated with more invasive spread, higher risk of positive surgical margins, tumor regrowth after ablation and worse RFS and OS.[36] We found a very strong association of *BRAF*^{V600E} mutations, and to a lesser extent for *RAS* mutations, with a lower probability to convert to resectable disease. After systemic and subsequent local treatment, both *RAS* and *BRAF*^{V600E} mutations lost their predictive value for early recurrence. These outcomes are in line with results from a previous phase 3 study.[37] A potential explanation may lie in the careful selection of patients undergoing local treatment including the assessment of tumor biology during systemic treatment and controlling micrometastatic disease by preoperative systemic treatment which may result in counteracting the biological aggressiveness of the genetic mutation.[37]

We acknowledge that our study has limitations. Firstly, the number of variables tested for association with postoperative outcomes was limited by the number of events. Secondly, all patients underwent panel resectability assessments. However, to objectively assess the added value of a panel, the outcomes should be compared with a matched cohort without intercurrent resectability assessments by a panel. Lastly, since the number of patients with *BRAF*^{V600E} mutations undergoing local treatment is relatively small in this study, results should be interpreted with caution.

The lack of predictive tumor-biological factors as found by our study warrants further research on novel predictive factors, such as the consensus molecular subtypes (CMS), which are strongly related with prognosis and response on treatment in colorectal cancer[38], and specific oncogenic driver mutations such as *KRAS* A146, which is associated with larger tumor burden and worse outcome in patients with CRLM.[39] In addition, preoperative and postoperative sampling of liquid biopsies for circulating tumor DNA (ctDNA) are reported to have a strong association with pathologic response on preoperative systemic treatment and survival outcomes after local CRLM treatment.[40,41]

In conclusion, a higher age and number of CRLM but not tumor-biological factors were independently associated with early recurrence without repeat local treatment in patients who received local treatment of CRLM after systemic induction therapy. Outcomes of patients with consensus on resectability of CRLM among panel surgeons are similar to patients for whom no consensus was present and panel conclusion was formed by majority vote. As such, the use of a liver panel as opposed to the opinion of a single surgeon allows a better selection of patients who are eligible for local treatment. Thus far, with current clinically available tumor biomarkers, resectability assessment remain a technical-anatomical decision.

Author contributions

Study conception and design: RJS, CJAP, TvG
Acquisition of data: KB, MB, MA, AK, TC, CD, ME, MG, DG, JH, KdJ, GK, JK, NK, WL, ML, KvL, IQM, UN, GP, AR, TR, RJS, AM, CV, JdW, CJAP, TvG
Analysis and interpretation of data: KB, MB, AM, CJAP, RJS
Drafting of manuscript: KB, MB, AM, CJAP, RJS
Critical revision: all authors

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References

1. Adam R, De Gramont A, Figueras J, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17(10):1225-1239.
2. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27(8):1386-1422.
3. Imai K, Adam R, Baba H. How to increase the resectability of initially unresectable colorectal liver metastases: A surgical perspective. Ann Gastroenterol Surg. 2019;3(5):476-486.
4. Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020;141:225-238.
5. Tomasello G, Petrelli F, Ghidini M, Russo A, Passalacqua R, Barni S. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol J. 2017;3(7):e170278.
6. Mima K, Beppu T, Chikamoto A, et al. Hepatic resection combined with radiofrequency ablation for initially unresectable colorectal liver metastases after effective chemotherapy is a safe procedure with a low incidence of local recurrence. Int J Clin Oncol. 2013;18(5):847-855.
7. Angelsen JH, Viste A, Loes IM, et al. Predictive factors for time to recurrence, treatment and post-recurrence survival in patients with initially resected colorectal liver metastases. World J Surg Oncol. 2015;13:328.
8. Imai K, Allard MA, Benitez CC, et al. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21(7):887-94.
9. Vigano 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. 2014;21(4):1276-1286.
10. Vigano L, Gentile D, Galvanin J, et al. Very Early Recurrence After Liver Resection for Colorectal Metastases: Incidence, Risk Factors, and Prognostic Impact. J Gastrointest Surg. 2021;26(3):570-582.
11. Takahashi S, Konishi M, Kinoshita T, et al. Predictors for early recurrence after hepatectomy for initially unresectable colorectal liver metastasis. J Gastrointest Surg. 2013;17(5):939-948.
12. Engstrand J, Nilsson H, Stromberg C, Jonas E, Freedman J. Colorectal cancer liver metastases - a population-based study on incidence, management and survival. BMC Cancer. 2018;18(1):78.
13. Huiskens J, Bolhuis K, Engelbrecht MR, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229(6):523-532 e2.
14. Isoniemi H, Uutela A, Nordin A, et al. Centralized repeated resectability assessment of patients with colorectal liver metastases during first-line treatment: prospective study. Br J Surg. 2021;108(7):817-825.
15. Osterlund P, Salminen T, Soveri LM, et al. Repeated centralized multidisciplinary team assessment of resectability, clinical behavior, and outcomes in 1086 Finnish metastatic colorectal cancer patients (RAXO): A nationwide prospective intervention study. Lancet Reg Health Eur. 2021;3:100049.
16. Ignatavicius P, Oberkofler CE, Chapman WC, et al. Choices of Therapeutic Strategies for Colorectal Liver Metastases Among Expert Liver Surgeons: A Throw of the Dice? Ann Surg. 2020;272(5):715-722.
17. Huiskens J, Bolhuis K, Engelbrecht MR, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229(6):523-532.e2.
18. Hallet J, Sa Cunha A, Adam R, et al. Factors influencing recurrence following initial hepatectomy for colorectal liver metastases. Br J Surg. 2016;103(10):1366-1376.
19. Choti MA, Sitzmann JV, Tiburi MF, et al. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg. 2002;235(6):759-766.
20. 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):309-18; discussion 318-21.
21. D'Angelica M, Kornprat P, Gonen M, et al. Effect on outcome of recurrence patterns after hepatectomy for colorectal metastases. Ann Surg Oncol. 2011;18(4):1096-1103.
22. Margonis GA, Sasaki K, Gholami S, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105(9):1210-1220.

23. Buisman FE, Galjart B, Buettner S, Groot Koerkamp B, Grunhagen 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.
24. Kobayashi S, Takahashi S, Takahashi N, et al. Survival Outcomes of Resected BRAF V600E Mutant Colorectal Liver Metastases: A Multicenter Retrospective Cohort Study in Japan. Ann Surg Oncol. 2020;27(9):3307-3315.
25. Schirripa M, Bergamo F, Cremolini C, et al. BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. Br J Cancer. 2015;112(12):1921-1928.
26. Bolhuis K, Wensink GE, Elferink MAG, et al. External Validation of Two Established Clinical Risk Scores Predicting Outcome after Local Treatment of Colorectal Liver Metastases in a Nationwide Cohort. Cancers (Basel). 2022;14(10):2356/
27. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
28. Mekenkamp LJ, Koopman M, Teerenstra S, et al. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010;103(2): 159-164.
29. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247.
30. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11(1):38-47.
31. Modest DP, Denecke T, Pratschke J, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer. 2018;88:77-86.
32. Paredes AZ, Hyer JM, Tsilimigras DI, et al. A Novel Machine-Learning Approach to Predict Recurrence After Resection of Colorectal Liver Metastases. Ann Surg Oncol. 2020;27(13):5139-5147.;
33. Brudvik KW, Jones RP, Giuliante F, et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;269(1):120-126.
34. Yamashita Y, Adachi E, Toh Y, et al. Risk factors for early recurrence after curative hepatectomy for colorectal liver metastases. Surg Today. 2011;41(4):526-532.
35. Ecker BL, Lee J, Saadat LV, et al. Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. Lancet Oncol. 2022;23(10):1332-1342.
36. Yamashita S, Chun YS, Kopetz SE, Vauthey JN. Biomarkers in colorectal liver metastases. Br J Surg. 2018;105(6):618-627.
37. Cremolini C, Casagrande M, Loupakis F, et al. Efficacy of FOLFOXIRI plus bevacizumab in liver-limited metastatic colorectal cancer: A pooled analysis of clinical studies by Gruppo Oncologico del Nord Ovest. Eur J Cancer. 2017;73:74-84.
38. Ten Hoorn S, de Back TR, Sommeijer DW, Vermeulen L. Clinical Value of Consensus Molecular Subtypes in Colorectal Cancer: A Systematic Review and Meta-Analysis. J Natl Cancer Inst. 2022;114(4):503-516.
39. van 't Erve I, Wesdorp NJ, Medina JE, et al. KRAS A146 Mutations Are Associated With Distinct Clinical Behavior in Patients With Colorectal Liver Metastases. JCO Precis Oncol. 2021;5:PO.21.00223.
40. Bidard FC, Kiavue N, Ychou M, et al. Circulating Tumor Cells and Circulating Tumor DNA Detection in Potentially Resectable Metastatic Colorectal Cancer: A Prospective Ancillary Study to the Unicancer Prodige-14 Trial. Cells. 2019;8(6):516.
41. 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. 2021;70:103498.

CHAPTER 6

External validation of two established clinical risk scores predicting outcome after local treatment of colorectal liver metastases in a nationwide cohort

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Summary

Patients with colorectal liver metastases (CRLM) are able to achieve long-term survival when they receive local treatment of CRLM (resection or tumor ablation). Existing clinical risk scores (CRSs) predicting prognosis of patients after resection of colorectal liver metastases were developed in highly specialized centers and thus may not function in the general population. We validated the Fong and GAME CRSs in a large population-based cohort, including two important subgroups: young/elderly and with/without perioperative chemotherapy. Both CRSs showed predictive ability. However, they were not able to discriminate preoperative risk sufficiently for clinical decision-making and, thus, require improvement.

Abstract

Optimized surgical techniques and systemic therapy have increased the number of patients with colorectal liver metastases (CRLM) eligible for local treatment. To increase postoperative survival, we need to stratify patients to customize therapy. Most clinical risk scores (CRSs) which predict prognosis after CRLM resection were based on the outcome of studies in specialized centers, and this may hamper the generalizability of these CRSs in unselected populations and underrepresented subgroups. We aimed to externally validate two CRSs in a population-based cohort of patients with CRLM. A total of 1105 patients with local treatment of CRLM, diagnosed in 2015/2016, were included from a nationwide population-based database. Survival outcomes were analyzed. The Fong and more recently developed GAME CRS were externally validated, including in pre-specified subgroups (≤70/>70 years and with/without perioperative systemic therapy). The three-year DFS was 22.8%, and the median OS in the GAME risk groups (high/moderate/low) was 32.4, 46.7, and 68.1 months, respectively (p<0.005). The median OS for patients with versus without perioperative therapy was 47.6 (95%CI [39.8, 56.2]) and 54.9 months (95%CI [48.8, 63.7]), respectively (p=0.152), and for below/above 70 years, it was 54.9 (95%CI [49.3-64.1]) and 44.2 months (95%CI [37.1-54.3]), respectively (p<0.005). The discriminative ability for OS of Fong CRS was 0.577 (95%CI [0.554, 0.601]), and for GAME, it was 0.596 (95%CI [0.572, 0.621]), and was comparable in the subgroups. In conclusion, both CRSs showed predictive ability in a population-based cohort and in predefined subgroups. However, the limited discriminative ability of these CRSs results in insufficient preoperative risk stratification for clinical decision-making.

Introduction

Approximately 30% of patients with colorectal cancer (CRC) develop liver metastases (CRLM).[1] Currently, local treatment of CRLM (e.g., resection or tumor ablation) offers the only chance for long-term survival, with 5-year overall survival (OS) rates of up to 55%.[2-4] Surgical techniques continue to evolve, with two-stage resections including associating liver partition and portal vein ligation for staged hepatectomy (ALPPS); and laparoscopic liver resections, including minor/major resections, robotic hepatectomy, anatomic resections, parenchymal sparing strategies, and minimally invasive procedures for simultaneous resections of liver metastases and primary CRC.[5,6] Improved surgical procedures, more lenient resection criteria, and optimization of induction systemic therapy have increased the number of patients with CRLM that are considered technically resectable.[7,8] However, relapse after liver resection occurs in up to 75% of patients[9-11], and a subgroup of patients have no long-term OS benefit, due to aggressive tumor biology. This underscores the urgent need to improve risk-stratification prior to surgery.[12]

An ideal clinical risk score (CRS) for these patients should identify patients with a high risk of early recurrence after surgery in order to prevent major surgery with associated risk of perioperative morbidity and mortality. Among earlier CRSs for patients with CRLM[2,13,14], the Fong score—developed in 1999[15]—is still used most frequently to predict prognosis after liver resection.[16] The Fong CRS incorporated lymph node status, CEA value, disease-free interval (DFI), and size and number of liver metastases.[15] However, essential validation efforts of these earlier CRSs are scarce[17-19], especially in populations receiving modern systemic therapies, improved surgical, and ablative treatment options.[2,13-15]

Novel CRSs[16,20-24] have been proposed with their own strengths and limitations, including the modified clinical score (m-CS)[20], Liverpool score[23], comprehensive evaluation of relapse risk score (CERR)[22], alternative clinical score (a-CS)[24], and the Genetic And Morphological Evaluation (GAME) score.[16] The GAME score incorporates recalibrated tumor markers such as *KRAS* mutational status, extrahepatic disease presence, and Tumor Burden Score (TBS). The TBS is suggested to better correlate with OS compared to separate information on the number and size of metastases.[25] The GAME score outperformed the Fong score in two single-institution patient cohorts but lacks external validation in more unselected patient cohorts.

Overall, the generalizability of these CRSs to routine care remains questionable. The scores were developed in single and/or specialized liver centers and validated in other specialized centers, potentially not reflecting results in a general population of patients with CRLM.[19,26] Furthermore, important subgroups were underrepresented in the

development and validation cohorts such as elderly patients, who represent 50% of the CRC population and who are increasingly offered local liver treatment, as long-term survival can also be achieved in these patients undergoing resection of CRLM.[27-29] Lastly, geographical differences in treatment guidelines might influence cohort characteristics and, therefore, risk score performance. For example, the GAME score was developed and validated in the United States of America, with the majority of patients receiving perioperative systemic therapy according to local guidelines[30], while other guidelines do not recommend standard (neo)adjuvant systemic therapy.[31,32]

The aim of this study was to evaluate the generalizability and clinical validity of two CRSs, the widely used Fong score and the more recent GAME score, in a nationwide population-based cohort of patients after local treatment of CRLM. Furthermore, we validated both CRSs in two pre-specified subgroups: with/without modern perioperative systemic therapy and age below/above 70 years.

Materials and methods

Population-based cohort

All patients initially diagnosed with CRC between 1 January 2015 and 31 December 2016 and who underwent local treatment (resection and/or local ablation) for CRLM were identified in the Netherlands Cancer Registry (NCR). The NCR is a population-based registry with clinical data of all newly diagnosed cancer patients in the Netherlands, based on notification of newly diagnosed malignancies in the Netherlands by the national automated pathological archive (PALGA[33]) or national registry of hospital discharge. PALGA comprises all patients with histologically confirmed cancer in the Netherlands. Patients with extrahepatic metastases before resection, R2 liver resections, appendix carcinoma, concomitant local liver treatments other than resection or ablation, and inadequate follow-up information were excluded. The research protocol and use of this data was approved by the Netherlands Comprehensive Cancer Organisation (IKNL). Written informed consent was not applicable according to national legislation. The study was performed in accordance with the Declaration of Helsinki.

Clinical data

Pseudonymized clinical data were retrieved from the NCR and PALGA, including age, sex, American Joint Committee on Cancer (AJCC) tumor status (T-status), nodal status (N-status; N0, N1, and N2), location of primary tumor (left, right, rectum), DFI between detection of primary tumor and metastases, size and number of metastases, serum carcinoembryonic antigen (CEA) level (ug/L) prior to liver resection, type of local treatment, resection margin status (R0 was defined as a microscopically tumor free

surgical margin), and *RAS*/*BRAFV600E* mutational status. TBS[25] was calculated. A major resection was defined as resection of ≥4 liver segments[34], synchronous disease as a DFI of ≤6 months[35], and perioperative systemic therapy as any systemic therapy administered within 100 days before and/or after local treatment of CRLM and initiated prior to progression of disease after resection. No distinction could be made between neo-adjuvant or induction systemic therapy in the NCR data, because intention of treatment was not registered. However, the Dutch guidelines for CRC[31] recommend not to administer perioperative systemic therapy in initially resectable CRLM contrary to the NCCN guidelines.[30] Thus, patients who have received preoperative systemic treatment are assumed to have undergone induction treatment for initially unresectable or potentially resectable CRLM. All assumptions regarding systemic treatment can be found in **Supplementary Table S1**.

Overall survival and disease-free survival

Follow-up data for recurrences were collected from medical records by trained data managers from the IKNL until May 2020, and vital status was obtained by linkage with the municipal population registry on 31 January 2021. OS was defined as the date of first CRLM resection/ablation till the date of vital status. Disease-free survival (DFS) was defined as the date of first CRLM resection/ablation till date of a DFS event, which was defined as recurrence of disease or death, whichever occurred first, or censored on last date of DFS. If the follow-up for recurrences was shorter than the follow-up for vital status, all vital status follow-up beyond the last follow-up for recurrences was discarded for assessment of DFS. All survival assumptions are included in **Supplementary Table S1**.

RAS and *BRAFV600E* mutational status

Tumor *KRAS* (codons 12, 13, 61, 117, and 146), *NRAS* (codons 12, 13, and 61) and *BRAF V600E* mutational status, as ascertained during routine clinical care, were retrieved from the NCR and PALGA.[33] As mutational status is generally only determined clinically if there is an indication for (palliative) systemic treatment, this information was not available for all patients. To further complement the *RAS/BRAFV600E* mutational status of the cohort, we aimed to sequence >170 available tumor tissues (the first 171 available of 250 requested) by Sequenom Massarray.[36] We specifically selected these 250 patients, as they had the lowest predicted chance of having a clinically assessed mutational status according to their clinicopathological profile (based on a logistic regression propensity score for mutational status with 16 clinicopathological variables). We used this strategy to improve the chance of successful multiple imputation and of accommodating the missing at random assumption (see below).

Statistical analysis and handling of missing data

The study population was described using standard descriptive statistics, overall, according to systemic treatment, and according to age, using median values and interquartile interval (IQI) for continuous variables and frequencies and percentages for categorical variables. Differences between systemic treatment and age groups were statistically tested by the Mann–Whitney U test or the Fisher's Exact Test. All reported *p*-values are two-sided and $p<0.05$ was considered statistically significant.

To handle missing data in the context of survival analysis, we performed multiple imputation by using a substantive model compatible fully conditional specification (SMC-FCS) approach[37], assuming missingness at random. The substantive model was a Cox proportional hazards model for OS which contained the following variables: T-status, N-status, *KRAS* mutational status, number and size of liver metastases, CEA, systemic perioperative treatment type, sidedness of the primary tumor, age, DFI, R-status, GAME CRS, Fong CRS, and TBS (with the last 3 being passively imputed in the model). We generated 53 imputed datasets based on the percentage of patients with at least one missing key variable.

Kaplan–Meier (KM) curves were created for OS and DFS. Using the multiple imputed dataset, pooled statistics were obtained by using Rubin's rules, including number at risk for given time points, log-rank subgroup comparison, and survival estimates with confidence intervals (using log–log transformation prior to pooling for the latter two).[38,39]

External validation of CRSs

The GAME[16] and Fong score[15] were externally validated following the TRIPOD guidelines sections pertinent to external validation studies [40]. Predictive performances were assessed by measures of calibration and discrimination. Calibration was evaluated by digitizing the originally published KM curves of scores by WebPlotDigitizer version 4.4[41] and plotted together with the observed KM curves of the NCR cohort. Discrimination was calculated by Harrell's concordance index (C-index) across each imputed dataset and pooled by using Rubin's rules. The C-index reflects the ability of the model to differentiate between patients who do and do not experience an event, with 0.5 representing a model without any discriminatory ability beyond chance and 1 perfect discrimination.[42]

Patients were assigned to low, moderate, or high CRS risk categories, as described previously[16]: low risk, 0–1 points; moderate risk, 2–3 points, and high risk, 4 or more points, with similar allocation for the GAME and Fong CRS points.

To analyze the overlap in risk groups following the two CRSs, a contingency table and heatmap were made. External validation was repeated for the following subgroups: perioperative systemic therapy (yes/no) and age (≤70/>70 years). An analysis was performed in IBM SPSS Statistics (Version 26) and R (Version 4.0.3 for Windows) with the mice (3.13.0), smcfcs (1.5.0), survival (3.2-7), and rms (6.2-0) packages.

Results

Patient characteristics

A total of 1105 patients fulfilled the eligibility criteria (1105/1489) (**Figure 1**). The cohort comprised 447 (40%) patients with and 658 (60%) patients without perioperative systemic therapy and 759 (69%) patients ≤70 and 346 (31%) patients >70 years. Among patients with perioperative systemic treatment, 334 (75%) received preoperative-only, 54 (12%) postoperative-only, and 59 (13%) received pre- and postoperative systemic treatment. The patient characteristics are displayed in **Table 1**. The median age of patients was 66 years, with 690 (62%) males, and 823 (75%) patients had synchronous disease. (**Table 1**). Patients were treated in a total of 39 hospitals, with 45% of patients treated in academic, 44% in teaching, and 11% in regional hospitals.

Figure 1 Flowchart of population-based NCR patients with local liver treatment for CRLM included in the study. Abbreviations: ACUP, adenocarcinoma with unknown primary; CRLM, colorectal liver metastases; mCRC, metastatic colorectal cancer; RFS, recurrence-free survival.

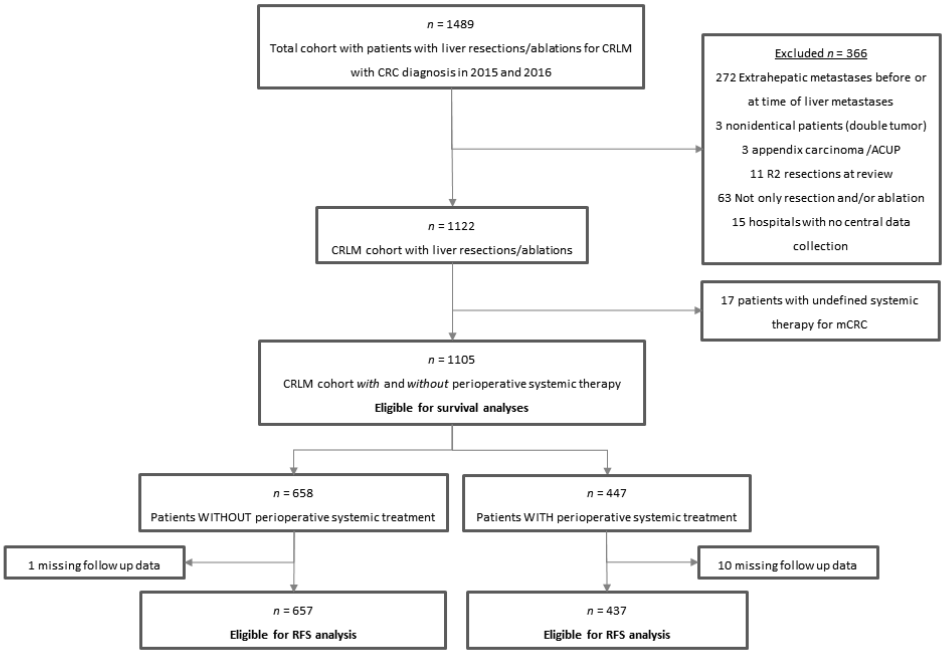


Table 1 Characteristics of total NCR cohort and patients with and without systemic therapy and below or above 70 years.

	NCR Cohort (n=1105)	Patients without Systemic Therapy (*n*=658)	Patients with Systemic Therapy (*n*=447)	*p*-Value	Patients ≤70 Years (*n*=759)	Patients >70 Years (*n*=346)	*p*-Value
Age Median (IQI)	66 (59-72)	68 (61-74)	63 (56-70)	<0.001	62 (56-66)	75 (72-78)	<0.001
Sex				0.60			<0.008
Male	690 (62)	415 (63)	275 (62)		454 (60)	236 (68)	
Female	415 (38)	243 (37)	172 (39)		305 (40)	110 (32)	
Side primary tumor				0.92			0.09
Right	261 (24)	154 (23)	107 (24)		167 (22)	94 (27)	
Left	473 (43)	285 (43)	188 (42)		324 (43)	149 (43)	
Rectum	371 (34)	219 (33)	152 (34)		268 (35)	103 (30)	
Chemoradiotherapy primary tumor				<0.001			0.67
No	977 (88)	556 (85)	421 (94)		669 (88)	308 (89)	
Yes	128 (12)	102 (15)	26 (6)		90 (12)	38 (11)	
T-status primary tumor				0.01			0.97
1	27 (3)	21 (3)	6 (1)		19 (3)	8 (2)	
2	128 (12)	86 (13)	42 (10)		89 (12)	39 (11)	
3	757 (69)	455 (69)	302 (69)		516 (69)	241 (70)	
4	185 (17)	96 (15)	89 (20)		129 (17)	56 (16)	
Missing	8 (-)	0 (-)	8 (-)		6 (-)	2 (-)	
Nodal status primary tumor				0.22			0.71
N0	408 (37)	257 (39)	151 (34)		277 (37)	131 (38)	
N1	389 (35)	224 (34)	165 (37)		265 (35)	124 (36)	
N2	306(28)	177 (27)	129 (29)		216 (29)	90 (26)	
Missing	2 (-)	0 (-)	2 (-)		1 (-)	1 (-)	
Stage of disease at diagnosis				<0.001			0.40
I	25 (2)	20 (3)	5 (1)		16 (2)	9 (3)	
II	102 (9)	87 (13)	15 (3)		65 (9)	37 (11)	
III	187 (17)	162 (25)	25 (6)		123 (16)	64 (19)	
IV	791 (72)	389 (59)	402 (90)		555 (73)	236 (68)	
Differentiation grade of CRC				0.12			0.77
Low	17 (2)	7 (1)	10 (3)		13 (2)	4 (1)	
Intermediate	936 (92)	577 (93)	359 (90)		642 (92)	294 (92)	
High	68 (7)	37 (6)	31 (8)		46 (7)	22 (7)	
Missing	84 (-)	37 (-)	47 (11)		58 (-)	26 (-)	
Time to metastases				<0.001			0.20
Synchronous	823 (75)	412 (63)	411 (92)		574 (76)	249 (72)	
Metachronous	282 (25)	246 (37)	36 (8)		185 (24)	97 (28)	
Number of liver metastases				<0.001			<0.001
Median (IQI)	2 (1-4)	1 (1-3)	3 (2-6)		2 (1-4)	2 (1-3)	
Missing	42	19	23		33	9	
CEA level				<0.001			0.90
Median (IQI)	9 (3.4–36)	6.3 (3.0–21)	14 (4.4–74)		18 (4.0-413)	17 (4.7-168)	
Unknown	231	180	51		160	71	

Table 1 (continued)

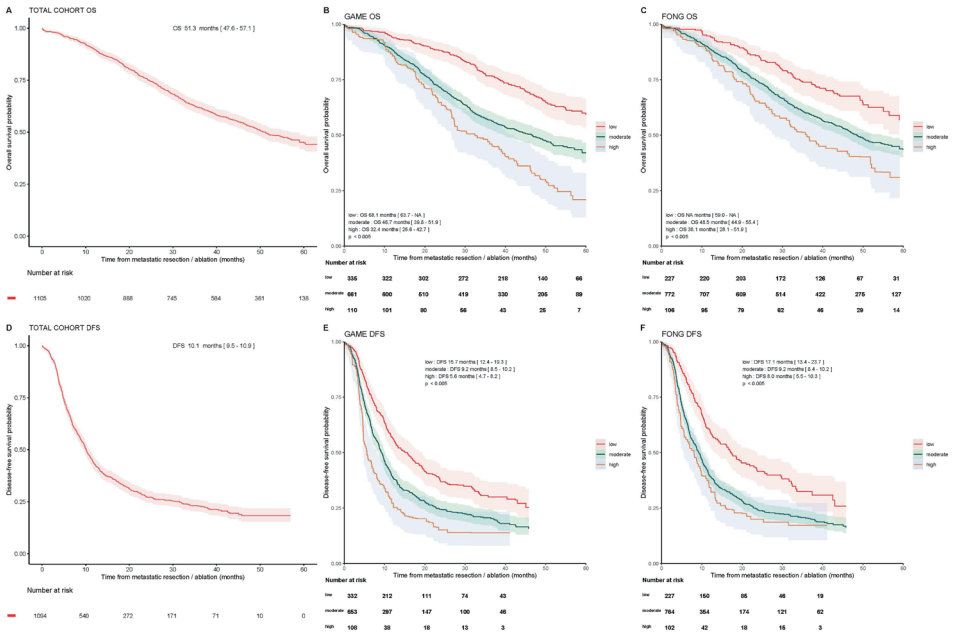
	NCR Cohort (n=1105)	Patients without Systemic Therapy (*n*=658)	Patients with Systemic Therapy (*n*=447)	*p*-Value	Patients ≤70 Years (*n*=759)	Patients >70 Years (*n*=346)	*p*-Value
Size largest liver metastasis, mm				0.002			0.30
Median (IQI)	25 (16-36)	23 (16-35)	27 (16-45)		25 (15-45)	26 (18-42)	
Missing	86	45	41		58	28	
Type of surgery				<0.001			0.34
Wedge/segment resection only	589 (53)	416 (63)	173 (39)		400 (53)	189 (55)	
Local ablative therapy only	95 (9)	63 (9)	32 (7)		59 (8)	36 (10)	
Wedge/segment and local ablative therapy	189 (17)	90 (14)	99 (22)		134 (18)	55 (16)	
Hemihepatectomy with/without ablation/wedge (major resection)	232 (21)	89 (14)	143 (32)		166 (22)	66 (19)	
One- or two-stage				<0.001			0.84
1-stage	1042 (94)	643 (98)	399 (89)		715 (94)	327 (95)	
2-stage	63 (6)	15 (2)	48 (11)		44 (6)	19 (6)	
R-status				0.07			
R0	866 (78)	521 (79)	345 (77)				0.37
R1	143 (13)	74 (11)	69 (15)		598 (79)	268 (78)	
Unknown because RFA/MWA	96 (9)	63 (10)	33 (7)		101 (13)	42 (12)	
Tumor mutational status				0.36			0.93
RAS mutation	362 (51)	221 (53)	141 (48)		247 (50)	115 (52)	
BRAF^{V600E} mutation	19 (3)	10 (2)	9 (3)		13 (3)	6 (3)	
RAS and BRAF^{V600E} wt	335 (47)	188 (45)	147 (50)		233 (47)	102 (46)	
Missing (*RAS* and/or *BRAF* status)	389 (-)	239 (-)	150 (-)		266 (-)	123 (-)	

Abbreviations: CEA, Carcinoembryonic Antigen; CRC, colorectal cancer; IQI, Interquartile range; NCR, Netherlands cancer registry; RFA, radiofrequency ablaction; MWA, microwave ablation

Follow-up and OS and DFS outcomes in total cohort

The median follow-up for OS and DFS was 53.7 and 35.0 months, with 556 (50%) and 807 (73%) documented events, respectively. The median OS was 51.3 months (95%CI [47.6, 57.1]), and the median DFS was 10.1 months (95%CI [9.5, 10.9], **Figure 2**). One-, three-, and five-year OS rates were 89.9% (95%CI [88.2, 91.7]), 61.8% (95%CI [59.0, 64.8]), and 44.9% (95%CI [41.6, 48.4]), whereas the one- and three-year DFS rates were 43.1% (95%CI [40.2, 46.1]) and 22.8% (95%CI [20.2, 25.8]).

Figure 2 Overall survival and disease-free survival in cohort and subgroups. Kaplan–Meier analysis showing OS and DFS curves and 95% confidence intervals of the total cohort and for the risk categories following the GAME and Fong scores. OS for total cohort (**A**), and OS for GAME CRS risk groups (**B**), OS for Fong CRS risk groups (**C**). DFS for total cohort (**D**), DFS for GAME CRS risk categories (**E**), and DFS for Fong CRS risk categories (**F**).



External validation of GAME and Fong CRSs in total cohort

The study characteristics of the development cohorts of the GAME and Fong CRSs were compared to the NCR validation cohort (**Table 2**). The percentage of patients with adjuvant systemic therapy was 71% in the GAME cohort compared to 6% in our NCR cohort; the percentage was not reported for the Fong cohort. In the development cohort of GAME CRS, patients with extrahepatic disease were included, while these patients were excluded in the Fong cohort and the NCR cohort.

The OS and DFS of the high, moderate, and low GAME and Fong risk groups are presented in **Figure 2**. The OS and DFS gradually decrease per point increase for both the GAME and Fong score (**Supplementary Figure S1**).

Table 2 Characteristics of original Fong and GAME CRS cohorts compared to Dutch NCR cohort used for external validation.

	GAME	Fong	NCR
Number of patients (design/validation)	502/747	1001/-	-/1105
Country (design/validation)	USA/USA	USA/-	-/Dutch
Study design	Single center	Single center	Nation-wide multicenter
Patients with liver-only metastases, %	90	100	100
Handling of missing data	Patients excluded with *KRAS* status missing	NR	No patients excluded based on missing data
Available mutation status	*KRAS* codon 12, 13, and 61	-	*RAS/BRAF*
Primary endpoint	OS	OS	OS
Preoperative systemic therapy, %	67	NR	55
Adjuvant systemic therapy, %	71	NR	6
DFI <12 months, %	74	49	84
Factors included in CRS, (points)	Nodal status (1) CEA >20 (1) TBS <9 (1) TBS ≤9 (2) *KRAS* mutation (1) Extrahepatic disease (2)	Nodal status (1) CEA >200 (1) DFI <1year (1) >1 Liver tumor (1) Largest tumor >5 cm (1)	-

Abbreviations: CEA, carcinoembryonic antigen; cm, centimeters; CRS, clinical risk score; DFI, disease-free interval; GAME, genetic and morphological evaluation score; NCR, Netherlands cancer registry; NR, not reported; OS, overall survival; TBS, tumor burden score; USA, United States of America.

By analyzing the calibration of the CRSs, we see that the original survival curves of low- and high-risk GAME groups overlapped well with the corresponding curves in our validation cohort. The GAME moderate-risk group, however, showed a shorter median OS compared to the development cohort, 46.7 versus 60 months (**Supplementary Figure S2**).

Overall, the discriminative ability of the GAME versus the Fong score, as measured by the Harrell's C-index for OS, was weak, 0.596 (95%CI [0.572, 0.621]) versus 0.577 (95%CI [0.554, 0.601]), respectively. The C-indexes of OS and DFS and the pooled survival estimates per risk group and per given time-point are depicted in **Table 3**.

In a head-to-head comparison of the GAME and Fong CRSs, 730 patients (66.0%) were categorized in the same risk group in both prediction models. Only three patients (0.3%) showed major discordance (categorized as GAME high risk and Fong low risk). The frequency distributions among the Fong/GAME combination risk categories and corresponding survival curves are shown in **Supplementary Figure S3**.

Table 3 Pooled Harrell's concordance index with 95% confidence intervals for 1-, 3-, and 5-year overall survival and disease-free survival outcomes for GAME and Fong risk scores and survival estimates at 1-, 3-, and 5 years for low-, moderate-, and high-risk groups according to GAME and Fong prediction model.

	GAME Score	Survival Estimates GAME Risk Categories			Fong Score	Survival Estimates Fong Risk Categories		
	C-Index [95% CI]	Low (%)	Moderate (%)	High (%)	C-Index [95% CI]	Low (%)	Moderate (%)	High (%)
OS								
1-yr	0.583 [0.531–0.636]	94	88	86	0.570[0.521–0.619]	95	89	87
3-yr	0.600 [0.573–0.627]	77	57	47	0.578 [0.552–0.604]	74	60	50
5-yr	0.597 [0.573–0.621]	50	42	21	0.577 [0.554–0.601]	57	40	31
DFS								
1-yr	0.585 [0.561–0.608]	57	39	27	0.586 [0.564–0.608]	60	39	34
3-yr	0.579 [0.557–0.600]	30	21	14	0.581 [0.561–0.602]	32	20	17

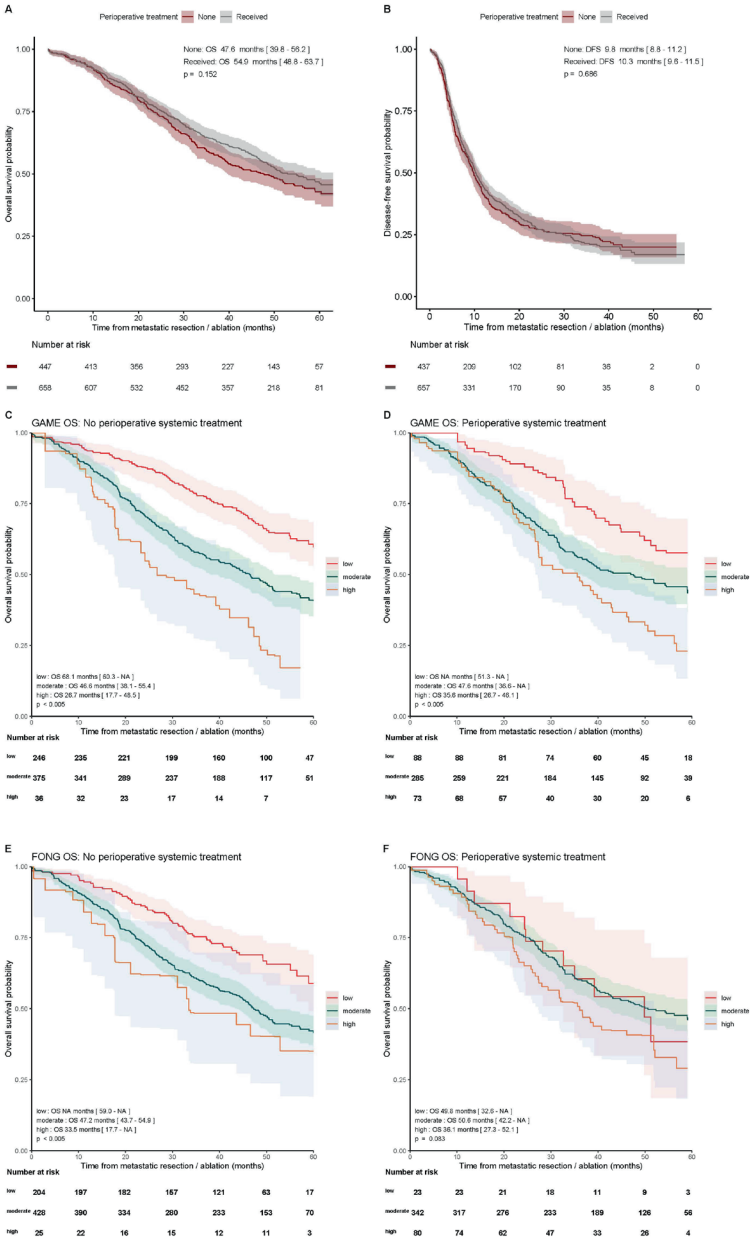
Abbreviations; C-index, concordance index; OS, overall survival; yr, year; DFS, disease-free survival.

External validation of GAME and Fong CRSs in pre-specified subgroups

With and without perioperative systemic therapy

Although prognostic patient characteristics were unfavorable for patients with perioperative systemic therapy (**Table 1**), comparable survival outcomes were found in patients with and without perioperative systemic treatment, with a median OS of 47.6 (95%CI [39.8, 56.2]) and 54.9 months (95%CI [48.8, 63.7]; *p*=0.152) and median DFS of 9.8 (95%CI [8.8, 11.2]) versus 10.3 months (95%CI [9.6, 11.5]; *p*=0.686), respectively. GAME high-risk patients with perioperative systemic therapy had a longer median OS of 35.6 (95%CI [26.7, 46.1]) compared to patients without systemic therapy (median OS 26.7 months, 95%CI [17.7, 48.5]) (**Figure 3**) and a longer median DFS of 5.9 months (95%CI [4.8, 10.9]) versus 4.6 months (95%CI [3.9, 10.0]) (**Supplementary Figure S4**). A survival advantage for patients receiving perioperative systemic therapy was not evident in the low- and moderate-risk groups (Supplementary Figure S4). The GAME C-index for patients with and without peri-operative systemic therapy for OS was 0.590 (95%CI [0.554, 0.626]) versus 0.602 (95%CI [0.569, 0.635]), and the Fong C-index was 0.556 (95%CI [0.519, 0.594]) versus 0.593 (95%CI [0.563, 0.624]), respectively (**Supplementary Table S2**).

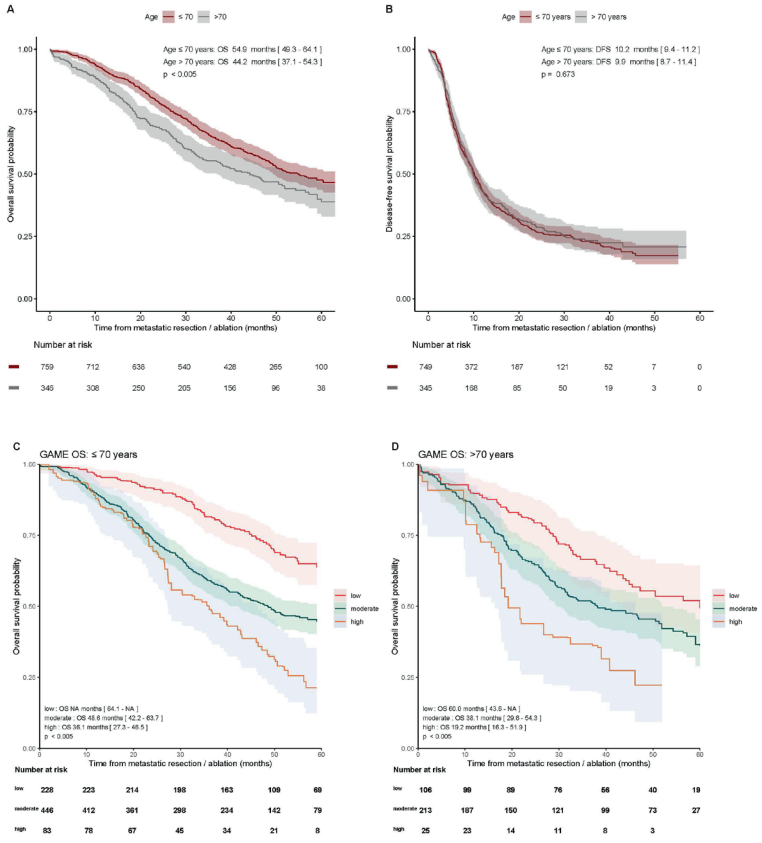
Figure 3 Kaplan–Meier analysis showing OS and DFS curves in patients with and without perioperative systemic therapy for the GAME and Fong risk categories. (**A**) OS and (**B**) DFS in patients with and without perioperative systemic therapy. OS outcomes of the GAME risk categories were analyzed in the subgroup without (**C**) and with perioperative systemic therapy (**D**) and OS outcomes of the Fong risk categories in subgroups of patients without (**E**) and with perioperative systemic therapy (**F**).

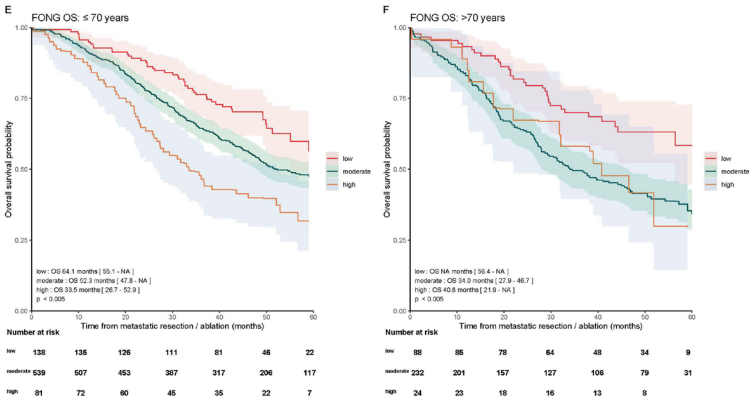


Age ≤70 years and >70 years

The median OS of 54.9 months (95 % CI [49.3-64.1]) was higher in patients ≤70 years compared to 44.2 months (95%CI [37.1-4.3) in patients >70 years (p<0.005). The median DFS was similar for 10.2 months (95%CI [9.4, 11.2]) versus 9.9 months (95%CI [8.7, 11.4]; p=0.673) (**Figure 4**). The discriminative ability for OS of GAME CRS and Fong CRS was comparable in both age groups, with GAME C-indexes of 0.613 (95%CI [0.584, 0.642]) and 0.575 (95%CI [0.531, 0.618]) and Fong C-indexes of 0.583 (95%CI [0.554, 0.612]) and 0.589 (95%CI [0.548, 0.630]), respectively, for below/above 70 years. The C-indexes for one-, three-, and five-year OS and DFS of GAME versus Fong in predefined subgroups are shown in **Supplementary Table S3**.

Figure 4 Kaplan–Meier analysis showing OS and DFS curves in patients with age ≤70 years and >70 years for the GAME and Fong risk categories. (**A**) OS and (**B**) DFS in patients with age ≤70 years and >70 years. Subsequently, the OS outcomes of the GAME risk categories were analyzed in the subgroup ≤70 years (**C**) and >70 years (**D**) and of the Fong risk categories in subgroups of patients ≤70 years (**E**) and >70 years (**F**).





Discussion

In this study, we externally validated and compared two established CRSs, the GAME and Fong score, for their ability to predict OS and DFS after resection of CRLM in the modern era in a real-life population-based cohort and in two pre-specified subgroups. Both CRSs showed predictive ability with a better performance of the GAME as compared to the traditional Fong CRS. The external validation in subgroups of both CRSs showed a comparable performance in patients with and without perioperative systemic therapy and in patients ≤70 and >70 years. However, the overall predictive performance remained suboptimal, with a high prognostic uncertainty which limits its utility in clinical decision-making.

The GAME score was originally validated in a cohort of patients from specialized institutes, while the Fong score was not validated in the original paper. This could hamper their generalizability to real-life patients. In our real-life cohort, we found a similar C-index for the GAME and Fong score for OS as compared to the C-indexes published by Margonis et al..[16] In our cohort, the GAME score outperformed the traditional Fong score. Both CRSs show discriminatory ability, but since C-indexes are 0.6 at most, a significant level of prognostic uncertainty remains. Furthermore, 25% of patients identified as “high-risk” according to the GAME score did achieve long-term survival, which exceeded five years, and this rate was even higher in the Fong high-risk group. This signifies that, although these CRSs might be used for risk counselling and managing expectations of patients, they cannot be used for clinical decision-making to select high-risk patients for whom surgery should be avoided or low-risk patient for whom extensive surgery may be justified.

To improve the prognostic performance of a CRS, categorizing variables should be avoided, and simplification of the CRS by a point system or classification in risk groups is not always desirable. While this strategy is performed to gain usability, it also results in the loss of information. One way to ensure model usability, while avoiding simplification, is to use a web calculator, along with a prediction model, which could be incorporated into electronic patient management systems for clinicians and patients.[24]

Evolving molecular research results in newly recognized tumor biomolecular prognostic markers and shows the heterogeneity of CRLM. The GAME CRS incorporated *KRAS* codon 12, 13, and 61 only. However, BRAFV600E mutation is recognized to be a strong prognostic factor, as well which negatively influences post-resection survival outcomes. Other molecular markers are proposed as prognostic markers too, such as mutations in the SMAD family, TP53, and PIK3CA. In future practice, by incorporating novel biomarkers and integrating molecular subtypes, clinical risk stratification may be improved.[43]
Other recently published CRSs were not externally validated on our cohort for various reasons. The m-CS[20] simplified the traditional CRS and replaced two risk factors by *RAS* mutational status, and the Liverpool score[23] did not incorporate *RAS* mutation status in its CRS, which is recognized to be the most promising prognostic factor in patients with CRLM.[44-46] The Chinese CERR[22] included two variables (serum CA 19.9 and bilobar liver distribution of metastatic disease) which were not available in our cohort. For the a-CS[24], discrepancies in the published survival outcomes and the web-based calculation tool of the a-CS complicate external validation.

When comparing the OS of our population-based cohort with the original GAME cohort, we found a lower median OS in our GAME moderate-risk group. Survival was similar in the GAME low- and high-risk groups. The difference in the moderate-risk group could potentially be influenced by treatment setting. The GAME cohort concerned a selected population treated in a tertiary center with potentially more (experimental) treatment options available, in contrast to our population-based cohort. We did not observe a survival difference between the moderate-risk groups in the subgroups with or without systemic treatment. Therefore, it is unlikely that the greater proportion of systemic therapy administered to the GAME cohort explains the survival differences in the moderate-risk group in our cohort versus the GAME cohort.

Furthermore, as our cohort consists of patients with and without perioperative systemic therapy, we could demonstrate additional interesting survival outcomes. Patients who received perioperative systemic therapy were found to have more prognostic unfavorable characteristics, while the median OS was similar in patients with and without perioperative systemic therapy. This could imply that, in these patients, systemic therapy compensates for the more unfavorable characteristics. This is

supported by the findings that patients in the high-risk CRS groups showed a longer median OS and DFS in the subgroup with versus without systemic therapy. Our results are consistent with studies suggesting that high-risk patients with CRLM could benefit from (neo)-adjuvant therapy[9,47-49] and is supported by the negative results of the EORTC 40983[50] study and the JCOG0603 study[51] for perioperative systemic treatment in, respectively, patients with low-risk disease with <4 CRLM and unselected patients with CRLM. Since the results of the treatment groups are based on retrospective data, this should be confirmed in prospective trials, randomizing high-risk CRLM patients between (neo-)adjuvant therapy or not. However, conducting a study such as this one has proved to be challenging.[52]

Another interesting finding is the OS difference in favor of patients ≤70 compared to >70 years after resection. Since it did not concern disease-specific but overall survival, other factors, such as comorbidity, might have influenced the OS in this group. This is supported by the result that DFS did not differ between these two subgroups. This OS difference should therefore not be used as an argument against liver resection in patients above 70 years.

The external validation of the CRSs in this study met the TRIPOD guidelines' methodological criteria [40]. Additional strengths include validation of CRS in a real-life population-based cohort which is representative of the whole CRLM population and the near-complete follow-up. Furthermore, the proportion of missing *RAS*/*BRAF* mutational status was low, and this was achieved by additional mutational analysis. Selection bias was avoided in correction for missing data by including propensity score matching to identify patients for additional mutational analysis and by using multiple imputation. One limitation of this study is that the patients in our cohort were selected based on primary tumor diagnosis in 2015 and 2016. Thus, our cohort does not include patients with metachronous disease with a long DFI.[53] In addition, selection and information bias is unavoidable given the retrospective nature of the study, although we believe we minimized bias by using a population-based cohort and by handling missing data by multiple imputation. For the validation of the GAME score, mutation status as risk factor was scored by the detection of *KRAS* codon 12, 13, and 61 mutations, meaning that other *RAS* mutations were ignored to meet the exact GAME criteria, as proposed by Margonis et al. Lastly, the GAME score incorporated patients with extrahepatic disease as a risk factor. As these patients were excluded from our study, the GAME high-risk groups in our validation cohort did not include patients with a maximum of five risk factors.

Conclusion

Two established CRSs, Fong and GAME, to predict outcome after CRLM resection were compared and externally validated in a real-life population-based cohort of patients with local treatment of CRLM, regardless of age or the administration of perioperative systemic therapy. Both CRSs showed predictive ability in the real-life cohort, with a better performance of the GAME as compared to the traditional Fong CRS. Although the novel CRS (GAME) outperformed the traditional CRS, the suboptimal predictive value of both CRSs limits the clinical utility of the CRSs. Surgical innovations increase the number of CRLM patients assessed as technically resectable, but high recurrence rates persist, and a significant group of patients has no long-term survival benefit of CRLM resection. Thus, there is still an unmet clinical need for a CRS with high discriminative ability that allows for a better stratification and counselling of patients before surgery and perioperative therapy in order to personalize therapy.

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Author contributions

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References

1. Van Der Geest LGM, Lam-Boer J, Koopman M, Verhoef C, Elferink MAG, De Wilt JHW. Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis. 2015;32:457–465.
2. 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. 2008;247: 125–135.
3. Kanas G, Taylor A, Primrose JN, Langeberg W, Kelsh M, Mowat F, Alexander D, Choti M, Poston G. Survival after liver resection in metastatic colorectal cancer: Review and meta-analysis of prognostic factors. Clin Epidemiol. 2012;4:283–301.
4. Andreou A, Aloia TA, Brouquet A, Dickson PV, Zimmitti G, Maru DM, Kopetz S, Loyer EM, Curley SA, Abdalla EK, et al. Margin Status Remains an Important Determinant of Survival After Surgical Resection of Colorectal Liver Metastases in the Era of Modern Chemotherapy. Ann Surg. 2013;257:1079–1088.
5. Chana P, Burns EM, Arora S, Darzi AW, Faiz OD. A Systematic Review of the Impact of Dedicated Emergency Surgical Services on Patient Outcomes. Ann Surg. 2016;263:20–27.
6. Rocca A, Cipriani F, Belli G, Berti S, Boggi U, Bottino V, Cillo U, Cescon M, Cimino M, Corcione F, et al. The Italian Consensus on minimally invasive simultaneous resections for synchronous liver metastasis and primary colorectal cancer: A Delphi methodology. Updat Surg. 2021;73:1247–1265.
7. Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent D, Larson DW, Grothey A, Vauthey JN, Nagorney DM, McWilliams RR. Improved Survival in Metastatic Colorectal Cancer Is Associated With Adoption of Hepatic Resection and Improved Chemotherapy. J Clin Oncol 2009;27:3677–3683.
8. Bolhuis K, Kos M, van Oijen MG, Swijnenburg RJ, Punt CJ. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020; 141:225–238.
9. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, 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. 2008;371:1007–1016.
10. de Jong MC, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, Choti MA, Aldrighetti L, Capussotti L, Pawlik TM. Rates and Patterns of Recurrence Following Curative Intent Surgery for Colorectal Liver Metastasis: An international multi-institutional analysis of 1669 patients. Ann Surg. 2009;250:440–448.
11. Jones RP, Jackson R, Dunne DFJ, Malik HZ, Fenwick SW, Poston GJ, Ghaneh P. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. Br J Surg. 2012;99: 477-486.
12. Imai K, Allard MA, Benitez CC, Vibert E, Cunha AS, Cherqui D, Castaing D, Bismuth H, Baba H, Adam R. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21:887–894.
13. Nordlinger B, Guiguet M, Vaillant JC, Balladur P, Boudjema K, Bachellier P, Jaeck D. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Française de Chirurgie. Cancer. 1996;77:1254–1262.
14. Iwatsuki S, Dvorchik I, Madariaga JR, Marsh JW, Dodson F, Bonham AC, Geller DA, Gayowski TJ, Fung JJ, Starzl TE. Hepatic resection for metastatic colorectal adenocarcinoma: A proposal of a prognostic scoring system. J Am Coll Surg. 1999;189:291–299.
15. 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; 230:309–318; discussion 318–321.
16. Margonis GA, Sasaki K, Gholami S, Kim Y, Andreatos N, Rezaee N, Deshwar A, Buettner S, Allen PJ, Kingham TP, et al. Genetic and Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105:1210–1220.
17. Zakaria S, Donohue JH, Que FG, Farnell MB, Schleck CD, Ilstrup DM, Nagorney DM. Hepatic Resection for Colorectal Metastases: Value for Risk Scoring Systems?. Ann Surg. 2007;246:183–191.

18. Creasy JM, Sadot E, Koerkamp BG, Chou JF, Gonen M, Kemeny NE, Balachandran VP, Kingham TP, DeMatteo RP, Allen PJ, et al. Actual 10-year survival after hepatic resection of colorectal liver metastases: What factors preclude cure? Surgery. 2018;163:1238–1244.
19. Mahar AL, Compton C, Halabi S, Hess KR, Weiser MR, Groome PA. Personalizing prognosis in colorectal cancer: A systematic review of the quality and nature of clinical prognostic tools for survival outcomes. J Surg Oncol. 2017;116:969–982.
20. Brudvik KW, Jones RP, Giuliante F, Shindoh J, Passot G, Chung MH, Song J, Li L, Dagenborg VJ, Fretland ÅA, et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;269:120–126.
21. Lang H, Baumgart J, Heinrich S, Tripke V, Passalaqua M, Maderer A, Galle PR, Roth W, Kloth M, Moehler M. Extended Molecular Profiling Improves Stratification and Prediction of Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;270:799–805.
22. Chen Y, Chang W, Ren L, Chen J, Tang W, Liu T, Jian M, Liu Y, Wei Y, Xu J. Comprehensive Evaluation of Relapse Risk (CERR) Score for Colorectal Liver Metastases: Development and Validation. Oncologist. 2020;25:e1031–e1041.
23. Dupré A, Berhane S, Chan A, Rivoire M, Chong C, Lai P, Cucchetti A, Poston GJ, Malik H, Johnson P. Multicentre validation of a clinical prognostic score integrating the systemic inflammatory response to the host for patients treated with curative-intent for colorectal liver metastases: The Liverpool score. Eur J Surg Oncol. 2019;45:999–1004.
24. Paredes AZ, Ms JMH, Tsilimigras DI, Moro A, Bagante F, Guglielmi A, Ruzzenente A, Alexandrescu S, Makris EA, Poultsides GA, et al. A Novel Machine-Learning Approach to Predict Recurrence After Resection of Colorectal Liver Metastases. Ann Surg Oncol. 2020;27:5139–5147.
25. Sasaki K, Morioka D, Conci S, Margonis GA, Sawada Y, Ruzzenente A, Kumamoto T, Iacono C, Andreatos N, Guglielmi A, et al. The Tumor Burden Score: A New "Metro-Ticket" Prognostic Tool For Colorectal Liver Metastases Based on Tumor Size and Number of Tumors. Ann Surg. 2018;267:132–141.
26. Meyer RM. Generalizing the Results of Cancer Clinical Trials. J Clin Oncol. 2010;28:187–189.
27. Booth CM, Nanji S, Wei X, Mackillop WJ. Management and Outcome of Colorectal Cancer Liver Metastases in Elderly Patients. JAMA Oncol. 2015;1:1111–1119.
28. Adam R, Frilling A, Elias D, Laurent C, Ramos E, Capussotti L, Poston GJ, Wicherts DA, de Haas RJ. Liver resection of colorectal metastases in elderly patients. Br J Surg. 2010;97:366–376.
29. Papamichael D, Audisio RA, Glimelius B, de Gramont A, Glynne-Jones R, Haller D, Köhne CH, Rostoft S, Lemmens V, Mitry E, et al. Treatment of colorectal cancer in older patients: International Society of Geriatric Oncology (SIOG) consensus recommendations 2013. Ann Oncol. 2015;26:463–476.
30. National Comprehensive Cancer Network Colon Cancer (Version 2.2021). Available online: https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf, accessed on 18 June 2021.
31. Nederlandse Vereniging voor Heelkunde Colorectaal Carcinoom (CRC) (Version 2019). Available online: File:///H:/Downloads/Colorectaal_carcinoom_CRC_ (1).pdf, accessed on 18 June 2021.
32. Van Cutsem E, Cervantes A, Adam R, Sobrero A, van Krieken JH, Aderka D, Aguilar EA, Bardelli A, Benson A, Bodoky G, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386–1422.
33. Casparie M, Tiebosch ATMG, Burger G, Blauwgeers H, van de Pol A, van Krieken JHJM, Meijer GA. Pathology Databanking and Biobanking in The Netherlands, a Central Role for PALGA, the Nationwide Histopathology and Cytopathology Data Network and Archive. Cell Oncol. 2007;29:19–24.
34. Reddy SK, Barbas AS, Turley RS, Steel JL, Tsung A, Marsh JW, Geller DA, Clary BM. A standard definition of major hepatectomy: Resection of four or more liver segments. HPB. 2011:13:494–502.
35. Mekenkamp LJM, Koopman M, Teerenstra S, Van Krieken JHJM, Mol L, Nagtegaal I, Punt CJA. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010;103:159–164.
36. Beelen K, Opdam M, Severson TM, Koornstra RHT, Vincent AD, Wesseling J, Muris JJ, Berns EMJJ, Vermorken JB, Van Diest PJ, et al. PIK3CA mutations, phosphatase and tensin homolog, human epidermal growth factor receptor 2, and insulin-like growth factor 1 receptor and adjuvant tamoxifen resistance in postmenopausal breast cancer patients. Breast Cancer Res. 2014;16:R13.

37. Bartlett JW, Seaman SR, White IR, Carpenter JR. Alzheimer's Disease Neuroimaging Ingitiative. Multiple imputation of covariates by fully conditional specification: Accommodating the substantive model. Stat. Methods Med Res. 2015;24:462–487.
38. Morisot A, Bessaoud F, Landais P, Rébillard X, Trétarre B, Daurès JP. Prostate cancer: Net survival and cause-specific survival rates after multiple imputation. BMC Med Res Methodol. 2015;15:54.
39. Marshall A, Altman DG, Holder RL, Royston P. Combining estimates of interest in prognostic modelling studies after multiple imputation: Current practice and guidelines. BMC Med Res Methodol. 2009;9:57.
40. Moons KGM, Altman DG, Reitsma JB, Ioannidis JPA, Macaskill P, Steyerberg EW, Vickers AJ, Ransohoff DF, Collins GS. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): Explanation and Elaboration. Ann Intern Med. 2015;162:W1–W73.
41. Rohatgi A. WebPlotDigitizer Version 4.4. Available online: https://automeris.io/WebPlotDigitizer, accessed on 3 November 2020.
42. Harrell FE, Jr, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the Yield of Medical Tests. JAMA. 1982; 247:2543–2546.
43. Pitroda SP, Khodarev NN, Huang L, Uppal A, Wightman SC, Ganai S, Joseph N, Pitt J, Brown M, Forde M, et al. Integrated molecular subtyping defines a curable oligometastatic state in colorectal liver metastasis. Nat Commun. 2018;9:1793.
44. Tsilimigras DI, Ntanasis-Stathopoulos I, Bagante F, Moris D, Cloyd J, Spartalis E, Pawlik TM. Clinical significance and prognostic relevance of KRAS, BRAF, PI3K and TP53 genetic mutation analysis for resectable and unresectable colorectal liver metastases: A systematic review of the current evidence. Surg Oncol. 2018;27:280–288.
45. Tosi FM, Magni E, Amatu A, Mauri G, Bencardino K, Truini M, Veronese SM, De Carlis L, Ferrari G, Nichelatti M, et al. Effect of KRAS and BRAF Mutations on Survival of Metastatic Colorectal Cancer After Liver Resection: A Systematic Review and Meta-Analysis. Clin Color Cancer. 2017;16:e153–e163.
46. Yamashita S, Chun YS, Kopetz S, Vauthey J. Biomarkers in colorectal liver metastases. Br J Surg. 2018; 105:618–627.
47. Rahbari NN, Reissfelder C, Schulze-Bergkamen H, Jäger D, Büchler MW, Weitz J, Koch M. 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. 2014;14:174.
48. Ayez N, Lalmahomed ZS, Van Der Pool AEM, Vergouwe Y, Van Montfort K, De Jonge J, Eggermont AMM, Ijzermans JNM, Verhoef C. Is the Clinical Risk Score for Patients with Colorectal Liver Metastases Still Useable in the Era of Effective Neoadjuvant Chemotherapy? Ann Surg Oncol. 2011;18:2757–2763.
49. Hirokawa F, Hayashi M, Miyamoto Y, Asakuma M, Shimizu T, Komeda K, Inoue Y, Uchiyama K. Reconsideration of the Indications for Adjuvant Chemotherapy for Liver Metastases from Colorectal Cancer After Initial Hepatectomy. Ann Surg Oncol. 2013;21:139–146.
50. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, 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. 2013;14:1208–1215.
51. Kanemitsu Y, Shimizu Y, Mizusawa J, Inaba Y, Hamaguchi T, Shida D, Ohue M, Komori K, Shiomi A, Shiozawa M, et al. A randomized phase II/III trial comparing hepatectomy followed by mFOLFOX6 with hepatectomy alone for liver metastasis from colorectal cancer: JCOG0603 study. J Clin Oncol. 2020;38: 4005.
52. Ayez N, Van Der Stok EP, De Wilt H, Radema SA, Van Hillegersberg R, Roumen RM, Vreugdenhil G, Tanis PJ, Punt CJ, Dejong CH, et al. Neo-adjuvant chemotherapy followed by surgery versus surgery alone in high-risk patients with resectable colorectal liver metastases: The CHARISMA randomized multicenter clinical trial. BMC Cancer. 2015;15:180.
53. Engstrand J, Nilsson H, Strömberg C, Jonas E, Freedman J. Colorectal cancer liver metastases – a population-based study on incidence, management and survival. BMC Cancer. 2018;18:78.

Supplementary materials

Table S1 Assumptions regarding baseline characteristics, systemic treatment, local treatment, and survival outcomes.

Assumptions regarding baseline characteristics:
- RAS and BRAF mutation are considered mutual exclusive, therefore patients with RAS mutations or BRAF mutations, were assumed to have BRAF wildtype or RAS wildtype status, respectively.
- Primary tumor nodal status was defined primarily on pathologic N-stadium. When pN stage was missing, cN stage (radiological) was used.
- If number of metastases was not given and code 77 was used (accounting for diffuse metastatic disease in the liver) then number of metastases was scored as 20.
Assumptions regarding systemic treatment regimens and strategies:
- Systemic treatment includes both chemotherapy and/or targeted therapy.
- A combination regimen is defined as all systemic agents starting within 4 weeks after start of the first agent and started before progression of disease.
- If bevacizumab was started more than 4 weeks after the start of the first agent but before stop of this agent and before progression of disease, we assume bevacizumab was part of this combination regimen.
- If a treatment line continues despite of progression, e.g., in case of reintroduction of the same or an equivalent regimen after a therapy break and detected progression, we regard this as continuation of the same treatment line.
- If oxaliplatin only is registered, we assume this was part of a capecitabine and oxaliplatin (CapOx) regimen of which capecitabine was not registered, so we add capecitabine. We assume this is due to a registration error, in which the administration of capecitabine has not been noticed by the data manager since it is registered differently as oral medication.
- Systemic therapy was considered adjuvant systemic therapy for primary tumor when started < 12 weeks after resection of primary tumor and started before diagnosis of metastases in patients with metachronous disease.
- Capecitabine monotherapy was considered radiosensitizer for primary tumor when started before primary tumor resection and before diagnosis of metastases and with notification to have received chemoradiotherapy.
- Systemic therapy was considered pre-operative therapy (neo-adjuvant or induction) before liver resection when the therapy ended within 120 days before liver resection. Adjuvant therapy after resection of primary tumor or chemotherapy as radiosensitizer was excluded.
- Systemic therapy was considered adjuvant therapy after liver resection when the therapy started within 120 days after liver resection. Chemotherapy as radiosensitizer was excluded.
- Systemic therapy was considered peri-operative therapy of liver resection when the systemic therapy was given < 120 days before and < 120 days after liver resection
- When systemic therapy was given between two liver procedures before progression of disease, the first liver procedure was considered as staging procedure and systemic therapy was considered as pre-operative systemic therapy (neo-adjuvant or induction) for surgery 2
- A treatment line is defined as systemic therapy (monotherapy or combination regimen) administered at the same time until suspension, regardless of reason for discontinuation.
- Treatment is considered as next line if an agent of a new drug group is started that is not applied in the previous systemic treatment regimen.
- If the same or an equivalent systemic treatment regimen is (re)started, this is considered continuation of the same treatment line, e.g., CapOx to 5-FU/oxaliplatin (FOLFOX).

Table S1 (continued)

Assumptions regarding local treatment
- Local treatments are categorized as follows: o 1 stage (1 procedure) o 2-stage (2 procedures < 120 days apart)
- R-status: when two stage procedure and first procedure was R2 resection and second procedure was R1/R0 resection than 2-stage resection considered as R-status of last procedure. - when 2-stage resection and one procedure was R1 resection and other local treatment was R0 resection than considered as R1 resection. *Table continued on next page.*
Assumptions regarding progression of disease and survival:
- Date of new episode is considered as time of progression.
- When disease progression is documented < 14 days of liver resection we assume this was part of the liver resection and first new episode is considered as time of progression.
- Disease-free survival is calculated from date of first liver procedure to date of progression. In case of 2-stage resection, DFS is calculated from last liver procedure.
- If no recurrence is registered: o If end of follow up is registered and reason end of follow up is: death, then date of death is registered as event of DFS; o If end of follow up is registered and reason end of follow up is other than death then DFS is censored on date of end of follow up; o If no date of end of follow up is registered then DFS is censored on date of last visit; o If none of these dates are registered then DFS is documented as missing.
- Lymph node metastases registered as abdominal lymph nodes at time of first liver metastases were considered extrahepatic disease and as so classified as not-liver only disease.
- Overall survival (OS) after resection was defined as date of first resection till date of last documented vital status as documented by the municipal population registry. In case of 2-stage resection, OS is calculated from date of last liver procedure o If the documented date of disease-free survival is after date of documented survival than the date of disease-free survival is date of last survival
- Patients who did not die are censored on the date last known to be alive in the municipal population registry.

Figure S1 Kaplan–Meier analysis showing overall survival and disease-free survival curves of the total cohort in scores following the GAME clinical risk score (A and B) or the Fong clinical risk score (C and D).

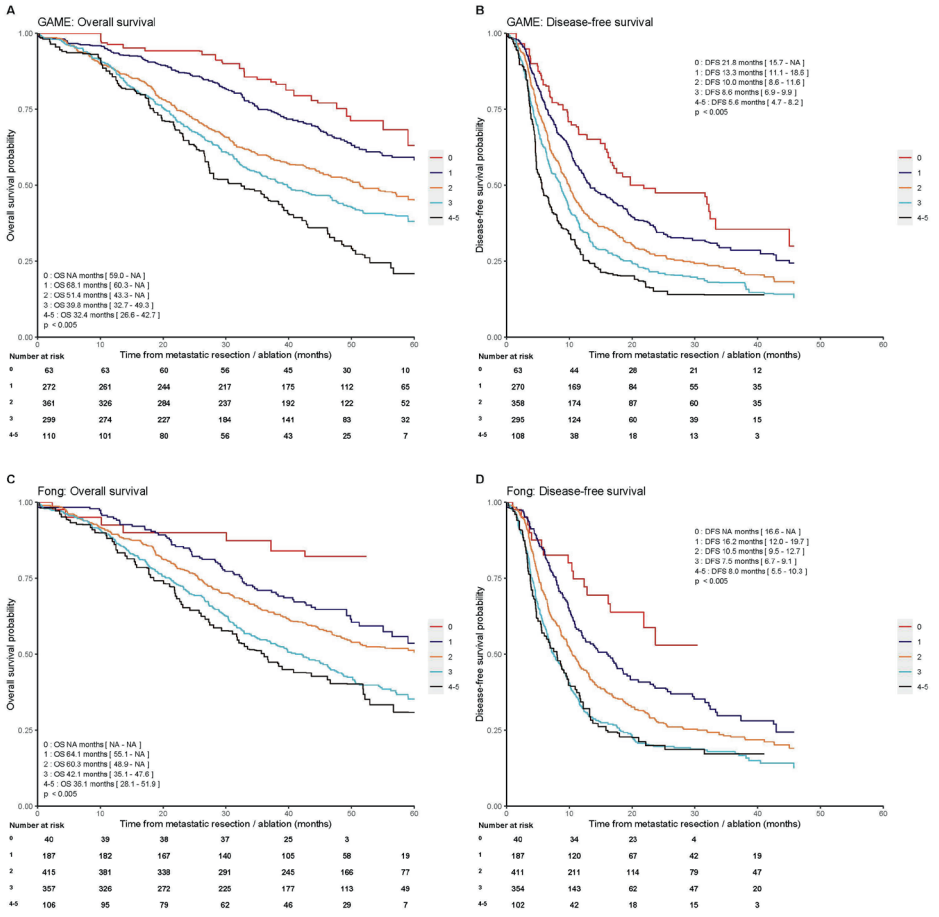


Figure S2 Calibration of the expected and observed survival outcomes in the NCR cohort of the risk groups (low, moderate, and high) according to (A) GAME and (B) Fong prediction model.

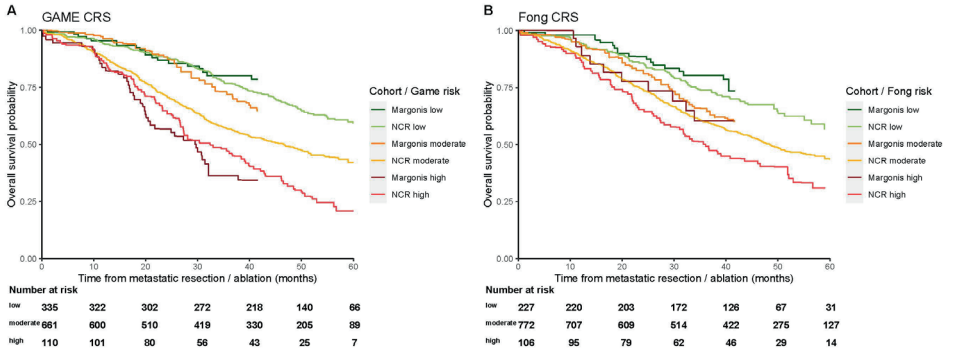
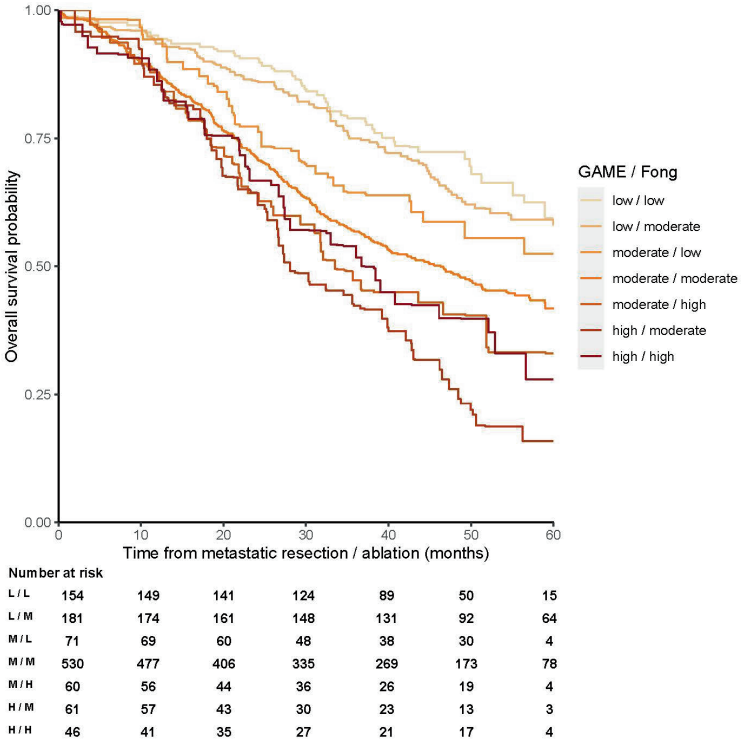


Figure S3 A combined figure containing, on the top, a contingency table showing the frequency distribution of patients among the risk categories (low, moderate, and high) following the Fong and GAME prediction model, as well as their corresponding 3-year survival rate estimate, which is also indicated by the heat map for each category. The corresponding survival curves for the groups are displayed in the KM plot in the figure on the bottom.

	Fong low	Fong moderate	Fong high	Total
GAME low	*n*=154 (13.9%) Survival: 79%	*n*=181 (16.4%) Survival: 75%	*n*=0 (0.0%) -	335
GAME moderate	*n*=71 (6.4%) Survival: 64%	*n*=530 (47.9%) Survival: 57%	*n*=60 (5.4%) Survival: 47%	661
GAME high	*n*=3 (0.3%)	*n*=61 (5.5%) Survival: 42%	*n*=46 (4.2%) Survival: 54%	110
Total	228	772	106	1106



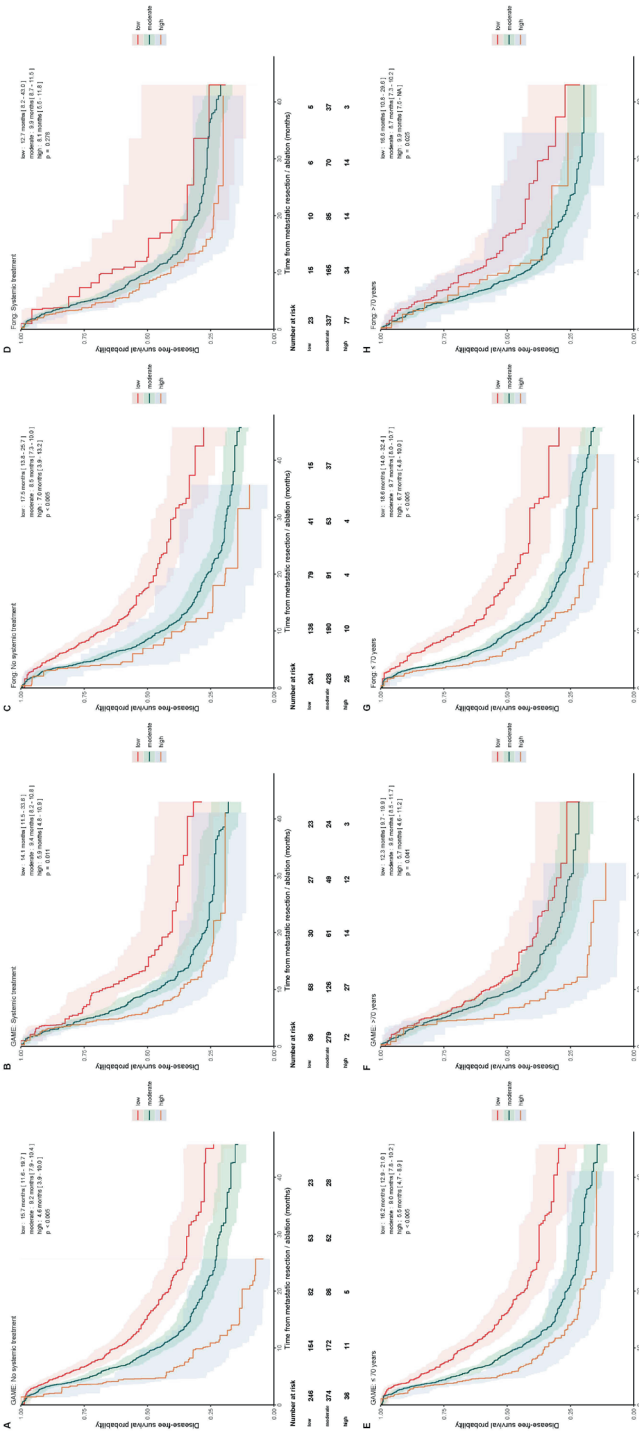


Figure S4 Kaplan–Meier analysis showing disease-free survival curves for GAME risk groups for patients without (A) and with (B) perioperative systemic therapy and for age groups ≤70 years (G) and >70 years (H). The DFS outcomes of the Fong risk groups are shown for patients without (D) and with (E) perioperative systemic therapy and for age groups ≤70 years (I) and >70 years (J).

Table S2 Pooled Harrell's concordance index with 95% confidence intervals for 1- and 3-year overall survival and disease-free survival outcomes for GAME and Fong risk scores in subgroups without and with perioperative systemic therapy. Survival estimates at 1- and 3- years for low, moderate and high risk groups according to GAME and Fong prediction model.

	WITHOUT PERIOPERATIVE SYSTEMIC THERAPY							
	GAME score	Survival estimates GAME risk categories			Fong score	Survival estimates Fong risk categories		
	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)
OS								
1-year OS	0.592 [0.518-0.667]	94	89	84	0.596 [0.532-0.661]	95	89	84
3-year OS	0.610 [0.574-0.647]	78	57	43	0.593 [0.559-0.628]	75	60	48
5-year OS	0.602 [0.569-0.635]	60	41	26	0.594 [0.563-0.624]	59	42	
DFS								
1-year DFS	0.584 [0.553-0.614]	56	39	21	0.606 [0.578-0.635]	61	37	31
3-year DFS	0.579 [0.551-0.606]	28	18	9	0.601 [0.575-0.627]	33	16	
	WITH PERIOPERATIVE SYSTEMIC THERAPY							
	GAME score	Survival estimates GAME risk categories			Fong score	Survival estimates Fong risk categories		
	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)
OS								
1-year OS	0.588 [0.511-0.664]	96	88	86	0.538 [0.460-0.617]	96	89	88
3-year OS	0.590 [0.549-0.631]	74	56	49	0.556 [0.515-0.598]	60	60	51
5-year OS	0.590 [0.554-0.627]	58	43	23	0.557 [0.519-0.594]		46	29
DFS								
1-year DFS	0.589 [0.551-0.626]	58	39	30	0.563 [0.528-0.598]	50	42	35
3-year DFS	0.581 [0.547-0.616]	34	23	19	0.559 [0.527-0.591]	26	26	20

When not indicated, the number of patients was too small to calculate the survival estimate.
Abbreviations; C-index, concordance index; DFS, disease-free survival; OS, overall survival.

Table S3 Pooled Harrell's concordance index with 95% confidence intervals for 1- and 3-year overall survival and disease-free survival outcomes for GAME and Fong risk scores in subgroups of ≤ 70 years and > 70 years. Survival estimates at 1- and 3-years for low, moderate and high risk groups according to GAME and Fong prediction model.

	AGE ≤ 70 YEARS							
	GAME score	Survival estimates GAME risk categories			Fong score	Survival estimates Fong risk categories		
	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)
OS								
1-year	0.611 [0.540-0.681]	96	90	88	0.583 [0.515-0.650]	96	92	86
3-year	0.618 [0.585-0.652]	82	59	50	0.588 [0.555-0.621]	76	64	48
5-year	0.613 [0.585-0.642]	64	45	21	0.584 [0.554-0.613]	56	48	32
DFS								
1-year	0.601 [0.573-0.628]	59	38	29	0.595 [0.569-0.621]	61	40	32
3-year	0.594 [0.568-0.619]	32	19	15	0.591 [0.567-0.614]	34	21	14
	AGE > 70 YEARS							
	GAME score	Survival estimates GAME risk categories			Fong score	Survival estimates Fong risk categories		
	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)	C-index [95% C.I.]	Low (%)	Moderate (%)	High (%)
OS								
1-year	0.572 [0.491-0.654]	90	85	79	0.601 [0.527-0.675]	93	83	89
3-year	0.580 [0.533-0.627]	67	52	37	0.590 [0.547-0.634]	70	50	58
5-year	0.575 [0.531-0.618]	49	36	37	0.589 [0.548-0.630]	58	34	
DFS								
1-year	0.554 [0.511-0.597]	51	41	22	0.584 [0.542-0.625]	58	37	41
3-year	0.547 [0.507-0.587]	26	23	22	0.575 [0.536-0.613]	31	20	

When not indicated, the number of patients was too small to calculate the survival estimate.
Abbreviations; C-index, concordance index; DFS, disease-free survival; OS, overall survival.

CHAPTER 7

Predicting early extrahepatic recurrence after local treatment of colorectal liver metastases

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Abstract

Objective

We aimed to develop and internally validate a prediction model for early extrahepatic recurrence (EHR) after local treatment of colorectal liver metastases (CRLM).

Summary background data

Patients who develop early EHR (≤6 months) may not benefit from local treatment of CRLM. However, prediction models for early EHR are not available.

Methods

We used a population-based cohort of 1077 patients locally treated for CRLM with curative intent to develop and internally validate a prediction model for EHR using Cox regression. Performance assessment included calibration, discrimination, net benefit, and generalizability by internal-external cross-validation. The prognostic relevance of 6-month EHR was evaluated by landmark analysis.

Results

During a median follow-up of 35 months, 557 patients developed EHR, and 249 died. The median OS for patients with EHR within six months after CRLM treatment was 19.5 months [95%C.I. 15.6-23.0] versus not reached [45.3-not reached]. The EHR prediction model included sidedness of primary tumour, T-stage and N-stage of primary tumour, RAS/BRAF^{V600E} mutational status, and number and size of CRLM. The model was well-calibrated, yielded overoptimism-corrected 6-month EHR risks between 5.9-56.0% (interquartile interval 12.9-22.0%). Harrell's C-index through 6 and 12 months was 0.663 [0.624-0.702] and 0.661 [0.632-0.689], respectively. Patients had an observed 6-month EHR risk of 32% (highest quartile) versus 6% (lowest quartile). Expected generalizability was good. EHR risk-informed CRLM treatment decisions yielded net benefit at 6-month EHR thresholds of 0-40%.

Conclusions

Early EHR after local treatment of CRLM has a major impact on prognosis and can be predicted with routine clinical information.

Introduction

Colorectal cancer liver metastases (CRLM) are the major cause of colorectal cancer (CRC)-related death.[1] Local treatment of CRLM without extrahepatic metastatic involvement, such as liver resection, offers the only chance for cure or long-term survival, with 5-year survival rates of up to 55%.[2–5] Improved surgical and ablation techniques, optimization of systemic neoadjuvant induction treatment with high response rates and more lenient eligibility criteria have increased the number of patients assessed as technically resectable and undergoing CRLM resection.[4,6]

However, relapse after local CRLM treatment occurs in up to 75% of patients, often with unresectable recurrences and subsequent decreased survival.[5,7,8] Numerous prediction models for (recurrence-free) survival after local treatment of CRLM exist[9–15], but these are not widely used to guide decision-making due to their inability to identify patients with a sufficiently short survival to render local treatment unjustified. Aspects which might contribute to this include suboptimal incorporation of prognostic factors and the use of (recurrence-free) survival as an endpoint.

A limitation of recurrence-free survival (RFS) as endpoint is its inability to discriminate between intra- and extrahepatic recurrences. Patients with liver-limited recurrences may be eligible for repeat local treatment, resulting in long-term survival.[8,16–18] In contrast, a minority of patients with extrahepatic recurrence (EHR) undergo repeated local treatment.[18–20] An early recurrence, usually defined as occurring within six months[21,22], and EHR are independently associated with poor overall survival (OS) in patients receiving local treatment for CRLM.[21–23] Therefore local treatment of CRLM may not be justified in patients who develop an early EHR. Being able to predict early EHR may spare patients an invasive treatment, treatment-related morbidity and avoid delay in starting systemic treatment which may effectively treat the systemic disease present. Moreover, early EHR estimates may stratify patients for perioperative systemic therapy, since patients receiving local CRLM treatment have no OS benefit from perioperative systemic therapy.[24,25]

Although early EHR after local treatment of CRLM is of major clinical importance, to our knowledge, no prediction models for early EHR exist. Also, novel prognostic factors, such as primary tumour localization and *RAS/ BRAF*^{V600E} tumour mutational status, may aid in better identifying patients at high risk for early EHR. Patients with right-sided primary tumours have a worse prognosis after local treatment of CRLM, more recurrences at multiple sites and less repeated local treatment as compared to patients with left-sided primaries.[26,27] The presence of *RAS* and *BRAF*^{V600E} mutations is associated with a higher recurrence rate of up to 94%, with EHR not amenable to local therapy and a shorter EHR-free survival (EHRFS).[8,28–30]

We aimed to develop and internally validate a prediction model, which incorporates primary tumour location and *RAS/ BRAF*^{V600E} mutational status as novel prognostic factors, for early EHR following local treatment of CRLM using a population-based cohort.

Methods

Patient cohort

All patients initially diagnosed with CRC between January 1st 2015 and December 31st 2016, who underwent local treatment (resection and/or local ablation) with curative intent for CRLM, were identified in the Netherlands Cancer Registry (NCR), a population-based registry of all newly diagnosed cancer patients in the Netherlands.[31] Patients with extrahepatic metastases before resection, R2 liver resections, appendix carcinoma, concomitant local liver treatment other than resection or ablation, and without any follow-up information were excluded. The scientific committee of the Netherlands Comprehensive Cancer Organisation (IKNL) approved the research protocol and use of this data, and the requirement of written informed consent was waived for this study. The study was performed in accordance with the Declaration of Helsinki and reported according to the TRIPOD guidelines.[32]

Candidate predictor variables

Data was extracted from the NCR including but not limited to: age, sex, American Joint Committee on Cancer (AJCC) tumour stage (T-stage), nodal stage (N-stage) of the primary tumour, location of the primary tumour (right-sided (coecum-transverse colon), left-sided (splenic flexure-rectosigmoid) and rectum), disease-free interval between detection of primary tumour and metastases (DFI), size (mm) and number of liver metastases, serum CEA level (ug/L) prior to local treatment of CRLM, type of local treatment, resection margin status (R0 versus R1) and administered perioperative systemic treatment. A major resection was defined as resection of ≥4 liver segments[33], synchronous disease as DFI ≤6 months[34] and perioperative systemic therapy as therapy administered ≤100 days before and/or after local CRLM treatment and initiated prior to progression of disease. Intent of systemic treatment was not registered, precluding a distinction between neo-adjuvant or induction systemic therapy. However, as Dutch guidelines for CRC[35] recommend not to administer perioperative systemic therapy in initially resectable CRLM, we assume that preoperative systemic treatment was given as induction treatment to achieve CRLM resectability. Further assumptions regarding systemic treatment are described in Supplementary Table 1.

RAS/BRAF^{V600E} mutational status was retrieved from the NCR and the national automated pathological archive (PALGA[36]), determined in daily practice on primary tumour or metastases at any time during the disease course. Missing *KRAS, NRAS* and *BRAF*^{V600E} mutation status was complemented by an additional Sequenom Massarray mutation analysis of tumour tissue of 250 patients. These 250 additional samples were selected in such a way to maximize mutation status information for patient subgroups in which that information was otherwise underrepresented, increasing the likelihood of successful multiple imputation.[37]

Patient outcomes

Follow-up data for recurrences was collected from medical records until May 2020 and survival was obtained by linkage with the municipal population registry on January 31st, 2021. OS was defined as date of first local treatment for CRLM till date of death or last follow-up. RFS and EHRFS was defined as date of first local treatment of CRLM till date of a RFS or EHRFS event, which was defined as first recurrence of disease or first EHR or death, whichever occurred first, or censored on last date of RFS or EHRFS without event, respectively. In two-stage resections, OS, RFS and EHRFS was calculated since the date of the last procedure. If follow-up for recurrences was shorter than follow-up for survival, all survival follow-up beyond the last follow-up for recurrences was discarded for assessment of RFS, EHRFS or OS. In all patients a minimum of one year RFS and two year OS follow-up was ensured. All assumptions regarding OS, RFS and EHRFS are included in Supplementary Table 1.

Statistical analysis

We used standard descriptive statistics to describe the study population, including medians and interquartile intervals (IQI) for continuous variables, and frequency and percentages for categorical variables. Follow-up data and patient outcomes were described using (reverse) Kaplan Meier approaches.

Prediction model development and performance assessment

Early EHR (≤6 months[21,22]) was defined as the clinically relevant primary endpoint of the model, due to the poor prognosis in patients with early EHR and lower chance of repeat local treatment, in contrast to patients with liver-only recurrences. We assessed the prognostic impact of our primary endpoint using landmark analysis at six months after CRLM treatment.

Based on published recommendations[38], we had sufficient data to model 17 coefficients. Nine candidate predictors were selected for model development by assessment of a multidisciplinary team based on literature of previous prediction models and novel

prognostic factors.[9–12,26,39] The predictors, including four continuous variables modeled non-linearly, were: neoadjuvant systemic treatment, primary tumour location, T-stage, N-stage, *RAS/BRAF*^{V600E} mutational status, number of liver metastases, size of largest liver metastasis, pre-operative CEA and DFI. We used multiple imputation using multivariate imputation by chained equations (MICE)[40] to account for missing data, generating 53 imputed datasets (based on the percentage of patients with any missing data in the candidate predictors).

A prediction model for EHRFS after local treatment of CRLM was developed using Cox regression, with a time-horizon of 12 months to improve the effective sample size, but with a primary evaluation of the model's performance for EHR ≤6 months. The prediction model was developed in the whole cohort, using Akaike Information Criterion (AIC)-based backward selection in each imputed dataset leading to a primary model only including predictors selected in ≥50% of imputed datasets, which was then refitted in each imputed dataset to obtain a pooled selection model using Rubin's rules ('EHR model'). Adjuvant systemic therapy was included in all models using an offset for expected therapeutic efficacy based on the pooled adjuvant systemic treatment effect from published randomized controlled trials.[24,25]

Model performance was assessed using calibration plots for 6- and 12-month EHR risk, discrimination (Harrell's C-index, Uno's C-index through 6-months and 12-months), time-dependent receiver operator characteristic (ROC) curve, Nagelkerke's R^2 and decision curve analysis. Each measure was determined for each imputed dataset separately and pooled using Rubin's rules, incorporating appropriate data-transformation steps. Decision curve analysis was used to assess the net benefit associated with CRLM treatment decisions based on a given threshold value for 6-month or 12-month EHRFS probability.[41] To visualize the model's potential relevance, we used Kaplan-Meier curves for EHRFS, RFS and OS, categorizing patients based on quartiles of predicted EHR risk. The reported performance measures were based on predicted EHR-risks which included the effect of adjuvant treatment (if given).

We used internal validation by 500-fold bootstrap resampling, repeating all model-development steps in each bootstrap sample, to obtain an overoptimism-corrected model (using uniform shrinkage) and C-index. We used internal-external cross-validation, including all modeling steps, to evaluate the generalizability of the model based on three geographic regions.

In an exploratory analysis, we tested whether the prognostic value of *RAS* mutation for EHRFS depended on the administration of preoperative systemic treatment as reported by others[28,29], using a multivariable model with a *RAS**preoperative systemic treatment interaction term.

A more detailed description of the methods is described in the **Supplementary Methods**. Analyses were performed using SPSS software version 25 (IBM, Armonk, New York, USA) and R version 4.0.3 (2020-10-10) with the following libraries: rms (V6.2-0), pec (V2022.03.0), survival (V3.3-1), mice (V3.14.0), survivalROC (V1.0.3).

Results

Patient cohort

All 1105 patients who underwent local treatment (resection and/or ablation) for CRLM were selected from the NCR for analysis, for which the primary endpoint regarding early EHR was available in 1077 patients. In 11 of the 1105 (<1%) patients, no follow-up data was available.

The patient characteristics of the cohort are displayed in **Table 1**. Overall, the median age was 66 years, 403 (37%) were females, 797 (74%) presented with synchronous disease and 256 (24%) with a right-sided primary tumour. A total of 427 (40%) patients received systemic treatment, and in 173 (16%) patients a major liver resection (hemihepatectomy with/without local ablation and/or wedge resection) was performed. The *RAS/BRAF* mutation status was available in 701 (65%) patients, of whom 352 (50%) harbored a *RAS* mutation and 19 (3%) a $BRAF^{V600E}$ mutation.

Table 1 Characteristics of 1105 Dutch CRC patients diagnosed in 2015-2016 who received local treatment for CRLM

		Overall *n*=1077
Age, years	Median (IQI)	66 [59-72]
Sex	Female, n (%)	403 (37.4)
Side primary tumour	Right, n (%)	256 (23.8)
	Left, n (%)	460 (42.7)
	Rectum, n (%)	361 (33.5)
Chemoradiotherapy primary tumour	Yes, n (%)	127 (11.8)
T-stage	1, n (%)	27 (2.5)
	2, n (%)	126 (11.8)
	3, n (%)	740 (69.1)
	4, n (%)	178 (16.6)
	Missing	6
N-stage	N0, n (%)	398 (37.0)
	N1, n (%)	380 (35.3)
	N2, n (%)	297 (27.6)
	Missing	2

Table 1 (continued)

		Overall *n*=1077
Stage of disease at diagnosis	I, n (%)	25 (2.3)
	II, n (%)	102 (9.5)
	III, n (%)	185 (17.2)
	IV, n (%)	765 (71.0)
Differentiation grade of CRC	Low, n (%)	15 (1.5)
	Intermediate, n (%)	916 (92.0)
	High, n (%)	65 (6.5)
	Missing	81
Time to metastases	Synchronous, n (%)	782 (72.6)
Number of liver metastases	Median (IQI)	2 [1- 4]
	Missing	41
Size largest liver metastasis, mm	Median (IQI)	24 [16-36]
	Missing, n (%)	83
CEA level, ug/L	Median (IQI)	9.00 [3.30, 36.00]
	Missing	225
Type of surgery	Local ablative therapy only, n (%)	97 (9.0)
	Wedge / segment resection only, n (%)	596 (55.3)
	Minor resection and local ablative therapy, n (%)	211 (19.6)
	Hemihepatectomy with/without ablation/wedge, n (%)	173 (16.1)
R-status	R0, n (%)	841 (78.1)
	R1, n (%)	141 (13.1)
	Unknown due to ablation	95 (8.8)
Perioperative systemic therapy	Neoadjuvant only, n (%)	322 (29.9)
	Adjuvant only, n (%)	51 (4.7)
	Peri-operative, n (%)	54 (5.0)
	None, n (%)	650 (60.4)
Tumour mutational status	*RAS*/*BRAF*^{V600E} wildtype, n (%)	330 (47.1)
	BRAF^{V600E} mutation, n (%)	19 (2.7)
	RAS mutation, n (%)	352 (50.2)
	Missing (*RAS* and/or *BRAF* status)	376
MMR-status	MMR-deficient, n (%)	15 (2.3)
	MMR-proficient, n (%)	632 (97.7)
	Missing	430

Characteristics of 1105 Dutch patients diagnosed in 2015-2016 with CRC who received local treatment for CRLM are shown for the cohort. The count (%) for categorical variables and median (IQI) for continuous variables is shown.

Abbreviations: CEA (carcinoembryonic antigen), CRC (colorectal cancer), CRLM (colorectal liver metastases), IQI (interquartile interval), mm (millimetres), MMR (mismatch repair), N-stage (nodal stage of primary tumour), R-status (resection margin status), T-stage (tumour stage of primary tumour), ug/L (microgram per liter).

Patient outcomes after local treatment of CRLM

In the cohort, 807 (73%) recurrences were observed during a median follow-up of 35 months. The median OS was 51.3 months [95%C.I. 49.3-not reached (NR)] and RFS was 10.1 months [95%C.I. 9.5-10.9; **Supplementary Figure 1**]. The site of first recurrence was

liver-only in 332 (43.3%) patients and extrahepatic (with/without intrahepatic metastases) in 399 (52.2%) patients (**Supplementary Table 2**). The median EHRFS was 20.4 months [95%C.I. 18.8-23.4]. In the cohort, there were 557 (51.7%) EHR events, of which 194 (18.0%) ≤6 months and 363 (33.7%) ≤12 months. First EHR concerned a multisite EHR in 127 (26.6%) patients. The site of first EHR was most frequently the lungs and lymph nodes in 213 (44.6%) and 55 (11.5%) patients, respectively, whereas the brain was affected in 6 (1.3%) patients. The site of first EHR was significantly correlated with post-recurrence survival, p<0.001, with the shortest post-recurrence survival in patients with brain metastases (1.9 months) and the longest in patients with EHR in the lymph nodes, intra-abdomen or lungs (23.4, 27.1 and 29.3 months, respectively; **Supplementary Figure 2**). The time to recurrence was similar for all extrahepatic sites, p=0.55. Notably, 40 (20.6%) patients with early EHR had a hemihepatectomy and 16 (8.2%) patients a two-stage resection.

Prognostic relevance of 6-month EHR

Patients who survived until the prespecified landmark time (6 months after local treatment of CRLM, n=982) were included in the landmark analysis to compare survival outcomes according to type of recurrence. Within the first six months after local treatment of CRLM, 726 (73.9%) patients had no recurrence, 123 (12.5%) suffered a liver-only recurrence and 133 (13.5%) an EHR (including 100 patients with an extra- and intrahepatic recurrence). The median OS from the landmark time for patients with 6-month EHR after CRLM treatment was 19.5 months [95%C.I. 15.6-23.0; **Supplementary Figure 3**]. The median OS from the landmark time in patients with liver-only recurrence was 30.7 months [95%C.I. 29.0-NR] and NR [95%C.I. 45.3-NR] in patients without a recurrence within the first six months.

Prognostic value of tumour mutational status and sidedness of primary tumour

The prognostic value of the novel candidate predictors, tumour mutational status and sidedness of the primary tumour, was first explored using univariable analysis for OS, EHRFS and RFS (**Supplementary Figure 4** and **Table 2**). The median EHRFS for patients with *BRAF*^{V600E} mutated, *RAS* mutated and *RAS/ BRAF*^{V600E} wildtype tumours was 11.4 months [95%C.I. 5.8-NR], 18.5 months [95%C.I. 14.3-20.9] and 28.2 months [95%C.I. 22.2-33.9, p<0.005], respectively. The EHRFS for patients with right-sided, rectum or left-sided localized tumours was 18.5 [95%C.I. 13.3-32.0], 18.6 [95%C.I. 14.5-23.8] and 23.0 [95%C.I. 20.4-32.3, p<0.05] months, respectively.

Table 2 Specifications of prediction model for extrahepatic recurrence-free survival.

Variable	Level	n	Univariable		Full multivariable		Selection model		
			HR	*p*	HR	*p*	HR	*p*	Shrunk HR
Neoadjuvant	Not received	*755*	REF		REF	0.371	-		
	Received	*322*	1.30 [1.05-1.62]	0.02	0.89 [0.69-1.15]		-		
Sidedness	Right	*256*	REF		REF	0.074	REF	0.080	
	Left	*460*	0.73 [0.56-0.95]	0.02	0.94 [0.71-1.25]		0.94 [0.71–1.25]		0.95
	Rectum	*361*	0.96 [0.74-1.26]	0.79	1.24 [0.94-1.65]		1.24 [0.93–1.64]		1.20
T-stage	T1-T2	*153*	REF		REF	0.007	REF	0.010	
	T3	*744*	1.29 [0.92-1.80]	0.15	1.23 [0.87-1.74]		1.22 [0.87–1.72]		1.19
	T4	*180*	1.96 [1.33-2.88]	<0.05	1.77 [1.18-2.65]		1.72 [1.16–2.56]		1.60
N-stage	N0	*399*	REF		REF	<0.005	REF	<0.005	
	N1	*380*	1.14 [0.88-1.48]	0.31	1.21 [0.93-1.58]		1.22 [0.94–1.59]		1.19
	N2	*298*	1.77 [1.37-2.28]	<0.05	1.64 [1.26-2.13]		1.66 [1.28–2.15]		1.55
Mutational status	*RAS/BRAF*-wildtype	*505*	REF		REF	<0.005	REF	<0.005	
	BRAF-mt	*44*	2.08 [1.20-3.59]	<0.05	2.16 [1.22-3.82]		2.13 [1.21–3.76]		1.92
	RAS-mt	*528*	1.48 [1.16-1.88]	<0.05	1.64 [1.26-2.13]		1.67 [1.28–2.16]		1.55
Number of liver metastases			Non-linearly		Non-linearly	<0.005	Non-linearly	<0.005	
Size of largest liver metastasis			Non-linearly		Non-linearly	<0.005	Non-linearly	<0.005	
Pre-operative CEA			Non-linearly		Non-linearly	0.264	-		
Disease-free interval			Non-linearly		Non-linearly	0.367	-		

Specifications for univariable and multivariable cox regression analyses for all candidate predictors for EHRFS within 12 months after local treatment of CRLM are shown, including the full multivariable models, the pooled selection model ('EHR model'). For the pooled selection model, the apparent model hazard ratio's and the overfitting-adjusted hazard ratio's (shrunken) are shown ($\beta_{adjusted} = \beta_{unadjusted}$ x shrinkage factor obtained via bootstrapping during internal validation). For the full multivariable and selection models, multivariable Wald D1 *p*-values are shown. Continuous variables were modeled non-linearly using restricted cubic splines, for which the hazard ratio's are shown in Supplementary Figure 5. The number of patients per category (*n*) indicated are the pooled number of patients for each level over the imputed datasets.
Abbreviations: β (regression coefficient), CEA (carcinoembryonic antigen), CRLM (colorectal liver metastases), EHRFS (extrahepatic recurrence-free survival), HR (hazard ratio), N-stage (nodal stage of the primary tumour), REF (reference), T-stage (tumour stage of the primary tumour).

EHR prediction model

Following AIC-informed backward selection, the model included 6/9 candidate predictor variables: sidedness of the primary tumour, T-stage, N-stage, *RAS/BRAF*^{V600E} mutational status, and number and size of liver metastases (preoperative systemic treatment,

preoperative CEA and DFI were excluded). The model's HR are shown in **Table 2** (non-linear HR plots for continuous variables in **Supplementary Figure 5**). In an exploratory analysis, including an interaction term between *RAS* mutational status and preoperative systemic treatment did not significantly improve the model fit (Wald's D1 test, *p*=0.194).

Performance and validation of model

The model was able to discriminate for EHRFS, RFS and OS, based on quartiles of predicted EHR risk (**Figure 1**). The 6-month EHR rates in the low, intermediate, high and very high risk patient groups were 6% [95%C.I. 4-10], 15% [95%C.I. 11-20], 20% [95%C.I. 16-25] and 32% [95%C.I. 26-38], respectively. Likewise, the model showed good discrimination for RFS and OS, with significant differences in survival among the risk groups.

The performance of the prediction model was assessed by calibration and discrimination. The estimated and observed risks for EHR or death were well-calibrated (**Figure 2**). The observed/expected ratio was 1.015 [95%C.I. 0.911-1.120], which is close to 1. For discrimination, Harrell's C-index through 6 and 12-months was 0.663 [95%C.I. 0.624-0.702] and 0.661 [95%C.I. 0.632-0.689], respectively, and similar for Uno's C-index. The 6 and 12-month area under the time-dependent ROC curves was 0.668 [95%C.I. 0.626-0.709] and 0.671 [95%C.I. 0.636-0.707], respectively (**Supplementary Figure 6**). Nagelkerke's R^2 was 0.094. The shrinkage factor obtained through internal validation was 0.86, with the shrunken HR in **Table 2**. The shrunken model yielded overoptimism-corrected 6-month risks for EHR or death between 5.9-56.0% (interquartile interval 12.9-22.0%). The optimism-adjusted Harrell's C-index through 6- and 12-months was 0.643 [95%C.I. 0.605-0.682] and 0.641 [95%C.I. 0.612-0.669]. The full model specifications are shown in **Appendix 1**.

Figure 1 Kaplan Meier plots for EHRFS (A), RFS (B) and OS (C) according to quartiles of predicted EHR risk groups (low, moderate, high and very high) .

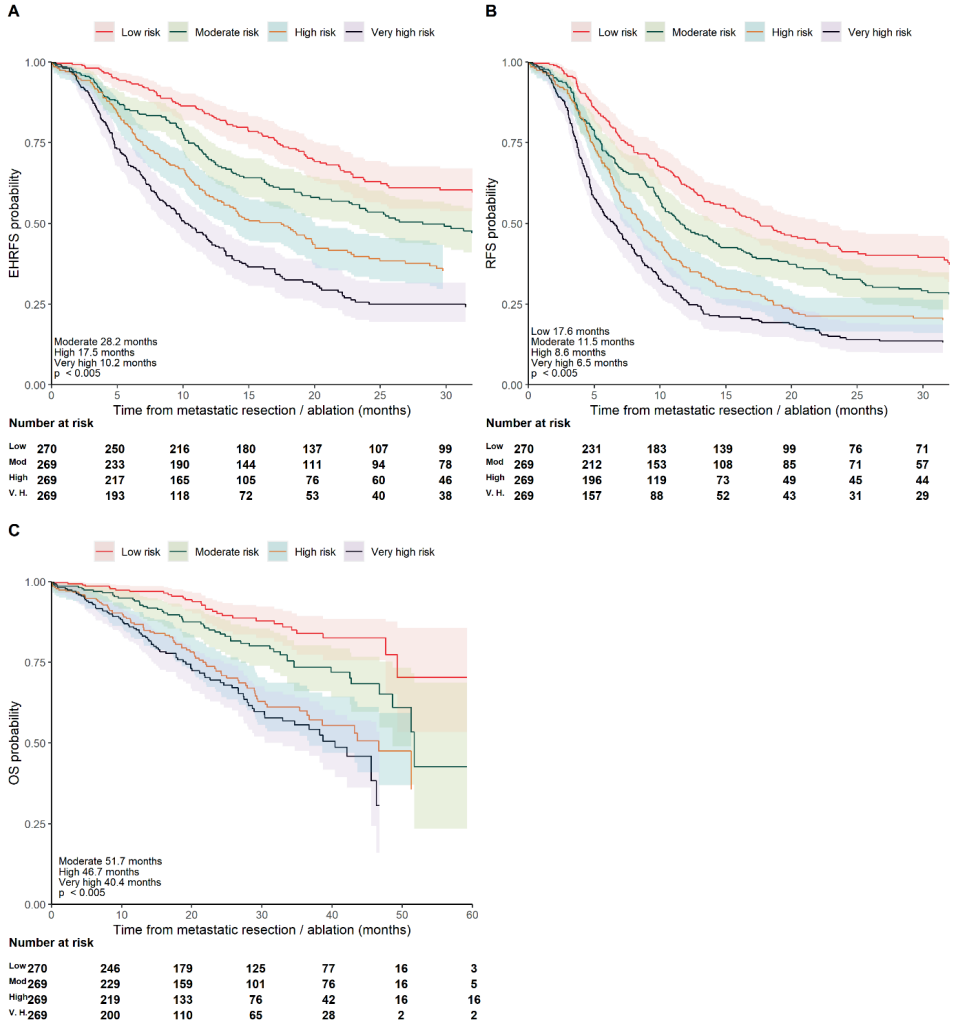

Figure 1. Kaplan Meier curves for quartiles of predicted EHR risk (low, moderate, high and very high risk) are shown for three survival endpoints (EHRFS, RFS and OS). Predicted EHR risk includes EHR or death as an event for EHRFS. The values in the plots summarize the median survival and 95% confidence intervals and the p-value for the log-rank test. The survival probabilities were pooled over the imputed datasets after complementary log-log transformation. If for a given group the median survival was not reached, it is not reported. *Abbreviations:* EHR (extrahepatic recurrence), EHRFS (extrahepatic recurrence-free survival), OS (overall survival), RFS (recurrence-free survival), V.H. (very high).

Figure 2 Calibration plot for extrahepatic recurrence.

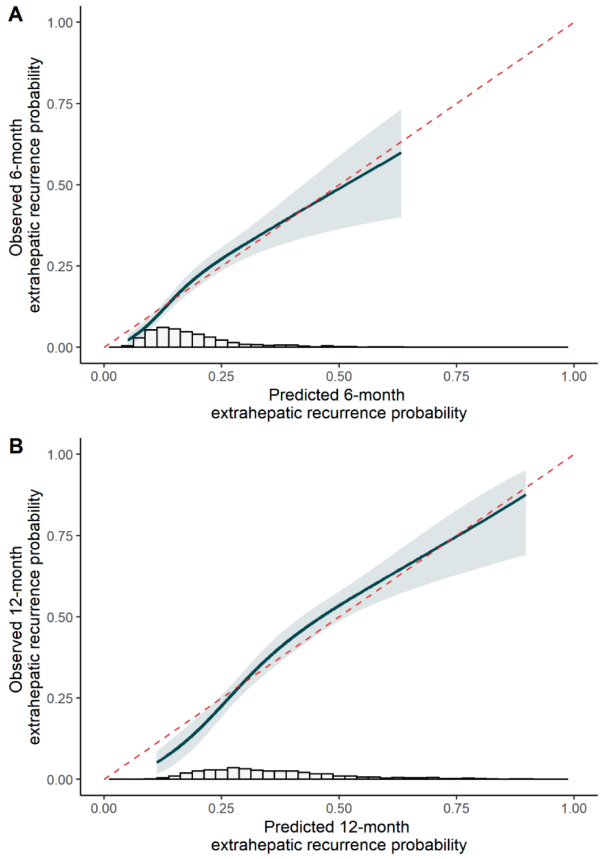

Figure 2. Calibration plots for the predicted 6-month (A) and 12-month (B) extrahepatic recurrence (EHR, which includes EHR or death as events for EHRFS) apparent probabilities versus the observed EHR probabilities are shown. The dashed red-line indicates perfect calibration, the solid green line the observed EHR probabilities with in light green the 95% confidence interval band. The histogram shows the distribution of the predicted EHR probabilities. The integrated calibration index is 0.015 (6-month EHR) and 0.028 (12-month EHR). The median absolute difference was 0.017 (6-month) and 0.030 (12-month), with a maximum absolute difference of 0.03 (6-month) and 0.06 (12-month). Abbreviations: EHR (extrahepatic recurrence), EHRFS (extrahepatic recurrence-free survival).

The model was further validated for generalizability by internal-external cross-validation using three geographical regions, which indicated that the models developed on the other regions showed adequate performance in each left-out geographical region, with some variation in the calibration slopes and observed/expected ratio, but less in the C-index (**Supplementary Figure 7**).

Decision curve analysis analyzing net benefit when using model-guided CRLM treatment decisions

We examined the potential net benefit of the model for clinical decision-making regarding local treatment of CRLM through decision curve analysis. EHR model-guided treatment of CRLM (compared to non-informed decision-making by treating all or no patients) results in net benefit for patients for 6-month EHR risk thresholds of 0-40% and for 12-month EHR risk thresholds of 0-60% (**Figure 3**).

Figure 3 Decision curve analysis for informed decision-making by selecting patients for local CRLM treatment according to the model's predicted EHR probability.

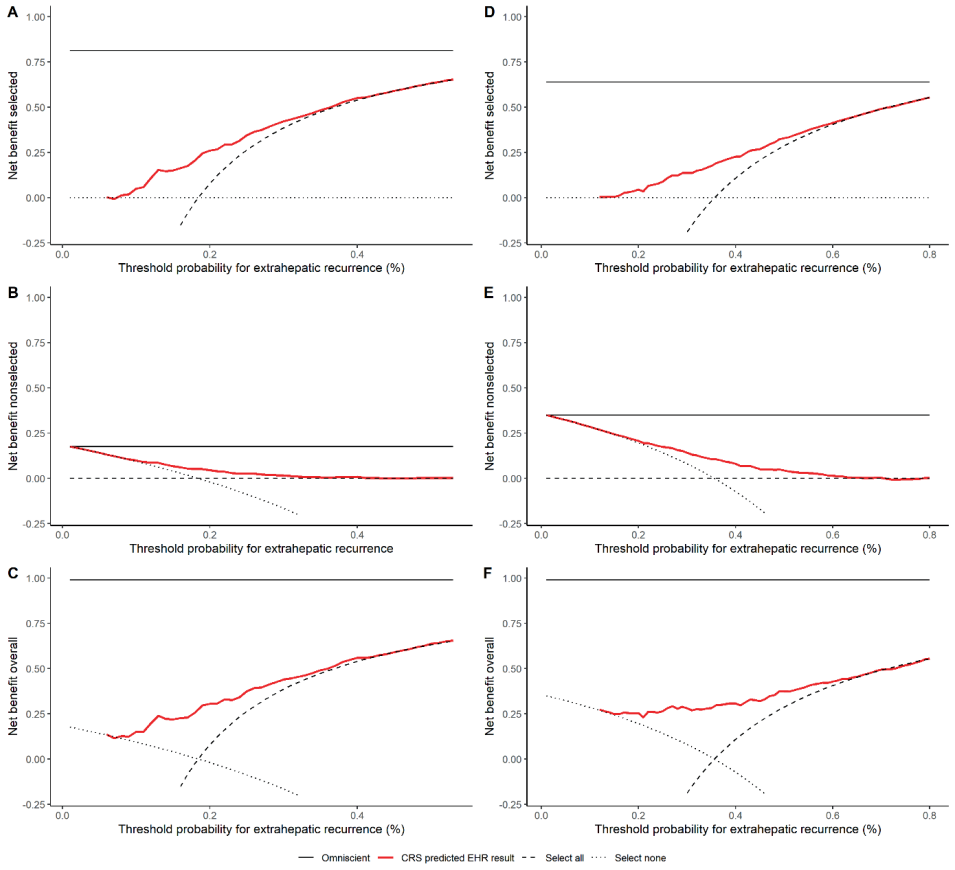

Figure 3. Decision curve analysis for informed decision-making by selecting patients for local CRLM treatment according to the model's predicted EHR probability. Decision curve analysis plots are shown indicating the net benefit obtained for a given threshold value for 6-month extrahepatic recurrence probability (A – C) and 12-month extrahepatic recurrence probability (D – F), which includes EHR or death as an EHRFS event. The net benefit was compared across 3 situations: non-informed decision-making (selecting all patients or selecting no patients (dashed

and dotted lines, respectively)) and for informed decision-making by selecting patients for local CRLM treatment according to the model's predicted EHRFS probability (red line). As comparison, the black line represents an omniscient model (all knowing model). **A & D.** The net benefit of treating patients with local treatment for CRLM ('selected patients') is determined using the true positives (patients with predicted EHRFS probability (p_{EHRFS}) above the threshold value and not having had an EHR) versus false positives (p_{EHRFS} > threshold and the patient did have an EHR) for a range of threshold values (0-1), with the benefit of false positives weighted relative to the threshold value. For consistency, the net benefit is shown for a range of thresholds for extrahepatic recurrence (extrahepatic recurrence probability = 1 – EHRFS probability). **B & E.** The net benefit of not treating patients with local treatment for CRLM ('nonselected patients') is determined using the true negatives (patients with p_{EHRFS} < threshold and having an EHR) versus false negatives (patients with p_{EHRFS} < threshold and not having had an EHR) for a range of threshold values (0-1), with the benefit of false negatives weighted relative to the threshold value. **C & F.** The overall net benefit is the sum of the net benefit of the selected and nonselected patients. *Abbreviations:* EHR (extrahepatic recurrence), EHRFS (extrahepatic recurrence-free survival).

Discussion

We developed a prediction model for early EHR in a nationwide, population-based cohort of patients with local treatment of CRLM. The model incorporated tumour *RAS/ BRAF*^{V600E} mutational status and sidedness of primary tumour alongside traditional prognostic factors. Early EHR after local CRLM treatment is of major clinical importance and can be meaningfully predicted with routine clinical information. Our EHR prediction model discriminates between patients based on EHR rates, reflected in differing EHRFS, RFS and OS. The EHR prediction model's expected generalizability is good.

Prediction models are increasingly used and can facilitate shared risk-informed decision-making for interventions, manage patient expectations or select patients for inclusion in trials. However, clinical application of available prediction models for local CRLM treatment is hampered by lack of generalizability, loss of predictive performance by simplification of models (e.g. categorizing continuous variables) and low clinical utility.[37] Published models were developed to predict RFS and OS. With increasing possibilities for repeated CRLM resections of recurrences with favorable survival outcomes[16,17], site-agnostic RFS and OS become a less relevant outcome for prediction models. Our study confirmed that about half of patients have a liver-limited first recurrence and experience long-term survival. Although RFS and OS are meaningful outcomes to manage expectations, EHRFS as outcome may guide clinical decisions for patients with CRLM.

To our knowledge, our model is the first to predict early EHR in patients after local treatment of CRLM. Local CRLM treatment should ideally be avoided in patients who experience an early EHR (18% of patients). These patients evidently have systemic disease, have a poor prognosis and are often not eligible for repeated local

treatment.[18-23] The poor OS we demonstrated in patients with early EHR (19.5 months in landmark analysis) is comparable to the expected OS of mCRC patients undergoing palliative systemic treatment.[42] Patients at high risk for early EHR are therefore unnecessarily exposed to potential perioperative risks and may be harmed by delaying palliative systemic treatment. The EHR prediction model can be used to confirm that local treatment should be pursued in low-risk patients. However, it is currently difficult for the EHR prediction model to identify patients with a sufficiently high predicted risk which would justify avoiding local CRLM treatment. The EHR prediction model may also aid clinical decision-making by identifying moderate/high-risk patients for early EHR who may benefit from perioperative systemic treatment. A treatment strategy for these patients may be to initiate long-lasting systemic treatment, and upon sustained response, perform local treatment of CRLM. Once externally validated, the EHR model will lend well for studies examining the optimal treatment by stratifying patients who are at moderate/high risk for early EHR.

The strength of our study is that our EHR prediction model was developed in a nationwide cohort of patients encompassing 39 academic, teaching and regional hospitals, and is thus representative for a general CRLM population undergoing local CRLM treatment. The cohort had minimal (<1%) loss to follow-up, likely not affecting its generalizability. Furthermore, the EHR prediction model included *RAS* and $BRAF^{V600E}$ mutational status, novel prognostic factors for outcomes after local treatment of CRLM. As not routinely available for all patients, we performed additional mutation analysis (resulting in 65% available) and handled remaining missing data by multiple imputation. *RAS* and $BRAF^{V600E}$ mutations are associated with an increased incidence of EHR.[29,30] Patients with $BRAF^{V600E}$ mutations and early unsalvageable recurrences have a poor survival after local treatment of CRLM.[13,29,43] Effective palliative systemic treatments are available for $BRAF^{V600E}$ mutated mCRC, further emphasizing the need to include *BRAF* mutational status in prediction models for local CRLM treatment. Only three prediction models included *RAS* and *BRAF* mutation status[13–15], potentially due to the low prevalence of *BRAF* mutations in patients with local treatment of CRLM (approximately 2%).[13] In contrast to previous studies[28,44], there was no interaction between neoadjuvant treatment status and *RAS* mutational status in our cohort.

However, our study has some limitations. Firstly, patients in our cohort were selected based on primary tumour diagnosis in 2015 and 2016 with subsequent local treatments of CRLM until January 2019. Thus, our cohort does not include metachronous disease with a DFI beyond four years. Secondly, our prediction model does not and could not robustly specify site of recurrence in patients, which may be relevant since patients with lung-only recurrences might be able to undergo local treatment and experience long-term survival, although this practice is based on retrospective highly selected patient series with small numbers.[45,46] Although we were unable to externally validate our

prediction model beyond the internal-external cross-validation, the full EHR prediction model specifications have been provided to facilitate external validation in other patient cohorts.

The performance of our model could be further improved by including additional promising histopathologic or tumour genetic features which may better identify high-risk patients.[15] Examples include distinct histopathological growth patterns, the Immunoscore (based on T-cell infiltration), a six-gene panel and liquid biopsies (detecting circulating tumour DNA).[47–50] Incorporating these additional features in an updated prediction model for local CRLM treatment may help identify patients at sufficiently high-risk for early EHR to optimize the treatment strategy for these patients.

Conclusion

We analysed population-based outcomes of patients after local treatment (resection and/or ablation) of CRLM. Early EHR is a valuable and informative alternative endpoint for prediction models. The EHR prediction model, including *RAS*/*BRAF*^{V600E} mutation status and sidedness, showed robust performance in discriminating between patients based on EHR probability, reflected in differing EHRFS, RFS and OS. After further external validation, the EHR prediction model might offer guidance in clinical decision-making in patients with resectable CRLM.

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References

1. Zhou H, Liu Z, Wang Y, Wen X, Amador EH, Yuan L, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther. 2022;7(70):1–25.
2. Kanas GP, Taylor A, Primrose JN, Langeberg WJ, Kelsh MA, Mowat FS, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. Clin Epidemiol. 2012;4:283–301.
3. Andreou A, Aloia TA, Brouquet A, Dickson PV, Zimmitti G, Maru DM, et al. Margin status remains an important determinant of survival after surgical resection of colorectal liver metastases in the era of modern chemotherapy. Ann Surg. 2013;257(6):1079–88.
4. Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020;141:225–38.
5. de Jong MC, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, 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. 2009;250(3):440–8.
6. Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol. 2009;27(22):3677–83.
7. Imai K, Allard MA, Benitez CC, Vibert E, Sa Cunha A, Cherqui D, et al. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21(7):887–94.
8. Okuno M, Goumard C, Kopetz S, Vega EA, Joechle K, Mizuno T, et al. RAS Mutation is Associated with Unsalvageable Recurrence Following Hepatectomy for Colorectal Cancer Liver Metastases. Ann Surg Oncol. 2018;25(8):2457–66.
9. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical Score for Predicting Recurrence After Hepatic Resection for Metastatic Colorectal Cancer. Ann Surg. 1999;230(3):309.
10. Margonis GA, Sasaki K, Gholami S, Kim Y, Andreatos N, Rezaee N, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105(9):1210–20.
11. Paredes AZ, Hyer JM, Tsilimigras DI, Moro A, Bagante F, Guglielmi A, et al. A Novel Machine-Learning Approach to Predict Recurrence After Resection of Colorectal Liver Metastases. Ann Surg Oncol. 2020;27(13):5139–47.
12. Brudvik K.W., Jones R.P., Giuliante F., Shindoh J., Passot G., Chung M.H., et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;269(1):120–6.
13. 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;168:25–33.
14. Chen Y, Chang W, Ren L, Chen J, Tang W, Liu T, et al. Comprehensive Evaluation of Relapse Risk (CERR) Score for Colorectal Liver Metastases: Development and Validation. Oncologist. 2020;25(7):e1031–41.
15. Lang H, Baumgart J, Heinrich S, Tripke V, Passalaqua M, Maderer A, et al. Extended Molecular Profiling Improves Stratification and Prediction of Survival After Resection of Colorectal Liver Metastases. Ann Surg. 2019;270(5):799–805.
16. Nanji S, Tsang ME, Wei X, Booth CM. Outcomes after repeat hepatic resection for recurrent metastatic colorectal cancer: A population-based study. Am J Surg. 2017;213(6):1053–9.
17. Matsuoka H, Morise Z, Tanaka C, Hayashi T, Ikeda Y, Maeda K, et al. Repeat hepatectomy with systemic chemotherapy might improve survival of recurrent liver metastasis from colorectal cancer-a retrospective observational study. World J Surg Oncol. 2019;17(1):33.
18. Isoniemi H, Uutela A, Nordin A, Lantto E, Kellokumpu I, Ovissi A, et al. Centralized repeated resectability assessment of patients with colorectal liver metastases during first-line treatment: prospective study. Br J Surg. 2021;108(7):817–25.
19. Battula N, Tsapralis D, Mayer D, Isaac J, Muiesan P, Sutcliffe RP, et al. Repeat liver resection for recurrent colorectal metastases: A single-centre, 13-year experience. HPB. 2014;16(2):157–63.

20. Butte JM, Gönen M, Allen PJ, Peter Kingham T, Sofocleous CT, DeMatteo RP, et al. Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection. Ann Surg Oncol. 2015;22(8):2761–71.
21. Malik HZ, Gomez D, Wong V, Al-Mukthar A, Toogood GJ, Lodge JPA, et al. Predictors of early disease recurrence following hepatic resection for colorectal cancer metastasis. Eur J Surg Oncol. 2007; 33(8):1003–9.
22. Vigano L, Capussotti L, Lapointe R, Barroso E, Hubert C, Giuliante F, 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. 2014;21(4):1276–86.
23. Stewart CL, Warner S, Ito K, Raoof M, Wu GX, Kessler J, et al. Cytoreduction for colorectal metastases: liver, lung, peritoneum, lymph nodes, bone, brain. When does it palliate, prolong survival, and potentially cure? Curr Probl Surg. 2018;55(9):330–79.
24. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, 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. 2013; 14(12):1208–15.
25. Kanemitsu Y, Shimizu Y, Mizusawa J, Inaba Y, Hamaguchi T, Shida D, 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. 2021;39(34):3789–99.
26. Buisman FE, Galjart B, Buettner S, Groot Koerkamp B, Grunhagen 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. 2020;22(3):351–7.
27. Russolillo N, Sperti E, Langella S, Menonna F, Allieta A, Di Maio M, et al. Impact of primary tumor location on patterns of recurrence and survival of patients undergoing resection of liver metastases from colon cancer. Hpb. 2020;22(1):116–23.
28. Takeda Y, Mise Y, Takahashi Y, Ito H, Inoue Y, Yoshioka R, et al. Limited Prognostic Value of KRAS in Patients Undergoing Hepatectomy for Colorectal Liver Metastases. Ann Surg Oncol. 2022;29(4):2383–91.
29. Kobayashi S, Takahashi S, Takahashi N, Masuishi T, Shoji H, Shinozaki E, et al. Survival Outcomes of Resected BRAF V600E Mutant Colorectal Liver Metastases: A Multicenter Retrospective Cohort Study in Japan. Ann Surg Oncol. 2020;27(9):3307–15.
30. Schirripa M, Bergamo F, Cremolini C, Casagrande M, Lonardi S, Aprile G, et al. BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. Br J Cancer. 2015;112(12):1921–8.
31. (IKNL) N.C.C.O. Netherlands Cancer Registry (NCR) [Internet]. Available from: https://iknl.nl/en/ncr
32. Moons KGM, Altman DG, Reitsma JB, Ioannidis JPA, Macaskill P, Steyerberg EW, et al. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): Explanation and elaboration. Ann Intern Med. 2015;162(1):W1–73.
33. Reddy SK, Barbas AS, Turley RS, Steel JL, Tsung A, Marsh JW, et al. A standard definition of major hepatectomy: resection of four or more liver segments. HPB. 2011;13(7):494–502.
34. Mekenkamp LJ, Koopman M, Teerenstra S, van Krieken JH, Mol L, Nagtegaal ID, et al. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010;103(2):159–64.
35. Nederlandse Vereniging voor Heelkunde. Colorectaal Carcinoom (CRC) (version 2019) [Internet]. Available from: file:///H:/Downloads/Colorectaal_carcinoom_CRC_ (1).pdf
36. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol. 2007;29(1):19–24.
37. Bolhuis K, Wensink GE, Elferink MAG, Bond MJG, Dijksterhuis WPM, Fijneman RJA, et al. External Validation of Two Established Clinical Risk Scores Predicting Outcome after Local Treatment of Colorectal Liver Metastases in a Nationwide Cohort. Cancers (Basel). 2022;14(10):2356.
38. Riley RD, Snell KIE, Ensor J, Burke DL, Harrell FE, Moons KGM, et al. Minimum sample size for developing a multivariable prediction model: PART II - binary and time-to-event outcomes. Stat Med. 2019;38(7): 1276–96.

39. Yamashita S, Chun YS, Kopetz SE, Vauthey JN. Biomarkers in colorectal liver metastases. Br J Surg. 2018;105(6):618–27.
40. van Buuren S, Groothuis-Oudshoorn K, Vink G, Doove L, Jolani S, Schouten R, et al. mice: Multivariate Imputation by Chained Equations in R. R package. J Stat Softw. 2011;45(3):1–67.
41. Rousson V, Zumbrunn T. Decision curve analysis revisited: Overall net benefit, relationships to ROC curve analysis, and application to case-control studies. BMC Med Inform Decis Mak. 2011;11(45):1–9.
42. Cremolini C, Antoniotti C, Rossini D, Lonardi S, Loupakis F, Pietrantonio F, et al. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, rand. Lancet Oncol. 2020;21(4):497–507.
43. Uutela A, Osterlund E, Halonen P, Kallio R, Ålgars A, Salminen T, et al. Resectability, conversion, metastasectomy and outcome according to RAS and BRAF status for metastatic colorectal cancer in the prospective RAXO study. Br J Cancer. 2022;127(4):686-94
44. Margonis GA, Kim Y, Sasaki K, Samaha M, Buettner S, Amini N, et al. Activating KRAS mutation is prognostic only among patients who receive preoperative chemotherapy before resection of colorectal liver metastases. J Surg Oncol. 2016;114(3):361–7.
45. Shah SA, Haddad R, Al-Sukhni W, Kim RD, Greig PD, Grant DR, et al. Surgical resection of hepatic and pulmonary metastases from colorectal carcinoma. J Am Coll Surg. 2006;202(3):468–75.
46. Neeff H, Hörth W, Makowiec F, Fischer E, Imdahl A, Hopt UT, et al. Outcome after resection of hepatic and pulmonary metastases of colorectal cancer. J Gastrointest Surg. 2009;13(10):1813–20.
47. Vigano L, Banciforte B, Laurenti V, Costa G, Procopio F, Cimino M, et al. The Histopathological Growth Pattern of Colorectal Liver Metastases Impacts Local Recurrence Risk and the Adequate Width of the Surgical Margin. Ann Surg Oncol. 2022;S709–10.
48. Wang R, Dai W, Gong J, Huang M, Hu T, Li H, et al. Development of a novel combined nomogram model integrating deep learning-pathomics, radiomics and immunoscore to predict postoperative outcome of colorectal cancer lung metastasis patients. J Hematol Oncol. 2022;15(1):4–9.
49. Wada Y, Shimada M, Morine Y, Ikemoto T, Saito Y, Baba H, et al. A transcriptomic signature that predicts cancer recurrence after hepatectomy in patients with colorectal liver metastases. Eur J Cancer. 2022;163:66–76.
50. Bidard FC, Kiavue N, Ychou M, Cabel L, Stern MH, Madic J, et al. Circulating tumor cells and circulating tumor DNA detection in potentially resectable metastatic Colorectal cancer: A prospective ancillary study to the Unicancer Prodige-14 Trial. Cells. 2019;8(516):1–13.

Supplementary materials

Table S1 Assumptions regarding systemic treatment and survival outcomes.

Assumptions regarding progression of disease and survival:
Date of new episode is considered as time of progression.
When disease progression is documented < 14 days of liver resection we assume this was part of the liver resection and first new episode is considered as time of progression.
Recurrence-free survival (RFS) is calculated from date of first liver procedure to date of progression. In case of 2-stage resection, RFS is calculated from last liver procedure.
RFS is calculated from date of first liver procedure to date of progression. In case of 2-stage resection, RFS is calculated from last liver procedure.
If no recurrence is registered: · If end of follow-up is registered and reason end of follow-up is: death, then date of death is registered as event of RFS; · If end of follow-up is registered and reason end of follow-up is other than death then RFS is censored on date of end of follow up; · If no date of end of follow-up is registered then RFS is censored on date of last visit; · If none of these dates are registered then RFS is documented as missing.
Extrahepatic recurrence-free survival (EHRFS) is calculated from date of first liver procedure to date of progression. In case of 2-stage resection, EHRFS is calculated from last liver procedure.
If no extrahepatic recurrence is registered: · If end of follow-up is registered and reason end of follow up is: death, then date of death is registered as event of EHRFS; · If end of follow-up is registered and reason end of follow up is other than death then EHRFS is censored on date of end of follow up; · If no date of end of follow-up is registered then EHRFS is censored on date of last visit; · If no last visit is registered but event for RFS is registered then EHRFS is censored on date of RFS; · If none of these dates are registered then EHRFS is documented as missing.
Extrahepatic disease was defined as presence of disease outside the liver or metastasectomy outside the liver.
Lymph node metastases registered as abdominal lymph nodes at time of first liver metastases were considered extrahepatic disease and as so classified as not-liver only disease.
Overall survival (OS) is calculated from date of diagnosis of metastatic disease.
Patients who did not die are censored on the date last known to be alive in the GBA (the municipal population registry).
OS after resection is calculated from date of first liver procedure. In case of 2-stage resection, OS is calculated from date of last liver procedure.
Assumptions regarding patient/tumour characteristics
Primary tumour nodal stage was defined primarily on pathologic N-stage. When pN stage was missing, cN stage (radiological) was used.
If number of metastases was not given and code 77 was used (accounting for diffuse metastatic disease in the liver) then number of metastases was scored as 20.
If performance status was missing, this was scored as 0-1 because patients were considered physically good enough for resection.
RAS and *BRAF* mutation are considered mutual exclusive, therefore patients with *RAS* mutations or *BRAF* mutations, were assumed to have *BRAF*-wildtype or *RAS*-wildtype status, retrospectively.
Assumptions regarding systemic treatment regimens and strategies:
Systemic treatment includes both chemotherapy and/or targeted therapy.
A combination regimen is defined as all systemic agents starting within 4 weeks after start of the first agent and started before progression of disease.
If bevacizumab was started more than 4 weeks after the start of the first agent but before stop of this agent and before progression of disease, we assume bevacizumab was part of this combination regimen.
If a treatment line continues despite of progression, e.g., in case of reintroduction of the same or an equivalent regimen after a therapy break and detected progression, we regard this as continuation of the same treatment line.
If oxaliplatin only is registered, we assume this was part of a capecitabine and oxaliplatin (CAPOX) regimen of which capecitabine was not registered, so we add capecitabine. We assume this is due to a registration error, in which the administration of capecitabine has not been noticed by the data manager.

Table S1 (continued)

Systemic therapy was considered adjuvant systemic therapy for primary tumour when started < 12 weeks after resection of primary tumour and started before diagnosis of metastases in patients with metachronous disease.
Capecitabine monotherapy was considered radiosensitizer for primary tumour when started before primary tumour resection and before diagnosis of metastases and with notification to have received chemoradiotherapy.
Systemic therapy was considered pre-operative therapy (neo-adjuvant or induction) before liver resection when the therapy ended within 120 days before liver resection. Adjuvant therapy after resection of primary tumour or chemotherapy as radiosensitizer was excluded.
Systemic therapy was considered adjuvant therapy after liver resection when the therapy started within 120 days after liver resection. Chemotherapy as radiosensitizer was excluded.
Systemic therapy was considered peri-operative therapy of liver resection when the systemic therapy was given <120 days before and < 120 days after liver resection
When systemic therapy was given between two liver procedures before progression of disease, the first liver procedure was considered as staging procedure and systemic therapy was considered as pre-operative systemic therapy (neo-adjuvant or induction) for surgery 2
Treatment strategies are categorized as follows: · Treatment regimens containing chemotherapy, without targeted therapy, subdivided in: monotherapy (1 chemotherapy agent), doublets (2 chemotherapy agents) and triplets (3 chemotherapy agents); · Treatment regimens containing targeted therapy with or without chemotherapy, subdivided in: bevacizumab-containing regimens, and anti-EGFR targeted therapy-containing regimens.
Systemic therapy regimens are categorized as follows: · Fluoropyrimidine monotherapy (e.g. 5-fluorouracil [5-FU], capecitabine); · Oxaliplatin-based doublet therapy (e.g. capecitabine + oxaliplatin (CAPOX), 5-FU/oxaliplatin [FOLFOX]); · Irinotecan-based doublet therapy (e.g. capecitabine + irinotecan (CAPIRI), 5-FU/irinotecan [FOLFIRI]) · Triplet systemic therapy (5-fluorouracil [5-FU], oxaliplatin and irinotecan) · Targeted therapy (anti-EGFR therapy; cetuximab or panitumumab, and bevacizumab)
A treatment line is defined as systemic therapy (monotherapy or combination regimen) administered at the same time until suspension, regardless of reason for discontinuation.
Treatment is considered as next line if an agent of a new drug group is started that is not applied in the previous systemic treatment regimen.
If the same or an equivalent systemic treatment regimen is (re)started, this is considered continuation of the same treatment line, e.g., CAPOX to FOLFOX.
Assumptions regarding systemic treatment lines:
A treatment line is defined as systemic therapy (monotherapy or combination regimen) administered at the same time until suspension, regardless of reason for discontinuation.
Treatment is considered as next line if an agent of a new drug group is started that is not applied in the previous systemic treatment regimen.
If the same or an equivalent systemic treatment regimen is (re)started, this is considered continuation of the same treatment line, e.g. CAPOX to FOLFOX.
Assumptions regarding local treatment
Local treatments are categorized as follows: 1 stage (1 procedure); 2-stage (2 procedures < 120 days apart)
R-status: · when two stage procedure and first procedure was R2 resection and second procedure was R1/R0 resection then 2-stage resection considered as R-status of last procedure. · when 2-stage resection and one procedure was R1 resection and other local treatment was R0 resection then considered as R1 resection.

Abbreviations: 5-FU (5-flouruoracil), CAPIRI (capecitabine + irinotecan), CAPOX (capecitabine + oxaliplatin), cN (radiological nodal-stage), EGFR (epidermal growth factor receptor), EHRFS (extrahepatic recurrence-free survival), FOLFIRI (5-FU + irinotecan), FOLFOX (5-FU + oxaliplatin), GBA (the municipal population registry), OS (overall survival), pN (pathological nodal-stage), RFS (recurrence-free survival).

Figure S1 Kaplan-Meier analysis showing RFS, EHRFS and OS curves of the total cohort.

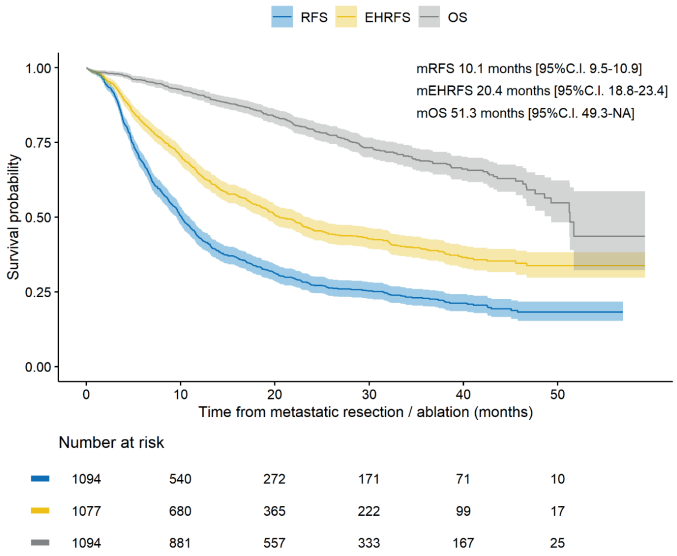


Figure S1. Kaplan Meier survival curves with 95% confidence intervals are shown for RFS, EHRFS and OS for the whole cohort. The number of patients at risk is indicated in the risk table. *Abbreviations:* EHRFS (extrahepatic recurrence-free survival), mEHRFS (median EHRFS), mOS (median OS), mRFS (median RFS), OS (overall survival), RFS (recurrence-free survival).

Table S2 Detailed information about first recurrence and first extrahepatic recurrence.

	Total cohort *n*=1105 n (%)
RFS data	
Event	
No	287 (26.0)
Yes	807 (73.0)
Recurrence	765
Death	42
Missing	11 (1.0)
Site of first recurrence, *n = 765*	
Liver-only	332 (43.3)
Extrahepatic	399 (52.2)
Missing site of recurrence	34 (4.4)
EHRFS data	
Extrahepatic event during follow up	
No	520 (48.3)
Yes	557 (51.7)
Recurrence	478
Death	79
Missing	28 (2.5)
Site of first extrahepatic recurrence, *n = 478*	
Lung	213 (44.6)
Intra-abdominal	55 (11.5)
Lymph nodes	55 (11.5)
Bone	5 (1.0)
Genito-urinary tract	5 (1.0)
Soft tissue	12 (2.5)
Brain with/without other sites	6 (1.3)
Multiple extrahepatic sites	127 (26.6)

Abbreviations: n (count), RFS (recurrence-free survival), EHRFS (extrahepatic recurrence-free survival).

Figure S2 Post-recurrence overall survival of patients according to site of extrahepatic recurrence.

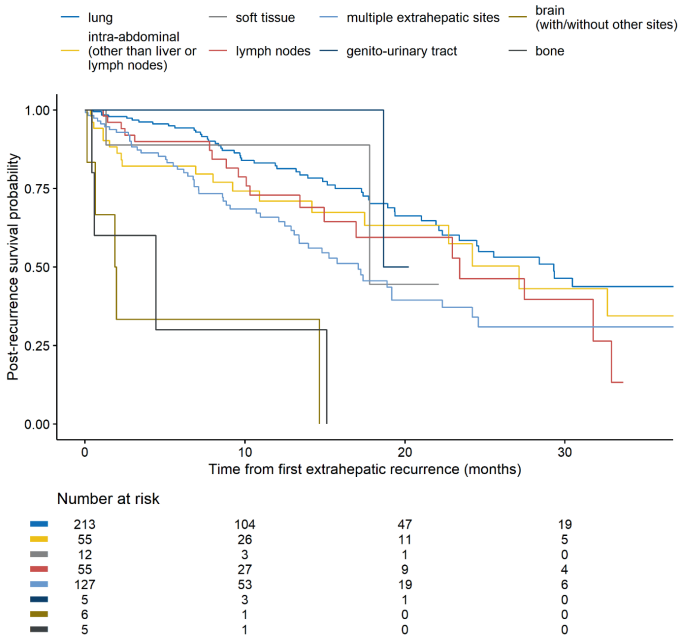


Figure S2. A KM plot of post-recurrence survival in patients according to the first site of extrahepatic recurrence. Categories indicate the site of extrahepatic metastasis, but may also include hepatic localization. A log-rank test for the post-recurrence survival probability per site of extrahepatic recurrence was performed (p<0.0001). A log-rank test for the time to recurrence per site of extrahepatic recurrence was performed (p=0.55).

Figure S3 Landmark analysis at six months showing Kaplan-Meier curves of patients with extrahepatic, intrahepatic-only and no recurrences within six months.

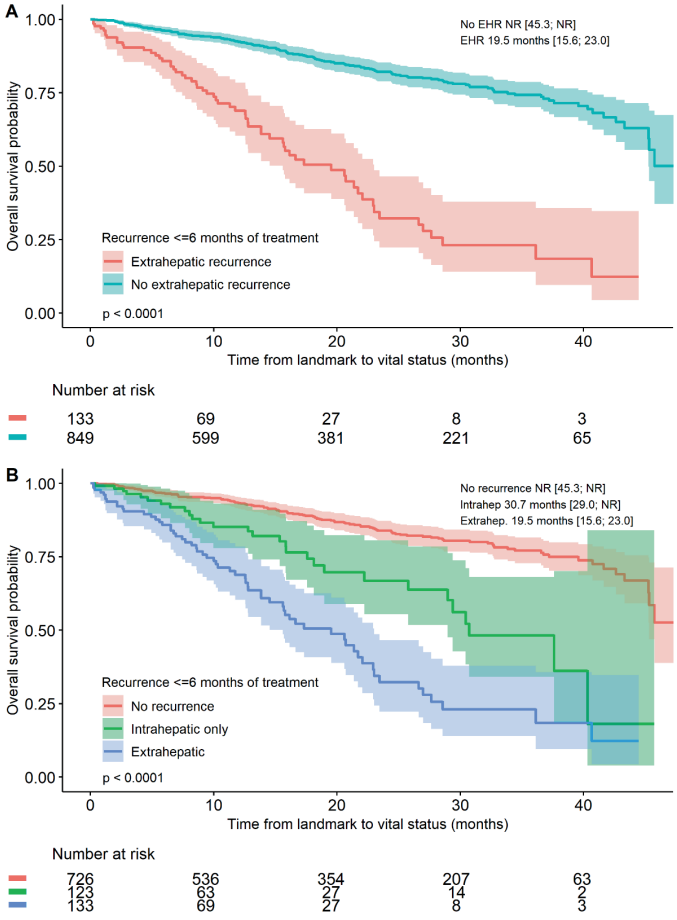
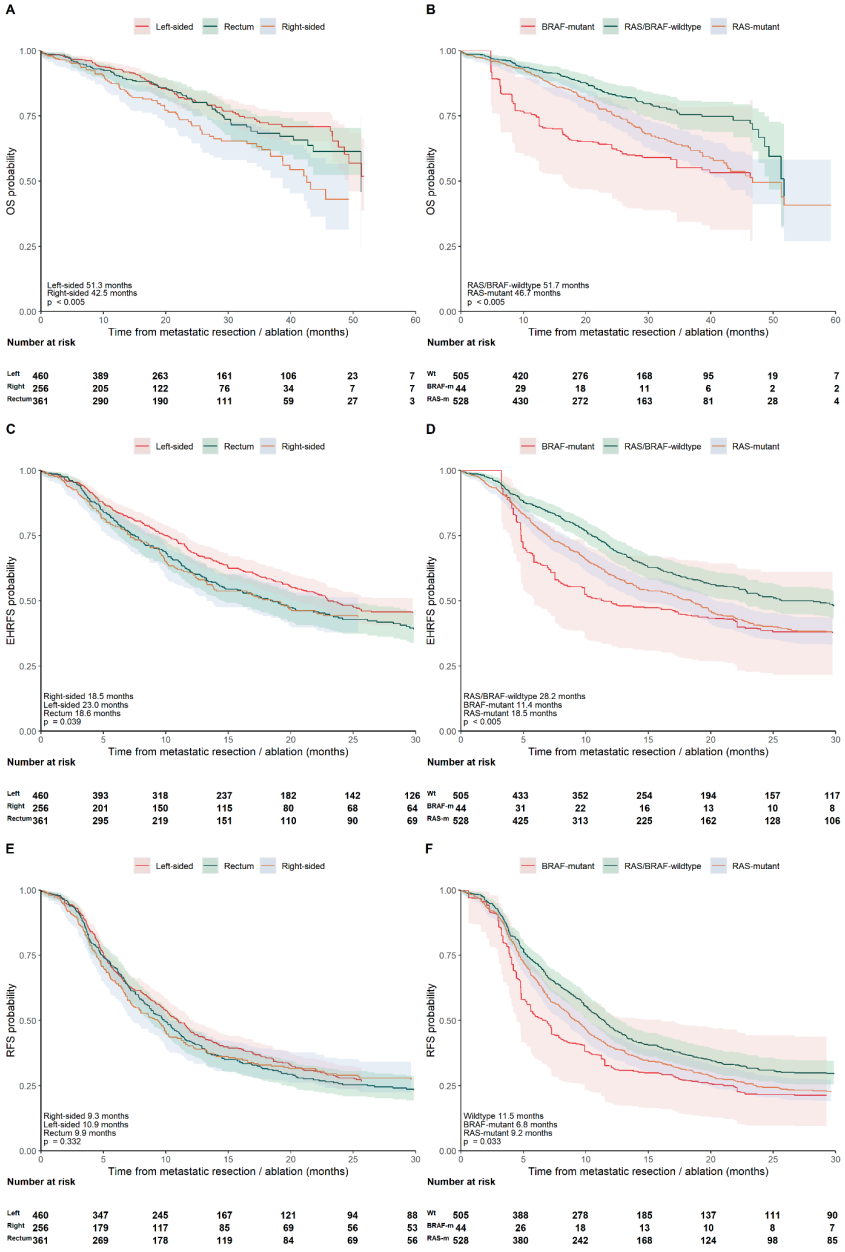


Figure S3. A landmark analysis Kaplan Meier survival plot is shown, indicating the overall survival of patients after the landmark point (6 months after local treatment of CRLM), according to the site of recurrence which patients had experienced within 6 months of local treatment of CRLM. A. The 2 groups are extrahepatic recurrence (which includes death as an event) versus no extrahepatic recurrence. B. The 3 recurrence site groups are: no recurrence, intrahepatic recurrence only, extrahepatic recurrence (including *n=100* intra + extrahepatic recurrence). The log-rank *p*-value is indicated in the plot along with the observed median survival and 95% confidence intervals. *Abbreviations:* EHR (extrahepatic recurrence), n (count), NR (not reached).

Figure S4 Kaplan-Meier curves describing post-resection overall survival (A and B), and recurrence-free survival (C and D) and extrahepatic recurrence-free survival (E and F) in the total cohort according to location of primary tumour (A, C, D) and *RAS/BRAF* mutational status (B, D, F) using the imputed dataset. The observed median survival and 95% confidence intervals are indicated in the plot and not indicated if not reached within the follow-up period.



Abbreviations: BRAF-m (*BRAF*-mutant), EHRFS (extrahepatic recurrence-free survival), OS (overall survival), *RAS*-m (*RAS*-mutant), RFS (recurrence-free survival), Wt (*RAS/BRAF*-wildtype).

Figure S5 Hazard ratio for EHRFS for continuous variables modeled non-linearly using restricted cubic splines.

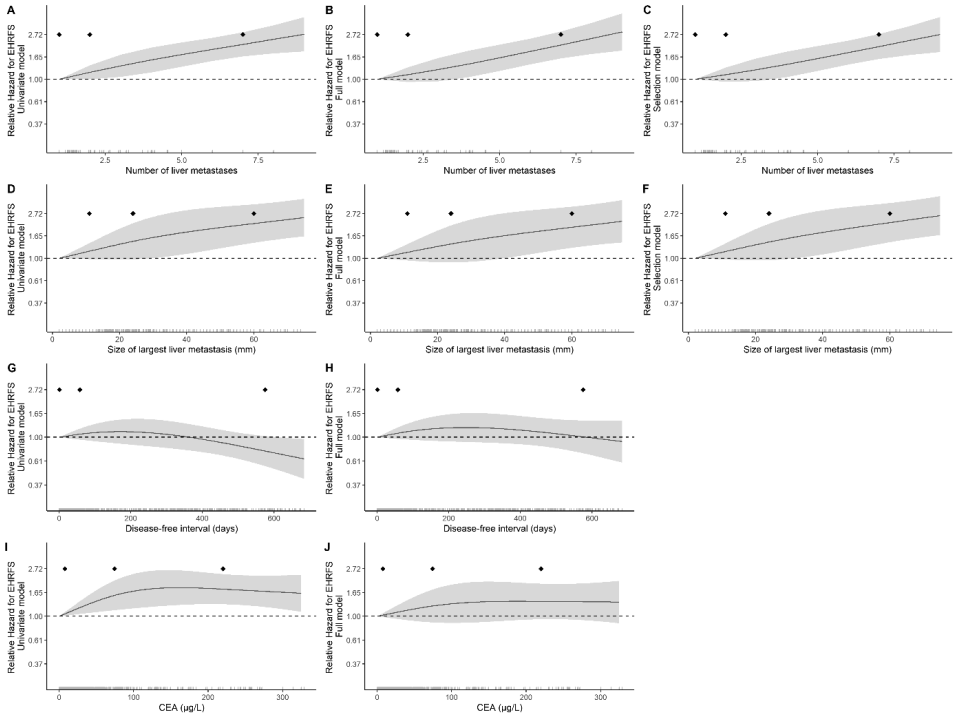


Figure S5**.** The pooled relative hazard and 95% confidence interval ribbon for EHRFS within 12 months from local treatment of CRLM according to one of three models (univariable, full multivariable and selection multivariable) are shown in the y-axis relative to the observed value of the continuous variable plotted on the x-axis. Each column indicates a model type with the univariable results in plots A, D, G, I; the full multivariable results in plots B, E, H, J; and the selection multivariable results in plots C and F (disease-free interval and pre-operative CEA were not included in the selection model). Each row shows a continuous variable, with number of liver metastases (A – C), size of largest liver metastasis (D – F), disease-free interval (G-H) and pre-operative CEA values (I-J). All 4 continuous variables were modeled using restricted cubic splines analysis with 3 knot positions (positions are indicated by the dots in the plot). The frequency of the observed values are indicated along the x-axis. *Abbreviations:* CEA (carcinoembryonic antigen), CRLM (colorectal liver metastasis), EHRFS (extrahepatic recurrence-free survival), mm (millimeter), µg/L (microgram per liter).

Figure S6 Time-dependent ROC curve for the EHRFS model indicating the true positives and false positives 6 and 12 months after local treatment for CRLM.

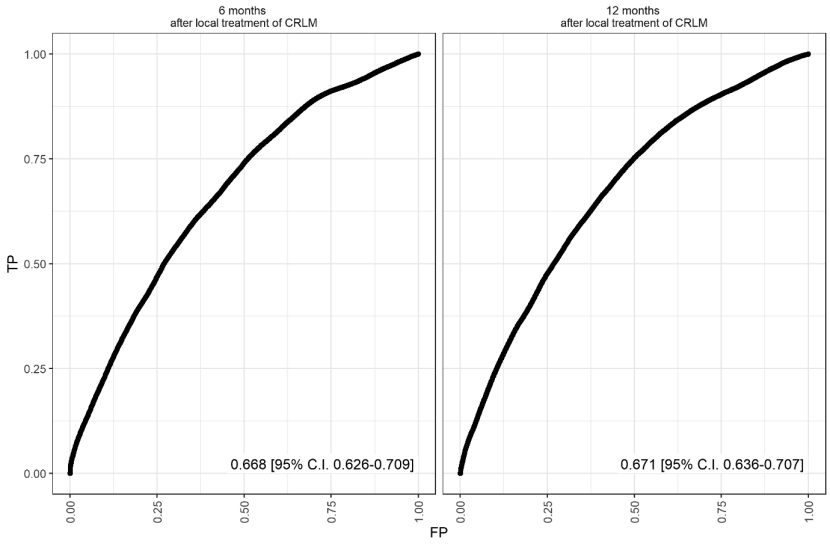


Figure S6. In a cumulative case/ dynamic control ROC analysis, the time-dependent receiver operator curve is shown with on the y-axis the true positive and the x-axis the false positive based on the model's linear predictor for each individual compared to the observed EHRFS at the given timepoint. The plot indicates how well the model predicts the survival time for the patients for 6 and 12 months after local treatment for CRLM, respectively. The AUC with 95% C.I. is indicated in each plot. The confidence intervals for AUC were calculated using 1000 bootstrap samples. *Abbreviations:* AUC (area under the (ROC) curve), C.I. (confidence interval), CRLM (colorectal liver metastasis), FP (false positive), ROC (receiver operator curve), TP (true positive).

Figure S7 Internal-external cross-validation results.

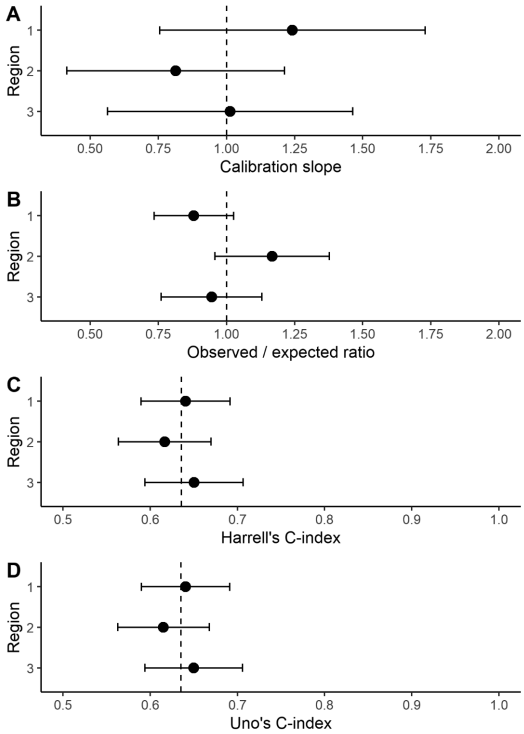


Figure S7**.** The results for internal-external cross-validation are shown, including the calibration slope (A), observed/expected ratio (B), Harrell's C-index (C) and Uno's C-index (D). We used internal-external cross-validation to evaluate the generalizability of the model. The data were split in three geographic regions and all modeling steps including backward selection of variables and internal validation were repeated in two of three regions, after which the performance of the overfitting-adjusted model was evaluated in the left-out geographical region (C-index, calibration slope and intercept). Each geographic region was left-out of model development once, resulting in three estimates of external validation. The reference line in plot C & D indicates the mean C-index. *Abbreviations:* C-index (calibration-index).

Supplemental methods

Early EHR as primary endpoint for the prediction model

By consensus of experts in the field (JR, RJS, KB, EW, JH, MK, CJAP), early EHR (within six months, conform previous publications[1,2]) was defined as the clinically relevant primary endpoint of the model, due to the poor prognosis in patients with early EHR and lower chance of repeat local treatment, in contrast to patients with liver-only recurrences. Thus, the added value of local treatment of CRLM may not be justified in patients with a rapid EHR after local treatment of CRLM by consensus of experts in the field.

Statistical analysis

We used standard descriptive statistics to describe baseline characteristics of the study population, including medians and interquartile intervals (IQI) for continuous variables, and frequency and percentages for categorical variables. Follow-up data and patient outcomes were described using (reverse) Kaplan Meier approaches. We assessed the prognostic impact of our primary endpoint occurrence of EHR ≤6 months after CRLM treatment using landmark analysis - which prevents immortal time bias - at six months after CRLM treatment and comparing the subsequent survival outcomes of three groups based on site of recurrence ≤6 months: no recurrence, intrahepatic only and EHR (which includes patients with intra- and extrahepatic recurrences).

We applied the recommendations published by Riley *et al.*[3], to determine the number and complexity of the candidate predictors (together amounting to the number of coefficients) to be evaluated in our prediction model. We used the C-index for the Comprehensive Evaluation of Relapse Risk (CERR) score[4] as the anticipated minimum C-index for our model, since it most closely represents our primary end-point. With an expected C-index of ≥0.695, and the observed EHR event rate within 12 months in our cohort, we had sufficient data to model 17 coefficients and fulfill the 3 criteria set by Riley *et al.*.[3]

Predictors were selected by assessment of a multidisciplinary team based on factors used in previous prediction models[5–8] and newly recognized prognostic factors.[9,10] Candidate predictors were blindly selected, prior to having analyzed the data. Nine candidate predictors were selected for model development, including 4 continuous variables that we aimed to model using three-knot restricted cubic splines (rcs) to allow for non-linearity (resulting in 17 coefficients): neoadjuvant systemic treatment, primary tumour location (left-sided, right-sided, rectum), T-status (T1-2, T3, T4), N-status (N0, N1, N2), *RAS/BRAF* mutational status (*RAS/BRAF*-wildtype, *RAS*-mutant, *BRAF*-mutant), number of liver metastases (continuous), size of largest liver metastasis (continuous),

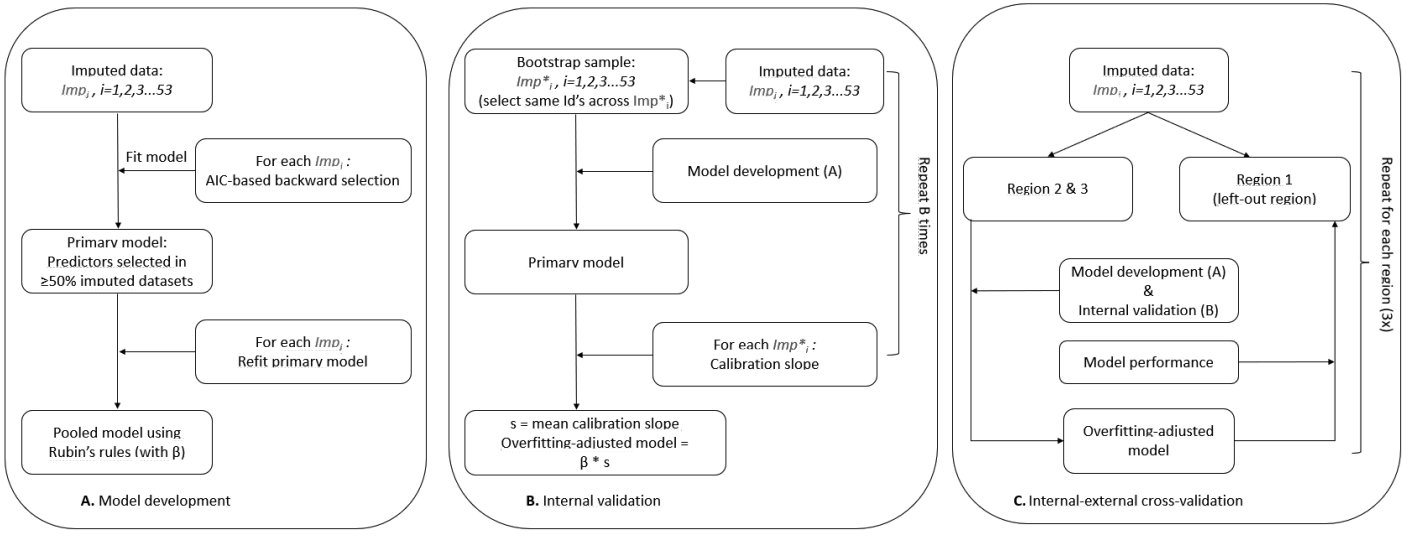
pre-operative CEA (continuous) and DFI (continuous). Continuous variables were Winsorized at the 95th percentile before analyses to decrease influential points. As certain candidate predictors had missing data, and merely removing patients with missing data leads to loss of information and potentially also to selection bias, we used multiple imputation using multivariate imputation by chained equations (MICE)[11], assuming missingness at random.

The imputation model contained all above selected candidate predictor variables including rcs transformations to accommodate congeniality, and included sidedness of the primary tumour, age, T-status, N-status, *RAS/BRAF^{V600E}* mutational status, number and size of liver metastases, serum CEA, DFI, systemic perioperative treatment type, R-status, tumour burden score (TBS[12], passively imputed in the model as auxiliary variables, as well as the primary outcome (EHRFS within 12 months using a Nelson-Aalen estimator and event indicator). We generated 53 imputed datasets, based on the percentage of patients with at least one missing variable in the candidate predictor variables set. To enable internal-external cross-validation without information leakage, we imputed the data separately for each geographical region.

Developing, validating and assessing performance of clinical risk score

Following multiple imputation, a prediction model for EHRFS within 12 months after local treatment of CRLM (EHRFS model) was created using Cox regression, ignoring follow-up information beyond 12 months. This 12-month time-horizon was chosen to allow a sufficient number of events for robust model-development based on the criteria by Riley *et al.*.[3] The primary evaluation of resulting model's performance was early EHR (≤6 months). Because of the limited evaluated follow-up period we did not evaluate deviations from the proportionality assumption.

The primary prediction model was developed in the whole cohort, using a Cox proportional hazards model with Akaike Information Criterion (AIC)-based backward selection in each imputed dataset leading to a primary model only including predictors selected in ≥50% of imputed datasets, which was then refitted in each imputed dataset to obtain a pooled selection model using Rubin's rules (**Supplementary Methods Figure 1**). The only variable that was not subjected to the above was adjuvant systemic therapy, which was included in all models using an offset for expected therapeutic efficacy (i.e. this effect was not estimated from the data but was imposed on the model by the offset). For the expected adjuvant systemic treatment effect we used the pooled random effects hazard ratio (HR) from known randomized controlled trials in the literature[13,14], resulting in a HR of 0.73.



Methods Figure S1 Model development, internal validation and internal-external cross-validation for development of prediction model.
A flowchart illustrating the steps in model development (A), internal validation (B) and internal-external cross-validation (C), adapted from 15,16. The EHRFS model was developed by performing AIC-based backward selection of all candidate predictors in each imputed dataset. The selection model included variables which were selected in ≥50% of the imputed datasets. The EHRFS model regression coefficients were pooled using Rubin's rules. Internal validation was performed using 500 bootstrap samples to determine the shrinkage factor and overfitting-adjusted model. The model development and internal validation was repeated for internal-external cross-validation, using geographical regions in the development and validation cohorts. The cross-validation was repeated for all regions, resulting in three performance measures. *Abbreviations:* AIC (Akaike Information Criteria), B (number of bootstrap samples), β (regression coefficient), *Imp** (bootstrap object of an imputed dataset), *Imp* (imputed dataset), s (shrinkage factor).

Model performance was assessed using calibration plots for 6 and 12 month EHR risk, discrimination (Harrell's C-index, Uno's C-index through 6 and 12 months), time-dependent receiver operator characteristic (ROC) curve, Nagelkerke's R^2 and decision-curve analysis. Each measure was determined for each imputed dataset separately and pooled using Rubin's rules (Supplementary Methods Figure 1). Model-predicted and Kaplan-Meier observed survival estimates (and 95% CI boundaries) were pooled after complementary log-log transformation, and Nagelkerke's R^2 was pooled after Fisher z-transformation. Decision curve analysis was used to assess the net benefit associated with CRLM treatment decisions based on a given threshold value for 6-month or 12-month EHRFS probability[17]. To visualize the potential relevance of the developed model we used Kaplan-Meier curves for EHRFS, RFS and OS, categorizing patients based on quartiles of (across-imputation dataset pooled) predicted EHR risk.

To quantify the overoptimism of the model regarding predicted risks and discriminative ability, we used internal validation by 500-fold bootstrap resampling, repeating all model-development steps in each bootstrap sample and testing the performance of the resulting models from each bootstrap in the original data. We derived a uniform shrinkage factor from internal validation that we applied to the apparent pooled regression coefficients of the primary model as fitted in the original data to create an overoptimism-corrected model (the offset for adjuvant systemic therapy was not shrunk). This overoptimism-corrected model yields predicted EHR probabilities that will agree more with actual risk in new patients. We similarly obtained overoptimism-corrected C-indexes that likely better reflect the actual discrimination of our model in new patients.

We used internal-external cross-validation to evaluate the generalizability of the model (Supplementary Methods Figure 1). The data were split in three geographic regions and all above described modeling steps including internal validation were repeated in two of three regions, after which the performance of the overfitting-adjusted model was evaluated in the left-out geographical region (C-index, calibration slope and intercept). Each geographic region was left-out of model development once, resulting in three estimates of external validation.

As an exploratory additional analysis, we tested whether the prognostic value of *RAS* mutation for EHRFS depended on the administration of preoperative systemic treatment which was reported[18,19], by using a D1 test between a multivariable model without and with a *RAS**preoperative systemic treatment interaction term. To avoid bias, two separate multiple imputation models were created for patients based on preoperative systemic treatment status solely for the exploratory analysis.

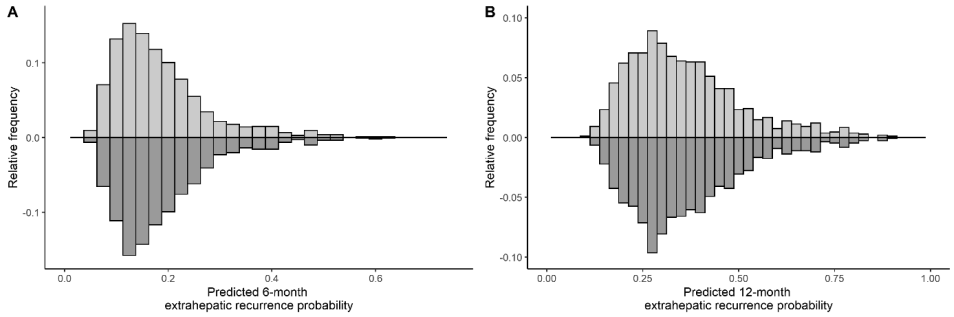
References

1. Malik H.Z., Gomez D., Wong V., Al-Mukthar A., Toogood G.J., Lodge J.P.A., et al. Predictors of early disease recurrence following hepatic resection for colorectal cancer metastasis. Eur J Surg Oncol. (2007);33(8):1003–9.
2. Vigano L., Capussotti L., Lapointe R., Barroso E., Hubert C., Giuliante F., 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. (2014);21(4):1276–86.
3. Riley R.D., Snell K.I.E., Ensor J., Burke D.L., Harrell F.E., Moons K.G.M., et al. Minimum sample size for developing a multivariable prediction model: PART II - binary and time-to-event outcomes. Stat Med. (2019);38(7):1276–96.
4. Chen Y., Chang W., Ren L., Chen J., Tang W., Liu T., et al. Comprehensive Evaluation of Relapse Risk (CERR) Score for Colorectal Liver Metastases: Development and Validation. Oncologist. (2020);25(7):e1031–41.
5. Fong Y., Fortner J., Sun R.L., Brennan M.F., Blumgart L.H. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. (1999);230(3):309–21.
6. Margonis G.A., Sasaki K., Gholami S., Kim Y., Andreatos N., Rezaee N., et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. (2018);105(9):1210–20.
7. Paredes A.Z., Hyer J.M., Tsilimigras D.I., Moro A., Bagante F., Guglielmi A., et al. A Novel Machine-Learning Approach to Predict Recurrence After Resection of Colorectal Liver Metastases. Ann Surg Oncol. (2020);27(13):5139–47.
8. Brudvik K.W., Jones R.P., Giuliante F., Shindoh J., Passot G., Chung M.H., et al. RAS Mutation Clinical Risk Score to Predict Survival After Resection of Colorectal Liver Metastases. Ann Surg. (2019);269(1):120–6.
9. Buisman F.E., Galjart B., Buettner S., Groot Koerkamp B., Grunhagen D.J., Verhoef C. Primary tumor location and the prognosis of patients after local treatment of colorectal liver metastases: a systematic review and meta-analysis. HPB. (2020);22(3):351–7.
10. Yamashita S., Chun Y.S., Kopetz S.E., Vauthey J.N. Biomarkers in colorectal liver metastases. Br J Surg. (2018);105(6):618–27.
11. van Buuren S., Groothuis-Oudshoorn K., Vink G., Doove L., Jolani S., Schouten R., et al. mice: Multivariate Imputation by Chained Equations in R. R package. J Stat Softw. (2011);45(3):1–67.
12. Sasaki K., Morioka D., Conci S., Margonis G.A., Sawada Y., Ruzzenente A., et al. The Tumor Burden Score: A New "Metro-ticket" Prognostic Tool For Colorectal Liver Metastases Based on Tumor Size and Number of Tumors. Ann Surg. (2018);267(1):132–41.
13. Nordlinger B., Sorbye H., Glimelius B., Poston G.J., Schlag P.M., Rougier P., 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. (2013);14(12):1208–15.
14. Kanemitsu Y., Shimizu Y., Mizusawa J., Inaba Y., Hamaguchi T., Shida D., et al. A randomized phase II/III trial comparing hepatectomy followed by mFOLFOX6 with hepatectomy alone for liver metastasis from colorectal cancer: JCOG0603 study. J Clin Oncol. (2020);38(15_suppl):4005.
15. Steyerberg E.W., Harrell F.E. Prediction models need appropriate internal, internal-external, and external validation. J Clin Epidemiol. (2016);69:245–7.
16. Musoro J.Z., Zwinderman A.H., Puhan M.A., Ter Riet G., Geskus R.B. Validation of prediction models based on lasso regression with multiply imputed data. BMC Med Res Methodol. (2014);14(1):1–13.
17. Rousson V., Zumbrunn T. Decision curve analysis revisited: Overall net benefit, relationships to ROC curve analysis, and application to case-control studies. BMC Med Inform Decis Mak. (2011);11(45):1–9.
18. Takeda Y., Mise Y., Takahashi Y., Ito H., Inoue Y., Yoshioka R., et al. Limited Prognostic Value of KRAS in Patients Undergoing Hepatectomy for Colorectal Liver Metastases. Ann Surg Oncol. (2022);29(4): 2383-91.
19. Kobayashi S., Takahashi S., Takahashi N., Masuishi T., Shoji H., Shinozaki E., et al. Survival Outcomes of Resected BRAF V600E Mutant Colorectal Liver Metastases: A Multicenter Retrospective Cohort Study in Japan. Ann Surg Oncol. (2020);27(9):3307–15.

Appendix 1

Formulas to predict extrahepatic recurrence risk at 6 and 12 months following local treatment of CRLM

This appendix is added to the manuscript in line with the TRIPOD recommendations.
The distribution of patients with 6-month (A) and 12-month (B) predicted extrahepatic recurrence risk according to the apparent model is illustrated below. The top light grey values include an offset for patients who received adjuvant systemic treatment, whereas the lower dark grey values indicate the predicted probabilities if patients had not received adjuvant systemic treatment.



Adjuvant systemic therapy was included in the model using an offset for expected therapeutic efficacy (i.e. this effect was not estimated from the data but was imposed on the model by the offset) based on the published hazard ratio, resulting in a HR of 0.73.

A. As observed in the analyzed dataset.

Baseline cumulative hazard at 6-months:

$H_0(t_{6\ months}) = 0.0416$

Baseline cumulative hazard at 12-months:

$H_0(t_{12\ months}) = 0.0947$

Prognostic index (PI; linear predictor):

$$\begin{aligned} PI = & - 0.061 * X_{\text{Left-sided primary tumour location}} \\ & + 0.212 * X_{\text{Rectum primary tumour location}} \\ & + 0.198 * X_{\text{T3 upon diagnosis}} + 0.543 * X_{\text{T4}} \\ & + 0.200 * X_{\text{N1 upon diagnosis}} + 0.507 * X_{\text{N2}} \\ & + 0.758 * X_{\textit{BRAF}\text{-mutant}} + 0.510 * X_{\textit{RAS}\text{-mutant}} \\ & + f(\text{Number of liver metastases}) \\ & + f(\text{Size of largest liver metastasis}) \\ & - 0.314 * X_{\text{Adjuvant systemic treatment}} \end{aligned}$$

Where number of liver metastases and size of largest liver metastasis are described with a restricted cubic spline function:

f (Number of liver metastases) =
+ 0.101*Number of liver metastases
+ 0.00194*max(Number of liver metastases -1,0)^3
- 0.0023*max(Number of liver metastases-2,0)^3
+ 0.000389*max(Number of liver metastases-7,0)^3

f (Size of largest liver metastasis) =
+ 0.017*Size of largest liver metastasis
- 3.332*10^{-6}*max(Size of largest liver metastasis-11,0)^3
+ 4.535*10^{-6}*max(Size of largest liver metastasis-24,0)^3
- 1.203*10^{-6}*max(Size of largest liver metastasis-60,0)^3

The absolute predicted extrahepatic recurrence risk at time t:

Risk = 1-exp(-(exp(PI)*$H_0(t)$))

Example:

The 6-month predicted extrahepatic recurrence risk for a patient with a right-sided primary tumour location, T3 tumour stage and N1 nodal stage upon diagnosis, a *RAS*-mutation and with 1 liver metastasis upon diagnosis of CRLM (size 23 mm), who received adjuvant systemic treatment:

$H_0(t=6)$ = 0.0416
PI = - 0.061*0 + 0.212*0 + 0.198*1 + 0.543*0 + 0.200*1 + 0.507*0 + 0.758*0 + 0.510*1 + 0.101*1 + 0.00194*max(1 -1,0)^3 - 0.0023*max(1-2,0)^3 + 0.000389*max(1-7,0)^3 + 0.017*23 - 3.332*10^{-6}*max(23-11,0)^3 + 4.535*10^{-6}*max(23-24,0)^3 - 1.203*10^{-6}*max(23-60,0)^3 - 0.314*1
= 1.08
Risk = 1-exp(-(exp(1.08)*0.0416))
= 0.115 = 11.5%

B. Following correction for overoptimism

Correction for overfitting was by 500-fold bootstrap resampling as internal validation. Since adjuvant systemic treatment was modelled using an offset term, its coefficient does not undergo shrinkage.

Baseline cumulative hazard at 6-months:

$H_0(t_{6\ months})$ = 0.1882

Baseline cumulative hazard at 12-months:

$H_0(t_{12\ months})$ = 0.4249

Prognostic index (PI; linear predictor):

$$\begin{aligned} PI = & - 0.053 * X_{\text{Left-sided primary tumour location}} + 0.183 * X_{\text{Rectum primary tumour location}} \\ & + 0.171 * X_{\text{T3 upon diagnosis}} + 0.469 * X_{\text{T4}} \\ & + 0.172 * X_{\text{N1 upon diagnosis}} + 0.438 * X_{\text{N2}} \\ & + 0.654 * X_{\textit{BRAF}\text{-mutant}} + 0.440 * X_{\textit{RAS}\text{-mutant}} \\ & + f(\text{Number of liver metastases}) \\ & + f(\text{Size of largest liver metastasis}) \\ & - 0.314 * X_{\text{Adjuvant systemic treatment}} \end{aligned}$$

Where number of liver metastases and size of largest liver metastasis are described with a restricted cubic spline function:

$$\begin{aligned} f(\text{Number of liver metastases}) = & \\ & + 0.087 * \text{Number of liver metastases} \\ & + 0.00167 * \max(\text{Number of liver metastases} - 1, 0)^3 \\ & - 0.0020 * \max(\text{Number of liver metastases} - 2, 0)^3 \\ & + 0.000333 * \max(\text{Number of liver metastases} - 7, 0)^3 \end{aligned}$$

$$\begin{aligned} f(\text{Size of largest liver metastasis}) = & \\ & + 0.015 * \text{Size of largest liver metastasis} \\ & - 2.915 * 10^{-6} * \max(\text{Size of largest liver metastasis} - 11, 0)^3 \\ & + 3.968 * 10^{-6} * \max(\text{Size of largest liver metastasis} - 24, 0)^3 \\ & - 1.053 * 10^{-6} * \max(\text{Size of largest liver metastasis} - 60, 0)^3 \end{aligned}$$

The absolute predicted extrahepatic recurrence risk at time t:

$$\text{Risk} = 1 - \exp(-(\exp(PI) * H_0(t)))$$

PART III

NOVEL DIAGNOSTIC STRATEGIES IN PATIENTS WITH COLORECTAL CANCER LIVER METASTASES

CHAPTER 8

Postoperative circulating tumour DNA is associated with pathologic response and recurrence-free survival after resection of colorectal cancer liver metastases

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Abstract

Background

Recurrence rates after resection of colorectal cancer liver metastases (CRLM) are high and correlate with worse survival. Postoperative circulating tumour DNA (ctDNA) is a promising prognostic biomarker. Focusing on patients with resected CRLM, this study aimed to evaluate the association between the detection of postoperative ctDNA, pathologic response and recurrence-free survival (RFS).

Methods

Twenty-three patients were selected from an ongoing phase-3 trial who underwent resection of *RAS*-mutant CRLM after induction systemic treatment. CtDNA analysis was performed by droplet digital PCR using blood samples collected at baseline, before and after resection. Pathologic response of CRLM was determined via the Tumour Regression Grading system.

Findings

With a median follow-up of 19.6 months, the median RFS for patients with detectable (N=6,[26%]) and undetectable (N=17,[74%]) postoperative ctDNA was 4.8 *versus* 12.1 months, respectively. Among 21 patients with available tumour tissue, pathologic response in patients with detectable compared to undetectable postoperative ctDNA was found in one of six (17%) and 15 of 15 (100%) patients, respectively (p<0.001). In univariable Cox regression analyses both postoperative detectable ctDNA (HR=3.3, 95%CI=1.1-9.6, p=0.03) and pathologic non-response (HR=4.6, 95%CI=1.4-15, p=0.01) were associated with poorer RFS and were strongly correlated (r=0·88, p<0.001). After adjusting for clinical characteristics in pairwise multivariable analyses, postoperative ctDNA status remained associated with RFS.

Interpretation

The detection of postoperative ctDNA after secondary resection of CRLM is a promising prognostic factor for RFS and appeared to be highly correlated with pathologic response.

Research in context

Evidence before this study

Recurrence rates after resection of colorectal liver metastases (CRLM) are high and caused by micro-metastases left *in situ* after resection. Currently available follow-up methods have limited accuracy for detecting this minimal residual disease (MRD). Studies in patients with stage I-III colorectal cancer demonstrated that postoperative circulating tumour DNA (ctDNA) is a strong independent prognostic biomarker for MRD and recurrence-free survival. Studies investigating postoperative ctDNA in stage IV disease are limited and mostly concern heterogeneous patient groups with both hepatic and extrahepatic disease and varying use of induction systemic treatment.

Added value of this study

This is a proof of concept study reporting on the prognostic value of ctDNA in an upfront carefully selected homogeneous population of patients with *RAS* mutant initially unresectable CRLM. In addition, this is the first study to analyse the association of postoperative ctDNA detection with pathologic response in patients with metastatic CRC. CtDNA analysis was performed using the relatively fast, inexpensive, and highly sensitive droplet digital PCR to facilitate translation to future clinical practice.

Implications of all the available evidence

The results of this study offer a perspective on the clinical relevance of the assessment of postoperative ctDNA in CRLM patients with a high risk of recurrence. Liquid biopsy ctDNA offers the possibility for longitudinal follow-up, whereas pathologic response can only be assessed after resection. This offers opportunities for the personalisation of postoperative disease management in this common subgroup of patients with metastatic CRC, *e.g.* by intensifying follow-up or providing adjuvant treatment.

Introduction

The liver is the primary metastatic site of colorectal cancer (CRC). In patients with metastatic CRC, 70 to 80% have liver metastases.[1] In patients with liver-limited colorectal cancer liver metastases (CRLM), resection offers the only chance for cure or long-term survival.[1] Approximately 20% of patients present with upfront resectable CRLM (primary resectable), and 20-40% of patients with initially unresectable CRLM may convert to resectable disease upon downsizing by induction systemic treatment (secondary resectable).[2] Nevertheless, reported 3-year recurrence rates for primary and secondary resectable CRLM are up to 60%[3,4] and 80%[4,5], respectively. The majority of recurrences occur within the first two years following resection.[4] Furthermore, over half of the CRLM patients die within five years following resection.[4,6] Pathologic response[7] and early recurrence[8] have been correlated with overall survival in patients with CRLM.

Recurrences are considered to be caused by minimal residual disease (MRD) consisting of micro-metastases left *in situ*. Currently, available follow-up methods like serum carcinogenic embryonic antigen (CEA) and cross-sectional clinical imaging such as CT- or PET-scans have limited accuracy for detecting MRD due to low sensitivity and specificity.[9] While magnetic resonance imaging (MRI) shows a higher sensitivity compared to CT-scan for detecting small and disappearing metastases in the liver after systemic therapy[10], CT-scan has a higher overall diagnostic accuracy for detecting extrahepatic disease and has clear logistical advantages compared to whole-body MRI. Determining MRD by detecting cell-free circulating tumour DNA (ctDNA) after local treatment of CRLM may offer an alternative approach with important prognostic and therapeutic implications.

Liquid biopsy-derived ctDNA represents a minimally invasive, cancer-specific biomarker with great potential to improve diagnosis and to better determine prognosis, predict drug responsiveness and monitor treatment response.[11-13] Its short half-life makes ctDNA a dynamic marker indicating the presence of cancer cells and may detect evidence of tumour response or recurrences earlier than imaging and clinical parameters.[14,15] In addition, ctDNA has the potential to provide information about the genomic changes of the tumour.[16] In patients with stage I-III CRC, postoperative ctDNA is a strong independent prognostic biomarker for MRD and recurrence-free survival (RFS).[17-19] These data suggest that ctDNA may be a potential marker for selecting early-stage CRC patients for adjuvant systemic therapy.[15,20-24] Compared to other tumour types, patients with metastatic CRC show among the highest levels of detectable ctDNA.[24,25] In unselected patients with metastatic CRC, multiple studies have shown that detectable postoperative ctDNA is also strongly correlated with recurrence rate.[26-29] However, most of these results were obtained from studies with a small and heterogeneous study population, with limited data on patients with liver-only metastatic

disease. Besides, there are no studies involving patients with metastatic CRC that correlated ctDNA results with pathologic response.

The present study makes use of a well-defined selected group of patients participating in a prospective randomised study and aims to determine the prognostic value of postoperative ctDNA for detection of MRD and RFS in patients with CRLM after induction systemic therapy and complete resection of liver metastases. Secondly, the association between postoperative ctDNA detection and pathologic tumour response in liver metastases was evaluated.

Methods

Patient selection

Patients were selected from the ongoing CAIRO5 randomised phase 3 trial of the Dutch Colorectal Cancer Group (DCCG), in which the currently most effective first-line systemic regimens of chemotherapy plus targeted therapy are being compared in patients with initially unresectable CRLM (registration number: NCT02162563). A total of 564 patients are planned to be enrolled in the CAIRO5 clinical trial based on statistical assumptions previously described.[30] CRLM are deemed initially unresectable after assessment following predefined baseline resectability criteria considering R0-resection cannot be achieved in one procedure with one surgical intervention only. Patients are stratified for *RAS* and *BRAF V600E* mutation status and sidedness of primary tumour. Mutation analyses were performed on DNA isolated from the primary tumour for most patients because tissue from metastases was rarely available (91% *versus* 9%, respectively). Patients are evaluated every two months by an expert panel of liver surgeons and abdominal radiologists for the possibility of local treatment of CRLM following current practice.[31] Patients in whom local treatment of CRLM is achieved continue postoperatively with the preoperative systemic regimen but without the targeted agent for a total duration of pre- and postoperative treatment of six months. After patients signed informed consent, formalin-fixed paraffin-embedded (FFPE) tumour tissue was collected prior to treatment for translational research. In addition, blood samples were collected longitudinally every two months until resection and every three months after resection. For the current observational translational research subgroup analysis patients were selected who were randomised between the start of the study (June 2014) and August 2018, with *RAS* mutated tumours treated with bevacizumab plus either doublet or triplet chemotherapy, complete (R0/R1) resection of the primary tumour and liver metastases (resection and/or local ablation), and available baseline, pre- and postoperative liquid biopsies. Follow-up was recorded until May 2020. ctDNA analyses were performed on the subset of patients with *RAS* hotspot mutations, which

can be analysed using the relatively fast, inexpensive and highly sensitive ddPCR test. Patients with a first postoperative liquid biopsy drawn after starting adjuvant systemic therapy were excluded to avoid the confounding effect of chemotherapy. After completing systemic treatment, follow-up was performed according to the standard of care, including a three-monthly clinical review, six-monthly serum CEA, and CT imaging.

Ethics

The medical ethical committee of the Amsterdam Medical Center approved the CAIRO5 study under reference number METC 2014_008, NL47650.018.14, and all patients signed written informed consent for study participation as well as liquid biopsy and tumour tissue collection for translational research.

Clinicopathological data

Baseline clinicopathological patient characteristics were prospectively collected, such as age, sex, characteristics of the primary tumour (sidedness of the tumour, type of *RAS* mutation), time to metastases (with metachronous disease defined as a disease-free interval of more than six months after diagnosis of the primary tumour[32]), size and number of metastases, serum CEA levels, clinical risk score (CRS)[33] (low risk 0-2 points and high risk 3-5 points), chemotherapy regimen (doublet or triplet), number of cycles and documented radiologic response according to the RECIST 1.1 criteria, type of local therapies for CRLM, and R-status of resections (R0 or R1).

Pathologic response assessment was done by evaluating hematoxylin- and eosin-stained slides by an independent pathologist blinded for ctDNA outcomes. Pathologic response was scored according to the tumour Regression Grading (TRG).[34] TRG was graded from 1 to 5, with TRG 4 and 5 indicating no or minor pathological response.

Previous studies have shown that early recurrence after resection of CRLM, defined as recurrence within six to eight months, correlates with prognosis.[8,35,36] Therefore, we defined early recurrence as occurring within eight months of local treatment of CRLM. RFS was calculated from the date of hepatic resection until documented progression or censored on the last clinical visit date. In the case of a two-stage hepatic resection, RFS was calculated from the last surgical procedure.

Cell-free DNA isolation and quantification

Prior to systemic treatment (baseline), preoperatively, a maximum of 100 days postoperatively, and during follow-up, 10 ml of blood was collected using a cell-stabilising BCT® tube (Streck, La Vista, USA) at the medical centre of inclusion. For analyses, all liquid biopsies were shipped to the Clinical Chemistry laboratory at the

Netherlands Cancer Institute (Amsterdam, the Netherlands). Cell-free plasma was collected in a two-step centrifugation process; 10 minutes at 1.700 g followed by 10 minutes at 20.000 g before storage at -80°C. Cell-free DNA (cfDNA) was isolated using the QIAsymphony (Qiagen, Germany) with an elution volume set to 60 µl. The concentration of the cfDNA was measured using the Qubit™ dsDNA High-Sensitivity Assay (TFS, Waltham, USA) and ranged from 0.12 to 60.4 ng/µl.

Cell-free DNA *RAS* mutation analyses

KRAS and *NRAS* mutation analyses using extracted cfDNA from plasma were performed by droplet digital PCR (ddPCR) (Bio-Rad, Hercules, USA). For these analyses, the ddPCR™ KRAS G12/G13 (#1863506), ddPCR™ KRAS Q61 (#12001626), ddPCR™ KRAS A146 (#10049550) and the ddPCR™ NRAS Q61 (#12001006) Screening Kits were used according to the manufacturer's instruction making use of 1 µl multiplex assay, 11 µl ddPCR supermix for probes (no dUTP), 9 µl sample and 1 µl H2O. When necessary, samples were diluted to 2 ng/µl. All measurements were performed in duplicate and included a blank (nuclease-free water) and an in-house positive control. Data were analysed using the QuantaSoft™ software version 1.6.6 (Bio-Rad, Hercules, USA). Individual wells with less than 10.000 total events (droplets) were excluded from the analysis, and all results were corrected based on a predefined false-positive rate, based on 60-fold analyses of commercial reference wildtype DNA (Promega; Fitchburg, WI, USA).[37]

Statistics

Patient and tumour characteristics were summarised as frequency counts and percentages, or as medians and range. Differences between groups were analysed using Pearson's chi-square test and Fisher exact test, as appropriate. Survival data were analysed using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Cox proportional hazards regression analysis was performed to analyse prognostic factors for RFS. Hazard ratios (HRs) and corresponding 95% confidence intervals (95% CI) were estimated. Given the small sample size and the limited number of events available, a maximum of two variables was introduced in multivariable analyses. Given the strong association between ctDNA and pathologic response, they were not analysed together in the same multivariable model. A multivariable Cox regression analysis including more than one covariate together with postoperative ctDNA was performed as sensitivity analysis. Spearman's correlation coefficient was estimated to evaluate the association between pathologic response and postoperative ctDNA status. Analyses were performed using SPSS software version 25 (IBM, New York, USA).

Role of the funding source

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Results

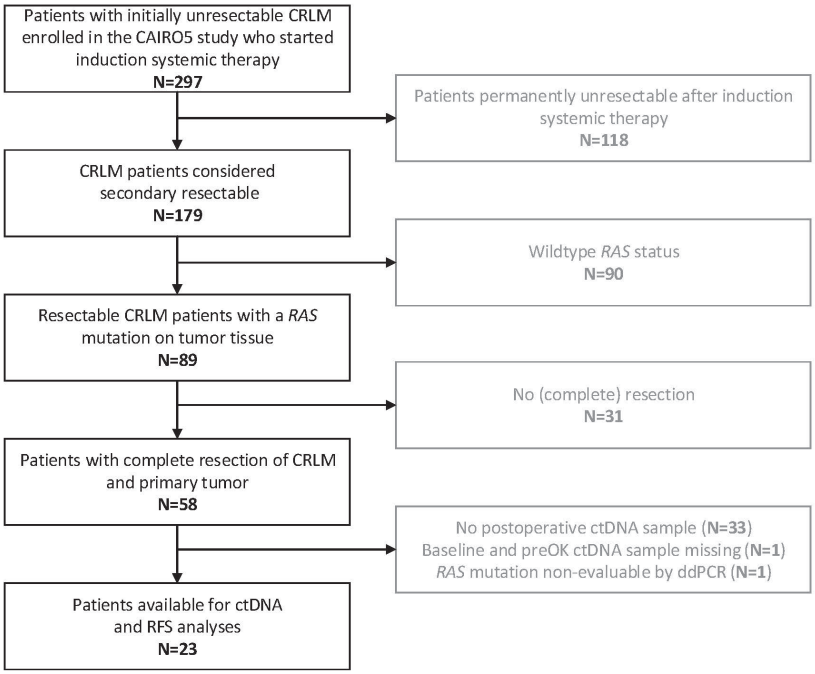
Patient characteristics

Patient selection and study overview are presented in **Figure 1**. Between November 2014 and August 2018, 297 patients with initially unresectable CRLM were enrolled in the CAIRO5 study. According to tumour tissue analyses, fifty-nine patients carried a *RAS* mutation and achieved a confirmed complete resection of liver metastases and primary tumour after systemic induction therapy. After exclusion of patients with unavailable preoperative and/or postoperative liquid biopsies, a total of 23 patients, one with a *NRAS* mutation and 22 with a *KRAS* mutation, were eligible for further ctDNA and RFS analysis. The follow-up was recorded until the 20th of April 2020. The baseline patient characteristics of this cohort are displayed in **Table 1** and show synchronous metastases in 19 (83%) patients, with a median number of metastases of eight (range 1-37), and 20 (87%) patients with a high CRS. Ten (44%) patients received doublet chemotherapy (FOLFOX or FOLFIRI) plus bevacizumab, and 13 (57%) patients triplet chemotherapy (FOLFOXIRI) plus bevacizumab.

Detection of ctDNA at baseline, preoperatively and postoperatively

Within the group of 23 patients, preoperative ctDNA analyses were performed on baseline blood samples in 20 patients (87%) and on preoperative blood samples in 22 patients (96%). Analyses of the postoperative liquid biopsies showed that six (26%) patients had detectable ctDNA compared to 17 (74%) patients with undetectable postoperative ctDNA. Patients with detectable *versus* undetectable postoperative ctDNA did not differ in baseline characteristics (**Supplementary Table 1**).

Figure 1 Flowchart of patient selection.



Association of ctDNA detection with recurrence of disease

At a median follow-up of 19.6 months (range 1.5 – 60 months), 17 patients (74%) had recurrence of disease, with 12 patients (52%) showing early disease recurrence (≤ eight months), see **Table 2**. In nine patients (53%), the first recurrence occurred at an extrahepatic site. In patients with postoperatively detectable ctDNA compared to undetectable ctDNA, early disease recurrence was observed in four (67%) patients *versus* eight (47%) patients, respectively. However, this was not significant (Pearson's chi-squared test, p=0·41, specificity 81% and sensitivity 33%). **Figure 2** presents the postoperative ctDNA status and lead-time to recurrence detected by ctDNA and imaging studies for all 23 patients. A detailed overview of both pre- and postoperative ctDNA detection per patient is presented in **Supplementary Figure 1**. In analysing the performance of ctDNA in the detection of MRD, we found that six patients (100%) with postoperative detectable ctDNA and 11 patients (65%) with undetectable postoperative ctDNA had a recurrence during follow-up. For a total of 15 patients, serum CEA was determined within 100 days following resection. Of patients with serum CEA levels within the normal range (N=14) *versus* elevated (>5 ng/ml) (N=1), 11 (79%) and one (100%) patient developed recurrences during follow-up, respectively. Postoperative ctDNA detection was significantly associated with poorer RFS, with a median RFS for

patients with postoperative undetectable *versus* detectable ctDNA of 12.1 and 4.8 months, respectively (HR 3·3, 95%CI 1.1-9.6, log-rank *p*=0·03), see **Figure 3.**

Table 1 Summary of clinicopathological patient characteristics.

Clinical characteristics	All patients (N = 23)
Age, median (range)	63 (54-76)
Sex, *n* (%)	
Male	15 (65)
Female	8 (35)
Tumour site, *n* (%)	
Left colon	17 (74)
Right colon	6 (26)
RAS mutation, *n* (%)	
KRAS mutation	22 (96)
NRAS mutation	1 (4)
Source tissue mutation analysis, n (%)	
Primary tumour	21 (91)
Liver metastases	2 (9)
Synchronous liver metastases, *n* (%)	
No	4 (17)
Yes	19 (83)
Number of metastases, median (range)	8 (1-37)
Prior resection of primary tumour, *n* (%)	
No	11 (48)
Yes	12 (52)
CEA, median (range)	9.5 (1-3469)
Fong risk score, *n* (%)	
Low (0-2)	3 (13)
High (3-5)	20 (87)
Perioperative systemic therapy, *n* (%)	
Doublet chemotherapy + target therapy	10 (44)
Triplet chemotherapy + target therapy	13 (57)
Cycles preoperative therapy, mean (range)	7.7 (4-13)
Cycles postoperative therapy, mean (range)	1.9 (0-7)
Best response (RECIST), *n* (%)	
Partial response	17 (74)
Stable disease	5 (22)
Progression of disease	1 (4)
Type of resection, *n* (%)	
1-stage	19 (83)
2-stage	3 (13)
R-status, *n* (%)	
R0	20 (83)
R1	3 (13)
Local ablative therapy	1 (4)
Baseline ctDNA, *n* (%)	
Undetectable	2 (9)
Detectable	18 (78)
Missing baseline sample	3 (13)
Histopathological response (TRG), *n* (%)	
Pathologic response (TRG 1-3)	16 (65)
No pathologic response (TRG 4-5)	5 (22)
Missing	2 (9)
Postoperative ctDNA, days after last surgery, median (range)	38 (1-99)

Abbreviations: CEA: carcinogenic embryonic antigen, RECIST: response evaluation criteria in solid tumours, ctDNA: circulating tumour DNA, TRG: tumour regression grade

Table 2 Follow-up and recurrence-free survival for patients with postoperative undetectable and postoperative detectable ctDNA.

	All patients (N=23)	Postoperative undetectable ctDNA (N=17)	Postoperative detectable ctDNA (N=6)
Median follow-up, months (95% CI)	19.6 (17.8– 21.4)		
Median RFS, months	7.4	12.1	4.8
Number of patients with recurrence, n (%)	17 (74)	11 (65)	6 (100)
Early recurrence (≤ 8 months), n (%)			
No	11 (48)	9 (53)	2 (33)
Yes	12 (52)	8 (47)	4 (67)
Site of recurrence			
Liver	8 (47)	6 (55)	2 (33)
Extrahepatic	9 (53)	5 (45)	4 (67)
No recurrence	6	6	-

Abbreviations: ctDNA: circulating tumour DNA, RFS: recurrence-free survival.

Figure 2 Overview of surveillance for disease recurrence in 23 patients with colorectal liver metastases (CRLM) after complete resection following induction systemic treatment. Clinical response evaluation is depicted until progression of disease (PD), where all liquid biopsy ctDNA ddPCR analysis results are showed. A distinction was made between four groups; patients with postoperative positive ctDNA with PD, patients with follow-up positive ctDNA with PD, patients with postoperative negative ctDNA with PD, and patients with postoperative negative ctDNA without PD.

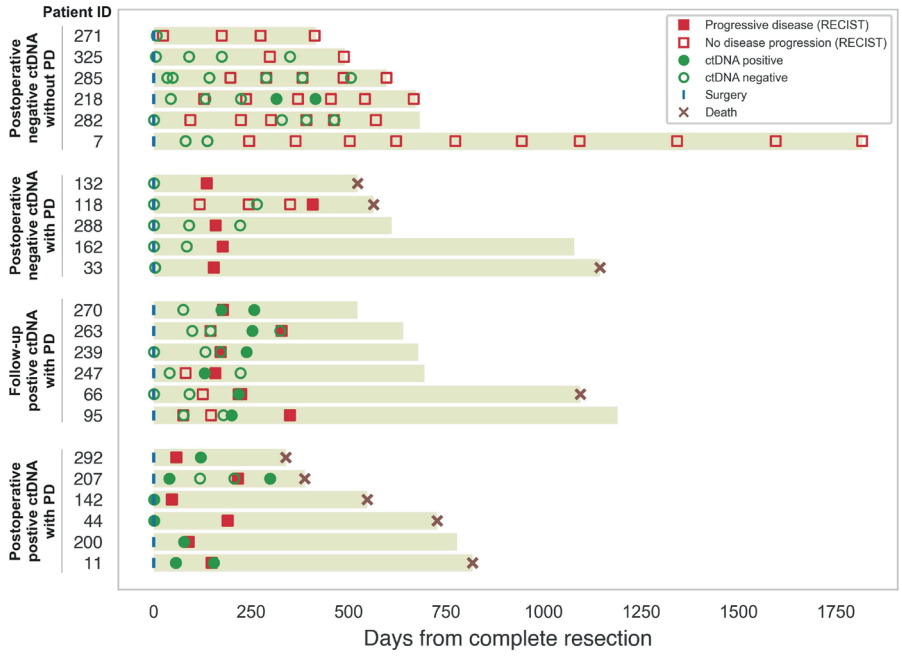
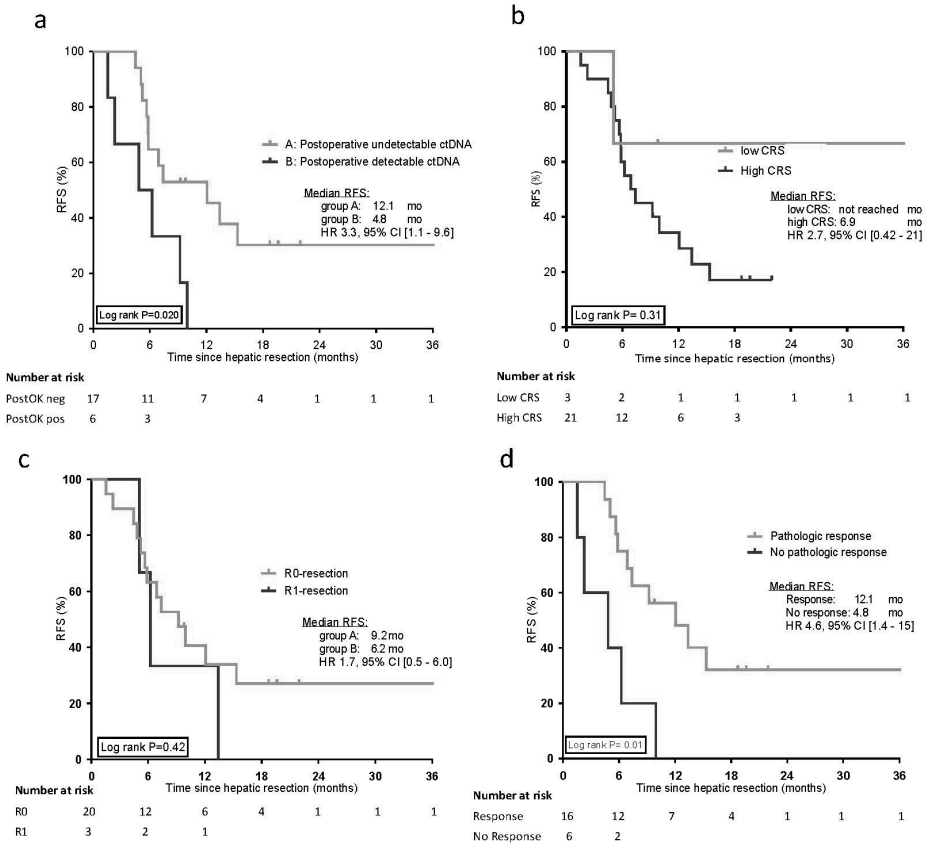


Figure 3 Kaplan-Meier curves showing recurrence-free survival according to: a) postoperative ctDNA mutation status (undetectable versus detectable), b) Fong clinical risk score (low versus high) c) resection margin (R0 versus R1), and d) pathologic response (TRG 1-3 versus TRG 4-5).



Postoperative ctDNA detection and association with pathologic response

For one patient only local ablative therapy was executed, and for one patient no HE-slides were available. Therefore, pathologic response was assessed on resected tissue from liver metastases of 21 (91%) patients using Slide Score.[38] In patients with liver metastases available for pathologic response assessment, major pathologic response (TRG 1 or 2), partial (TRG 3), and no pathologic response (TRG 4 or 5) was scored in 10 (48%), six (29%), and five (24%) patients, respectively. Postoperative ctDNA status was strongly correlated with pathologic response (TRG 1-3) (Spearman's correlation, r=0.88, p<0.001). All patients (N=15, 100%) with undetectable ctDNA had partial or major pathologic response compared to only one (17%) patient with detectable ctDNA (Pearson's Chi-squared test, p<0.001).

Postoperative ctDNA and pathologic non-response are associated with poor RFS

Univariable survival analysis showed detectable postoperative ctDNA (HR 3.3, 95%CI 1.1-9.6, log-rank *p*=0.03) and pathologic non-response (TRG 4-5) (HR 4.6, 95%CI 1.4-15, log-rank *p*=0.01) to be associated with poorer RFS (see **Table 3**). After adjusting postoperative ctDNA for age, sex, Fong CRS, radiological response, sidedness and R-status in separate pairwise multivariable analyses, detectable postoperative ctDNA remained significantly associated with poorer RFS. The association between postoperative ctDNA and RFS remained strong in the sensitivity analysis adjusting for all the aforementioned variables simultaneously in a multivariable model (HR 4·1, 95%CI 1.19-14.47, log-rank *p*=0.026). No indications of an association between RECIST response or non-response and pathologic response (Fisher's Exact, *p*=0.761), detection of postoperative ctDNA (Fisher's Exact, *p*=0.083), or recurrence of disease (Fisher's Exact, *p*=0.217) was found.

Table 3 Cox regression univariable recurrence-free survival analysis by clinicopathological variables and postoperative ctDNA status.

Variable	Number patients	Event RFS	Univariable analysis		
	n (%)	n	HR	95% CI	Log-rank *P*
Age, years					
<60	8 (35)	6			
>60	15 (65)	11	1.2	0.4 – 3.1	0.78
Sex					
Male	15 (65)	11			
Female	8 (35)	6	0.98	0.4 – 3.7	0.98
Sidedness primary tumour					
Left	17 (74)	13			
Right	6 (26)	4	1.2	0.4 – 3.8	0.74
Clinical risk score*					
Low	3 (13)	1			
High	20 (87)	16	2.7	0.4 – 21	0.33
Postoperative serum CEA					
Normal	15 (94)	10			
Elevated (>5 ng/ml)	1 (6)	1	0.9	0.1 – 6.8	0.90
Resection status					
R0-resection	19 (86)	13			
R1-resection	3 (14)	3	1.7	0.5 – 6.0	0.43
Radiological response on induction treatment					
Response	17 (74)	14			
No Response	6 (26)	3	2.5	0.7 – 8.7	0.16
Tumour regression grade					
Response (TRG 1-3)	16 (76)	10			
No response (TRG 4-5)	5 (24)	5	4.6	1.4 – 15	0.01
Postoperative ctDNA status					
Undetectable	17 (74)	11			
Detectable	6 (26)	6	3.3	1.1 – 9.6	0.03

Abbreviations: RFS: recurrence-free survival, CEA: carcinogenic embryonic antigen, TRG: tumour regression grade, ctDNA: circulating tumour DNA, *Clinical risk groups are classified according to Fong.

Discussion

This study analysed the association between postoperative ctDNA and both pathologic response and RFS in patients with initially unresectable CRLM after radical resection of both CRLM and primary tumour. The results indicate that postoperative ctDNA analysis within a high-risk cohort may potentially identify patients with a higher risk of disease recurrence after secondary resection. In addition, postoperative ctDNA showed a strong association with pathologic response on systemic therapy as assessed by the tumour regression grade and is an independent prognostic factor for RFS.

Liquid biopsies are a rich source of minimal invasive biomarkers such as circulating tumour cells (CTCs) and ctDNA, which have the potential to be applied for the clinical management of patients with CRC.[39] In this study we focused on the analysis of ctDNA, considering the higher detection rate of ctDNA compared to CTCs in patients with metastatic CRC.[40] Limited data is available on the value of ctDNA in patients with CRLM.[40-42] Narayan *et al.* showed an association of preoperative ctDNA with overall survival in patients with upfront resectable CRLM.[41] The PRODIGE-14 METHEP-2 trial showed in initially unresectable CRLM patients that preoperative ctDNA levels correlate with R0/R1 resections and overall survival.[40] The trial of He *et al.* involving twenty CRLM patients, not clearly defined as initially resectable or unresectable and with approximately 50% receiving neo-adjuvant systemic therapy, demonstrated a prolonged RFS for patients with low preoperative ctDNA.[42] Further studies in patients with resected CRLM concerned heterogeneous populations in terms of CRC stage among the whole population, presence of extrahepatic metastases, first presentation and relapse of CRLM[27,28], inclusion of both radical and non-radical resections[28], primary and secondary resectable CRLM and types of local therapy.[29,42]

To our knowledge, this is the first study to analyse the association of postoperative ctDNA detection and pathologic response in resected liver metastases in CRLM patients. Pathologic response is a well-known independent prognostic factor for overall survival in patients with CRLM[7] and can therefore be used as an early surrogate marker for survival. Our results show a strong association between postoperative ctDNA status and pathologic response. After adjusting for clinical characteristics, both postoperative ctDNA and pathologic response were independent prognostic factors for RFS in separately conducted pairwise multivariable analysis. The added value of ctDNA compared to pathologic response is the ability to perform serial ctDNA analyses in longitudinal follow-up, whereas pathologic response is only possible after resection. Additionally, ctDNA is analysed by a simple blood draw while pathologic response requires tumour tissue. These factors combined with the results of this study might have clinically relevant implications since ctDNA could be used as a surrogate marker for pathologic response and clinical outcome in metastatic CRC patients without available

tumour tissue after systemic therapy, such as patients treated with local ablative therapy only or patients on palliative systemic therapy.

The promising monitoring and prognostic value of ctDNA have raised major interest in ctDNA driven adjuvant trials.[43] Adjuvant systemic therapy in CRLM patients has failed to show a 5 year survival benefit.[6] However, this study concerned relatively low-risk CRLM patients (with four or fewer metastases), and retrospective studies suggest that an adequate selection of patients with a high risk of recurrence could help select the patients who might benefit from adjuvant treatment.[44,45] Our results show that postoperative ctDNA status is an independent prognostic factor for RFS and might be a promising biomarker in future trials to select very high-risk CRLM patients for adjuvant trials or otherwise for individualised therapy.

Liquid biopsy ctDNA is a promising biomarker to optimize strategies for monitoring disease recurrence after resection of CRLM. Early detection of a recurrence limited to the liver might offer an opportunity for repeated local treatments with curative intent. Further studies are needed to determine if patients with detectable postoperative ctDNA have clinical benefit from intensified follow-up strategies, like more frequent evaluations or additional imaging methods such as MRI or PET-CT, resulting in better survival outcomes than the current standard of care follow-up strategies. With the additional advantage of liquid biopsies providing the ability for longitudinal monitoring of disease recurrence, having less burden to patients and lower costs than radiological imaging, ctDNA is an interesting biomarker to investigate in future prospective trials. Furthermore, combining radiologic and ctDNA assessments might also help interpret indeterminate radiological findings such as nonspecific liver or lung nodules. Currently, serum CEA is used after resection of CRLM to monitor disease recurrence. However, serum CEA has a low sensitivity and specificity, which might be explained by expression in both neoplastic and normal cells.[45-47] Liquid biopsy ctDNA was shown to perform better[41,48] with higher sensitivity compared to serum CEA, 100% *versus* 56% (p=0.01).[26] In our population with high-risk CRLM patients, we confirmed that ctDNA is a stronger prognostic marker for RFS than CEA. Secondly, in pairwise multivariable analysis with other potential clinicopathological risk factors for disease recurrence (*e.g.* CEA, CRS, R-status), we found indications that postoperative ctDNA status was an independent prognostic factor for RFS in patients with secondary resection of CRLM.

An ideal test to diagnose MRD after resection, and further tailor adjuvant systemic treatment, has high sensitivity and specificity.[49] Previously, postoperative ctDNA in metastatic CRC was shown to have high specificity but relatively low sensitivity, since a considerable number of patients with undetectable postoperative ctDNA still developed a recurrence.[27-29] Similarly, in our study investigating a homogeneous group of CRLM patients, we found a high specificity, where all patients with postoperative detectable

ctDNA had a recurrence during follow-up, but lower sensitivity, since 65% of the patients with undetectable postoperative ctDNA also developed a recurrence. A possible factor contributing to our study's sensitivity is the use of ddPCR as a hotspot detection method (detection of one mutation). Our study focused on patients whose *RAS* mutation status was determined as part of the clinical diagnostic workflow, to establish their eligibility for anti-EGFR treatment. Methodologically, ddPCR-based assays for detecting ctDNA hotspot mutations have high sensitivity and are relatively cheap.[37] This ensures more widespread applicability in daily clinical practice as compared to NGS analyses of gene panels and rendered ddPCR a logical choice for detecting ctDNA in this subset of patients in the present study. Another explanation for the phenomenon of undetectable postoperative ctDNA in patients with MRD leading to recurrence might be the use of preoperative systemic therapy in all patients in our study. This could have (temporarily) reduced the proliferation and apoptosis of minimal residual tumour cells postoperatively, thereby reducing the shedding of ctDNA.[49] Also the time window from postoperative blood draw till disease recurrence might have been too long. Lastly, the site of recurrence might have an impact on ctDNA detection in the circulation.[25] Future studies should determine the optimal time window for the sampling of ctDNA after surgery. Liquid biopsy cfDNA levels after tissue damage resulting from the surgery itself can be elevated up to four weeks, which may result in masking ctDNA with false-negative outcomes. It has been recommended that a second blood sample, collected after four weeks, is analysed for patients with postoperative undetectable ctDNA.[50]

Limitations of our study include the small sample size, in part caused by the exclusion of patients with missing postoperative blood samples. The challenging logistics of blood sampling for translational research are well established.[40] Also, the sample size was limited to patients with a known *RAS* mutation, present in only 40-56% of patients with metastatic CRC.[51-53] A strength of our study is the homogeneous study population relative to other studies assessing the value of postoperative ctDNA in CRLM patients.[28,29,41,42] Integrating clinical, pathological and molecular markers can help to improve and customise therapy.

In conclusion, the detection of postoperative ctDNA is a promising prognostic factor for disease recurrence and median RFS in patients after secondary resection of *RAS* mutated colorectal cancer liver-only metastases. In addition, postoperative ctDNA showed a strong association with pathologic response. Further analysis with a bigger sample size would be needed to confirm these promising findings.

Author contributions

Study concept and design: KB, IvtE, CJAP, RJAF
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Data collection: KB, IvtE, CM, PMDvD, AK, RJS

Statistical analyses: KB, IvtE, MLY
Data interpretation: KB, IvtE, CJAP, RJAF
Manuscript writing: KB, IvtE, CJAP, RJAF
Critical revision of the manuscript: All authors

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References

1. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3(1):50-56.
2. Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020;141:225-238.
3. Hallet J, Sa Cunha A, Adam R, Goere D, Bachellier P, Azoulay D, et al. Factors influencing recurrence following initial hepatectomy for colorectal liver metastases. Br J Surg. 2016;103(10):1366-1376.
4. Angelsen JH, Viste A, Loes IM, Eide GE, Hoem D, Sorbye H, et al. Predictive factors for time to recurrence, treatment and post-recurrence survival in patients with initially resected colorectal liver metastases. World J Surg Oncol. 2015;13:328.
5. Mima K, Beppu T, Chikamoto A, Miyamoto Y, Nakagawa S, Kuroki H, et al. Hepatic resection combined with radiofrequency ablation for initially unresectable colorectal liver metastases after effective chemotherapy is a safe procedure with a low incidence of local recurrence. Int J Clin Oncol. 2013;18(5):847-855.
6. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, 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. 2013;14(12):1208-1215.
7. Blazer DG, 3rd, Kishi Y, Maru DM, Kopetz S, Chun YS, Overman MJ, et al. Pathologic response to preoperative chemotherapy: a new outcome end point after resection of hepatic colorectal metastases. J Clin Oncol. 2008;26(33):5344-5351.
8. Imai K, Allard MA, Benitez CC, Vibert E, Sa Cunha A, Cherqui D, et al. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21(7):887-894.
9. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. J Clin Oncol. 2009;27(36):e279-280; author reply e281.
10. Barimani D, Kauppila JH, Sturesson C, Sparrelid E. Imaging in disappearing colorectal liver metastases and their accuracy: a systematic review. World J Surg Oncol. 2020;18(1):264.
11. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Bruhm DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570(7761):385-389.
12. Phallen J, Sausen M, Adleff V, Leal A, Hruban C, White J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med. 2017;9(403): eaan2415.
13. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223-238.
14. Tie J, Kinde I, Wang Y, Wong HL, Roebert J, Christie M, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. Ann Oncol. 2015;26(8):1715-1722.
15. Wang Y, Li L, Cohen JD, Kinde I, Ptak J, Popoli M, et al. Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer. JAMA Oncol. 2019;5(8):1118-1123.
16. Bai HX, Lee AM, Yang L, Zhang P, Davatzikos C, Maris JM, et al. Imaging genomics in cancer research: limitations and promises. Br J Radiol. 2016;89(1061):20151030.
17. Wang R, Zhao A, Cao N, Li Z, Zhang G, Liu F. The value of circulation tumor DNA in predicting postoperative recurrence of colorectal cancer: a meta-analysis. Int J Colorectal Dis. 2020;35(8): 1463-1475.
18. Tie J, Cohen JD, Lo SN, Wang Y, Li L, Christie M, et al. Prognostic significance of postsurgery circulating tumor DNA in nonmetastatic colorectal cancer: Individual patient pooled analysis of three cohort studies. Int J Cancer. 2021;148(4):1014-1026.
19. Reinert T, Henriksen TV, Christensen E, Sharma S, Salari R, Sethi H, et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. JAMA Oncol. 2019;5(8): 1124-1131.

20. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92.
21. Tarazona N, Gimeno-Valiente F, Gambardella V, Zuniga S, Rentero-Garrido P, Huerta M, et al. Targeted next-generation sequencing of circulating-tumor DNA for tracking minimal residual disease in localized colon cancer. Ann Oncol. 2019;30(11):1804-1812.
22. Fleming CA, O'Leary DP, Wang J, Redmond HP. Association of Observed Perioperative Cell-Free DNA Dynamics With Early Recurrence in Patients With Colon Cancer. JAMA Surg. 202-;155(2):168-170.
23. Tie J, Cohen JD, Wang Y, Li L, Christie M, Simons K, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut. 2019;68(4):663-671.
24. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra24.
25. Vidal J, Muinelo L, Dalmases A, Jones F, Edelstein D, Iglesias M, et al. Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. Ann Oncol. 2017;28(6):1325-1332.
26. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008;14(9):985-990.
27. Scholer LV, Reinert T, Orntoft MW, Kassentoft CG, Arnadottir SS, Vang S, et al. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res. 2017;23(18):5437-5445.
28. Benesova L, Halkova T, Ptackova R, Semyakina A, Menclova K, Pudil J, et al. Significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA. World J Gastroenterol. 2019;25(48):6939-6948.
29. Boysen AK, Pallisgaard N, Andersen CSA, Spindler KG. Circulating tumor DNA as a marker of minimal residual disease following local treatment of metastases from colorectal cancer. Acta Oncol. 2020:1-6.
30. Huiskens J, van Gulik TM, van Lienden KP, Engelbrecht MR, Meijer GA, van Grieken NC, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
31. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27(8):1386-1422.
32. Mekenkamp LJ, Koopman M, Teerenstra S, van Krieken JH, Mol L, Nagtegaal ID, et al. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010;103(2):159-164.
33. 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):309-318; discussion 318-321.
34. Rubbia-Brandt L, Giostra E, Brezault C, Roth AD, Andres A, Audard V, 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. 2007;18(2):299-304.
35. Takahashi S, Konishi M, Nakagohri T, Gotohda N, Saito N, Kinoshita T. Short time to recurrence after hepatic resection correlates with poor prognosis in colorectal hepatic metastasis. Jpn J Clin Oncol. 2006;36(6):368-375.
36. Vigano L, Capussotti L, Lapointe R, Barroso E, Hubert C, Giuliante F, 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. 2014;21(4):1276-1286.
37. Vessies DCL, Greuter MJE, van Rooijen KL, Linders TC, Lanfermeijer M, Ramkisoensing KL, et al. Performance of four platforms for KRAS mutation detection in plasma cell-free DNA: ddPCR, Idylla, COBAS z480 and BEAMing. Sci Rep. 2020;10(1):8122.
38. Slide Score. [https://www.slidescore.com] 2020

39. Marcuello M, Vymetalkova V, Neves RPL, Duran-Sanchon S, Vedeld HM, Tham E, et al. Circulating biomarkers for early detection and clinical management of colorectal cancer. Mol Aspects Med. 2019;69:107-122.
40. Bidard FC, Kiavue N, Ychou M, Cabel L, Stern MH, Madic J, et al. Circulating Tumor Cells and Circulating Tumor DNA Detection in Potentially Resectable Metastatic Colorectal Cancer: A Prospective Ancillary Study to the Unicancer Prodige-14 Trial. Cells. 2019;8(6):516.
41. Narayan RR, Goldman DA, Gonen M, Reichel J, Huberman KH, Raj S, et al. Peripheral Circulating Tumor DNA Detection Predicts Poor Outcomes After Liver Resection for Metastatic Colorectal Cancer. Ann Surg Oncol. 2019;26(6):1824-1832.
42. He Y, Ma X, Chen K, Liu F, Cai S, Han-Zhang H, et al. Perioperative Circulating Tumor DNA in Colorectal Liver Metastases: Concordance with Metastatic Tissue and Predictive Value for Tumor Burden and Prognosis. Cancer Manag Res. 2020;12:1621-1630.
43. Schraa SJ, van Rooijen KL, van der Kruijssen DEW, Rubio Alarcon C, Phallen J, Sausen M, et al. Circulating tumor DNA guided adjuvant chemotherapy in stage II colon cancer (MEDOCC-CrEATE): study protocol for a trial within a cohort study. BMC Cancer. 2020;20(1):790.
44. Rahbari NN, Reissfelder C, Schulze-Bergkamen H, Jager D, Buchler MW, Weitz J, 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. 2014;14:174.
45. Hirokawa F, Hayashi M, Miyamoto Y, Asakuma M, Shimizu T, Komeda K, et al. Reconsideration of the indications for adjuvant chemotherapy for liver metastases from colorectal cancer after initial hepatectomy. Ann Surg Oncol. 2014;21(1):139-146.
46. Benson AB, 3rd, Desch CE, Flynn PJ, Krause C, Loprinzi CL, Minsky BD, et al. 2000 update of American Society of Clinical Oncology colorectal cancer surveillance guidelines. J Clin Oncol. 2000;18(20): 3586-3588.
47. Ayez N, Lalmahomed ZS, van der Pool AE, Vergouwe Y, van Montfort K, de Jonge J, et al. Is the clinical risk score for patients with colorectal liver metastases still useable in the era of effective neoadjuvant chemotherapy? Ann Surg Oncol. 2011;18(10):2757-2763.
48. Wong R, Tie J, Lee M, Cohen J, Wang Y, Li L, et al. The potential role of circulating tumor DNA (ctDNA) in the further investigation of colorectal cancer patients with nonspecific findings on standard investigations. Int J Cancer. 2019;145(2):540-547.
49. Coakley M, Garcia-Murillas I, Turner NC. Molecular Residual Disease and Adjuvant Trial Design in Solid Tumors. Clin Cancer Res. 2019;25(20):6026-6034.
50. Henriksen TV, Reinert T, Christensen E, Sethi H, Birkenkamp-Demtroder K, Gogenur M, et al. The effect of surgical trauma on circulating free DNA levels in cancer patients - implications for studies of circulating tumor DNA. Mol Oncol. 2020;14(8):1670-1679.
51. Peeters M, Kafatos G, Taylor A, Gastanaga VM, Oliner KS, Hechmati G, et al. Prevalence of RAS mutations and individual variation patterns among patients with metastatic colorectal cancer: A pooled analysis of randomised controlled trials. Eur J Cancer. 2015;51(13):1704-1713.
52. Hsu HC, Thiam TK, Lu YJ, Yeh CY, Tsai WS, You JF, et al. Mutations of KRAS/NRAS/BRAF predict cetuximab resistance in metastatic colorectal cancer patients. Oncotarget. 2016;7(16):22257-22270.
53. Therkildsen C, Bergmann TK, Henrichsen-Schnack T, Ladelund S, Nilbert M. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis. Acta Oncol. 2014;53(7):852-864.

Supplementary materials

Supplementary Table 1 Comparison of patient characteristics between patients with postoperative undetectable and postoperative detectable ctDNA.

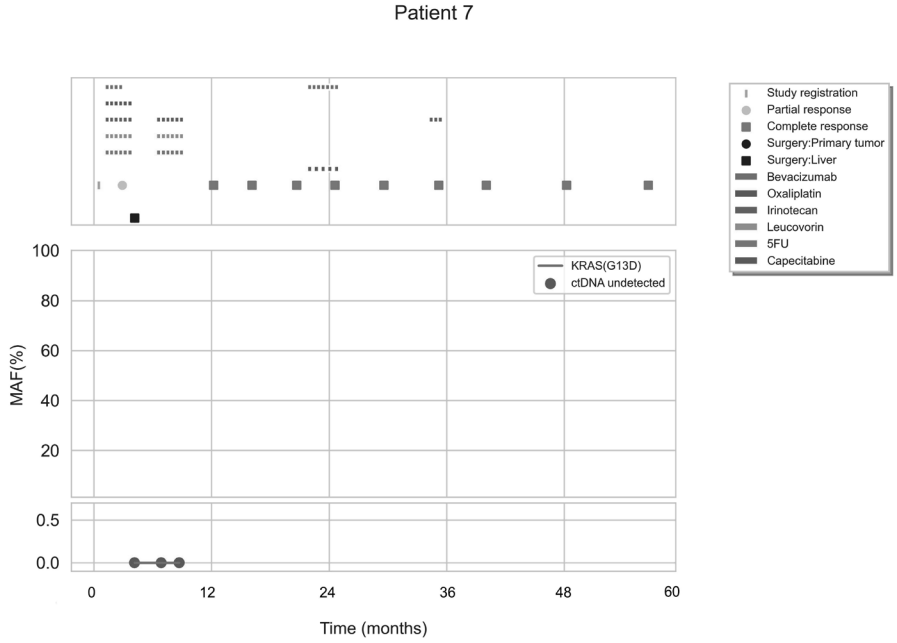
	All patients (N=23)	Postoperative undetectable ctDNA (N=17)	Postoperative detectable ctDNA (N=6)	p-value*
Age, median (range)	63 (54-76)	64	58	0·76
Sex, *n* (%)				0·93
Male	15 (65)	11 (65)	4 (67)	
Female	8 (35)	6 (35)	2 (33)	
Tumour site, *n* (%)				0·54
Left colon	17 (74)	12 (71)	5 (83)	
Right colon	6 (26)	5 (29)	1 (17)	
RAS mutation, *n* (%)				0·26
KRAS mutation	22 (96)	17 (100)	5 (83)	
NRAS mutation	1 (4)	0	1 (17)	
Synchronous liver metastases, *n* (%)				0·19
No	4 (17)	4 (24)	0	
Yes	19 (83)	13 (77)	6 (100)	
Number of metastases, median (range)	7·5 (1-37)	8·5	6·5	0·81
Prior resection of primary tumour, *n* (%)				0·90
No	11 (46)	8 (47)	3 (50)	
Yes	13 (54)	9 (5530)	3 (50)	
CEA, median (range)	10·8 (1-3469)			
Fong risk score, n (%)				0·74
Medium (2-3)	14 (59)	10 (59)	4 (67)	
High (4-5)	7 (41)	7 (41)	2 (33)	
Perioperative systemic therapy, *n* (%)				0·18
Doublet + target therapy	10 (42)	6 (35)	4 (67)	
Triplet + target therapy	14 (58)	11 (65)	2 (33)	
Cycles neo-adjuvant therapy, mean (range)	7·8 (4-13)	7·6	8·7	0·87
Cycles adjuvant therapy, mean (range)	1·9 (0-7)	2·4	0·3	0·39
Best response, *n* (%)				0·10
Partial response	17 (74)	12 (71)	5 (83)	
Stable disease	5 (22)	5 (29)	0	
Progression of disease	1 (4)	0	1 (17)	
Type of resections, *n* (%)				0·80
1-stage	20 (87)	15 (88)	5 (83)	
2-stage	3 (13)	2 (12)	1 (17)	
R-status, *n* (%)				0·76
R0	19 (86)	14 (88)	5 (83)	
R1	3 (14)	2 (12)	1 (17)	
RFA/MWA	1	1	0	

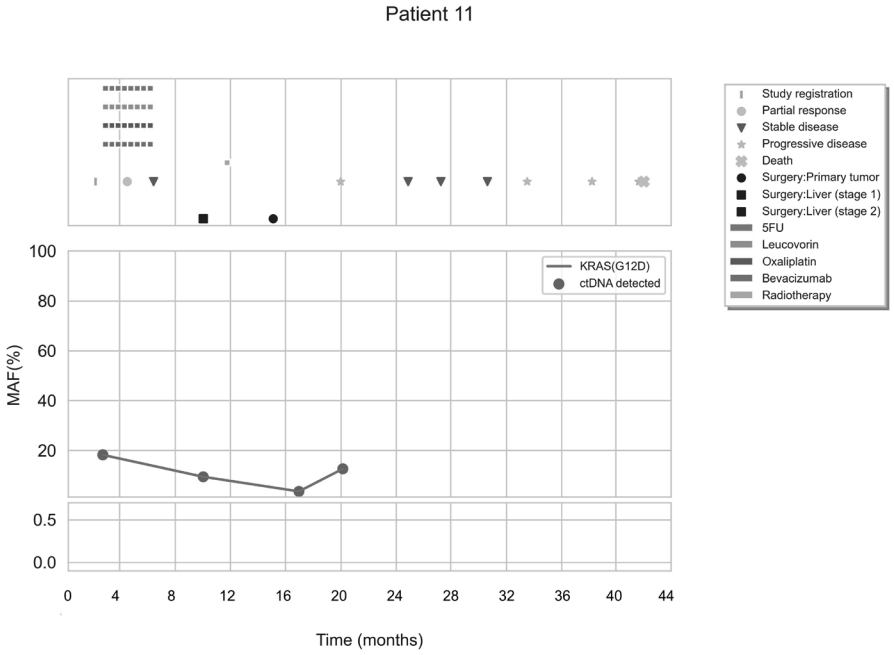
Supplementary Table 1 (continued)

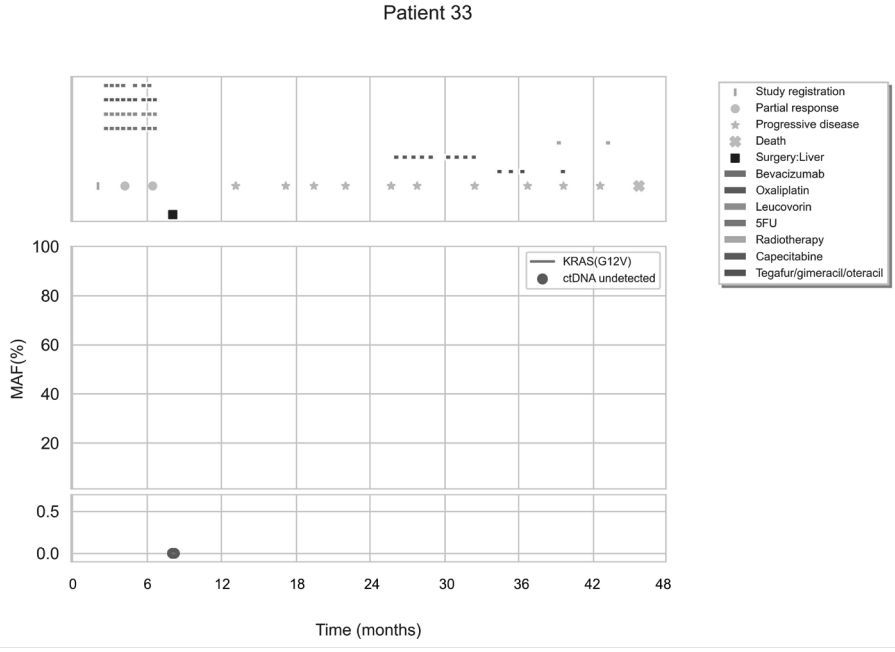
	All patients (N=23)	Postoperative undetectable ctDNA (N=17)	Postoperative detectable ctDNA (N=6)	p-value*
Baseline ctDNA, *n* (%)				0·48
Undetectable	2 (10)	2 (14)	0	
Detectable	18 (90)	12 (86)	6 (100)	
Missing baseline sample	3	3	0	
Histopathological response (TRG) , *n* (%)				< 0·001
Pathologic response (TRG 1-3)	16 (76)	15 (100)	1 (17)	
No pathologic response (TRG 4-5)	5 (24)	0 (0)	5 (83)	
Missing	2	2	-	

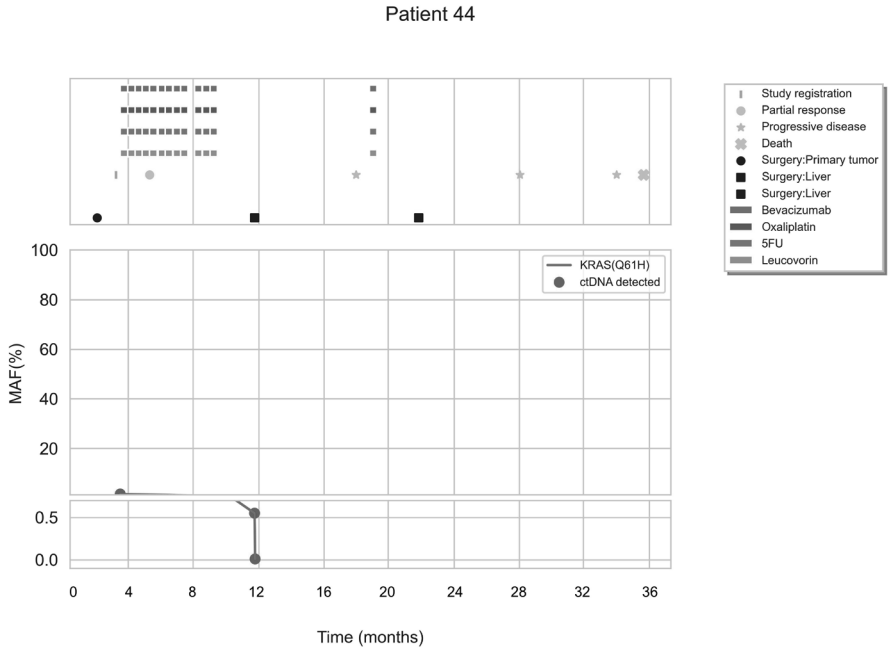
Abbreviations: ctDNA: circulating tumour DNA, CEA: carcinogenic embryonic antigen, RECIST: response evaluation criteria in solid tumours, TRG: tumour regression grade. *Categorical variables were compared with the Pearson's chi-square test and continuous variables were compared using the Mann-Whitney U test.

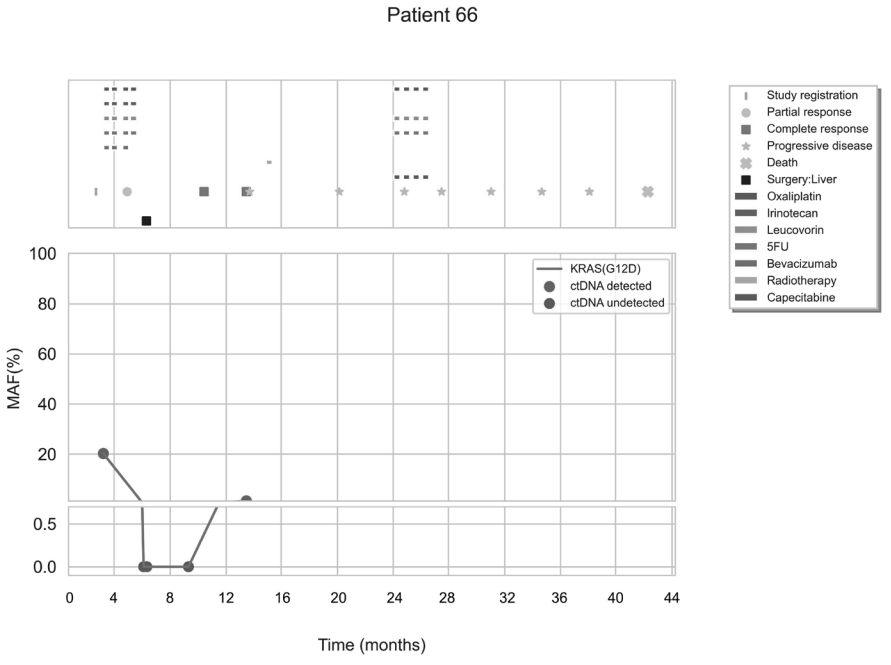
Supplementary Figure 1 Individual plots of all 23 patients showing ctDNA dynamics in the bottom panel by depicting the mutant allele frequencies (MAF) of the identified somatic *RAS* mutation. Detected mutations are shown in red, whereas undetected mutations are shown in green. Following the same x-axis, the top panel provides information about the treatment, surgeries, and radiological assessments of the patient.

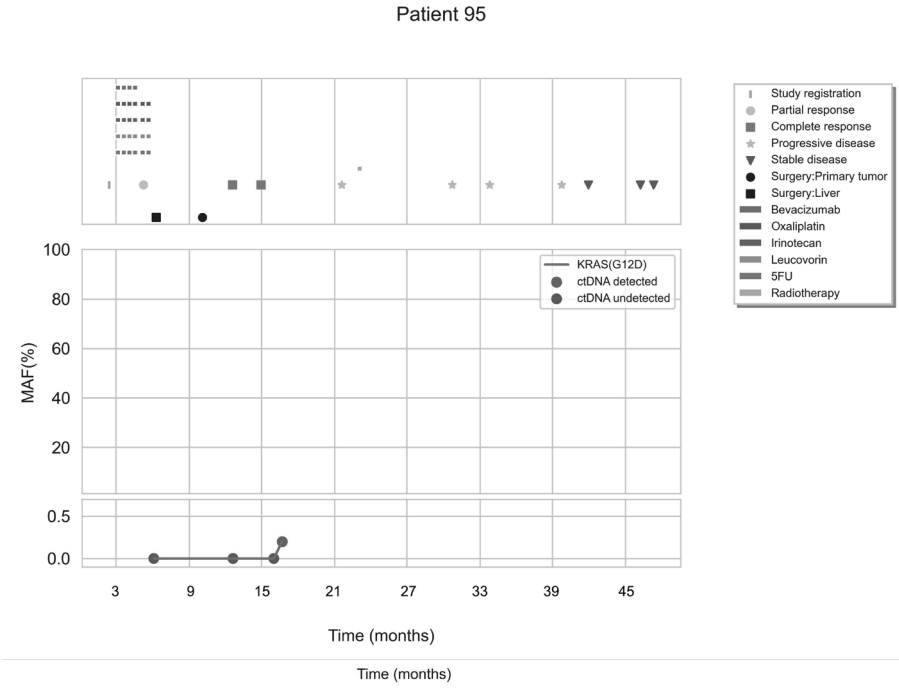


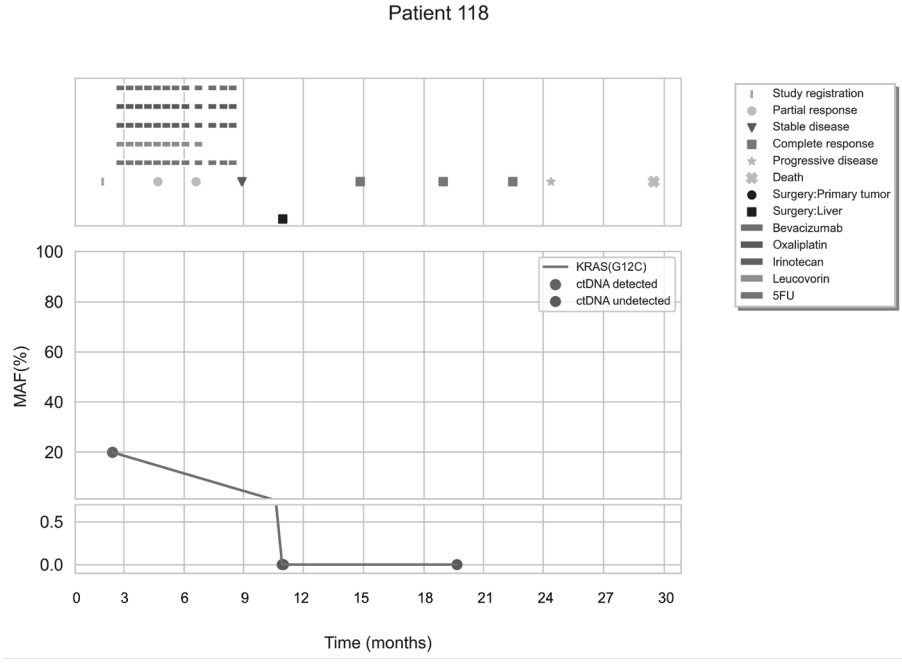

Patient 11
Study registration
Partial response
Stable disease
Progressive disease
Death
Surgery:Primary tumor
Surgery:Liver (stage 1)
Surgery:Liver (stage 2)
5FU
Leucovorin
Oxaliplatin
Bevacizumab
Radiotherapy
KRAS(G12D)
ctDNA detected
MAF(%)
100
80
60
40
20
0.5
0.0
0
4
8
12
16
20
24
28
32
36
40
44
Time (months)

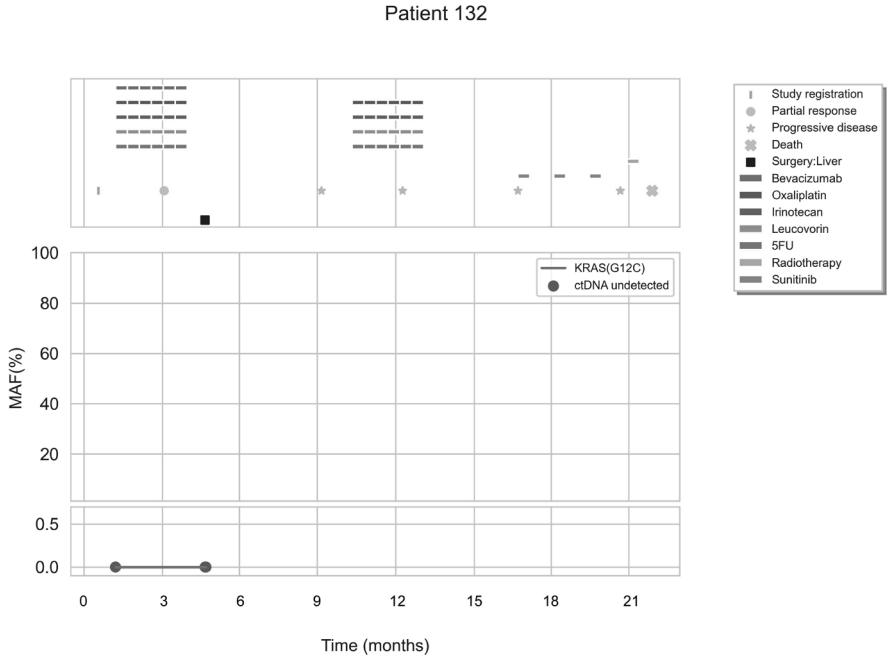

Patient 33
Study registration
Partial response
Progressive disease
Death
Surgery:Liver
Bevacizumab
Oxaliplatin
Leucovorin
5FU
Radiotherapy
Capecitabine
Tegafur/gimeracil/oteracil
KRAS(G12V)
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
6
12
18
24
30
36
42
48
Time (months)

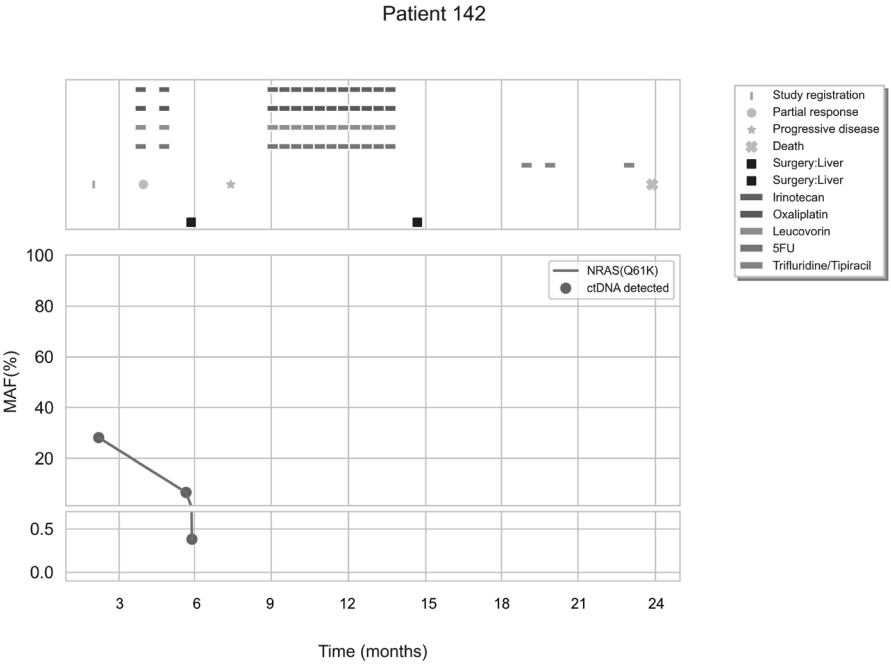

Patient 44
Study registration
Partial response
Progressive disease
Death
Surgery:Primary tumor
Surgery:Liver
Surgery:Liver
Bevacizumab
Oxaliplatin
5FU
Leucovorin
KRAS(Q61H)
ctDNA detected
MAF(%)
100
80
60
40
20
0.5
0.0
0
4
8
12
16
20
24
28
32
36
Time (months)

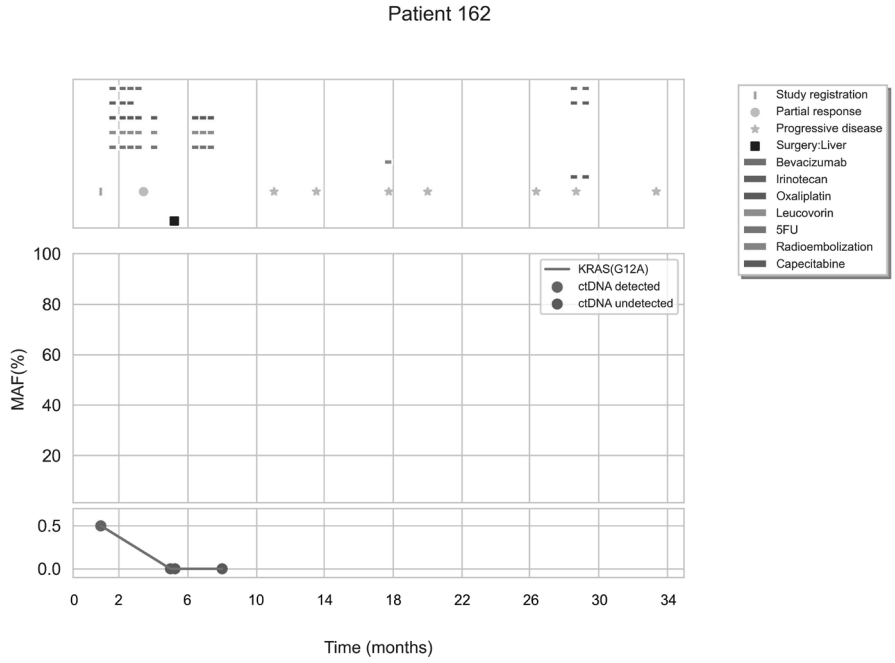

Patient 66
Study registration
Partial response
Complete response
Progressive disease
Death
Surgery:Liver
Oxaliplatin
Irinotecan
Leucovorin
5FU
Bevacizumab
Radiotherapy
Capecitabine
KRAS(G12D)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
4
8
12
16
20
24
28
32
36
40
44
Time (months)

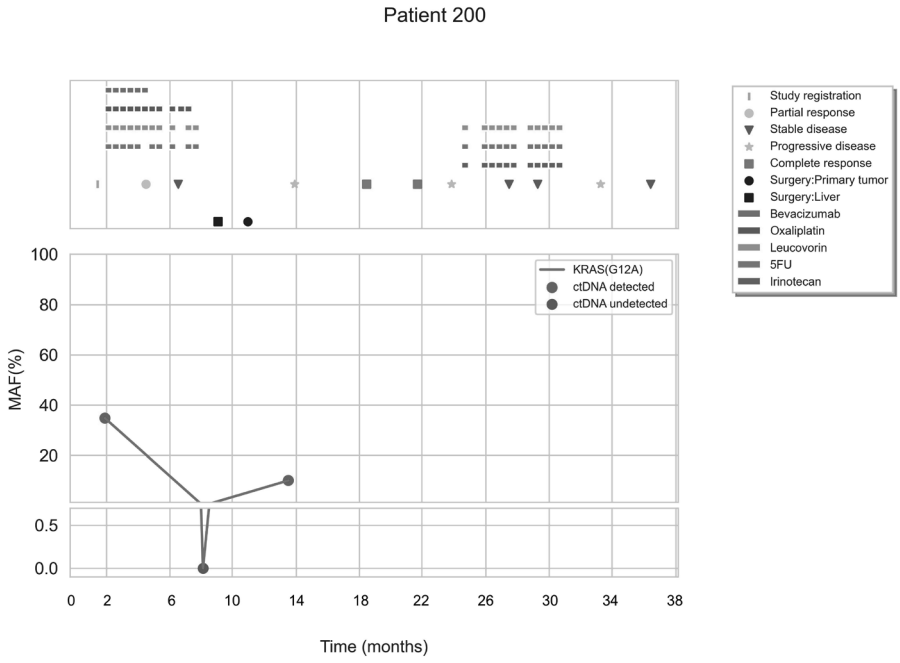

Patient 95
Study registration
Partial response
Complete response
Progressive disease
Stable disease
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
Oxaliplatin
Irinotecan
Leucovorin
5FU
Radiotherapy
KRAS(G12D)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
3
9
15
21
27
33
39
45
Time (months)

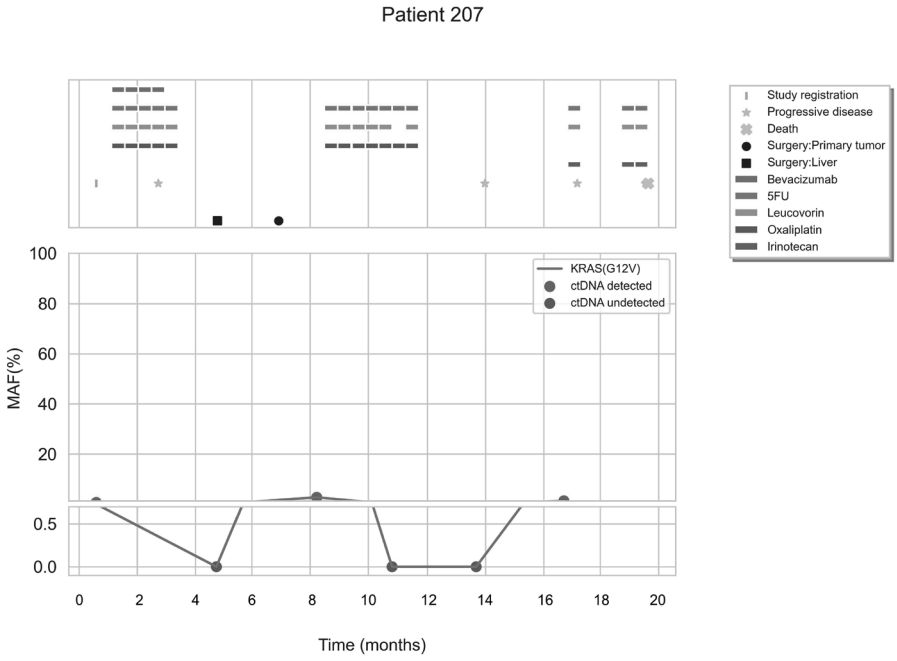

Patient 118
Study registration
Partial response
Stable disease
Complete response
Progressive disease
Death
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
Oxaliplatin
Irinotecan
Leucovorin
5FU
KRAS(G12C)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
3
6
9
12
15
18
21
24
27
30
Time (months)

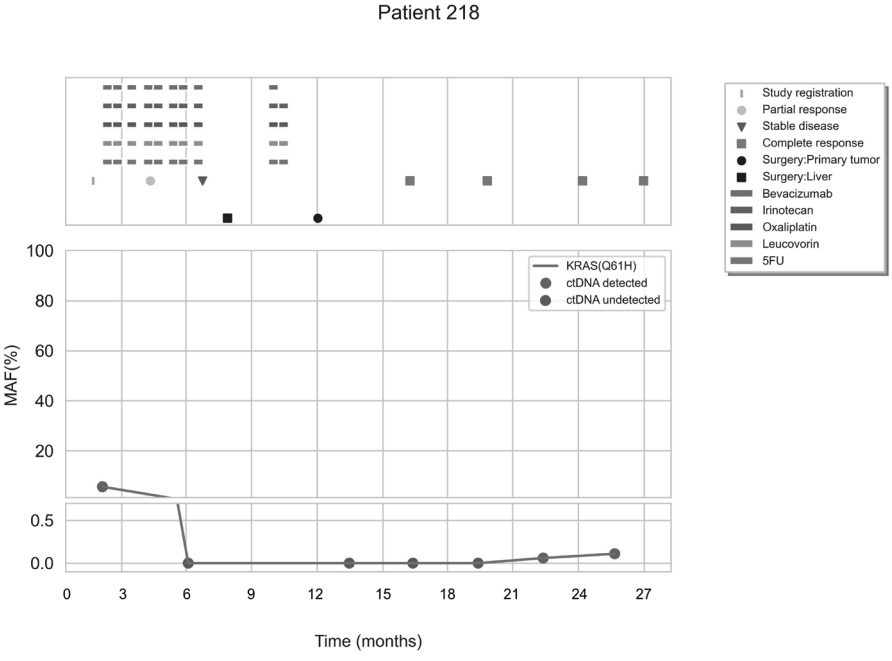

Patient 132
Study registration
Partial response
Progressive disease
Death
Surgery:Liver
Bevacizumab
Oxaliplatin
Irinotecan
Leucovorin
5FU
Radiotherapy
Sunitinib
KRAS(G12C)
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
3
6
9
12
15
18
21
Time (months)

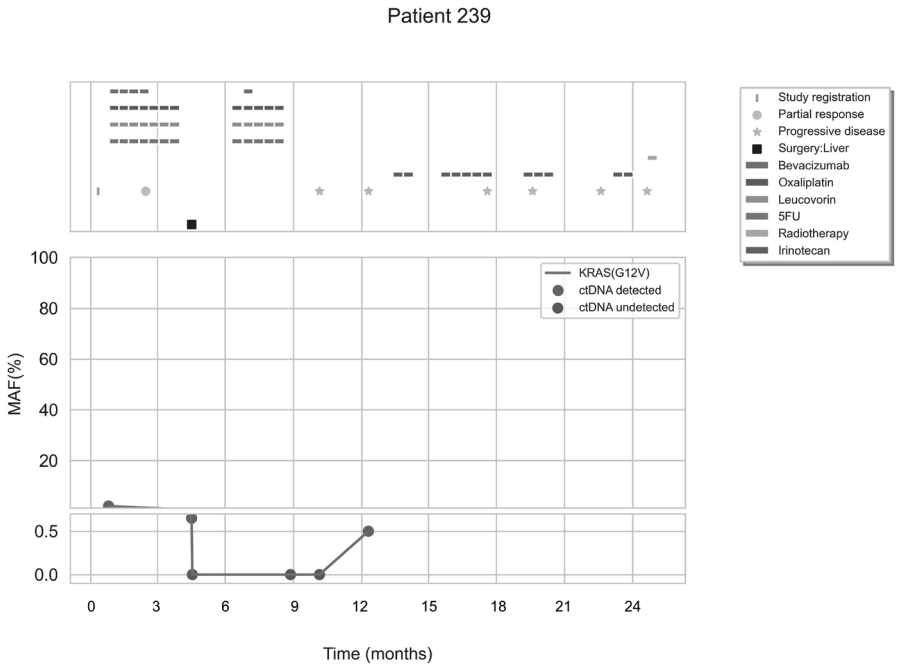

Patient 142
Study registration
Partial response
Progressive disease
Death
Surgery:Liver
Surgery:Liver
Irinotecan
Oxaliplatin
Leucovorin
5FU
Trifluridine/Tipiracil
NRAS(Q61K)
ctDNA detected
MAF(%)
100
80
60
40
20
0.5
0.0
3
6
9
12
15
18
21
24
Time (months)

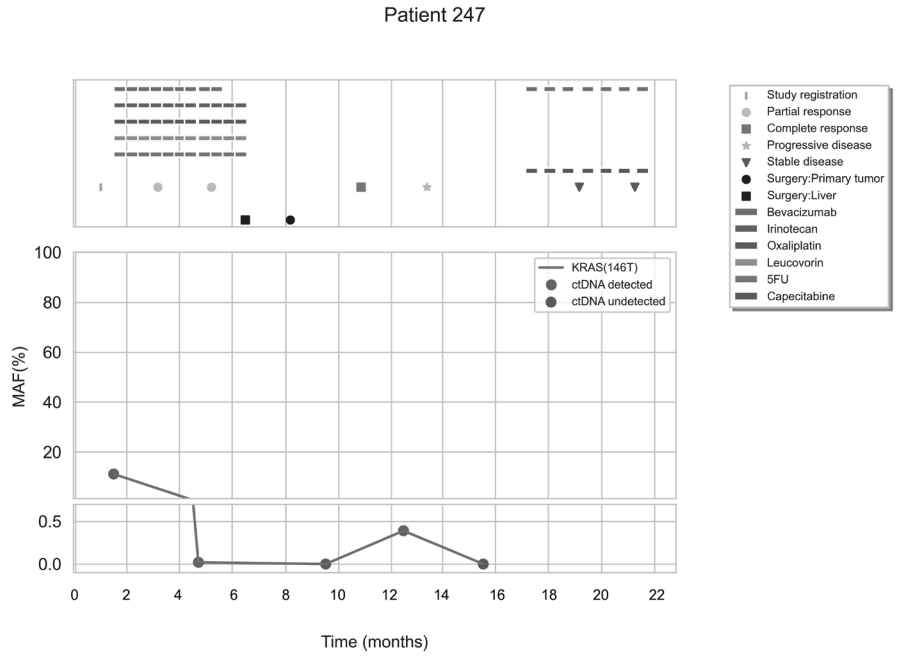

Patient 162
Study registration
Partial response
Progressive disease
Surgery:Liver
Bevacizumab
Irinotecan
Oxaliplatin
Leucovorin
5FU
Radioembolization
Capecitabine
KRAS(G12A)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
6
10
14
18
22
26
30
34
Time (months)

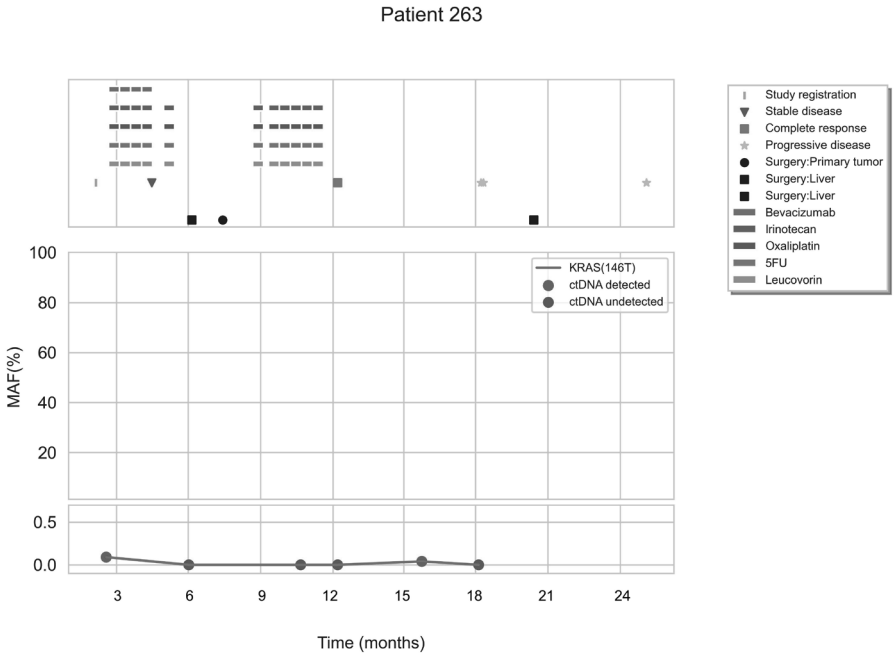

Patient 200
Study registration
Partial response
Stable disease
Progressive disease
Complete response
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
Oxaliplatin
Leucovorin
5FU
Irinotecan
KRAS(G12A)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
6
10
14
18
22
26
30
34
38
Time (months)

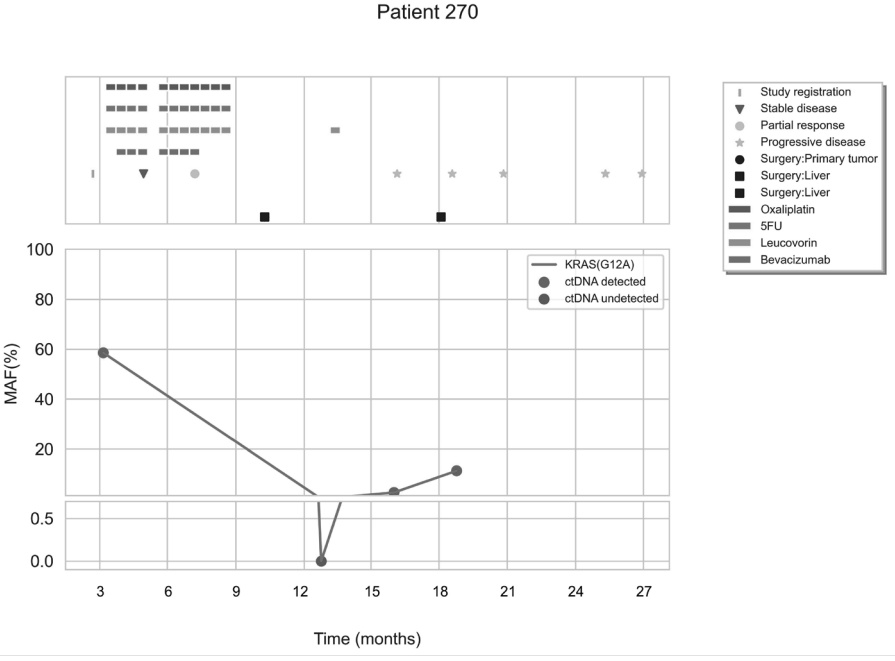

Patient 207
Study registration
Progressive disease
Death
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
5FU
Leucovorin
Oxaliplatin
Irinotecan
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (months)

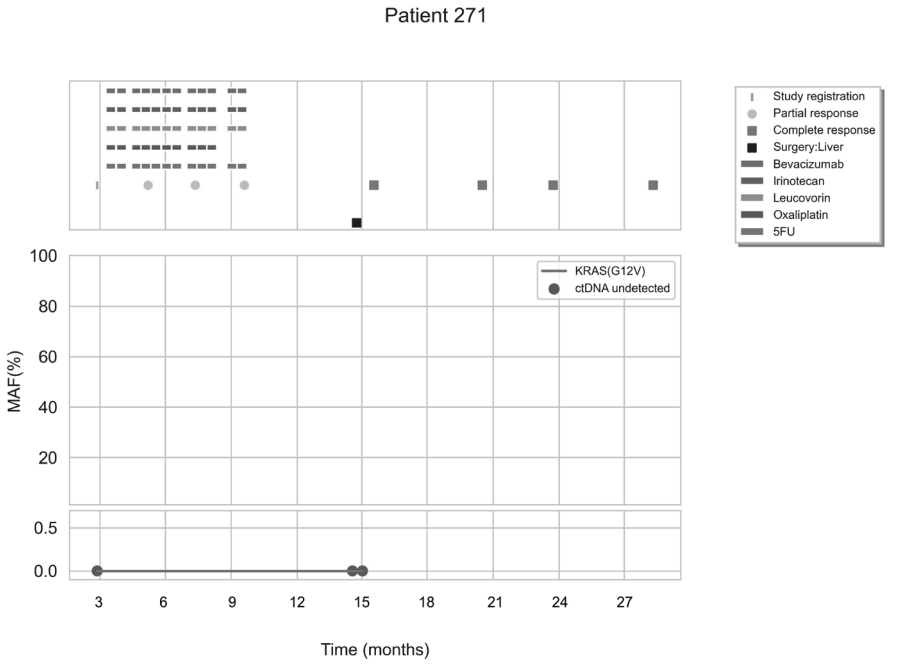

Patient 218
Study registration
Partial response
Stable disease
Complete response
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
Irinotecan
Oxaliplatin
Leucovorin
5FU
KRAS(Q61H)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
3
6
9
12
15
18
21
24
27
Time (months)

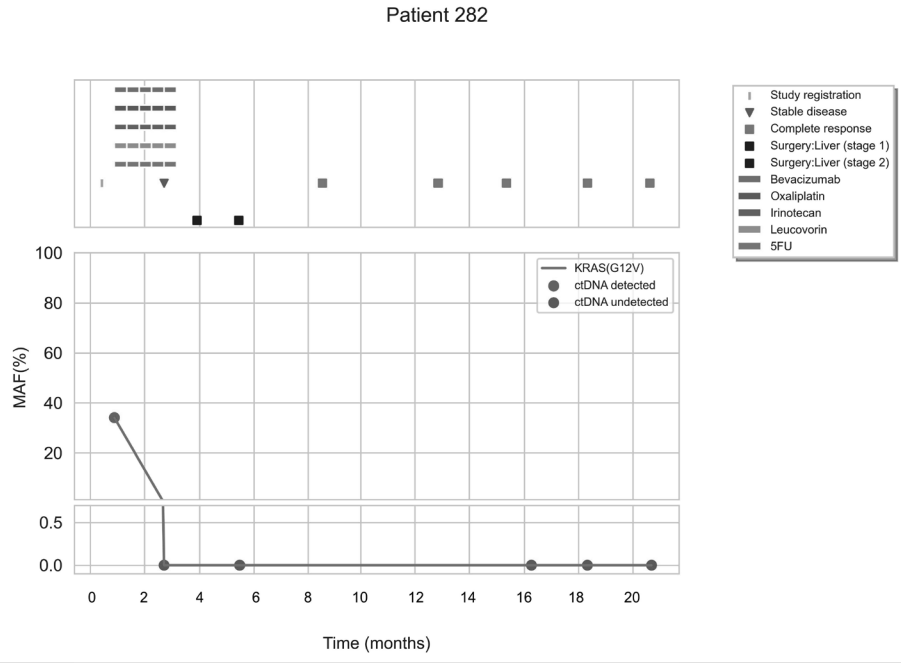

Patient 239
Study registration
Partial response
Progressive disease
Surgery:Liver
Bevacizumab
Oxaliplatin
Leucovorin
5FU
Radiotherapy
Irinotecan
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
3
6
9
12
15
18
21
24
Time (months)

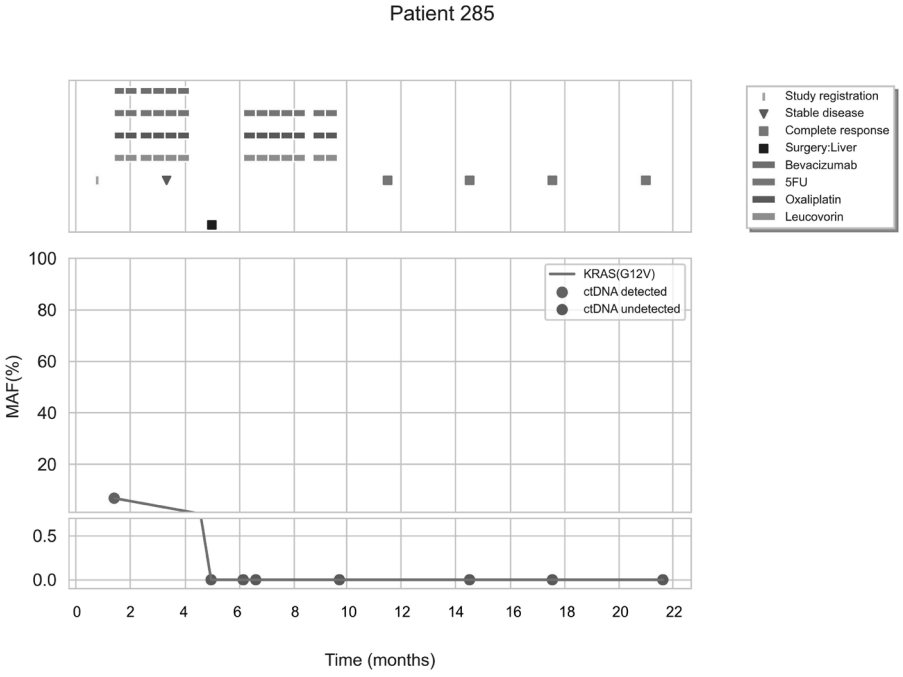

Patient 247
Study registration
Partial response
Complete response
Progressive disease
Stable disease
Surgery:Primary tumor
Surgery:Liver
Bevacizumab
Irinotecan
Oxaliplatin
Leucovorin
5FU
Capecitabine
KRAS(146T)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
22
Time (months)

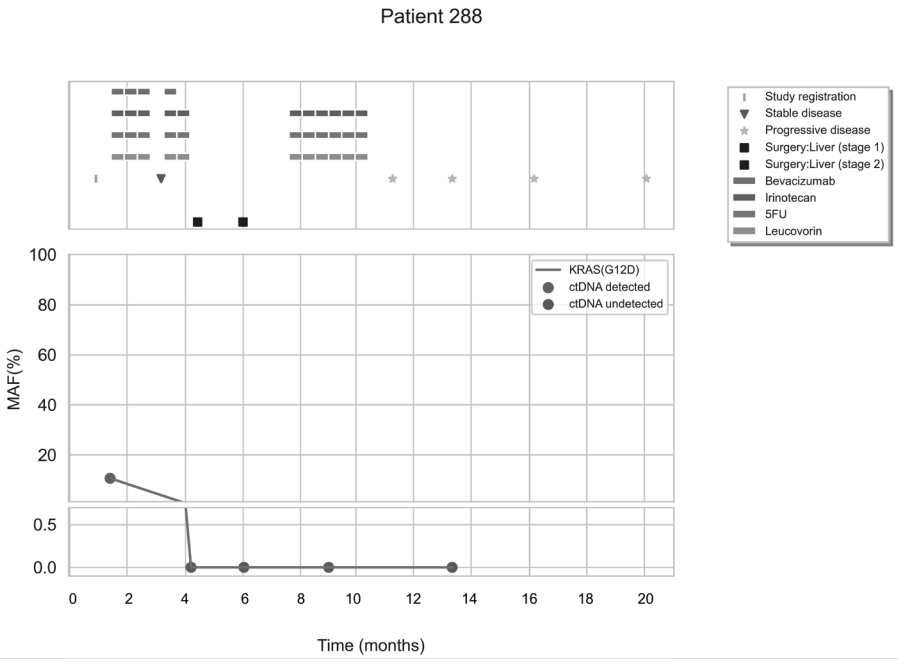

Patient 263
Study registration
Stable disease
Complete response
Progressive disease
Surgery:Primary tumor
Surgery:Liver
Surgery:Liver
Bevacizumab
Irinotecan
Oxaliplatin
5FU
Leucovorin
KRAS(146T)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
3
6
9
12
15
18
21
24
Time (months)

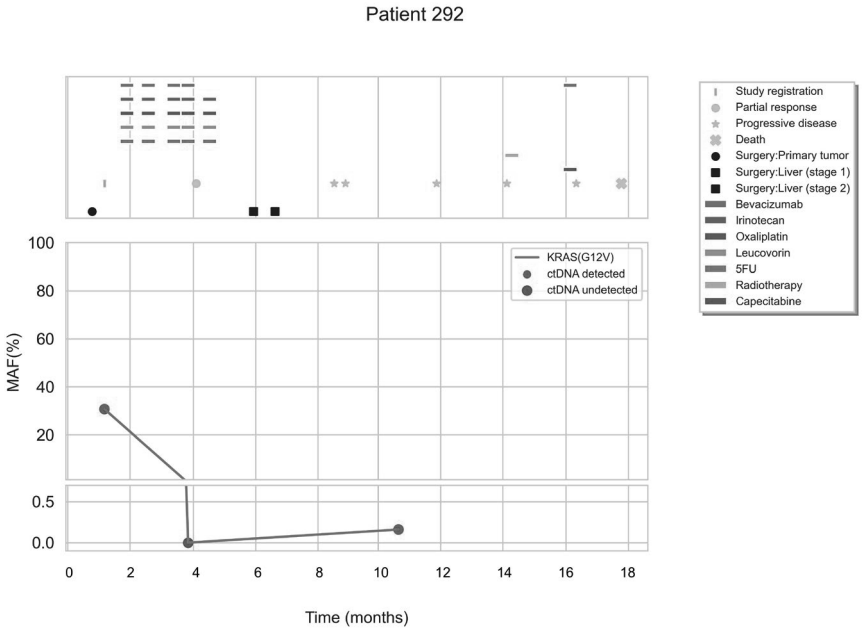

Patient 270
Study registration
Stable disease
Partial response
Progressive disease
Surgery:Primary tumor
Surgery:Liver
Surgery:Liver
Oxaliplatin
5FU
Leucovorin
Bevacizumab
KRAS(G12A)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
3
6
9
12
15
18
21
24
27
Time (months)

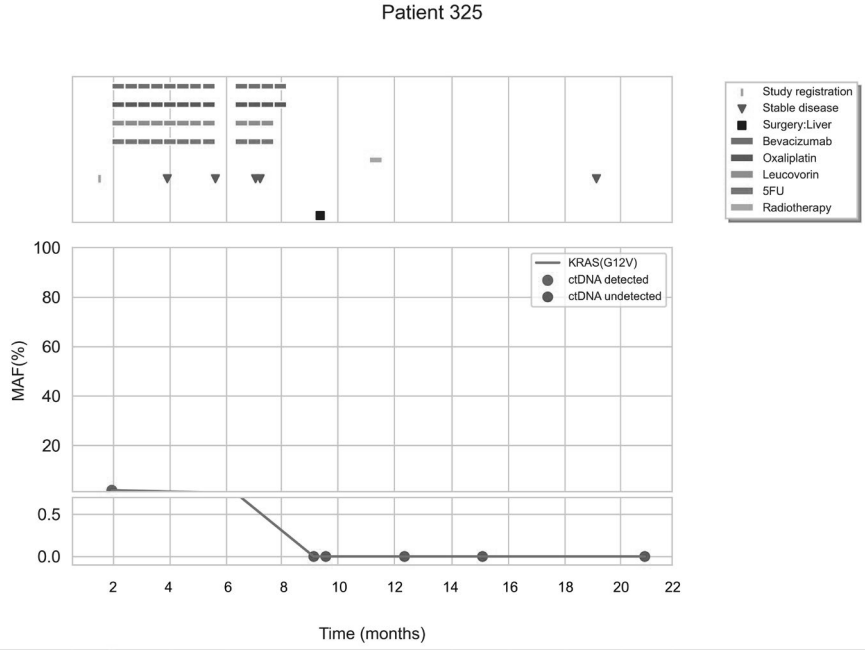

Patient 271
Study registration
Partial response
Complete response
Surgery:Liver
Bevacizumab
Irinotecan
Leucovorin
Oxaliplatin
5FU
KRAS(G12V)
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
3
6
9
12
15
18
21
24
27
Time (months)


Patient 282
Study registration
Stable disease
Complete response
Surgery:Liver (stage 1)
Surgery:Liver (stage 2)
Bevacizumab
Oxaliplatin
Irinotecan
Leucovorin
5FU
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (months)


Patient 285
Study registration
Stable disease
Complete response
Surgery:Liver
Bevacizumab
5FU
Oxaliplatin
Leucovorin
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
22
Time (months)


Patient 288
Study registration
Stable disease
Progressive disease
Surgery:Liver (stage 1)
Surgery:Liver (stage 2)
Bevacizumab
Irinotecan
5FU
Leucovorin
KRAS(G12D)
ctDNA detected
ctDNA undetected
MAF(%)
100
80
60
40
20
0.5
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (months)


Patient 292
Study registration
Partial response
Progressive disease
Death
Surgery:Primary tumor
Surgery:Liver (stage 1)
Surgery:Liver (stage 2)
Bevacizumab
Irinotecan
Oxaliplatin
Leucovorin
5FU
Radiotherapy
Capecitabine
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
Time (months)


Patient 325
Study registration
Stable disease
Surgery:Liver
Bevacizumab
Oxaliplatin
Leucovorin
5FU
Radiotherapy
KRAS(G12V)
ctDNA detected
ctDNA undetected
MAF(%)
Time (months)

CHAPTER 9

The prognostic value of total tumor volume response compared with RECIST1.1 in patients with initially unresectable colorectal liver metastases undergoing systemic treatment

Nina J Wesdorp, Karen Bolhuis, Joran Roor, Jan Hein TM van Waesberghe, S van Dieren, Martin J van Amerongen, Thiery Chapelle, Cornelis HC Dejong, Marc RW Engelbrecht, Michael F Gerhards, Dirk J Grunhagen, Thomas M van Gulik, John J Hermans, Koert P de Jong, Joost M Klaase, Mike SL Liem, Krijn P van Lienden, I Quintus Molenaar, Gijs A Patijn, Arjen M Rijken, Theo M Ruers, Cornelis Verhoef, Johannes HW de Wilt, Rutger-Jan Swijnenburg, Cornelis JA Punt, Joost Huiskens, Geert Kazemier, for the Dutch Colorectal Cancer Group Liver Expert Panel

Annals of Surgery Open 2021;2(4):e103

Abstract

Objectives

Compare total tumor volume (TTV) response after systemic treatment to Response Evaluation Criteria in Solid Tumors (RECIST1.1) and assess the prognostic value of TTV change and RECIST1.1 for recurrence-free survival (RFS) in patients with colorectal liver-only metastases (CRLM).

Background

RECIST1.1 provides unidimensional criteria to evaluate tumor response to systemic therapy. Those criteria are accepted worldwide but are limited by interobserver variability and ignore potentially valuable information about TTV.

Methods

Patients with initially unresectable CRLM receiving systemic treatment from the randomized, controlled CAIRO5 trial (NCT02162563) were included. TTV response was assessed using software specifically developed together with SAS analytics. Baseline and follow-up CT-scans were used to calculate RECIST1.1 and TTV response to systemic therapy. Different thresholds (10%, 20%, 40%) were used to define response of TTV as no standard currently exists. RFS was assessed in a subgroup of patients with secondarily resectable CRLM after induction treatment.

Results

A total of 420 CT-scans comprising 7820 CRLM in 210 patients were evaluated. In 30-50% (depending on chosen TTV threshold) of patients, discordance was observed between RECIST1.1 and TTV change. A TTV decrease of >40% was observed in 47(22%) patients who had stable disease according to RECIST1.1. In 118 patients with secondarily resectable CRLM, RFS was shorter for patients with less than 10% TTV decrease compared to patients with more than 10% TTV decrease (p= 0.015), whilst RECIST1.1 was not prognostic (p=0.821).

Conclusion

TTV response assessment shows prognostic potential in the evaluation of systemic therapy response in patients with CRLM.

Introduction

Patients with colorectal cancer develop metastases in the majority of cases, which are confined to the liver in about 30%.[1,2] For patients with colorectal liver-only metastases (CRLM), surgical resection and/or local ablative therapy is considered to be the only potentially curative treatment, with 5 year survival rates of 40% (range 16–71%).[3-5] Unfortunately, only 20% of patients diagnosed with CRLM present with resectable disease.[5,6] Patients with initially unresectable CRLM however, can become eligible for local treatment after downsizing by systemic therapy, allowing secondary resections with comparable survival rates as primary resections.[7-9] Accurate response evaluation and classification is crucial to these patients, but also to patients receiving palliative treatment, as the effect of initial treatment often determines the following treatment strategy.[10,11]

Efficacy of systemic therapy is most commonly measured on computer tomography scans (CT-scans) according to the Response Evaluation Criteria in Solid Tumors (RECIST1.1).[12,13] If RECIST1.1 is used, response is measured manually by radiologists and expressed as diameter change in a maximum of two selected target lesions per affected organ.[12] Despite that RECIST1.1 is most commonly applied for response evaluation, the validity of RECIST1.1 has been questioned.[14,15] Manual measurements are vulnerable to subjectivity, thus RECIST1.1 is hampered by inter- and intra-observer variability.[16,17]

More importantly, RECIST1.1 ignores potential valuable information about tumor volume and grayscale values provided by modern imaging techniques, as it only includes unidimensional size changes (diameter) of just two target lesions per organ.[18,19] In addition, the RECIST1.1 response category of stable disease is limited by its very broad range (30% decrease to 20% increase in sum of diameters), potentially grouping together patients with different prognoses.[12] RECIST1.1 was created acknowledging time and technology constraints of cross-sectional imaging at that time.[13] However, due to advances in diagnostic imaging and computer techniques over the past years, possibilities have evolved to measure three-dimensional (3D) volumes of tumors or total tumor volume (TTV).[20-22] Especially for patients with multiple CRLM, TTV assessment could represent a more complete evaluation of tumor burden, as the 3D effect on all tumors is considered. TTV already showed potential as predictor of survival and hepatic recurrence in patients with resectable CRLM.[22]

Nevertheless, consensus regarding response criteria for volume assessment are lacking, as different thresholds to define response of tumor volume are being used based on tumor shape.[13,21,23] Guidelines for volumetric assessment within RECIST1.1 state that thresholds are based on a spherical shape of a tumor, assuming that tumors are perfectly round objects.[13] Other studies based their criteria on the ellipsoid shape of

tumors.[19,21,23] Both types of criteria were based on the assessment of two target lesions only and not on the assessment of the total tumor burden. Currently no criteria for TTV response assessment exist and it is therefore difficult to compare TTV response to RECIST1.1. As a result, it remains unclear if TTV response assessment could potentially lead to different treatment strategies.

This study aims to compare TTV response after systemic treatment to RECIST1.1 and to assess the prognostic value of TTV and RECIST1.1 for recurrence-free survival (RFS) in patients with initially unresectable CRLM undergoing induction systemic treatment.

Methods

Study population

All patients registered between November 2014 and August 2018 from the ongoing multicenter randomized clinical trial of the Dutch Colorectal Cancer Group (DCCG), CAIRO5 (NCT02162563) were selected for this study.[(24)] The ongoing CAIRO5 trial aims to select the optimal systemic treatment strategy for patients with initially unresectable CRLM. In this trial, patients are randomized between different systemic therapy combinations based on primary tumor site and *RAS* and *BRAF* gene mutations. Patients are evaluated for resectability at baseline and during systemic treatment by an expert panel consisting of hepatobiliary surgeons and radiologists, using a digital online platform particularly created for this trial (ALEA FormsVision BV, Abcoude, The Netherlands).[(24)] Uniform criteria for (un)resectability were applied for this study.[24,25] All patients signed a written consent form and the study was conducted according to the ethical standards of the Helsinki Declaration of 1975. The following patient data were collected: patients' baseline characteristics, including demographics, genetic mutation status (*RAS/BRAF*) and serum markers (CEA and LDH level), medical images, radiology reports, and study outcome measures, including resection rate and recurrence-free survival. Liver segments were classified according to Couinaud.[26] Patients with Fong risk scores ranging between 0 and 2 points were categorized as low-risk score and patients with 3 to 5 points as high-risk score.[27]

Imaging

The CAIRO5 dataset used for the present research consisted of contrast-enhanced thorax-abdomen CT-scans at baseline and subsequently every two months during systemic therapy. All scans were performed in one of the 54 centers responsible for inclusion, resulting in difference in quality of scans. Every baseline and follow-up CT-scan was evaluated by one of the radiologists from the expert radiology panel, consisting of 5 radiologists, for tumor response analysis according to RECIST1.1 on a digital platform

(ALEA FormsVision BV, Abcoude, The Netherlands). Use of additional magnetic resonance imaging (MRI) or positron emission tomography (PET) scans was at the discretion of the local treatment team. Based on the available imaging scans and accompanying radiology reports, the hepatobiliary surgeon expert panel assessed resectability every eight weeks during systemic therapy, according to predefined criteria.[24,25] In the current study, only contrast-enhanced abdominal CT-scans in the portal-venous phase were included.

Data processing

Pre- and post-treatment CT-scans from the CAIRO5 trial were used for semi-automatic segmentation in the Tumor Tracking Modality of IntelliSpace Portal 9.0® (Philips Medical Systems, Best, The Netherlands), which is Conformité Européene (CE) certified software. First, the liver and the CRLM of all included patients were segmented by two trained members of the research team (NJW and SP) with the use of radiology reports from the CAIRO5 trial. All present CRLM on CT scans were segmented, including potential new lesions in the follow-up scan. Lesions were roughly outlined, which resulted in a semi-automatic contour or region of interest based on differences in density and were subsequently manually adjusted in every CT slice. Afterwards, all segmentations were adjusted and verified by a radiologist expert in abdominal imaging (JHTMW). Segmentation is the delineation of structures (e.g. tumors) on diagnostic imaging, resulting in 3D contours of these structures. The 3D segmentations and related CT-scans were combined to create grayscale segmentations on the SAS analytical platform® (SAS Institute Inc., Cary, North Carolina, USA).

Quantification of volume

Total tumor volume was calculated before and after systemic therapy in the SAS analytical platform® using the 'quantifyBioMedImages' action.[28] This action was specially developed by SAS and is not yet CE approved for use in clinical practice. This action calculates TTV directly out of the tumor segmentation from all CRLM present in the liver. A CT scan is built up by voxels, the 3D equivalent of a pixel, each containing a gray value. First the 'quantifyBioMedImages' action determined the volume of the box that one voxel represents. This was done by multiplying the length in the X, Y and Z direction of this box. These lengths are extracted from the pixel spacing and slice thickness attributes of the DICOM file, which are dependent of the type and settings of the CT-scanner.[(29)] After the volume of this box was calculated, the number of voxels included in the tumor segmentation were counted. Multiplying this number of voxels with the volume that one voxel represents, resulted in the TTV. The volume of the liver was measured in the same manner. For each patient, the change in TTV or delta TTV before and after systemic therapy was measured. In addition, the percentage TTV of the total liver volume, including TTV was calculated before and after therapy.

Tumor response according to RECIST1.1

Tumor response to systemic treatment was assessed routinely according to RECIST1.1 by the radiologists of the CAIRO5 expert panel.[25] Two target lesions were selected, and the longest diameter of both lesions were measured. The sum of diameters at baseline was used as reference for further response assessment. RECIST1.1 classification criteria for objective response were defined as complete response (disappearance of all target lesions), partial response (at least 30% decrease in the sum of diameters of target lesions), progressive disease (at least 20% increase in the sum of diameters of target lesions, including an absolute increase of 5 mm in diameter, or appearance of one or more new lesions) and stable disease (neither progressive disease or partial/complete response).[12]

Total tumor volume response groups

TTV response groups were determined based on the following thresholds: 10%, 20%, 40% increase or decrease in TTV. This resulted in the following TTV response groups:

- 10% thresholds: response (at least 10% decrease in TTV), progression (at least 10% increase in TTV), stable disease (less than 10% change in TTV from baseline)
- 20% thresholds: response (at least 20% decrease in TTV), progression (at least 20% increase in TTV), stable disease (less than 20% change in TTV from baseline).
- 40% thresholds: response (at least 40% decrease in TTV), progression (at least 40% increase in TTV), stable disease (less than 40% change in TTV from baseline).

Survival analysis

Patients undergoing local therapy (complete resection of CRLM or successful ablative therapy, or a combination of both) after successful downsizing of CRLM by systemic therapy were included for recurrence-free survival (RFS) analysis. Patients who did not undergo local therapy or underwent an incomplete resection were excluded. Complete resection was defined as R0 or R1 resection. R0 resection indicates a microscopically tumor margin-negative resection, in which no microscopic tumor cells have remained in the resection margins, and R1 resection was defined as the removal of all macroscopic disease, but with margins microscopically positive for tumor cells (<1 mm of the margin). Recurrence-free survival (RFS) was calculated from the date of surgery until progression of disease, defined as new metastases detected on the CT-scan, or death. In case of R0 or R1 resection, the follow-up was performed until disease progression according to the protocol and current national guideline: CT-scan of the liver every 6 months for 2 years, then every 12 months up to 5 years after surgery.[30] RFS was compared between response groups based on change in TTV and RECIST1.1. For the RFS analysis, the response groups were dichotomized into two groups per threshold (10%, 20%, 40%) and per RECIST1.1 classification. As a result, the following response groups were compared:

response (equal to and more than 10% TTV decrease) vs. stable/progressive (less than 10% TTV decrease), response (equal to and more than 20% TTV decrease) vs. stable/progressive (less than 20% TTV decrease), response (equal to and more than 40% TTV decrease) vs. stable/progressive (less than 40% TTV decrease), RECIST1.1 response vs. RECIST1.1 stable/ progression.

Statistics

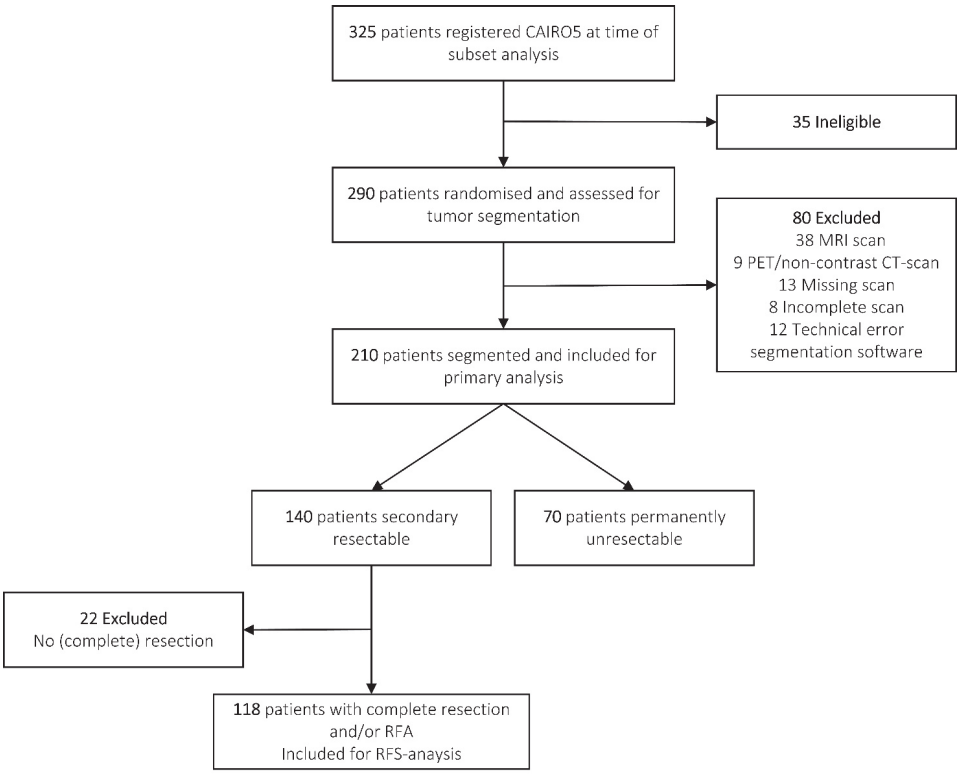
Statistical analyses were performed using SAS® Studio (version 5.2, SAS® Viya® release V.03.05, SAS Institute Inc., Cary, North Carolina, USA). Continuous variables were displayed as median with interquartile range (IQR) or range and categorical variables by number with percentages. TTV response was reported as continuous and categorical variables. TTV response and RECIST1.1 were compared by calculating the discordant patients. In addition, survival curves were generated separately for RECIST1.1 and TTV response using the Kaplan-Meier method and compared with the log-rank test. Survival analysis was considered statistically significant with a p-value <0.05. The relation between baseline parameters and baseline TTV was assessed using univariable and multivariable linear regression models, with backward elimination. Categorical variables were compared between different TTV response groups with Chi-Square Test, and continuous variables with Kruskal-Wallis Test. The Bonferroni correction was applied for multiple testing for the linear regression analyses and for the comparison of the baseline parameters between the TTV response groups (critical p-value=0.05/13=0.004).

Results

Study population

Between June 2014 and August 2018, 325 patients were registered and screened for eligibility for the CAIRO5 trial. Of these patients, 291 were randomized for the CAIRO5 study and after assessment for eligibility for tumor segmentation, a total of 210 patients were included in the current study. Most common reason for exclusion was use of MRI-scan (**Figure 1**). In primary analysis, TTV assessment was compared to RECIST1.1 using baseline and first follow-up CT-scan. On 420 evaluated baseline and first follow-up CT-scans a total of 7280 CRLM were segmented. Baseline characteristics of the included patients are shown in **Table 1**. Median age was 62 years (IQR 55–70) and one third of the patients was female. Most patients had a left-sided primary colon tumor and synchronous metastases. *RAS/BRAF* mutation was present in 59% of the patients. The median number of metastases at baseline was 11 (IQR 7–22) and 93% of patients had bilobar metastatic disease, with a median of six (IQR 4–7) liver segments involved.

Figure 1 Flow diagram of patient inclusion.



Abbreviations: PET indicates positron emission tomography; RFA, radio-frequency ablation.

Total tumor volume assessment

Radiological parameters are summarized in **Table 2**. The median TTV at baseline was 100 cm^3, ranging between 1.44 and 2530 cm^3. Median TTV at first follow-up scan was 49 cm^3 (range 0.54 cm^3–3827 cm^3). The median change in TTV was a 47% decrease (range 92% decrease to 658% increase), with a median delta TTV of 27 cm^3 decrease (range 1340 cm^3 decrease to 2662 cm^3 increase). The percentage TTV of the total liver volume at baseline ranged between 0.09% and 59%, with a median of 6%. The association between baseline parameters and baseline TTV was examined **Table 3**). In multivariable analysis, the following parameters were independently correlated with larger TTV at baseline: serum LDH level (*β 0.310*, p<0.001), serum CEA level (*β 0.038*, p=0.001), number of liver metastases (*β 4.741*, p<0.001), and diameter of largest tumor (*β 5.770*, p<0.001).

Table 1 Baseline patient characteristics.

Baseline parameters	Total cohort N=210
Age - yr	
Median [IQR]	62 [55.0–70.0]
Sex - no (%)	
Male	138 (65.7)
Female	72 (34.3)
Site of primary tumor – no (%)	
Right colon	56 (34.3)
Left colon or rectum	154 (65.7)
pN status primary tumor – no (%)	
Negative	51 (24.3)
Positive	69 (32.9)
Missing	90 (42.9)
Time to metastases – no (%)	
Synchronous	185 (88.1)
Metachronous	25 (11.9)
Mutational status – no (%)	
RAS mutation	111 (52.9)
BRAF^{V600E} mutation	12 (5.7)
RAS & *BRAF* wild-type	87 (41.4)
Baseline serum LDH level	
Median [IQR]	292 [209–530]
Baseline serum CEA level	
Median [IQR]	44 [11–258]
Number of liver metastases	
Median [IQR]	11 [7–22]
Diameter of largest metastasis (mm)	
Median [IQR]	41.5 [28–71]
Number of liver segments involved	
Median [IQR]	6 [4–7]
Distribution of liver metastases – no (%)	
Unilobar	15 (7.1)
Bilobar	195 (92.5)
Fong risk score – no (%)	
Low	10 (4.8)
High	200 (95.2)
Induction systemic therapy – no (%)	
FOLFOX / FOLFIRI and Bevacizumab	107 (51.0)
FOLFOX / FOLFIRI and Panitumumab	42 (20.0)
FOLFOXIRI and Bevacizumab	61 (29.0)

Table 2 Radiological parameters (baseline & first follow-up scan).

Radiological parameters	Total cohort N=210
Total tumor volume	
TTV (cm^3) - median [range]	
Baseline	100 [1.44–2530]
Follow-up 1	49 [0.54–3827]
TTV delta (cm^3)	
Median [range]	-27 [-1340–2662]
TTV change (%)	
Median [range]	-47 [-92–658]
TTV percentage of liver volume - median [range]	
Baseline	6 [0.09–59]
Follow-up 1	3 [0.04–65]
TTV response groups (TH 10%) – no (%)	
Response	171 (81.4)
Stable	11 (5.2)
Progression	28 (13.3)
TTV response groups (TH 20%) – no (%)	
Response	165 (78.6)
Stable	25 (11.9)
Progression	20 (9.5)
TTV response groups (TH 40%) – no (%)	
Response	125 (59.5)
Stable	70 (33.3)
Progression	15 (7.1)
RECIST	
Sum of TL (mm) - median [range]	
Baseline	72 [17–282]
Follow-up 1	54 [8–244]
Diameters change (%)	
Median [range]	-24 [-76–40]
RECIST classification – no (%)	
Response	84 (40.0)
Stable	110 (52.4)
Progression	16 (7.6)

Abbreviations: RECIST, Response Evaluation Criteria in Solid Tumors; TH, threshold; TL, target lesions; TTV, total tumor volume.

Patient characteristics of TTV response groups

Patients were classified in TTV response groups based on the volumetric thresholds (**Table 2**). Baseline parameters were compared between the different TTV response groups using the same thresholds (**Supplementary Table S1-3**). No statistically significant differences in baseline parameters were found between the different TTV response groups (**Supplementary Table S1-3**). In the TTV response groups using thresholds of 40%, trends towards differences were found in age, number of liver metastases, and number of liver segments involved (**Supplementary Table S1**). A similar trend towards difference in the number of liver segments involved was observed between the TTV response groups using thresholds of 20% (**Supplementary Table S2**).

Table 3 Univariable and multivariable linear regression analyses for relation between baseline parameters and total tumor volume at baseline.

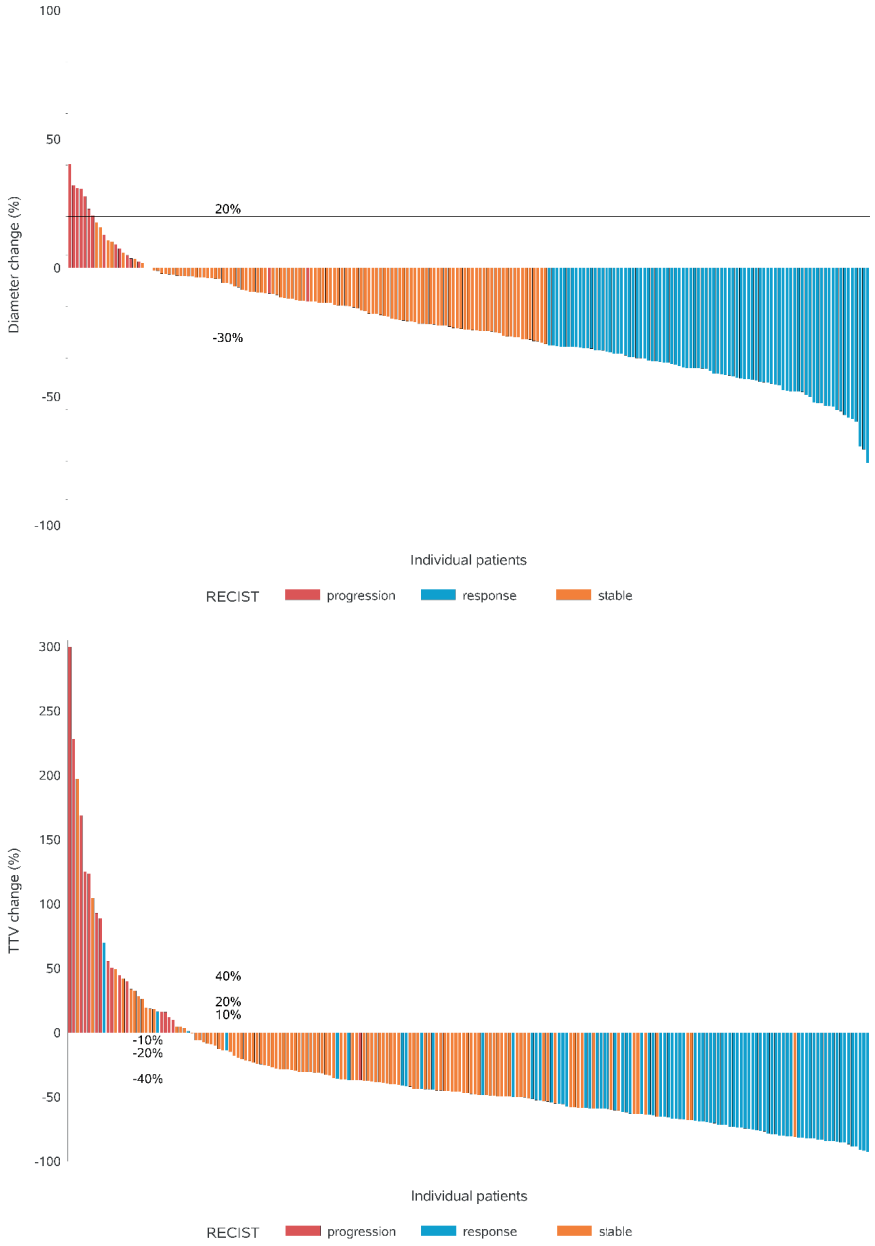
		Univariable analyses			Multivariable analysis		
Baseline parameters	**Patients**	**B**	**95% CI**	***P* value**	**B**	**95% CI**	***P* value**
Age, years	210	-4.438	-9.723–0.846	0.099	-		
Sex; Female vs. Male	210	102.577	-6.004–211.158	0.064	-		
Sidedness primary tumor, right vs. left	210	-23.470	-140.942–94.002	0.694	-		
Primary tumor nodal status, positive vs. negative	120	109.965	-17.362–237.293	0.090	-		
Time to metastases, synchronous vs. metachronous	210	224.986	67.490–382.482	0.005	-		
RAS/BRAF mutational status, wild-type vs. mutation	210	30.301	-75,114–135.716	0.567	-		
LDH level at baseline	210	0.630	0.530–0.731	<0.001	0.310	0.211–0.409	<0.001
CEA level at baseline	210	0.103	0.072–0.135	<0.001	0.038	0.016–0.061	0.001
Number of metastases	210	5.508	2.433–8.582	0.001	4.741	2.668–6.814	<0.001
Diameter of largest metastasis (mm)	210	7.925	6.702–9.149	<0.001	5.770	4.545–6.994	<0.001
Number of liver segments involved	210	25.642	-2.789–54.073	0.077	-		
Location metastases, bilobar vs. unilobar	210	-170,711	-371.142–29.719	0.095	-		
Fong risk score, high vs. low	210	-43.191	-287.145–200.763	0.727	-		

Critical p-value = 0.004 (Bonferroni corrected). *Abbreviations:* B, unstandardized coefficients beta; BRAF, v-Raf murine sarcoma viral oncogene homolog B; CEA, carcinoembryonic antigen; CI, confidence interval; LDH, lactate dehydrogenase; RAS, rat sarcoma oncogene.

RECIST1.1 versus TTV change

According to RECIST1.1, CRLM were classified as having an objective response to treatment, stable disease, or progression of disease in 84 (40%), 110 (52%), and 16 (8%) patients, respectively (**Table 2**). Based on the 10% TTV thresholds, CRLM were classified as having response, stable disease, or progression of disease in 171 (81%), 11 (5%), and 28 (13%), respectively. According to 20% TTV thresholds, CRLM were classified as response, stable disease, or progression in 165 (79%), 25 (12%), and 20 (10%) patients, respectively. Based on the 40% TTV thresholds, CRLM were classified as having response, stable disease, or progression of disease in 125 (60%), 70 (33%), and 15 (7%) patients, respectively (**Table 2**). The percentual changes in sum of diameters and the change in TTV for the included patients are shown in **Figure 2**.

Figure 2 Change in TTV and in sum of diameters (RECIST1.1). The percentual change in sum of diameters is depicted on the y axis for the individual patients on the x axis (A). In the same manner, the percentual change in TTV is depicted on the y axis for the individual patients on the x axis (B). Patients are classified according to RECIST1.1 as response, stable, and progression. One patient classified as progression by RECIST1.1 experienced a TTV increase of 658%, which is depicted as 300% TTV increase in (B).



The change in TTV using the different TTV thresholds was compared to RECIST1.1 (**Table 4**). According to the 10%, 20%, and 40% TTV thresholds, discordance between RECIST1.1 and TTV change was observed in 104 (50%), 101 (48%), and 63 (30%) patients, respectively. The majority of discordant cases were observed in the RECIST1.1 stable group for all TTV thresholds. In particular, response to treatment was more often classified when applying the TTV thresholds than following RECIST1.1. A total of 47 (22%) patients classified as stable according to RECIST1.1, experienced a TTV decrease of more than 40% (range 40–81%). In 11 (5%) patients, TTV increased with more than 10%, while classified as stable according to RECIST1.1. In four (2%) of these RECIST1.1 stable patients, TTV even increased by more than 40% (range 42–197%). The majority of cases in concordance were found in the RECIST1.1 response and RECIST1.1 progression patients. Nevertheless, three patients classified as responsive according to RECIST1.1 showed TTV increase of 1%, 17%, and 70%. An illustration of TTV before and after therapy for one of the discordant patients is shown in Supplementary **Figure S1**.

Recurrence-free survival analysis

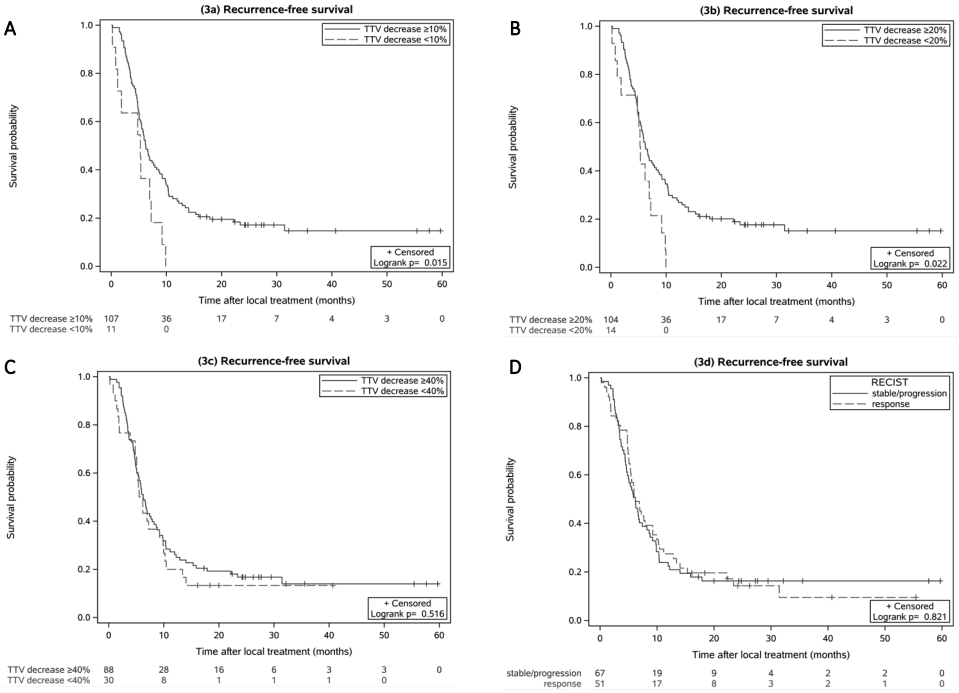
Of the 210 included patients, CRLM of 140 patients were evaluated as secondarily resectable after successful downsizing of the CRLM by the expert panel from the CAIRO5 trial. Of these patients, 118 patients underwent local therapy (complete resection of CRLM or a successful ablative therapy, or a combination of both) and were included for the RFS analysis (**Figure 2**). Median follow-up of these patients was 27 months. Patients were allocated in different response groups based on change in TTV and RECIST1.1 upon induction systemic treatment and RFS was compared between these groups. Patients with less than 10% TTV decrease had significantly shorter RFS compared to patients with more than 10% TTV decrease, with median RFS time of 5.3 and 6.2 months respectively (p=0.015). Similar results were found for patients with less than 20% TTV decrease compared to patients with more than 20% TTV decrease (median RFS 5.3 months vs. 6.3 months, respectively (p=0.022)). No significant differences in RFS were found between the patients with more than 40% TTV decrease compared to patients with less than 40% decrease in TTV (p=0.516). In addition, no significant differences in RFS were observed between responsive patients according to RECIST1.1 compared to patients with stable or progressive disease by RECIST1.1 (p=0.821). Survival curves of the different response groups are depicted in **Figure 3**.

Table 4 RECIST versus TTV change at first follow-up scan

TTV response groups	RECIST Response N=84	RECIST Stable N=110	RECIST Progression N=16	Total cohort N=210 Discordant – no (%)
TTV change (TH 10%) – no				
Response	81	89	1	
Stable	1	10	0	
Progression	2	11	15	
Discordant - no	*3*	*100*	*1*	104 (50)
TTV change (TH 20%) – no				
Response	80	84	1	
Stable	3	18	4	
Progression	1	8	11	
Discordant - no	*4*	*92*	*5*	101 (48)
TTV change (TH 40%) – no				
Response	78	47	0	
Stable	5	59	6	
Progression	1	4	10	
Discordant - no	*6*	*51*	*6*	63 (30)

Abbreviations: RECIST, Response Evaluation Criteria in Solid Tumors; TH, threshold; TTV, total tumor volume.

Figure 3 Kaplan Meier analysis of recurrence-free survival of patients with secondarily resectable CRLM according to TTV change and RECIST1.1. Survival curves and life tables of patients with: (A) response (equal to and more than 10% TTV decrease) versus stable/progressive (less than 10% TTV decrease), (B) response (equal to and more than 20% TTV decrease) versus stable/progressive (less than 20% TTV decrease), (C) response (equal to and more than 40% TTV decrease) versus stable/progressive (than 40% TTV decrease), (D) RECIST1.1 response versus RECIST1.1 stable/progression.



Discussion

This study demonstrates that change in TTV after systemic treatment differed from RECIST1.1 in 30–50% of CRLM patients followed by CT who were deemed unresectable according to predefined criteria. Furthermore, change in TTV was found prognostic for RFS in a subgroup of patients who became eligible for resection after induction with systemic therapy, whilst RECIST1.1 was not.

Since response to therapy is considered to be an important prognostic factor predicting long-term outcomes after liver surgery, difference in response assessment could potentially lead to a different treatment strategy.[31] Patients with CRLM classified as responsive or stable by RECIST1.1, but experiencing TTV progression may benefit from an earlier switch of treatment regimen in daily care. In addition, patients classified stable by RECIST1.1 but showing large decrease in TTV (e.g. >40%) may potentially be selected earlier for tumor resection if anatomically feasible. These findings suggest that TTV response assessment could be of added value in the evaluation of systemic therapy in a subgroup of patients with initially unresectable CRLM.

In this study, baseline TTV was positively associated with number and diameter of metastases, and serum marker levels (CEA and LDH). Since high TTV equals large tumor burden, it is not surprising that these baseline characteristics were associated. High levels of baseline CEA and LDH are prognostic risk factors for disease extensiveness and poor survival in patients with CRLM.[32-34] High TTV could also reflect a more aggressive underlying tumor biology, with potentially decreased sensitivity to systemic therapy. Patients with TTV progression of more than 40% showed a trend of younger age compared to the other TTV response groups. Interestingly, a recent study of Jácome *et al.* demonstrated that in patients with CRLM undergoing resection, earlier onset of disease (i.e. younger age) in combination with *RAS* mutation was prognostic for poorer overall survival in comparison to patients with late age onset.[35] These results indicate that TTV might be a reliable prognostic factor also in these patient groups. This is in agreement with the study of Tai *et al.,* where TTV at baseline was prognostic for both overall survival and recurrence-free survival in patients with multiple CRLM, whilst RECIST1.1 was not.[22]

Currently, consensus regarding volumetric thresholds defining response to treatment, stable disease or progression is lacking, since different thresholds for volumetric response based on tumor shape are being used.[21,23,36] Guidelines within RECIST1.1 for volumetric assessment defined the geometrical relationship between change in diameter and volume based on the spherical shape of tumors, and stated that 30% decrease in diameter correlated geometrically to 65% decrease in volume, while 20% increase in diameter correlated with 73% increase in volume.[13] Other studies based their

volumetric criteria on the ellipsoid shape of tumors, using the thresholds of 30% decrease in volume and 20% increase in volume other studies.[19,21,23] Moreover, no criteria for the assessment of TTV are defined yet, as only assessments of target lesions have been described.[19,36,37]

In this study, different thresholds for TTV response assessment were examined as no standard currently exists and response was more often classified when applying those thresholds than following RECIST1.1 response classification. As expected, the majority of patients with initially unresectable CRLM that became secondarily resectable after systemic treatment, showed TTV decrease (>10%) and only a small number of patients had progressive or stable TTV. The results showed that change in TTV was prognostic for RFS, while no significant differences were observed between the RECIST1.1 response groups. These findings strengthen the hypothesis that change in TTV could be a reliable prognostic factor. However, the clinical relevance of one month difference in RFS using the 10% TTV thresholds in a small number of patients could be debated. Therefore, the applied TTV thresholds in this study should be validated in a larger study population of an external dataset. Additionally, thresholds should be investigated with receiver operating characteristics curve analysis based on survival outcomes, such as overall survival and progression-free survival.

Median RFS of 5 months and maximum RFS of 10 months in patients with small decrease in TTV (less than 10%) were observed in this study. The benefit of local treatment for these patients could be argued, as median progression-free survival of unresectable metastatic colorectal cancer patients treated with systemic therapy, is up to 12 months.[38] In trials restricted to patients with initially unresectable liver-only metastases, similar progression-free survival around 12 months (range 10–18) is described.[38-43]

Accurate response evaluation is pivotal to the treatment of patients with initially unresectable CRLM. However, the current RECIST1.1 guidelines only include unidimensional size changes of maximum two target lesions per organ, thereby ignoring potentially valuable other information of the tumors, such as TTV, morphological changes, early tumor shrinkage, and depth of response. These alternative radiological metrics have been found prognostic for overall survival, progression-free survival, or pathologic response in patients with CRLM treated with systemic therapy.[18,22,44,45)] Total tumor volume assessment could represent a more complete evaluation of the tumor burden, as the effect on the overall tumor load is evaluated and 3D measurements may capture size changes better than unidimensional measurements. A recent study demonstrated that the limited number of target lesions following RECIST1.1 may not be an accurate representation of the overall tumor load in patients with metastatic cancer, including liver-only metastases.[14]

In this study, TTV was measured based on the semi-automatic segmentations of all CRLM with specially developed software, enabling assessment of the whole tumor burden. The positive results presented in this study suggest that TTV assessment appears a promising method to improve tumor response evaluation in patients with CRLM. Nevertheless, the semi-automatic segmentations used for volumetric assessment in this study are still time-consuming and advanced volumetric software is not yet widely available in every radiology department. To actually implement TTV assessment in clinical practice, fully automatic volumetric algorithms should be developed. Another important factor to implement TTV assessment in clinical practice, is the CE marking or Food and Drug Administration approval for such tool. The specially developed action used to calculate TTV in this study is not CE or Food and Drug Administration approved for clinical practice. In fact, this action is part of the development of an automatic tumor response pipeline conducted by this research group. This pipeline is still under development and will be externally validated in future studies. With the use of automatic algorithms, volume measurements of individual tumors could also play a role in the assessment of mixed tumor response in future studies. This mixed response is common and observed in approximately 35% of patients with multiple CRLM treated with systemic therapy, showing poorer prognosis than patients with homogeneous response, and might point towards different tumor biology of different CRLM.[46,47]

This study had several limitations. First, survival outcomes were only investigated in a subgroup of patients who became eligible for hepatic resection after induction treatment, because the CAIRO5 trial is ongoing, and no analysis could be performed on the whole study group. As a result, the prognostic value of TTV change could only be investigated in a selection of patients, excluding potentially interesting patients with large TTV increase or other specific patient groups. Therefore, the relation of TTV response assessment with survival needs to be further established in a future study when the CAIRO5 trial is completed. Second, the evaluation of non-target lesions was not included in the assessment of RECIST1.1 in current study. This may have resulted in a selective comparison between RECIST1.1 and TTV. The evaluation of non-target lesions was often not described in the CAIRO5 radiology reports but should be included in future studies. Third, the results of this study were based on the tumor response assessment of the first follow-up scan only. The assessment of tumor response using TTV and RECIST1.1 on the second follow-up scan may also be valuable.[48,49] In future studies, implementation of more additional imaging features, such as morphological changes, in tumor response assessment is recommended, because patients with CRLM may also show a morphological response to systemic treatment without affecting tumor size.[18,50]

In conclusion, TTV response assessment shows prognostic potential in the evaluation of systemic therapy response in patients with initially unresectable CRLM and could contribute to the improvement of treatment selection. Change in TTV differed from

RECIST1.1 in 30–50% of initially unresectable CRLM patients, using different TTV thresholds. Furthermore, change in TTV (using 10% and 20% TTV thresholds) was found prognostic for RFS in patients with secondarily resectable CRLM after induction therapy, whilst RECIST1.1 was not. The actual benefit of TTV response assessment needs to be validated in future studies.

Author contributions

Study concept and design: NW, KB, CJAP, RJS, JH, GK,
Data collection: all authors
Statistical analyses: NW, KB, SvD, RJS, CJAP, JH, GK
Data interpretation: all authors
Manuscript writing: NW, KB, CJAP, JH, GK
Critical revision of the manuscript: All authors

References

1. Elferink MA, de Jong KP, Klaase JM, et al. Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis. 2015;30:205–212.
2. van der Geest LG, Lam-Boer J, Koopman M, et al. Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis. 2015;32:457–465.
3. Adam R, De Gramont A, Figueras J, et al; Jean-Nicolas Vauthey of the EGOSLIM (Expert Group on OncoSurgery management of LIver Metastases) group. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17: 1225–1239.
4. Simmonds PC, Primrose JN, Colquitt JL, et al. Surgical resection of hepatic metastases from colorectal cancer: a systematic review of published studies. Br J Cancer. 2006;94:982–999.
5. de Ridder JAM, van der Stok EP, Mekenkamp LJ, et al. Management of liver metastases in colorectal cancer patients: a retrospective case-control study of systemic therapy versus liver resection. Eur J Cancer. 2016;59:13–21.
6. Norén A, Eriksson HG, Olsson LI. Selection for surgery and survival of synchronous colorectal liver metastases; a nationwide study. Eur J Cancer. 2016;53:105–114.
7. Adam R, Delvart V, Pascal G, et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. Ann Surg. 2004;240:644–657.
8. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3:50–56.
9. Lam VW, Spiro C, Laurence JM, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol. 2012;19:1292–1301.
10. Adam R. Developing strategies for liver metastases from colorectal cancer. Semin Oncol. 2007;34(2 suppl 1):S7–S11.
11. Poston GJ, Figueras J, Giuliante F, et al. Urgent need for a new staging system in advanced colorectal cancer. J Clin Oncol. 2008;26:4828–4833.
12. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–247.
13. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205–216. criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–247.
14. Kuhl CK, Alparslan Y, Schmoee J, et al. Validity of RECIST Version 1.1 for response assessment in metastatic cancer: a prospective, multireader study. Radiology. 2019;290:349–356.
15. Sosna J. Is RECIST Version 1.1 reliable for tumor response assessment in metastatic cancer? Radiology. 2019;290:357–358.
16. Yoon SH, Kim KW, Goo JM, et al. Observer variability in RECISTbased tumour burden measurements: a meta-analysis. Eur J Cancer. 2016;53:5–15.
17. Beaumont H, Evans TL, Klifa C, et al. Discrepancies of assessments in a RECIST 1.1 phase II clinical trial - association between adjudication rate and variability in images and tumors selection. Cancer Imaging. 2018;18:50.
18. Chun YS, Vauthey JN, Boonsirikamchai P, et al. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. JAMA. 2009;302:2338–2344.
19. Schiavon G, Ruggiero A, Schöffski P, et al. Tumor volume as an alternative response measurement for imatinib treated GIST patients. PLoS One. 2012;7:e48372.
20. Rothe JH, Grieser C, Lehmkuhl L, et al. Size determination and response assessment of liver metastases with computed tomography–comparison of RECIST and volumetric algorithms. Eur J Radiol. 2013;82:1831–1839.
21. Hayes SA, Pietanza MC, O'Driscoll D, et al. Comparison of CT volumetric measurement with RECIST response in patients with lung cancer. Eur J Radiol. 2016;85:524–533.

22. Tai K, Komatsu S, Sofue K, et al. Total tumour volume as a prognostic factor in patients with resectable colorectal cancer liver metastases. BJS open. 2020;4:456–466.
23. Schiavon G, Ruggiero A, Bekers DJ, et al. The effect of baseline morphology and its change during treatment on the accuracy of Response Evaluation Criteria in Solid Tumours in assessment of liver metastases. Eur J Cancer. 2014;50:972–980.
24. Huiskens J, van Gulik TM, van Lienden KP, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases, a study protocol of the randomised phase 3 CAIRO5 study of the Dutch Colorectal Cancer Group (DCCG). BMC Cancer. 2015;15:365.
25. Huiskens J, Bolhuis K, Engelbrecht MR, et al; Dutch Colorectal Cancer Group. Outcomes of resectability assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229:523–532.e2.
26. Couinaud Foie Etudes anatomiques et chirurgicales. Mason;1957:400–409.
27. Fong Y, Fortner J, Sun RL, et al. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg. 1999;230:309–318.
28. SAS Visual Data Mining and Machine Learning Programming Guide: The quantifyBioMedImages Action 2020. Available at: https://go.documentation.sas.com/?cdcId=pgmsascdc&cdcVersion=9.4_3.5&docsetId=casactml&docsetTarget=casactml_biomedimage_details05.htm&locale=en. Accessed May 1, 2021.
29. Innolitics. DICOM Standard Browser: Image Plane Module 2020. Available at: https://dicom.innolitics.com/ciods/ct-image/image-plane. Accessed May 1, 2021.
30. Bolhuis K, Huiskens J, Bond MJG, et al. Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases CAIRO5 a randomised phase 3 study of the Dutch Colorectal Cancer Group (DCCG) 2020. Available at: https://dccg.nl/files/C1.CAIRO5%20protocol%20amendement%20V.10.0%20%20final%2007-10-2020%20 clean.pdf. Accessed January 3, 2021.
31. Wang K, Liu W, Yan XL, et al. Long-term postoperative survival prediction in patients with colorectal liver metastasis. Oncotarget. 2017;8:79927–79934.
32. Margonis GA, Sasaki K, Gholami S, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105:1210–1220.
33. Sasaki K, Margonis GA, Andreatos N, et al. Pre-hepatectomy carcinoembryonic antigen (CEA) levels among patients undergoing resection of colorectal liver metastases: do CEA levels still have prognostic implications? HPB (Oxford). 2016;18:1000–1009.
34. Connell LC, Boucher TM, Chou JF, et al. Relevance of CEA and LDH in relation to *KRAS* status in patients with unresectable colorectal liver metastases. J Surg Oncol. 2017;115:480–487.
35. Jácome AA, Vreeland TJ, Johnson B, et al. The prognostic impact of *RAS* on overall survival following liver resection in early versus late-onset colorectal cancer patients. Br J Cancer. 2021;124:797–804.
36. van Kessel CS, van Leeuwen MS, Witteveen PO, et al. Semi-automatic software increases CT measurement accuracy but not response classification of colorectal liver metastases after chemotherapy. Eur J Radiol. 2012;81:2543–2549.
37. Lee IS, Choi SJ, Seo CR, et al. Comparison of the response evaluation criteria in solid tumors with volumetric measurement for evaluation of response and overall survival with liver metastases from colorectal cancer. J Korean Soc Radiol. 2019;80:906–918.
38. Bolhuis K, Kos M, van Oijen MGH, et al. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: a systematic review. Eur J Cancer. 2020;141:225–238.
39. Ye LC, Liu TS, Ren L, et al. Randomized controlled trial of cetuximab plus chemotherapy for patients with *KRAS* wild-type unresectable colorectal liver-limited metastases. J Clin Oncol. 2013;31:1931–1938.
40. Tang W, Ren L, Liu T, et al. Bevacizumab plus mFOLFOX6 versus mFOLFOX6 alone as first-line treatment for *RAS* mutant unresectable colorectal liver-limited metastases: the BECOME randomized controlled trial. J Clin Oncol. 2020;38:3175–3184.
41. Carrato A, Abad A, Massuti B, et al; Spanish Cooperative Group for the Treatment of Digestive Tumours (TTD). First-line panitumumab plus FOLFOX4 or FOLFIRI in colorectal cancer with multiple or unresectable liver metastases: a randomised, phase II trial (PLANET-TTD). Eur J Cancer. 2017;81:191-202.

42. Folprecht G, Gruenberger T, Bechstein WO, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11:38–47.
43. Gruenberger T, Bridgewater J, Chau I, et al. Bevacizumab plus mFOLFOX-6 or FOLFOXIRI in patients with initially unresectable liver metastases from colorectal cancer: the OLIVIA multinational randomised phase II trial. Ann Oncol. 2015;26:702–708.
44. Cremolini C, Loupakis F, Antoniotti C, et al. Early tumor shrinkage and depth of response predict long-term outcome in metastatic colorectal cancer patients treated with first-line chemotherapy plus bevacizumab: results from phase III TRIBE trial by the Gruppo Oncologico del Nord Ovest. Ann Oncol. 2015;26:1188–1194.
45. Primavesi F, Fadinger N, Biggel S, et al. Early response evaluation during preoperative chemotherapy for colorectal liver metastases: combined size and morphology-based criteria predict pathological response and survival after resection. J Surg Oncol. 2019;121:382–391.
46. van Kessel CS, Samim M, Koopman M, et al. Radiological heterogeneity in response to chemotherapy is associated with poor survival in patients with colorectal liver metastases. Eur J Cancer. 2013;49: 2486-2493.
47. Brunsell TH, Cengija V, Sveen A, et al. Heterogeneous radiological response to neoadjuvant therapy is associated with poor prognosis after resection of colorectal liver metastases. Eur J Surg Oncol. 2019; 45:2340–2346.
48. White RR, Schwartz LH, Munoz JA, et al. Assessing the optimal duration of chemotherapy in patients with colorectal liver metastases. J Surg Oncol. 2008;97:601–604.
49. Heinemann V, Stintzing S, Modest DP, et al. Early tumour shrinkage (ETS) and depth of response (DpR) in the treatment of patients with metastatic colorectal cancer (mCRC). Eur J Cancer. 2015;51:1927–1936.
50. Shindoh J, Loyer EM, Kopetz S, et al. Optimal morphologic response to preoperative chemotherapy: an alternate outcome end point before resection of hepatic colorectal metastases. J Clin Oncol. 2012;30:4566–4572.

Supplementary materials

Figure S1 Visualization of the three-dimensional (3D) liver segmentation (blue) and the 3D tumor segmentations (orange) depicted in the baseline (a) and follow-up (b) CT-scans. This patient with colorectal liver metastases showed a 20% decrease of diameters in target lesions and was classified as stable according to RECIST, while total tumor volume decreased with 81%.

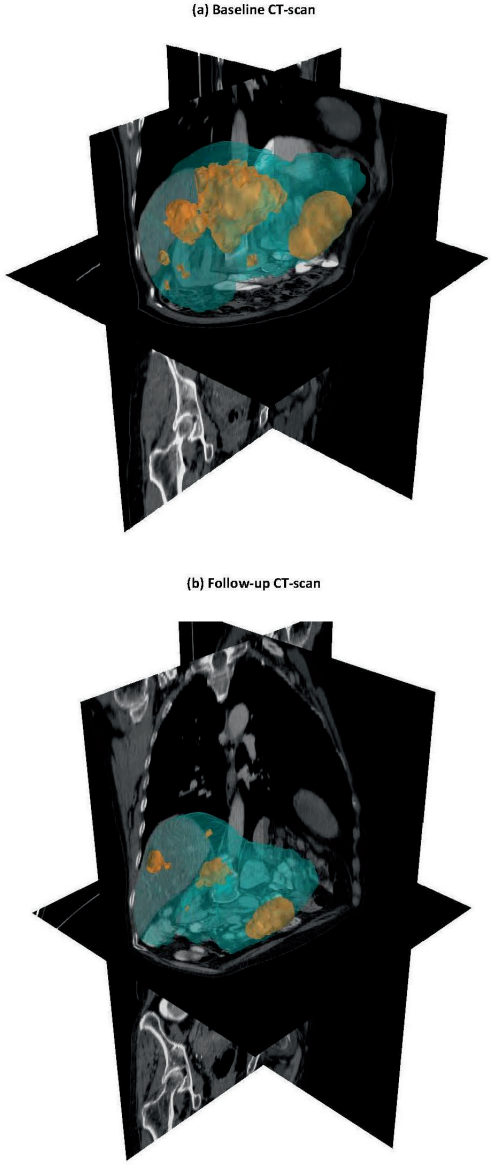


Table S1 Comparison of patient characteristics in TTV response groups.

Patient characteristics	*Total cohort*	TTV response ≥40% decrease	TTV stable -40% to +40%	TTV progression ≥40% increase	P-value
	N=210	N=125	N=70	N=15	
Age - yr					0.042
Median	63	63	60	54	
IQR	55 - 69	57 - 70	55 - 71	49 - 60	
Sex, - no (%)					0.805
Male	138 (66)	81 (65)	46 (66)	11 (73)	
Female	72 (34)	44 (35)	24 (34)	4 (27)	
Site of primary tumor – no (%)					0.203
Left colon	154 (73)	96 (77)	46 (66)	12 (80)	
Right colon or rectum	56 (27)	29 (23)	24 (34)	3 (20)	
pN status primary tumor – no (%)					0.464
Negative	51 (43)	35 (42)	12 (40)	4 (67)	
Positive	69 (58)	49 (58)	18 (60)	2 (33)	
Missing	90				
Time to metastases – no (%)					0.144
Synchronous	25 (12)	19 (15)	4 (6)	2 (13)	
Metachronous	185 (88)	106 (85)	66 (94)	13 (87)	
Baseline LDH Level					0.208
Median	265	279	319	222	
IQR	13 - 436	208 - 429	21 - 667	189 - 845	
Baseline serum CEA level					0.286
Median	23	49	61	16	
IQR	7 - 185	11 - 263	12 - 290	6 - 82	
Number of liver metastases - no					0.014
Median	12	10	15	19	
IQR	7 - 22	6 - 20	8 - 29	11 - 32	
Number of liver segments - no					0.012
Median	6	5	6	7	
IQR	4-7	4-7	4-7	5-8	
Diameter of largest metastasis					0.934
Median	43	41	43	41	
IQR	28 - 58	28 - 71	28 - 72	27 - 64	
Distribution of liver metastases					0.078
Unilobar	15 (7)	13 (10)	2 (3)	0 (0)	
Bilobar	195 (93)	112 (90)	68 (97)	15 (100)	
Fong risk score					0.359
Low	10 (5)	8 (6)	2 (3)	0	
High	196 (95)	117 (94)	68 (97)	15 (100)	
TTV baseline (cm^3)					0.312
Median	100	67	139	153	
IQR	25 – 426	23 – 334	29 – 504	16 – 493	

Critical p-value=0.004 (Bonferroni corrected). Abbreviations: CEA, carcinoembryonic antigen; IQR, interquartile range; LDH, lactate dehydrogenase; pN, nodal status primary tumor; TTV, total tumor volume.

Table S2 Comparison of patient characteristics in TTV response groups.

Patient characteristics	*Total cohort*	TTV response ≥20% decrease	TTV stable -20% to +20%	TTV progression ≥ 20% increase	P-value
	N=210	N=165	N=25	N=20	
Age - yr					0.095
Median	63	62	62	54	
IQR	55–69	56–71	57–72	50–65	
Sex, - no (%)					0.541
Male	138 (66)	111 (67)	14 (56)	13 (65)	
Female	72 (34)	54 (33)	11 (44)	7 (35)	
Site of primary tumor – no (%)					0.275
Left colon	154 (73)	124 (75)	15 (60)	15 (75)	
Right colon or rectum	56 (27)	41 (25)	10 (40)	5 (25)	
pN status primary tumor – no (%)					0.459
Negative	51 (43)	40 (40)	6 (46)	5 (63)	
Positive	69 (58)	59 (60)	7 (54)	3 (38)	
Missing	90				
Time to metastases – no (%)					0.411
Synchronous	25 (12)	144 (87)	24 (96)	17 (85)	
Metachronous	185 (88)	21 (13)	1 (4)	3 (15)	
Baseline LDH Level					0.740
Median	265	283	312	343	
IQR	13–436	208–504	229–428	200–801	
Baseline serum CEA level					0.497
Median	23	50	33	26	
IQR	7–185	12–284	9–147	10–164	
Number of liver metastases - no					0.120
Median	12	11	15	14	
IQR	7–22	7–21	9–31	8–30	
Number of liver segments - no					0.045
Median	6	5	6	7	
IQR	4–7	4–7	5–7	5–8	
Diameter of largest metastasis					0.820
Median	43	41	39	46	
IQR	28–58	28–71	24–72	27–90	
Distribution of liver metastases					0.110
Unilobar	15 (7)	15 (9)	0	0	
Bilobar	195 (93)	150 (91)	25 (100)	20 (100)	
Fong risk score					0.547
low	10 (5)	9 (6)	1 (4)	0	
high	196 (95)	156 (94)	24 (96)	20 (100)	
TTV baseline (cm^3)					0.883
Median	100	79	129	166	
IQR	25–426	25–419	28–378	18–537	

Critical p-value=0.004 (Bonferroni corrected). Abbreviations: CEA, carcinoembryonic antigen; IQR, interquartile range; LDH, lactate dehydrogenase; pN, nodal status primary tumor; TTV, total tumor volume.

Table S3 Comparison of patient characteristics in TTV response groups.

Patient characteristics	Total cohort	TTV response ≥10% decrease	TTV stable -10% to +10%	TTV progression ≥10% increase	P-value
	N=210	N=171	N=11	N=28	
Age - yr					0.149
Median	63	63	61	58	
IQR	55–69	56–70	56–72	52–70	
Sex, - no (%)					0.079
Male	138 (66)	117 (68)	4 (36)	17 (61)	
Female	72 (34)	54 (32)	7 (64)	11 (39)	
Site of primary tumor – no (%)					0.179
Left colon	154 (73)	130 (76)	7 (64)	17 (61)	
Right colon or rectum	56 (27)	41 (24)	4 (36)	11 (39)	
pN status primary tumor – no (%)					0.470
Negative	51 (43)	42 (42)	2 (33)	7 (58)	
Positive	69 (58)	60 (58)	4 (67)	5 (42)	
Missing	90				
Time to metastases – no (%)					0.436
Synchronous	25 (12)	150 (88)	11 (100)	24 (86)	
Metachronous	185 (88)	21 (12)	0 (0)	4 (14)	
Baseline LDH Level					0.599
Median	265	283	314	343	
IQR	13–436	208–497	223–436	209–801	
Baseline serum CEA level					0.596
Median	23	50	95	30	
IQR	7–185	11–290	8–137	13–164	
Number of liver metastases - no					0.078
Median	12	11	19	15	
IQR	7–22	7–21	9–29	8–32	
Number of liver segments - no					0.070
Median	6	5	6	6	
IQR	4–7	4–7	5–8	4–7	
Diameter of largest metastasis					0.688
Median	43	41	35	46	
IQR	28–58	28–71	22–56	27–73	
Distribution of liver metastases					0.159
Unilobar	15 (7)	15 (9)	0	0	
Bilobar	195 (93)	156 (91)	11 (100)	15 (100)	
Fong risk score					0.302
low	10 (5)	10 (6)	0	0	
high	196 (95)	161 (94)	11 (100)	28 (100)	
TTV baseline (cm^3)					0.812
Median	100	79	156	149	
IQR	25–426	25–413	20–435	24–537	

Critical p-value=0.004 (Bonferroni corrected). Abbreviations: CEA, carcinoembryonic antigen; IQR, interquartile range; LDH, lactate dehydrogenase; pN, nodal status primary tumor; TTV, total tumor volume.

CHAPTER 10

English summary and general discussion

English summary

Colorectal cancer (CRC) is the third most common cancer worldwide, with the liver as primary metastatic site.[1] Colorectal cancer liver metastases (CRLM) is the major cause of colorectal cancer-related deaths.[2,3] However, in patients with metastases confined to the liver, local treatment of CRLM may offer a chance of long-term survival with 5-year-survival rates of 45-60% or even cure.[4].As such, optimizing treatment outcomes of CRLM patients may help to improve outcomes in the overall CRC population. At initial diagnosis, 20% of patients with CRLM are considered upfront resectable.[5] Systemic treatment with the combination of chemotherapy and targeted therapy converts up to 57% of patients with initially unresectable CRLM to secondary resectable CRLM.[5-9] In this thesis we focus on survival outcomes and comparison of established first line systemic conversion treatments and safety-outcomes of local treatments of advanced initially unresectable CRLM. Furthermore, we evaluate prognostic and predictive technical-anatomical and tumor-biological preoperative factors and focus on clinically relevant endpoints of prediction models to help guide clinical-decision making in CRLM patients. Finally, we analyze the performance of promising novel diagnostic techniques in CRLM patients. The aim of the research in this thesis is to contribute to a more individualized treatment of patients with CRLM by providing valuable disease- and treatment-related insights of CRLM.

Part I of this thesis focuses on outcomes of currently most active systemic conversion treatments and local treatment in patients with CRLM. There is no consensus regarding the optimal first line systemic conversion therapy although the globally established treatments of choice are fluoropyrimidine-based (doublet or triplet) treatments combined with either anti-EGFR targeted therapy or bevacizumab (or biosimilar).[10,11] Translation of outcomes to clinical practice of prospective studies in CRLM patients, is hampered by heterogeneity in trial design and study populations due to lack of consensus on critieria for (un)resectability, lack of long-term survival outcomes as primary outcome in trials and by bias induced by unplanned retrospective subgroup analyses of CRLM patients in phase 3 studies with unselected patients with metastatic CRC (mCRC). In addition, the continuously evolving field of metastatic CRC with new predictive factors emerging over the years, such as (*K)RAS, BRAF* and sidedness of primary tumor, renders outcomes of previous studies to be outdated.[6,12,13] Comparison of trials considering these factors is both challenging and crucial for drawing conclusions from CRLM studies and for future research directions. In **Chapter 2** a systematic review is presented of randomized studies in (subgroups of) patients with initially unresectable CRLM, with focus on patient characteristics and basic methodology including clinical endpoints, criteria for (un)resectability, and long-term survival outcomes. A total of 20 Phase II/III randomized trials, regarding first line systemic conversion therapy in patients or subgroups of patients with CRLM were included. Seven trials comprised

CRLM patients only and 13 trials involved subgroup analyses of CRLM patients in mCRC studies. We noted that the majority of trials did not provide unresectability criteria at baseline, and criteria differed among the remaining studies. This results in heterogeneity of study populations and subsequently affects resection rates which indeed varied considerably between CRLM studies and mCRC studies, 22-57% and 11-38%, respectively. Trials and study populations proved to be also heterogeneous in prognostic/predictive factors ((*K*(*RAS*)/*BRAF* and sidedness of primary tumor*)*, use of primary endpoints, and reporting on long-term clinical outcomes. Notably, among trials with CRLM patients only, all studies except for one used short-term resection outcomes as primary outcome (conversion rate, (R0-)resection rate and objective response rate). With this systematic review we provide an overview of the available short-term and long-term outcomes after CRLM resection and pooled results of studies with bevacizumab containing regimens in both the unselected population and (*K*)*RAS* wildtype population and of anti-EGFR therapy containing regimens in the (*K*)*RAS* wildtype population. The conclusion of our research presented in **Chapter 2** is that as a result of abovementioned issues, no optimal conversion systemic treatment can be selected from available trials. Recommendations for future trial design are provided and are also incorporated in the CAIRO5 study design; a multicenter, randomized, phase 3 trial of the Dutch Colorectal Cancer Group (DCCG) investigating the optimal systemic conversion treatment in patients with initially unresectable CRLM.[14] An innovative aspect of the CAIRO5 study design is that all patients are prospectively evaluated for resectability according to predefined baseline (un)resectability criteria by an online central liver expert panel consisting of experienced hepatobiliary surgeons and radiologists. We hypothesized that the use of this panel which operates by an online platform may decrease individual subjectivity in defining (un)resectability, and subsequently may improve consensus on criteria for resection of CRLM. In **Chapter 3** we present an analysis of the feasibility and outcomes of the resectability assessments by the DCCG liver expert panel. The median time to panel conclusion was 7 days, which is considerably faster than the preconceived maximum of 14 days, and thereby allowing efficient assessment by multiple experienced liver surgeons in these very complex patients. Intersurgeon disagreement was observed in 50% of evaluations, with major disagreement (resectable *vs.* permanently unresectable) in 11% of evaluations. The high intersurgeon variation at follow up assessments reflects the complexity in defining treatment strategies for CRLM and the urgent need for a consensus on resectability criteria based on strong predictive and prognostic factors. Until consensus is reached on resectability criteria, use of a panel is recommended rather than a single-surgeon decision to reach a balanced decision and to prevent patients being wrongly denied from surgery. Among patients with CRLM defined as permanent unresectable at baseline, approximately 15% of patients converted to resectable CRLM during systemic conversion treatment. This underlines the importance of repeated resectability assessments, as these patients can still have a chance of local liver treatment and, as a

consequence, long-term survival. In conclusion, we show that the DCCG CAIRO5 Liver Expert Panel is feasible and provides a platform for prospective initial and follow-up assessments on resectability in patients with advanced CRLM on a national level.

The increase of complex hepatic resections of CRLM, technical innovations pushing boundaries of resectability, and use of intensified induction systemic regimens warrant for safety data in a homogeneous multicenter prospective cohort. In **Chapter 4** the short-term outcomes of liver surgery in patients with initially unresectable CRLM downsized by chemotherapy plus targeted agents in the CAIRO5 study are presented. Severe postoperative morbidity and 90-day mortality were noticed in 15.6% and 2.9% of patients, respectively. After multivariable analysis, blood transfusion, major resection, and triplet chemotherapy were independently correlated with severe postoperative complications. No association was found between number of cycles of systemic conversion treatment and severe complications and as such, length of first line systemic treatment should not be a contraindication for liver surgery. The acceptable postoperative morbidity and mortality rates support the increase of complex liver surgery. Careful patient selection considering the type of preoperative systemic treatment as well as efforts to perform parenchymal-sparing resections might help to further reduce the severe complication and mortality rate.

Part II of this thesis focuses on preoperative risk stratification of patients with CRLM. While CRLM is recognized as a heterogeneous disease based on prognostic clinical features and biomarkers, resectability-assessments of CRLM remain a technical-anatomical decision.[15,16] Surgical innovations increase the number of CRLM patients assessed as technically resectable, but high recurrence rates persist and a significant group of patients have no long-term survival benefit of CRLM resection.[17] Thus, there is an unmet clinical need for a prediction model with high discriminative ability allowing better stratification and counselling of patients before surgery in order to personalize therapy. While multiple models which predict prognosis after CRLM resection are proposed over the years, most prediction models are developed in retrospective analysis of highly selected single institution cohorts and lack external validation with adherence to methodological guidelines, which may hamper the generalizability of these models in unselected populations and underrepresented subgroups in clinical practice.[18-20]

In **Chapter 5** we present a subgroup analysis in 482 patients from the multicenter, randomized CAIRO5 study focusing on the association of tumor-biological and technical-anatomical preoperative factors with consensus among panel surgeons (i.e. same vote for (un)resectability of CRLM), conversion to resectable disease, early recurrence defined as recurrence within 6 months and early recurrence without curative-intent repeat local treatment. Higher number of CRLM (OR 1.09 [95%CI 1.03-1.15]) and age (OR 1.03 [95%CI 1.00-1.07]) but not tumor-biological factors were independently associated

with early recurrence without repeat local treatment. Furthermore, both *RAS* and *BRAF V600E* as compared to *RAS* and *BRAF* wildtype tumors were strong prognostic factors for conversion to resectable disease (OR 0.38 [95%CI 0.21-0.69], p=0.002 and OR 0.10 [95%CI 0.03-0.30], p<0.001, respectively). After systemic and subsequent local treatment, both *RAS* and $BRAF^{V600E}$ mutations lost their predictive value for early recurrence. Disagreement among panel surgeons, with a panel conclusion by majority vote, existed in more than 50% of patients. No factors could predict disagreement at first follow up while in the subgroup of patients who underwent local liver treatment, more advanced CRLM was associated with more disagreement prior to local treatment of CRLM. After adjustment for other factors, postoperative outcomes of patients with agreement and disagreement prior to local treatment was comparable. In **Chapter 5** we conclude that considering the lack of clinically available predictive tumor-biological factors for clinically relevant postoperative outcomes, resectability assessment of CRLM remains a primarily technical-anatomical decision where an expert panel as opposed to the opinion of a single surgeon allows a better selection of patients who are eligible for local treatment.

In **Chapter 6** two established prediction models, the traditional Fong score and Genetic And Morphological Evaluation (GAME)[21,22], predicting outcome after CRLM resection are compared and externally validated in a nationwide real-life population-based cohort of patients with local treatment of CRLM, including in pre-specified subgroups (≤70/>70 years and with/without perioperative systemic therapy). Both CRSs showed predictive ability in the real-life cohort. Although the novel CRS (GAME) outperformed the traditional CRS, the suboptimal predictive value of both CRSs limits the clinical utility of the CRSs for clinical decision making. Furthermore, we concluded that the proposed endpoints, overall survival (OS) and RFS, are suboptimal endpoints for CRS since OS is confounded by potential sequential local and/or multiple systemic treatments after resection and RFS is a weak surrogate marker for long-term survival mainly because it does not distinguish between liver-limited and extrahepatic recurrences or mono- or multisite recurrences.[23,37] Early recurrences within six months and extrahepatic recurrences are both independently associated with poor overall survival.[24-26] We hypothesized that prediction of early extrahepatic recurrence-free survival (EHRFS) defined as extrahepatic recurrence within six months would present a clinically relevant endpoint to patients and clinicians and could help to adequately risk stratify patients and guide clinical decisions in patients with technically resectable CRLM. In **chapter 7** a new prediction model is developed and internally validated based on a nationwide population-based cohort of 1077 patients. This model is developed to predict extrahepatic recurrence (EHR) within 6 months after local treatment of CRLM. Performance assessment included calibration, discrimination, net benefit, and generalizability by internal-external cross-validation. Extrahepatic recurrences were reported in 52% of patients. To assess the relevance of EHR within 6 months, a landmark

analysis was performed and the median OS for patients with EHR within six months after CRLM treatment was 19.5 months (95% C.I. 15.6-23.0) versus not reached (45.3-not reached). The EHR prediction model includes sidedness of primary tumour, T-stage and N-stage of primary tumour, RAS/BRAF^{V600E} mutational status, and number and size of CRLM. The model was well-calibrated, yielded overoptimism-corrected 6-month EHR risks between 5.9-56.0% (interquartile interval 12.9-22.0%). Harrell's C-index through 6 and 12 months was 0.663 (0.624-0.702) and 0.661 (0.632-0.689), respectively. Patients in the highest risk quartile had an observed 6-month EHR risk of 32% versus 6% in the lowest quartile. The conclusion of **Chapter 7** is that early EHR after local treatment of CRLM has a major impact on prognosis and can be predicted with routine clinical information.

Part III focuses on promising novel diagnostic techniques in patients with CRLM. Recurrence rates after resection of CRLM are high and caused by micro-metastases left *in situ* after resection. Currently available follow-up methods, like serum carcinogenic embryonic antigen (CEA) and cross-sectional clinical imaging such as CT- or PET-scans, have limited accuracy for detecting this minimal residual disease (MRD).[27] Liquid biopsy-derived ctDNA represents a minimally invasive, cancer-specific biomarker with great potential as diagnostic, prognostic and disease-monitoring marker.[28] In patients with early stage CRC, postoperative ctDNA showed to be a strong independent prognostic biomarker for MRD and recurrence-free survival (RFS).[29,30] Studies investigating postoperative ctDNA in stage IV disease were limited and mostly concern heterogeneous patient groups with both hepatic and extrahepatic disease and varying use of induction systemic treatment.[31,32]

In **Chapter 8** we show the prognostic value of postoperative ctDNA in an upfront carefully selected homogeneous population of patients with *RAS* mutant initially unresectable CRLM after systemic conversion treatment and complete resection and showed that the detection of postoperative ctDNA is a strong independent prognostic factor for disease recurrence and recurrence-free survival in patients after secondary resection of *RAS* mutated CRLM. In addition, this was the first study to analyze and subsequently determine the strong association of postoperative ctDNA with pathologic response which is highly correlated with survival outcomes in CRC. In contrast to pathologic response evaluation, liquid biopsy ctDNA offers the possibility for longitudinal follow-up, whereas pathologic response can only be assessed after resection. This offers opportunities for the personalization of postoperative disease management in this subgroup of patients with metastatic CRC, e.g. by intensifying follow-up or providing adjuvant treatment.

Patients with initially unresectable or permanently unresectable CRLM receive systemic treatment with the intention to convert unresectable disease to resectable CRLM or to

reduce cancer-related symptoms, maintain quality of life and/or prolong survival. Response evaluation is crucial in these patients since decisions regarding further treatment strategy (e.g. resection or switch of systemic treatment) is based on these radiologic evaluations. RECIST1.1 provides unidimensional radiological criteria to evaluate tumor response to systemic therapy.[33] Those criteria are accepted worldwide but are limited by interobserver variability and ignore potentially valuable information about total tumor volume (TTV).[34-36] In Chapter 9 a total of 420 CT scans in 210 CAIRO5 patients with initially unresectable CRLM receiving systemic conversion treatment are evaluated. TTV response is assessed. Baseline and follow-up CT scans were used to calculate RECIST1.1 and TTV response to systemic therapy. Different thresholds (10%, 20%, 40%) were used to define response of TTV as no standard currently exists. RFS was assessed in the subgroup of CRLM patients who underwent secondary resection. In 30% to 50% (depending on chosen TTV threshold) of patients, discordance was observed between RECIST1.1 and TTV change. The study showed that among patients with RECIST 1.1 stable disease, in 47 (22%) patients a TTV decrease of more than 40% (range 40%–81%) was observed and in 4 (2%) patients TTV was increased by more than 40% (range 42%–197%). In 3 (2%) patients classified as responsive according to RECIST 1.1 showed TTV increase up to 70%. In patients after secondary resection of CRLM, TTV decrease of less or more than 10% and 20% was associated with RFS (p=0.015 and p=0.022, respectively), while RFS was not associated with response or non-responsive disease according to RECIST1.1 (p=0.821). In conclusion, TTV response assessment showed prognostic potential in the evaluation of systemic treatment response in patients with initially unresectable CRLM and could contribute to the improvement of treatment selection and precision management.

References

1. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol.2019;16:713-732.
2. Zhou H, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther. 2022;7:70.
3. Manfredi S, et al. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. 2006;244:254-259.
4. Morris EJ, et al. Surgical management and outcomes of colorectal cancer liver metastases. Br J Surg. 2010;97:1110-1118.
5. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3:50-56.
6. Folprecht G, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11: 38-47.
7. Tomasello G, et al. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol. 2017;3:e170278.
8. Cremolini C, et al. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. J Clin Oncol. 2020:Jco2001225.
9. Ychou M, et al. Chemotherapy (doublet or triplet) plus targeted therapy by RAS status as conversion therapy in colorectal cancer patients with initially unresectable liver-only metastases. The UNICANCER PRODIGE-14 randomised clinical trial. Br J Cancer. 2022;126:1264-1270.
10. Benson AB, 3rd, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15:370-398.
11. Van Cutsem E, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386-1422.
12. Holch JW, Ricard I, Stintzing S, Modest DP, Heinemann V. The relevance of primary tumour location in patients with metastatic colorectal cancer: A meta-analysis of first-line clinical trials. Eur J Cancer. 2017; 70:87-98.
13. Tsilimigras DI, et al. Clinical significance and prognostic relevance of KRAS, BRAF, PI3K and TP53 genetic mutation analysis for resectable and unresectable colorectal liver metastases: A systematic review of the current evidence. Surg Oncol. 2018;27:280-288.
14. Huiskens J, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229:523-532 e522.
15. Adam R, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17:1225-1239.
16. Guinney J, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21:1350-1356.
17. Lam VW, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol. 2012;19:1292-1301.
18. Mahar AL, et al. Personalizing prognosis in colorectal cancer: A systematic review of the quality and nature of clinical prognostic tools for survival outcomes. J Surg Oncol. 2017;116:969-982.
19. Meyer RM. Generalizing the results of cancer clinical trials. J Clin Oncol. 2010;28:187-189.
20. Zamanipoor Najafabadi AH, et al. TRIPOD statement: a preliminary pre-post analysis of reporting and methods of prediction models. BMJ Open. 2020;10:e041537.
21. 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;230:309-318; discussion 318-321.
22. Margonis GA, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105:1210-1220.

23. Oba M, et al. Discrepancy between recurrence-free survival and overall survival in patients with resectable colorectal liver metastases: a potential surrogate endpoint for time to surgical failure. Ann Surg Oncol. 2014;21:1817-1824.
24. Creasy JM, et al. Actual 10-year survival after hepatic resection of colorectal liver metastases: what factors preclude cure? Surgery. 2018;163:1238-1244.
25. Imai K, et al. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21:887-894.
26. Vigano L, 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. 2014;21:1276-1286.
27. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. J Clin Oncol. 2009;27:e279-280; author reply e281.
28. Cristiano S, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature 2019;570: 385-389.
29. Wang R, et al. The value of circulation tumor DNA in predicting postoperative recurrence of colorectal cancer: a meta-analysis. Int J Colorectal Dis. 2020;35:1463-1475.
30. Tie J, et al. Prognostic significance of postsurgery circulating tumor DNA in nonmetastatic colorectal cancer: Individual patient pooled analysis of three cohort studies. Int J Cancer. 2021;148;1014-1026.
31. Benesova L, et al. Significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA. World J Gastroenterol. 2019;25:6939-6948.
32. Scholer LV, et al. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res. 2017;23:5437-5445.
33. Eisenhauer EA, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-247.
34. Beaumont H, et al. Discrepancies of assessments in a RECIST 1.1 phase II clinical trial - association between adjudication rate and variability in images and tumors selection. Cancer Imaging. 2018;18:50.
35. Yoon SH, Kim KW, Goo JM, Kim DW, Hahn S. Observer variability in RECIST-based tumour burden measurements: a meta-analysis. Eur J Cancer. 2016;53:5-15.
36. Kuhl CK, et al. Validity of RECIST Version 1.1 for Response Assessment in Metastatic Cancer: A Prospective, Multireader Study. Radiology. 2019;290:349-356.
37. Ecker, B.L., Lee, J., Saadat, L.V., *et al.* Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. *Lancet Oncol* (2022).

General discussion

The last decade, progress has been made in the systemic treatment of patients with metastatic colorectal cancer (mCRC) from a one-size-fits-all approach towards more individualized treatment based on molecular profiling and sidedness of primary tumor.[1,2] Guidelines prescribe monoclonal antibodies that inhibit the epithelial growth factor receptor (cetuximab and panitumumab) in patients with *KRAS/NRAS* wildtype and/or left-sided primary tumors[3-5], encorafenib with cetuximab as second line treatment is approved for patients with *BRAFV600E* mutated tumors and anti-programmed death-1 (PD-1) monoclonal antibodies is offered to patients with mismatch repair deficient (MMRd) or microsatellite-stability-high (MSI-h) metastatic colorectal cancer.[6-9] In addition, promising targeted therapy is available in studies or in compassionate use programs for patients with genetic mutations of the *ERBB2*, *NTRK*, *MET* and *PIK3CA* genes. However, although survival outcomes of mCRC patients have improved in clinical trials as was shown in **Chapter 2**[9,10], population-based studies show less favorable outcomes and state that these survival advantages are attributed to only subgroups of patients including patients with colorectal liver metastases.[9,11] The increase of local treatment rate in CRLM patients with primary resectable or secondary resectable CRLM, has contributed to these improved survival outcomes.[11,12] In the absence of resection criteria, all patients with technically resectable CRLM should be discussed in an MDT for local liver treatment. However, nodal infiltration and occult micrometastatic dissemination is common resulting in high recurrence rates with the majority not amenable for repeat local treatment with curative intent.[13-15] This underlines the need for risk stratification prior to surgery to further individualize treatment. The chapters in this thesis showed a valuable overview of established care for patients with CRLM but also exposed several limitations of current practice.

Clinical precision oncology based on individual clinical characteristics and molecular profiling may guide further improvement of treatment and outcomes of patients with CRLM. At the same time, multimodal treatment and multidisciplinary cooperation remain of critical importance to warrant for optimal treatment[16] as is also reflected by the outcomes of the multidisciplinary projects included in this thesis.

Prognosis of patients with CRLM is multifactorial influenced and could roughly be divided into three topics: prevention, early detection, management and disease-monitoring of CRLM. As such, this general discussion provides future perspectives on challenges in these topics by discussing the following fields: tumorgenetics, (un)resectability criteria and risk stratification, systemic treatment, local treatment, circulating tumor DNA (ctDNA), and imaging techniques.

Development and early detection of colorectal liver metastases

As early as 1889, the predisposed state of certain organs for dissemination of cancer according to origin of primary tumor is claimed to reach further than anatomical explanations such as portal blood supply in colorectal cancer and the development of CRLM.[17] Furthermore, CRLM development is recognized to be associated with sidedness of primary tumor, sex, ethnicity, histologic type of the tumor and multiple gene mutations: *BRAF*, *KRAS*, *NRAS*, *PI3KCA*, *TP53*, *NRAS*, *CDK12* and *EBF1*.[18] Meanwhile evidence at cell level is accumulating that interaction of internal factors of cells (by activation of proto-oncogenes and inactivation of tumor suppressor genes) and external mciroenvironment (with involvement of immune cells, cytokines, chemokines an exosomes) creates a supportive tumor microenvironment in the liver which jointly initiates and drives the occurrence of CRLM.[18] Knowledge about the complex development of metastases and the molecular mechanisms which drive it, can advance care and might play a role in developing methods to prevent CRLM or contribute to early detection.[19]

The risk of developing metastases increases by stage, with recurrence rates of less than 10% in stage I, 15% in stage II and up to 50% in stage III CRC.[20] However, also among patients with stage II CRC, some subgroups have a much higher risk of reccurence.[21,22] This was demonstrated by *Tie et al*., who showed a high recurrence rate of nearly 80% in the subgroup of stage II patients with detectable postoperative circulating tumor DNA (ctDNA).[21] In **Chapter 8** of this thesis we showed a strong association of detectable postoperative ctDNA with pathological response and RFS. As such, ctDNA provides a chance for selecting patients at high-risk for recurrence and creates the ability to modify and individualize treatment or intensify follow-up. Major challenges to overcome before wide clinical application of ctDNA after resection of stage II to IV disease is defining the most cost-effective technique[23], the most accurate liquid biopsy according to site of metastatic disease[24] and the optimal timeframe to draw blood after resection to reduce false-negative results.[25] Furthermore, the major advantages of liquid biopsies as compared to tissue biopsies is the minimally invasive character and the possibility to perform serial testing. Multiple randomized controlled trials are ongoing offering adjuvant systemic treatment in patients with detectable postoperative ctDNA such as MEDOCC-CREATE in the Netherlands.[26]

Management of colorectal liver metastases

High response rates of systemic treatment in mCRC are reported.[27] This comes at a cost of toxicity including: nausea, vomiting, hair loss and fatigue. To improve health related quality of life, reduce the number of patients needlessly exposed to toxicity without benefit of treatments and improve survival outcomes of CRLM, advances in systemic treatments to a more individualized approach are crucial.[28] Further understanding of

molecular mechanisms might offer insights of primary and secondary systemic therapy resistance and might help to develop (combinations of) targeted therapies bypassing this protective mechanism of the tumor cell.[29,30] In addition, drug test by in vitro culture of circulating tumor cells may facilitate access to personalized medicine.[31] However required time to obtain results out of this technique limit its current application. Patient-derived organoids is a promising ex vivo culture technique and are stem-cell derived, three-dimensional self-organizing structures, shown to accurately represent the heterogeneity of the original tumor and has great potential to further individualize treatment in mCRC. This technique showed promising results in evaluating drug responses and resistance of standard-of-care chemotherapy treatment and to novel drug therapies in patients with mCRC.[32-34] However, since growing organoids is still very time-consuming and as it brings high costs the current clinical application is limited. Second, it remains challenging to perfectly mimic the tumor microenvironment with respect to stromal, vascular, endothelial and immune cells which are present and influence the original tumor and treatment effects.

An actual topic regarding systemic treatment options in CRLM, is immunotherapy in the subgroup of patients with CRLM with deficient mismatch repair status (dMMR). Although this subgroup comprises only 2-3% of patients with CRLM[35], immunotherapy is a relevant treatment option to consider as it has a favorable toxicity profile as compared to standard first line systemic therapy and as encouraging clinical outcomes of anti-PD-1 immunotherapy in dMMR mCRC patients are reported.[6] The role of immunotherapy in dMMR CRLM patients as neo-adjuvant, induction, adjuvant or even as substitute treatment for resection is still unanswered. Since the incidence of dMMR CRLM is low, answers to these questions can be provided by large international collaborations or by a study following the Trial within cohorts (TwiCs) design. By randomizing patients between adjuvant or no adjuvant immunotherapy after CRLM resection and, after careful selection, randomizing between continuation of induction immunotherapy versus local treatment of CRLM the role of immunotherapy in these patients can be further elaborated. A potential drawback of immunotherapy as induction therapy is the phenomenon of crossing of the curves in trials comparing immunotherapy and chemotherapy with a shorter time to response on standard first-line chemotherapy compared to immunotherapy hereby potentially inducing a risk to miss out on resection of CRLM.[6]

Other upcoming treatments in CRLM patients focus on further intensifying treatment of the liver. As was confirmed in our results presented in **Chapters 5** and **7**, liver-only recurrences are reported in up to 50% of CRLM patients after systemic treatment[36], improvement of local therapy to the liver, such as hepatic arterial infusion pump (HAIP) or chemo- or radio-embolization, might have substantial effect on prognosis with less systemic side-effects than systemic treatment.[37]

A different and major issue regarding the management of CRLM patients is inter-surgeon variability resulting in practice variation in local treatment of CRLM. This starts by lack of (un)resectability criteria, followed by lack of high-quality prospective RCTs comparing evolving surgical techniques and local ablative treatments.[38] This was emphasized by Chapter 3 and chapter 5 showing high inter-surgeon variation in all patients undergoing resectability assessment for CRLM and in one out of ten patients total disagreement among surgeons existed (defined by at least one surgeon assessed the CRLM as resectable while one other surgeon called for a permanently unresectable conclusion). Furthermore, in the subgroup of patients in whom a resection was performed, we showed low adherence to the local treatment plan as proposed by the panel. The magnitude of this issue was confirmed by a global effort in which ten cases with CRLM were proposed to highly experienced liver surgeons from all continents.[38] The impact and relevancy of this practice variation on outcomes was shown by a retrospective surgical review of the FIRE-3 trial[39], in which more than 70% of centrally reviewed study patients with CRLM were in retrospect considered resectable at best response on systemic treatment, but only 36% of these patients actually underwent resection. Patients with centrally reviewed resectable CRLM who actually underwent resection had a significantly better median OS compared to patients without resection.[39] In addition to the Dutch initiative of centralized resectable assessments conducted by the DCCG liver expert panel, other national initiatives with repeated centralized resectability assessments have been set up in the meanwhile. An example is the Finish RAXO study. This study showed a high resection rate of 37% in a patient cohort with partially patients with multisite metastatic disease.[40] Although these initiatives are important to have a more balanced decision on technical resectability and to not miss out on the chance for resection, prediction models, combining strong preoperative prognostic and predictive factors to predict a poor outcome after CRLM resection, might help to define unresectibility criteria and support a panel decision. In chapter 6 prediction models were validated and tested on different outcomes after local treatment of CRLM. However, we noted that the CRS predicting OS and RFS, lack discriminative power and the endpoints used in these prediction models are confounded (OS) or are weak surrogate marker for OS (RFS) which was supported recently by a meta-analysis.[41] In Chapter 7, we proposed a new more clinically relevant endpoint for CRS: early extrahepatic RFS (EHRFS). This prediction model proved to be informative for patients and clinicians, and has great potential to guide clinical decision making in the future as demonstrated by the net benefit analysis. However, this model should first be externally validated in other patient cohorts and we consider the selection of patients and the chosen endpoint as potential variables to further improve. To move forward on this issue, we suggest selecting a patient cohort with advanced CRLM requiring major surgery and improve the CRS by the method described in Chapter 7 and further refine the predicted outcome by early extrahepatic recurrences not amenable for repeat local treatment with curative-intent. Combination of this CRLM prediction model with other prognostic methods or factors

such as preoperative ctDNA, the immunoscore or histopathological growth pattern might help to further improve the discriminative power of the model.[42,43] In addition, medical experts should define a cut-off of this endpoint at which local treatment of CRLM is considered futile.

To decrease practice variation and improve outcomes in CRLM patients we argue for three goals:

1. All CRLM patients should be reviewed by an expert liver surgical team to ensure that long-term survival by surgery is not denied
2. Performing high-quality prospective RCTs comparing evolving local treatment of CRLM (e.g. local ablative therapy, nanoknife, SABR) with surgery
3. Ascertain (un)resectability criteria based on prognostic and predictive factors

Disease monitoring of colorectal liver metastases

Currently, available follow-up methods like serum carcinoembryonic antigen (CEA) and cross-sectional clinical imaging such as CT- or PET-scans have limited accuracy for detecting MRD due to low sensitivity and specificity.[44]

Blood-based biomarkers such as circulating tumor cells (CTCs), circulating free tumor DNA (cfDNA) and microRNAs (miRNAs) are potential indicators for the tumor burden of patients with cancer and allows to receive real-time information relevant to cancer diagnosis and therapy. Derivation of these markers from blood may offer an additional valuable tool for modern cancer therapy: apart from being of high importance when tissue biopsies are not accessible or to invasive, blood-based tests may allow a close follow-up of disease markers offering longitudinal follow-up of the efficacy of treatment and potentially improve the choice of treatment options. Furthermore, ctDNA gives a detailed picture of the tumor, while biopsies can provide false negative results by tissue sampling due to heterogeneity of the tumor[45]. As patients with CRC shed high levels of ctDNA, the clinical applicability of ctDNA in CRC patients is widely researched worldwide and is closely monitored by clinicians.[46] In chapter 8 we offered a perspective on the clinical relevance of the assessment of postoperative ctDNA in CRLM patients by showing the association with pathologic response and postoperative recurrence. This offers opportunities for the individualization of postoperative disease management. However, the method described is suitable for approximately 50% of CRLM patients only, since *RAS* hot-spot mutations were used to guide detection for ctDNA. Future research challenges lay within the determination of robust validated and fast assays by conduct of translational and clinical research to bring the liquid biopsy concept into the clinic for all CRLM patients.

CT is the standard imaging method for patients with suspected CRLM in the diagnostic workup for local treatment. Diffusion-weighted and gadoxetic-acid-enhanced magnetic resonance imaging (MRI) of the liver is increasingly used to improve the detection rate and characterization of liver lesions. MRI is superior in detection and characterization of CRLM as compared to CT. However, it is unknown if MRI actually provides a clinically relevant impact on patient management compared to CT scan. The Dutch CAMINO study is ongoing and will provide answers to that.[47]

Current disease monitoring is performed by RECIST 1.1 guidelines, which depend on unidimensional size changes of maximum two target lesions per organ, thereby ignoring potentially valuable other information of the tumors such as morphological changes, early tumor shrinkage, depth of response and mixed response or total tumor volume (TTV).[48-52] These alternative radiological metrics have been found prognostic for overall survival, progression-free survival, or pathologic response in patients with CRLM treated with systemic therapy. In Chapter 9 the results were presented of response evaluation by TTV and compared to RECIST 1.1 outcomes. TTV appeared to be associated with RFS in contrast to RECIST 1.1. These developments in imaging techniques are encouraging and have the potential to perform more accurate response evaluations in the future by combining TTV with morphological changes. Challenges to overcome for this technique are the definition of thresholds defining response or progression of disease in larger prospective studies. In addition, advanced volumetric software is not yet widely available in most radiology departments and lastly, the method is still very time-consuming. Despite these issues to resolve, these new imaging techniques show great potential and in combination with radiogenomics, an imaging method to assess tumor genetics, a non-invasive technique with major diagnostic, prognostic and therapeutic consequences could be effectuated.[53] These methods can play a major role in further individualizing treatment of CRLM and cancer in general.

References

1. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda Aguilar E, Bardelli A, Benson A, Bodoky G, et al: ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27(8):1386-1422.
2. Punt CJ, Koopman M, Vereulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol. 2017;14(4):235-246.
3. Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011;29(15):2011-2019.
4. Venook AP, Niedzwiecki D, Lenz HJ, Innocenti F, Fruth B, Meyerhardt JA, Schrag D, Greene C, O'Neil BH, Atkins JN, et al. Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. JAMA. 2017;317(23):2392-2401.
5. Arnold D, Lueza B, Douillard JY, Peeters M, Lenz HJ, Venook A, Heinemann V, Van Cutsem E, Pignon JP, Tabernero J, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol. 2017;28(8):1713-1729.
6. Diaz LA, Jr, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. Lancet Oncol. 2022;23(5):659-670.
7. Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, Wasan H, Ciardiello F, Loupakis F, Hong YS, et al. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-Mutated Colorectal Cancer. N Engl J Med. 2019;381(17):1632-1643.
8. Cremolini C, Antoniotti C, Moretto R, Masi G, Falcone A. First-line therapy for mCRC - the influence of primary tumour location on the therapeutic algorithm. Nat Rev Clin Oncol. 2017;14(2):113.
9. Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA. 2021; 325(7):669-685.
10. Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. Eur J Cancer. 2020; 141:225-238.
11. Hamers PAH, Elferink MAG, Stellato RK, Punt CJA, May AM, Koopman M, Vink GR. Informing metastatic colorectal cancer patients by quantifying multiple scenarios for survival time based on real-life data. Int J Cancer. 2021;148(2):296-306.
12. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3(1):50-56.
13. Jones RP, Jackson R, Dunne DF, Malik HZ, Fenwick SW, Poston GJ, Ghaneh P. Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases. Br J Surg. 2012;99(4): 477-486.
14. Angelsen JH, Viste A, Loes IM, Eide GE, Hoem D, Sorbye H, Horn A. Predictive factors for time to recurrence, treatment and post-recurrence survival in patients with initially resected colorectal liver metastases. World J Surg Oncol. 2015;13:328.
15. Okuno M, Goumard C, Kopetz S, Vega EA, Joechle K, Mizuno T, Omichi K, Tzeng CD, Chun YS, Vauthey JN, et al. RAS Mutation is Associated with Unsalvageable Recurrence Following Hepatectomy for Colorectal Cancer Liver Metastases. Ann Surg Oncol. 2018;25(8):2457-2466.
16. Isoniemi H, Uutela A, Nordin A, Lantto E, Kellokumpu I, Ovissi A, Kosunen J, Kallio R, Soveri LM, Salminen T, et al. Centralized repeated resectability assessment of patients with colorectal liver metastases during first-line treatment: prospective study. Br J Surg. 2021;108(7):817-825.
17. Paget S. The distribution of secondary growths in cancer of the breast. Lancet. 1889; 133(3421):571-573.

18. Zhou H, Liu Z, Wang Y, Wen X, Amador EH, Yuan L, Ran X, Xiong L, Ran Y, Chen W, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther. 2022; 7(1):70.
19. Wang LG, Gu J. Serum microRNA-29a is a promising novel marker for early detection of colorectal liver metastasis. Cancer Epidemiology. 2012;36(1):e61-e67.
20. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, et al. SEER Cancer Statistics Review, 1975-2016, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2016/, based on November 2018 SEER data submission, posted to the SEER web site, April 2019.
21. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra392.
22. Tie J, Cohen JD, Lo SN, Wang Y, Li L, Christie M, Lee M, Wong R, Kosmider S, Skinner I, et al. Prognostic significance of postsurgery circulating tumor DNA in nonmetastatic colorectal cancer: Individual patient pooled analysis of three cohort studies. Int J Cancer. 2021;148(4):1014-1026.
23. van 't Erve I, Greuter MJE, Bolhuis K, Vessies DCL, Leal A, Vink GR, van den Broek D, Velculescu VE, Punt CJA, Meijer GA, et al. Diagnostic Strategies toward Clinical Implementation of Liquid Biopsy RAS/BRAF Circulating Tumor DNA Analyses in Patients with Metastatic Colorectal Cancer. J Mol Diagn. 2020; 22(12):1430-1437.
24. Vidal J, Muinelo L, Dalmases A, Jones F, Edelstein D, Iglesias M, Orrillo M, Abalo A, Rodriguez C, Brozos E, et al. Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. Ann Oncol. 2017;28(6):1325-1332.
25. Henriksen TV, Reinert T, Christensen E, Sethi H, Birkenkamp-Demtroder K, Gogenur M, Gogenur I, Zimmermann BG, Group IS, Dyrskjot L, et al. The effect of surgical trauma on circulating free DNA levels in cancer patients-implications for studies of circulating tumor DNA. Mol Oncol. 2020;14(8):1670-1679.
26. Schraa SJ, van Rooijen KL, van der Kruijssen DEW, Rubio Alarcon C, Phallen J, Sausen M, Simmons J, Coupe VMH, van Grevenstein WMU, Elias S, et al. Circulating tumor DNA guided adjuvant chemotherapy in stage II colon cancer (MEDOCC-CrEATE): study protocol for a trial within a cohort study. BMC Cancer. 2020;20(1):790.
27. Cremolini C, Antoniotti C, Stein A, Bendell J, Gruenberger T, Rossini D, Masi G, Ongaro E, Hurwitz H, Falcone A, et al. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. J Clin Oncol. 2020; 38(28):3314-3324.
28. Van Cutsem E, De Gramont A, Henning G, Rougier P, Bonnetain F, Seufferlein T. Improving Outcomes in Patients with CRC: The Role of Patient Reported Outcomes-An ESDO Report. Cancers. (Basel) 2017;9(6):59.
29. Huang L, Guo Z, Wang F, Fu L. KRAS mutation: from undruggable to druggable in cancer. Signal Transduct Target Ther. 2021;6(1):386.
30. Siena S, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, Yamaguchi K, Nishina T, Fakih M, Elez E, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. Lancet Oncol. 2021; 22(6):779-789.
31. Greene BT, Hughes AD, King MR. Circulating tumor cells: the substrate of personalized medicine? Front Oncol. 2012;2:69.
32. Ooft SN, Weeber F, Dijkstra KK, McLean CM, Kaing S, van Werkhoven E, Schipper L, Hoes L, Vis DJ, van de Haar J, et al. Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. Sci Transl Med. 2019;11(513):eaay2574.
33. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernandez-Mateos J, Khan K, Lampis A, Eason K, Huntingford I, Burke R, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018, 359(6378):920-926.
34. Wensink GE, Elias SG, Mullenders J, Koopman M, Boj SF, Kranenburg OW, Roodhart JML. Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. NPJ Precis Oncol 2021, 5(1):30.

35. Thorstensen L, Qvist H, Nesland JM, Giercksky KE, Lothe RA. Allelotype profiles of local recurrences and distant metastases from colorectal-cancer patients. Int J Cancer 1996, 69(6):452-456.
36. Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, Kemeny N, Brennan MF, Blumgart LH, D'Angelica M. Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol 2007, 25(29):4575-4580.
37. Buisman FE, Galjart B, van der Stok EP, Kemeny NE, Balachandran VP, Boerner T, Cercek A, Grunhagen DJ, Jarnagin WR, Kingham TP, 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, 22(9):1271-1279.
38. Ignatavicius P, Oberkofler CE, Chapman WC, DeMatteo RP, Clary BM, D'Angelica MI, Tanabe KK, Hong JC, Aloia TA, Pawlik TM, et al. Choices of Therapeutic Strategies for Colorectal Liver Metastases Among Expert Liver Surgeons: A Throw of the Dice? Ann Surg 2020, 272(5):715-722.
39. Modest DP, Denecke T, Pratschke J, Ricard I, Lang H, Bemelmans M, Becker T, Rentsch M, Seehofer D, Bruns CJ, et al. Surgical treatment options following chemotherapy plus cetuximab or bevacizumab in metastatic colorectal cancer-central evaluation of FIRE-3. Eur J Cancer 2018, 88:77-86.
40. Osterlund P, Salminen T, Soveri LM, Kallio R, Kellokumpu I, Lamminmaki A, Halonen P, Ristamaki R, Lantto E, Uutela A, et al. Repeated centralized multidisciplinary team assessment of resectability, clinical behavior, and outcomes in 1086 Finnish metastatic colorectal cancer patients (RAXO): A nationwide prospective intervention study. Lancet Reg Health Eur 2021, 3:100049.
41. Ecker, B.L., Lee, J., Saadat, L.V., *et al.* Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. *Lancet Oncol* (2022).
42. Hoppener DJ, Galjart B, Nierop PMH, Buisman FE, van der Stok EP, Coebergh van den Braak RRJ, van Amerongen MJ, Balachandran VP, Jarnagin WR, Kingham TP, et al. Histopathological Growth Patterns and Survival After Resection of Colorectal Liver Metastasis: An External Validation Study. JNCI Cancer Spectr 2021, 5(3).
43. Wang Y, Lin HC, Huang MY, Shao Q, Wang ZQ, Wang FH, Yuan YF, Li BK, Wang DS, Ding PR, et al. The Immunoscore system predicts prognosis after liver metastasectomy in colorectal cancer liver metastases. Cancer Immunol Immunother 2018, 67(3):435-444.
44. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. J Clin Oncol 2009, 27(36):e279-280; author reply e281.
45. Sveen A, Loes IM, Alagaratnam S, Nilsen G, Holand M, Lingjaerde OC, Sorbye H, Berg KC, Horn A, Angelsen JH, et al. Intra-patient Inter-metastatic Genetic Heterogeneity in Colorectal Cancer as a Key Determinant of Survival after Curative Liver Resection. PLoS Genet 2016, 12(7):e1006225.
46. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014; 6(224):224ra224.
47. Gorgec B, Hansen I, Kemmerich G, Syversveen T, Abu Hilal M, Belt EJT, Bisschops RHC, Bollen TL, Bosscha K, Burgmans MC, et al. Clinical added value of MRI to CT in patients scheduled for local therapy of colorectal liver metastases (CAMINO): study protocol for an international multicentre prospective diagnostic accuracy study. BMC Cancer. 2021;21(1):1116.
48. Brunsell TH, Cengija V, Sveen A, Bjornbeth BA, Rosok BI, Brudvik KW, Guren MG, Lothe RA, Abildgaard A, Nesbakken A. Heterogeneous radiological response to neoadjuvant therapy is associated with poor prognosis after resection of colorectal liver metastases. Eur J Surg Oncol. 2019;45(12):2340-2346.
49. Tai K, Komatsu S, Sofue K, Kido M, Tanaka M, Kuramitsu K, Awazu M, Gon H, Tsugawa D, Yanagimoto H, et al. Total tumour volume as a prognostic factor in patients with resectable colorectal cancer liver metastases. BJS Open. 2020;4(3):456-466.
50. Chun YS, Vauthey JN, Boonsirikamchai P, Maru DM, Kopetz S, Palavecino M, Curley SA, Abdalla EK, Kaur H, Charnsangavej C, et al. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. JAMA. 2009; 302(21):2338-2344.

51. van Kessel CS, Samim M, Koopman M, van den Bosch MA, Borel Rinkes IH, Punt CJ, van Hillegersberg R. Radiological heterogeneity in response to chemotherapy is associated with poor survival in patients with colorectal liver metastases. Eur J Cancer. 2013;49(11):2486-2493.
52. Cremolini C, Loupakis F, Antoniotti C, Lonardi S, Masi G, Salvatore L, Cortesi E, Tomasello G, Spadi R, Zaniboni A, et al. Early tumor shrinkage and depth of response predict long-term outcome in metastatic colorectal cancer patients treated with first-line chemotherapy plus bevacizumab: results from phase III TRIBE trial by the Gruppo Oncologico del Nord Ovest. Ann Oncol. 2015;26(6):1188-1194.
53 Ji GW, Wang K, Xia YX, Li XC, Wang XH. Decoding Tumor Biology of Colorectal Liver Metastases With Radiogenomics: A Novel Insight Into Surgical Approach Selection. Ann Surg. 2019;269(1):e3-e4.

APPENDICES

Nederlandse samenvatting

Dikkedarmkanker, of colorectaal carcinoom (CRC) is de op twee na meest voorkomende vorm van kanker wereldwijd en uitzaaiingen (metastasen) van CRC ontstaan meestal in de lever.[1] Levermetastasen van colorectaal carcinoom (CRLM) zijn de belangrijkste oorzaak van sterfte door CRC.[2,3] Bij patiënten met metastasen beperkt tot de lever kan lokale behandeling van CRLM een kans bieden op lange termijn overleving, met een 5-jaars overlevingspercentage van 45-60%, of zelfs genezing.[4] Hierdoor kan het optimaliseren van de behandeling van CRLM-patiënten leiden tot verbetering van de uitkomsten in de algehele CRC-populatie. Bij diagnose van CRLM wordt in 20% van de patiënten de ziekte als direct resectabel beschouwd[5]. In de populatie met patiënten met initieel niet-resectabele CRLM kan systemische behandeling met een combinatie van chemotherapie en doelgerichte therapie leiden tot een conversie naar secundair resectabele CRLM in 57% van de patiënten.[5-9]

In dit proefschrift worden in de groep patiënten met initieel niet-resectabele CRLM de uitkomsten vergeleken van de meest effectieve eerstelijns systemische conversie-behandelingen. Daarnaast worden de postoperatieve uitkomsten betreffende de veiligheid van lokale behandelingen geanalyseerd. Verder evalueren we technisch-anatomische en tumor-biologische factoren die invloed hebben op de prognose of uitkomst van behandeling (prognostische en predictieve factoren) en richten we ons op klinisch relevante eindpunten van predictiemodellen om de klinische besluitvorming bij CRLM-patiënten te ondersteunen. Ten slotte analyseren we de prestaties van veelbelovende nieuwe diagnostische technieken bij CRLM-patiënten. Het doel van het onderzoek in dit proefschrift is om een bijdrage te leveren aan een betere en meer geïndividualiseerde behandeling van patiënten met CRLM door waardevolle inzichten in ziekte en therapie van CRLM te verschaffen.

Deel I van dit proefschrift richt zich op de uitkomsten van de momenteel meest effectieve eerstelijns systemische conversiebehandelingen en lokale behandeling bij patiënten met CRLM. Er is internationaal geen consensus over de optimale eerstelijns systemische conversiebehandeling, alhoewel de voorkeursbehandelingen bestaat uit fluoropyrimidine gebaseerde chemotherapie (doublet of triplet chemotherapie) in combinatie met doelgerichte therapie middels anti-EGFR- of anti-VEGF-gerichte therapie.[10,11] De vertaling van uitkomsten van prospectieve studies naar systemische conversiebehandeling bij CRLM patiënten naar de klinische praktijk wordt belemmerd door heterogeniteit in onderzoeksopzet en studiepopulaties, gebrek aan criteria voor (niet-)resectabiliteit, gebrek aan langetermijnuitkomsten van studies en door bias veroorzaakt door ongeplande retrospectieve subgroepanalyses. Bovendien maken de voortgaande ontwikkelingen op het gebied van CRC, zoals *(K)RAS*, *BRAF* mutatiestatus en zijdigheid van de primaire tumor, dat de resultaten van eerdere studies snel achterhaald

zijn.[6,12,13] Vergelijking van deze studies waarbij predictieve factoren wel in aanmerking worden genomen, is zowel uitdagend als cruciaal voor het trekken van conclusies uit CRLM-onderzoeken en voor toekomstig onderzoek.

In **Hoofdstuk 2** wordt een systematische review gepresenteerd van gerandomiseerde studies bij (subgroepen van) patiënten met aanvankelijk niet-resectabele CRLM, met focus op patiëntkenmerken en methodologie van de studies, inclusief klinische eindpunten, criteria voor (niet-)resectabiliteit en langetermijnoverleving. Een totaal van 20 gerandomiseerde fase II/III-onderzoeken met betrekking tot eerstelijns systemische conversietherapie bij patiënten of subgroepen van patiënten met CRLM werden geïncludeerd. Zeven onderzoeken betroffen alleen CRLM-patiënten en 13 onderzoeken hadden betrekking op subgroep analyses van CRLM-patiënten in niet geselecteerde patiënten met gemetastaseerd CRC (mCRC). We hebben geconstateerd dat de meeste onderzoeken bij inclusie geen criteria voor resectabiliteit hadden gesteld en dat als er wel criteria werden genoemd deze onderling verschilden tussen de onderzoeken. Dit resulteert in heterogeniteit van onderzoekspopulaties en beïnvloedt vervolgens de resectiepercentages, die inderdaad aanzienlijk bleken te variëren tussen studies bij CRLM populaties versus mCRC populaties, respectievelijk 22-57% en 11-38%. Studies en onderzoekspopulaties bleken ook heterogeen te zijn in selectie volgens prognostische en predictieve factoren zoals *K(RAS)/BRAF* mutatiestatus en zijdigheid van primaire tumor, gebruik van primaire eindpunten en rapportage van langetermijnoverleving van patiënten. Op één studie na waren de primaire uitkomstmaten bij alle studies korte termijn resectie-uitkomsten (conversiepercentage, (R0-)resectiepercentage en objectieve respons op therapie). Met deze systematische review geven we een overzicht van de korte en lange termijn uitkomsten na CRLM-resectie van onderzoeken met bevacizumab-bevattende regimes in zowel de ongeselecteerde populatie als in de *(K)RAS*-wildtype-populatie, en van anti-EGFR-therapie-bevattende regimes in de *(K)RAS*-wildtype-populatie. Als gevolg van bovengenoemde beperkingen kon er geen optimale systemische conversiebehandeling worden gekozen uit beschikbare studies. Aanbevelingen voor toekomstig onderzoeksopzet worden gegeven en zijn ook opgenomen in het ontwerp van de CAIRO5 studie; een multicenter, gerandomiseerde, fase 3-studie van de Dutch Colorectal Cancer Group (DCCG), een studie die de optimale systemische conversiebehandeling onderzoekt bij patiënten met aanvankelijk niet-resectabele CRLM.[14] Een innovatief aspect van het CAIRO5 studie is dat de CT scans van alle patiënten prospectief worden beoordeeld op resectabiliteit van CRLM volgens vooraf gedefinieerde baseline criteria door een centraal online lever expert panel bestaande uit ervaren leverchirurgen en radiologen. We veronderstelden dat het gebruik van dit panel, dat opereert via een online platform, de individuele subjectiviteit bij het definiëren van resectabiliteit kan verminderen en vervolgens kan leiden tot consensus over criteria voor resectie van CRLM.

In Hoofdstuk 3 is een analyse van de haalbaarheid van het DCCG lever expert panel en resultaten van de resectabiliteitsbeoordelingen gepresenteerd. De mediane tijd tot conclusie van het panel was 7 dagen, wat aanzienlijk sneller is dan het voorspelde maximum van 14 dagen, waardoor een efficiënte beoordeling door meerdere ervaren leverchirurgen bij deze zeer complexe patiënten mogelijk is. Gebrek aan consensus tussen panel chirurgen werd waargenomen in ongeveer 50% van de patiënten, met majeure discrepantie tussen chirurgen (resectabel versus permanent niet-resectabel) in 11% van de evaluaties. De grote variatie tussen chirurgen bij follow-up beoordelingen weerspiegelt de complexiteit bij het definiëren van behandelstrategieën voor CRLM en de dringende behoefte aan consensus over resectabiliteitscriteria op basis van predictieve en prognostische factoren. Totdat consensus is bereikt over de criteria voor resectabiliteit wordt het gebruik van een panel aanbevolen in plaats van een beslissing van een enkele chirurg om daarmee tot een evenwichtige beslissing te komen en te voorkomen dat patiënten ten onrechte de mogelijkheid van een operatie wordt onthouden. Van de patiënten met CRLM gedefinieerd als permanent niet-resectabel bij inclusie, converteerde de CRLM van ongeveer 15% van de patiënten naar resectabele CRLM tijdens systemische conversiebehandeling. Dit onderstreept het belang van herhaalde resectabiliteitsbeoordelingen, aangezien deze patiënten nog kans kunnen hebben op lokale leverbehandeling en daarmee op lange termijn overleving. Concluderend laten we zien dat een lever expert panel zoals gebruikt in de DCCG CAIRO5 studie op nationaal niveau haalbaar is en een platform biedt voor prospectieve beoordelingen van resectabiliteit bij patiënten met gevorderde CRLM.

De toename van complexe lokale lever behandelingen van CRLM, technische innovaties die de grenzen van resectabiliteit verleggen en het gebruik van steeds intensievere systemische conversieregimes vragen om gegevens betreffende de veiligheid van deze combinatie behandelingen in een homogeen multicenter prospectief cohort. In Hoofdstuk 4 worden de korte-termijn postoperatieve uitkomsten van lokale behandeling van patiënten met aanvankelijk niet-resectabele CRLM, na chemotherapie plus doelgerichte therapie in de CAIRO5 studie, gepresenteerd. Ernstige postoperatieve morbiditeit en mortaliteit na 90 dagen werden waargenomen bij respectievelijk 15,6% en 2,9% van de patiënten. Na multivariabele analyse waren bloedtransfusie, majeure resectie en triplet chemotherapie onafhankelijk gecorreleerd met ernstige postoperatieve complicaties. Er werd geen verband gevonden tussen het aantal cycli van eerstelijns systemische conversiebehandeling binnen de CAIRO5 studie en ernstige complicaties en daarom mag de duur van de eerstelijns systemische behandeling geen contra-indicatie zijn voor leverchirurgie. In deze groep patiënten met zeer gevorderde ziekte en complexe lokale leverbehandelingen (mediaan aantal CRLM van 9, 51% majeure leverresecties en 21% two-stage resecties) tonen wij met deze studie acceptabele postoperatieve kortetermijnuitkomsten (morbiditeit en mortaliteit). Zorgvuldige selectie van patiënten, rekening houdend met het preoperatief systemisch

regime (doublet versus triplet chemotherapie), en inspanningen om parenchym-sparende resecties uit te voeren, kunnen helpen om de ernstige postoperatieve complicaties en mortaliteit verder te verminderen.
Deel II van dit proefschrift richt zich op preoperatieve risicostratificatie van patiënten met CRLM. Hoewel CRLM wordt erkend als een heterogene ziekte op basis van prognostische klinische kenmerken en biomarkers, blijven resectabiliteitsbeoordelingen van CRLM een technisch-anatomische beslissing.[15,16] Chirurgische innovaties vergroten het aantal patiënten van wie de CRLM als technisch resectabel wordt beoordeeld, maar de hoge recidiefpercentages blijven bestaan en een aanzienlijk aantal patiënten heeft geen voordeel qua langetermijnoverleving van CRLM-resectie.[17] Er is dus een grote klinische behoefte aan een predictiemodel met een hoog onderscheidend vermogen dat een betere stratificatie en counseling van patiënten vóór de operatie mogelijk maakt om de therapie te personaliseren. Hoewel er in de loop der jaren meerdere modellen zijn voorgesteld die de prognose na CRLM-resectie voorspellen, zijn de meeste predictiemodellen ontwikkeld op basis van retrospectieve studies met een cohort van sterk geselecteerde patiënten behandeld in gespecialiseerde ziekenhuizen. Vaak ontbreekt een externe validatie met inachtneming van methodologische richtlijnen. Deze factoren belemmeren de generaliseerbaarheid van deze modellen in niet-geselecteerde populaties en in ondervertegenwoordigde subgroepen in de klinische praktijk.[18-20]

In Hoofdstuk 5 presenteren we een subgroepanalyse van de CAIRO5-studie naar de associatie van tumor-biologische en technisch-anatomische factoren met: conversie naar resectabele ziekte, vroeg recidief gedefinieerd als recidief binnen 6 maanden, en vroeg recidief zonder lokale behandeling met curatieve intentie. Na inductie systemische therapie en lokale behandeling van CRLM kreeg 43% van de patiënten een vroeg recidief en 31% een vroeg recidief zonder mogelijkheid tot lokale behandeling met curatieve intentie. Aantal CRLM was onafhankelijk geassocieerd met vroeg recidief. Aantal CRLM en leeftijd waren onafhankelijk geassocieerd met vroeg recidief zonder lokale behandeling. In een minderheid van de patiënten was er volledige consensus tussen panelchirurgen in individuele resectabiliteitsbeoordelingen. Postoperatieve uitkomsten waren gelijk tussen de patiënten met en zonder panel consensus. Wij concluderen dat gezien het ontbreken van voorspellende tumor-biologische factoren voor vroeg recidief en vroeg recidief zonder lokale vervolgbehandeling, de beoordeling van resectabiliteit van CRLM een primair technisch-anatomische beslissing blijft. Het gebruik van een expertpanel is van toegevoegde waarde om tot een meer afgewogen beslissing te komen waarbij ook meer CRLM patiënten in aanmerking komen voor lokale behandelingen.

In Hoofdstuk 6 wordt het voorspellend vermogen voor postoperatieve uitkomsten in CRLM patiënten vergeleken tussen twee gevestigde predictiemodellen, de traditionele

Fong-score en de Genetic And Morphological Evaluation score (GAME).[21,22] De predictiemodellen zijn extern gevalideerd in een nationaal real-life populatie-gebaseerd cohort van patiënten met lokale behandeling van CRLM, inclusief in vooraf gespecificeerde subgroepen (≤70/>70 jaar en met/zonder perioperatieve systemische therapie). Beide predictiemodellen vertoonden voorspellend vermogen in ons cohort. Hoewel het nieuwe predictiemodel (GAME) beter presteerde dan het traditionele model, beperkt de suboptimale voorspellende waarde van beide modellen de bruikbaarheid in klinische besluitvorming. Verder concluderen we dat de voorgestelde eindpunten, totale overleving (OS) en recidief-vrije overleving na lokale behandeling (RFS), suboptimale eindpunten zijn voor predictiemodellen. OS kan sterk worden beïnvloed door heterogeniteit in latere lijns behandelingen (lokaal en/of systemisch) en RFS is een zwakke surrogaatmarker voor overleving op lange termijn voornamelijk omdat het geen onderscheid maakt tussen recidieven die tot de lever beperkt blijven of zich buiten de lever manifesteren (extrahepatisch).[23,37] Vroege recidieven binnen zes maanden en extrahepatische recidieven zijn beide onafhankelijk geassocieerd met een kortere overleving.[24-26]

Onze hypothese in hoofdstuk 7 was dat voorspelling van extrahepatisch recidief binnen zes maanden, een klinisch relevant eindpunt zou vormen voor patiënten en clinici en zou kunnen helpen om patiënten adequaat te stratificeren en klinische beslissingen te sturen bij patiënten met technisch resectabele CRLM. In hoofdstuk 7 wordt een nieuw predictiemodel ontwikkeld en intern gevalideerd op basis van een populatie-gebaseerd cohort van 1077 patiënten. Dit model is ontwikkeld om extrahepatisch recidief (EHR) te voorspellen binnen 6 maanden na lokale behandeling van CRLM. Tijdens een follow-up van 35 maanden werden extrahepatische recidieven gevonden bij 52% van de patiënten. Om de relevantie van EHR binnen 6 maanden te beoordelen werd een landmark analyse uitgevoerd waarbij we toonden dat patiënten met een EHR event <6 maanden een mediane overleving hadden van 19,5 maanden (95% BI 15,6-23,0) versus niet bereikt (45,3-niet bereikt) zonder event. Het EHR-predictiemodel werd gevormd door de volgende predictieve factoren: zijdigheid van de primaire tumor, het T-stadium en het N-stadium van de primaire tumor, de *RAS/BRAFV600E*-mutatiestatus van de tumor en het aantal en de afmeting van CRLM. Het model was goed gekalibreerd en het 6-maand EHR-risico varieerde tussen 5,9-56,0%. Harrell's C-index voor 6 en 12 maanden was respectievelijk 0,663 (0,624-0,702) en 0,661 (0,632-0,689). Patiënten in het kwartiel met het hoogste risico hadden een waargenomen 6-maandsen EHR-risico van 32% versus 6% in het laagste kwartiel. De conclusie van het in Hoofdstuk 7 beschreven onderzoek is dat vroeg EHR na lokale behandeling van CRLM een grote invloed heeft op de prognose en kan worden voorspeld met routinematige klinische informatie.

Deel III richt zich op veelbelovende nieuwe diagnostische technieken bij patiënten met CRLM. Het recidiefpercentage na resectie van CRLM is hoog en wordt veroorzaakt door

micrometastasen die na resectie zijn achtergebleven in situ. Momenteel hebben beschikbare follow-upmethoden, zoals serum carcinogeen embryonaal antigeen (CEA) en beeldvorming zoals CT- of PET-scans, een beperkte nauwkeurigheid voor het detecteren van deze minimale residuele ziekte (MRD)[27]. Bepaling van circulerend tumor DNA (ctDNA) in bloed vertegenwoordigt een minimaal invasieve, kankerspecifieke biomarker met een groot potentieel als diagnostische, prognostische en ziekte-monitorende marker.[28] Bij patiënten met CRC in een vroeg stadium bleek postoperatief ctDNA een sterke onafhankelijke prognostische biomarker voor MRD en RFS.[29,30] Studies naar postoperatief ctDNA bij mCRC zijn beperkt en betreffen meestal heterogene patiëntengroepen met zowel lever- als extrahepatische ziekte en wisselend gebruik van systemische behandeling.[31,32]

In **Hoofdstuk 8** laten we de prognostische waarde zien van postoperatief ctDNA in een vooraf zorgvuldig geselecteerde homogene populatie van patiënten met *RAS*-gemuteerd CRLM na inductie systemische behandeling en complete lokale behandeling. We tonen in deze groep aan dat de detectie van postoperatief ctDNA een sterke onafhankelijke prognostische factor is voor terugkeer van de ziekte. Bovendien is dit de eerste studie waarin een sterke associatie van postoperatief ctDNA met pathologische respons wordt aangetoond, waarvan bekend is dat pathologische respons sterk gecorreleerd is met lange termijn overleving in patiënten met CRC. In tegenstelling tot pathologische responsevaluatie biedt ctDNA de mogelijkheid tot minimaal invasieve longitudinale follow-up, terwijl pathologische respons pas kan worden beoordeeld na biopt of resectie. Dit biedt kansen voor het personaliseren van postoperatief ziektemanagement in deze subgroep van patiënten met mCRC, bjivoorbeeld door intensievere follow-up of adjuvante behandeling.

Patiënten met aanvankelijk niet-resectabele CRLM ondergaan systemische behandeling met als doel conversie naar resectabele CRLM of om kankergerelateerde symptomen te verminderen, kwaliteit van leven te behouden en/of voor levensverlenging. Evaluatie van de tumorrespons op therapie is cruciaal bij deze patiënten, aangezien beslissingen over verdere behandelstrategie (bijv. resectie of het stoppen of verandering van systemische behandeling) gebaseerd zijn op deze radiologische evaluaties. RECIST1.1 biedt unidimensionale radiologische criteria om de tumorrespons op systemische therapie te evalueren.[33] Alhoewel deze criteria wereldwijd worden gebruikt, zijn er ook tekortkomingen gerapporteerd zoals de grote interobserver variabiliteit en het niet betrekken van het totaal tumorvolume (TTV).[34-36]

In **Hoofdstuk 9** wordt een studie besproken waarin in totaal 420 CT-scans zijn geëvalueerd voor en tijdens systemische conversiebehandeling bij 210 CAIRO5 patiënten met aanvankelijk niet-resectabele CRLM. Baseline- en follow-up CT-scans werden gebruikt om de RECIST1.1- en TTV-respons op systemische therapie te berekenen. Er

werden verschillende drempels (10%, 20%, 40%) gebruikt om de respons van TTV te definiëren aangezien er momenteel geen standaard bestaat. RFS werd beoordeeld in de subgroep van CRLM-patiënten die secundaire resectie ondergingen. Bij 30% tot 50% (afhankelijk van de gekozen TTV-drempel) van de patiënten werd een discordantie waargenomen tussen RECIST1.1 en TTV-verandering. De studie toonde aan dat bij patiënten met stabiele ziekte volgens RECIST 1.1 bij 47 (22%) patiënten een TTV-daling van meer dan 40% (spreiding 40%–81%) werd waargenomen en bij 4 (2%) patiënten TTV was gestegen met meer dan 40% (bereik 42%-197%). Bij 3 (2%) patiënten die volgens RECIST 1.1 als partiële respons waren geclassificeerd, vertoonden TTV-toename tot 70%. Bij patiënten na secundaire resectie van CRLM was een TTV-afname van minder dan 10% en of meer dan 20% geassocieerd met RFS (respectievelijk p=0.015 en p=0.022), terwijl RFS niet geassocieerd was met (non)-respons volgens RECIST1.1 (p=0.821). Concluderend lijkt TTV-responsbeoordeling prognostisch veelbelovend bij de evaluatie van respons op systemische behandeling bij patiënten met aanvankelijk niet-resectabele CRLM en zou dit kunnen bijdragen aan het optimaliseren van de systemische behandeling door een nauwkeurigere responsevaluatie.

References

1. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16:713-732.
2. Zhou H, et al. Colorectal liver metastasis: molecular mechanism and interventional therapy. Signal Transduct Target Ther. 2022;7:70.
3. Manfredi S, et al. Epidemiology and management of liver metastases from colorectal cancer. Ann Surg. 2006;244:254-259.
4. Morris EJ, et al. Surgical management and outcomes of colorectal cancer liver metastases. Br J Surg. 2010;97:1110-1118.
5. Adam R, Kitano Y. Multidisciplinary approach of liver metastases from colorectal cancer. Ann Gastroenterol Surg. 2019;3:50-56.
6. Folprecht G, et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol. 2010;11: 38-47.
7. Tomasello G, et al. FOLFOXIRI Plus Bevacizumab as Conversion Therapy for Patients With Initially Unresectable Metastatic Colorectal Cancer: A Systematic Review and Pooled Analysis. JAMA Oncol. 2017;3:e170278.
8. Cremolini C, et al. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. J Clin Oncol. 2020;Jco2001225.
9. Ychou M, et al. Chemotherapy (doublet or triplet) plus targeted therapy by RAS status as conversion therapy in colorectal cancer patients with initially unresectable liver-only metastases. The UNICANCER PRODIGE-14 randomised clinical trial. Br J Cancer. 2022;126:1264-1270.
10. Benson AB, 3rd, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017;15:370-398.
11. Van Cutsem E, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386-1422.
12. Holch JW, Ricard I, Stintzing S, Modest DP, Heinemann V. The relevance of primary tumour location in patients with metastatic colorectal cancer: A meta-analysis of first-line clinical trials. Eur J Cancer. 2017;70:87-98.
13. Tsilimigras DI, et al. Clinical significance and prognostic relevance of KRAS, BRAF, PI3K and TP53 genetic mutation analysis for resectable and unresectable colorectal liver metastases: A systematic review of the current evidence. Surg Oncol. 2018;27:280-288.
14. Huiskens J, et al. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. J Am Coll Surg. 2019;229:523-532 e522.
15. Adam R, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012;17:1225-1239.
16. Guinney J, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21:1350-1356.
17. Lam VW, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol. 2012;19:1292-1301.
18. Mahar AL, et al. Personalizing prognosis in colorectal cancer: A systematic review of the quality and nature of clinical prognostic tools for survival outcomes. J Surg Oncol. 2017;116:969-982.
19. Meyer RM. Generalizing the results of cancer clinical trials. J Clin Oncol. 2010;28:187-189.
20. Zamanipoor Najafabadi AH, et al. TRIPOD statement: a preliminary pre-post analysis of reporting and methods of prediction models. BMJ Open. 2020;10:e041537.
21. 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;230:309-318; discussion 318-321.
22. Margonis GA, et al. Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases. Br J Surg. 2018;105:1210-1220.

23. Oba M, et al. Discrepancy between recurrence-free survival and overall survival in patients with resectable colorectal liver metastases: a potential surrogate endpoint for time to surgical failure. Ann Surg Oncol. 2014;21:1817-1824.
24. Creasy JM, et al. Actual 10-year survival after hepatic resection of colorectal liver metastases: what factors preclude cure? Surgery. 2018;163:1238-1244.
25. Imai K, et al. Early Recurrence After Hepatectomy for Colorectal Liver Metastases: What Optimal Definition and What Predictive Factors? Oncologist. 2016;21:887-894.
26. Vigano L, 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. 2014;21:1276-1286.
27. Chao M, Gibbs P. Caution is required before recommending routine carcinoembryonic antigen and imaging follow-up for patients with early-stage colon cancer. J Clin Oncol. 2009;27:e279-280; author reply e281.
28. Cristiano S, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570: 385-389.
29. Wang R, et al. The value of circulation tumor DNA in predicting postoperative recurrence of colorectal cancer: a meta-analysis. Int J Colorectal Dis. 2020;35:1463-1475.
30. Tie J, et al. Prognostic significance of postsurgery circulating tumor DNA in nonmetastatic colorectal cancer: Individual patient pooled analysis of three cohort studies. Int J Cancer. 2021;148:1014-1026.
31. Benesova L, et al. Significance of postoperative follow-up of patients with metastatic colorectal cancer using circulating tumor DNA. World J Gastroenterol. 2019;25:6939-6948.
32. Scholer LV, et al. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res. 2017;23:5437-5445.
33. Eisenhauer EA, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-247.
34. Beaumont H, et al. Discrepancies of assessments in a RECIST 1.1 phase II clinical trial - association between adjudication rate and variability in images and tumors selection. Cancer Imaging. 2018;18:50.
35. Yoon SH, Kim KW, Goo JM, Kim DW, Hahn S. Observer variability in RECIST-based tumour burden measurements: a meta-analysis. Eur J Cancer. 2016;53:5-15.
36. Kuhl CK, et al. Validity of RECIST Version 1.1 for Response Assessment in Metastatic Cancer: A Prospective, Multireader Study. Radiology. 2019;290:349-356.
37. Ecker BL, Lee J, Saadat LV, et al. Recurrence-free survival versus overall survival as a primary endpoint for studies of resected colorectal liver metastasis: a retrospective study and meta-analysis. Lancet Oncol. 2022;23:1332–1342.

List of publications

Van Bree SH, Vlug MS, Bemelman WA, Hollmann MW, Ubbink DT, Zwinderman AH, de Jonge WJ, Snoek SA, **Bolhuis K**, van der Zanden E, The FO, Bennink RJ, Boeckxstaens GE. Faster recovery of gastrointestinal transit after laparoscopy and fast-track care in patients undergoing colonic surgery. *Gastroenterology*. Sep 2011;141(3):872-880.e1-4. doi:10.1053/j.gastro.2011.05.034

Bolhuis K, Bakker LJ, Keijer JT, de Vries PJ. Implementing a hospital-wide protocol for Staphylococcus aureus bacteremia. *Eur J Clin Microbiol Infect Dis*. Aug 2018;37(8):1553-1562. doi:10.1007/s10096-018-3284-9

Bolhuis K[*], Huiskens J[*], Engelbrecht MR, De Jong KP, Kazemier G, Liem MS, Verhoef C, de Wilt JH, Punt CJ, van Gulik TM, Dutch Colorectal Cancer G. Outcomes of Resectability Assessment of the Dutch Colorectal Cancer Group Liver Metastases Expert Panel. *J Am Coll Surg*. Dec 2019;229(6):523-532 e2. doi:10.1016/j.jamcollsurg.2019.08.1445

Bolhuis K, Kos M, van Oijen MGH, Swijnenburg RJ, Punt CJA. Conversion strategies with chemotherapy plus targeted agents for colorectal cancer liver-only metastases: A systematic review. *Eur J Cancer*. Dec 2020;141:225-238. doi:10.1016/j.ejca.2020.09.037

Van 't Erve I, Greuter MJE, **Bolhuis K**, Vessies DCL, Leal A, Vink GR, van den Broek D, Velculescu VE, Punt CJA, Meijer GA, Coupé VMH, Fijneman RJA. Diagnostic Strategies toward Clinical Implementation of Liquid Biopsy RAS/BRAF Circulating Tumor DNA Analyses in Patients with Metastatic Colorectal Cancer. *J Mol Diagn*. Dec 2020;22(12):1430-1437. doi:10.1016 /j.jmoldx. 2020.09.002

Bolhuis K[*], van 't Erve I[*], Mijnals C, Delis-Van Diemen PM, Huiskens J, Komurcu A, Lopez-Yurda M, van den Broek D, Swijnenburg RJ, Meijer GA, Punt CJA, Fijneman RJA. 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. doi:10.1016/j.ebiom.2021.103498

Van 't Erve I, Wesdorp NJ, Medina JE, Ferreira L, Leal A, Huiskens J, **Bolhuis K**, van Waesberghe JTM, Swijnenburg RJ, van den Broek D, Velculescu VE, Kazemier G, Punt CJA, Meijer GA, Fijneman RJA. KRAS A146 Mutations Are Associated With Distinct Clinical Behavior in Patients With Colorectal Liver Metastases. *JCO Precis Oncol*. 2021;5doi:10.1200/po.21.00223

Van't Erve I, Rovers KP, Constantinides A, **Bolhuis K**, Wassenaar EC, Lurvink RJ, Huysentruyt CJ, Snaebjornsson P, Boerma D, van den Broek D, Buffart TE, Lahaye MJ, Aalbers AG, Kok NF, Meijer GA, Punt CJ, Kranenburg O, de Hingh IH, Fijneman RJ. Detection of tumor-derived cell-free DNA from colorectal cancer peritoneal metastases in plasma and peritoneal fluid. *J Pathol Clin Res*. May 2021;7(3):203-208. doi:10.1002/cjp2.207

Bolhuis K, Grosheide L, Wesdorp NJ, Komurcu A, Chapelle T, Dejong CHC, Gerhards MF, Grünhagen DJ, van Gulik TM, Huiskens J, De Jong KP, Kazemier G, Klaase JM, Liem MSL, Molenaar IQ, Patijn GA, Rijken AM, Ruers TM, Verhoef C, de Wilt JHW, Punt CJA, Swijnenburg R-J. Short-Term Outcomes of Secondary Liver Surgery for Initially Unresectable Colorectal Liver Metastases Following Modern Induction Systemic Therapy in the Dutch CAIRO5 Trial. *Annals of Surgery Open*. 2021;2(3):e081. doi:10.1097/as9.0000000000000081

Wesdorp NJ, **Bolhuis K**, Roor J, van Waesberghe J-HTM, van Dieren S, van Amerongen MJ, Chapelle T, Dejong CHC, Engelbrecht MRW, Gerhards MF, Grunhagen D, van Gulik TM, Hermans JJ, de Jong KP, Klaase JM, Liem MSL, van Lienden KP, Molenaar IQ, Patijn GA, Rijken AM, Ruers TM, Verhoef C, de Wilt JHW, Swijnenburg R-J, Punt CJA, Huiskens J, Kazemier G. The Prognostic Value of Total Tumor Volume Response Compared With RECIST1.1 in Patients With Initially Unresectable Colorectal Liver Metastases Undergoing Systemic Treatment. *Annals of Surgery Open*. 2021;2(4):e103. doi:10.1097/as9.0000000000000103

Bolhuis K[*], Wensink GE[*], Elferink MAG, Bond MJG, Dijksterhuis WPM, Fijneman RJA, Kranenburg OW, Rinkes I, Koopman M, Swijnenburg RJ, Vink GR, Hagendoorn J, Punt CJA, Elias SG, Roodhart JML. External Validation of Two Established Clinical Risk Scores Predicting Outcome after Local Treatment of Colorectal Liver Metastases in a Nationwide Cohort. *Cancers (Basel)*. May 10 2022;14(10)doi:10.3390/cancers14102356

Wesdorp NJ, Kemna R, **Bolhuis K**, van Waesberghe J, Nota I, Struik F, Oulad Abdennabi I, Phoa S, van Dieren S, van Amerongen MJ, Chapelle T, Dejong CHC, Engelbrecht MRW, Gerhards MF, Grünhagen D, van Gulik TM, Hermans JJ, de Jong KP, Klaase JM, Liem MSL, van Lienden KP, Molenaar IQ, Patijn GA, Rijken AM, Ruers TM, Verhoef C, de Wilt JHW, Swijnenburg RJ, Punt CJA, Huiskens J, Stoker J, Kazemier G. Interobserver Variability in CT-based Morphologic Tumor Response Assessment of Colorectal Liver Metastases. *Radiol Imaging Cancer*. May 2022;4(3):e210105. doi:10.1148/rycan.210105

Van 't Erve I, Medina JE, Leal A, Papp E, Phallen J, Adleff V, Jiayuee Chiao E, Arun AS, **Bolhuis K**, Simmons JK, Kandikar A, Valkenburg KC, Sausen M, Angiuoli SV, Scharpf RB, Punt CJA, Meijer GA, Velculescu VE, Fijneman RJA. Metastatic colorectal cancer treatment response evaluation by ultra-deep sequencing of cell-free DNA and matched white blood cells. *Submitted*

Bolhuis K*, Wensink GE*, Elferink MAG, Fijneman RJA, Kranenburg OW, Rinkes I, Koopman M, Swijnenburg RJ, Vink GR, Hagendoorn J, Punt CJA, Roodhart JML, Elias SG. Predicting early extrahepatic recurrence after local treatment of colorectal liver metastases. *Submitted*

Bolhuis K*, Bond M*, Van Amerongen MJ, Komurcu A, Chapelle T, Dejong CHC, Engelbrecht MRW, Gerhards MF, Grünhagen DJ, Van Gulik T, Hermans JJ, Huiskens J, De Jong KP, Kazemier G, Klaase JM, Kok NFM, May AM, Leclercq WKG, Liem MSL, Van Lienden KP, Molenaar IQ, Neumann UP, Patijn GA, Rijken AM, Ruers TM, Verhoef C, De Wilt JHW, Punt CJA, Swijnenburg RJ. The role of tumor-biological factors in technical-anatomical resectability assessment of colorectal liver metastases following induction systemic treatment: An analysis of the Dutch CAIRO5 trial. *Submitted*

Bond MJG, Kuiper BI, **Bolhuis K**, Komurcu A, Van Amerongen MJ, Chapelle T, Dejong CHC, Engelbrecht MRW, Gerhards MF, Grünhagen DJ, Van Gulik T, Hermans JJ, De Jong KP, Klaase JM, Kok NFM , Leclercq WKG, Liem MSL, Van Lienden KP, Molenaar IQ, Neumann UP, Patijn GA, Rijken AM, Ruers TM, Verhoef C, De Wilt JHW, Kazemier G, May AM, Punt CJA, Swijnenburg RJ. Intersurgeon variability in local treatment planning for patients with initially unresectable colorectal liver metastases (CRLM): Analysis of the Dutch Colorectal Cancer Group Liver Expert Panel. *Submitted*

Zwart K, Van der Baan FH, Punt CJA, Wensink GE, **Bolhuis K**, Laclé MM, Van Grevenstein W, Hagendoorn J, De Hingh I, Koopman M, Vink G, Roodhart J. Characteristics and survival of metastatic colorectal cancer patients receiving local treatment with deficient mismatch repair versus proficient mismatch repair tumors. *Submitted*

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PhD portfolio

Name PhD student:	Karen Bolhuis
PhD period:	May 2017 – July 2022
PhD supervisor:	Prof. dr. C.J.A. Punt
PhD co-supervisor:	Dr. R.J. Swijnenburg

1. PhD training

	Year	Workload (ECTS)
General courses		
- BROK	2018	1.5
- Project Management	2019	1.0
Specific courses		
- Searching for systematic review	2017	0.6
- Practical biostatistics	2018	1.4
Seminars, workshops and master classes		
- ESMO Preceptorship on Colorectal Cancer. *Valencia, Spain*	2018	0.6
- Weekly department seminars discussing landmark studies. *Amsterdam, NL*	2017-2022	4.0
Presentations		
- Evaluation of feasibility and outcomes of the liver expert panel. One-yearly meetings liver expert panel DCCG. *Utrecht*, *NL.*	2017-2020	1.0
- Update of CAIRO5 Central liver expert panel reviewing resectability in initially unresectable liver-only colorectal cancer metastases. CCA Seminar series, *Amsterdam, NL.*	2018	1.0
- Treatment strategies in colorectal cancer patients with initially unresectable CRLM. 5D's Congress. *NL.*	2018	1.0
- Progress CAIRO5. Research meetings DCCG. *Utrecht, NL.*	2017–2020	1.0
- E-special. Het optimaliseren van de behandeling van mensen met dikke darmkanker die is uitgezaaid in de lever. Landelijke Werkgroep Darmkanker.	2019	0.1
- Treatment strategies in colorectal cancer patients with initially unresectable CRLM. HPB meeting. *Utrecht, NL.*	2020	0.5
- CAIRO5 study presentation. BSHPBS Board meeting. *Brussels, BE.*	2019	0.5
- Seminar on Metastatic colorectal cancer: CAIRO studies, influence on current practice. Servier Nederland Farma BV, *Leiden, NL.*	2018	0.5
- Implementation and ouctomes of a national liver expert panel to determine resectability of CRLM. EMCC, *Lisbon, Portugal.*	2019	1.0
- CAIRO5 presentation, Dutch Study Group for Liver Surgery. *Utrecht, NL.*	2019	0.5
- Poster presentation. Feasibility of a national expert panel to determine secondary resectability in patients with Colorectal Cancer Liver Metastases (CRLM) in the CAIRO5 study. ASCO, *Chicago, USA.*	2019	1.0

(Inter)national conferences		
- 5D's Multidisciplinary Gastro-intestinal Oncology Congress, *Netherlands*	2018	0.6
- European-African Hepato-Pancreato-Biliary Association (E-AHPBA) congress, *Amsterdam, Netherlands*	2019	0.5
- European Multidisciplinary Colorectal Cancer Congress (EMCCC), *Lisbon, Portugal*	2019	1.1
- American Society of Medical Oncology (ASCO) Annual Meeting, *Chicago, USA*	2019	1.1
Other		
- Weekly meetings study team	2017–2022	4.0
- Organizing two-monthly translational research meetings for researchers in colorectal cancer of AMC, VUmc and NKI-AvL	2018–2020	1.0
- Study coordinator of the multicentre randomized controlled phase 3 CAIRO5 study	2017–2020	6.0
- Coordinator of the liver expert panel of the Dutch Colorectal Cancer Group	2017–2020 2019	3.0 2.0
- Coordinating the audit of Health and Youthcare inspectorate (IGJ) of the Dutch Colorectal Cancer Group, CAIRO5 trial and participating hospital		
2. Teaching		
Supervising		
- Bachelor Thesis Medicine Student	2017	1.0
- Bachelor Medicine Student	2019	2.0

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Graag bedank ik alle patiënten en ziekenhuizen die actief deelnemen of hebben deelgenomen aan wetenschappelijk onderzoek en in het bijzonder aan de CAIRO5 studie. Jullie inzet is van groot belang en kan de wetenschap en de zorg vooruit helpen.

Alle coauteurs die hebben bijgedragen aan de artikelen: veel dank voor de feedback en de samenwerking.

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Milan, Ali en Esther, het was fantastisch om de kamer met jullie te delen na de eenzame tijd in de cubicle. Veel gezamenlijke koffie, grappen, ommetjes om de dag door te komen en steun bij tegenslagen. Will, dank voor je hulp bij het organiseren van het oerwoud van NKR data. Milan, zo ontzettend fijn om met jou samen te werken als mens en als onderzoeker bij mijn eerste grote project, het systematic review. Dapper door allemaal!

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Curriculum Vitae

Karen Bolhuis is geboren op 17 maart 1985 te Nieuwegein, waarna zij verhuisde naar Tiel waar zij woonde met haar ouders, twee jongere zusjes en broertje. In 2003 behaalde zij het VWO diploma aan het RSG Lingecollege en verhuisde zij naar Amsterdam. Vanaf 2003 studeerde zij Geneeskunde aan de Universiteit van Amsterdam en was zij actief lid, waaronder een jaar bestuurslid, van Studentenvereniging L.A.N.X.. In 2011 was zij gedurende drie maanden werkzaam op de afdeling Interne Geneeskunde in het Onandjokwe Lutheran Medical Hospital in Namibië. Haar eerste contact met uitvoeren van wetenschappelijk onderzoek was in het kader van een wetenschappelijke stage bij de maag darm lever afdeling van het Academische Medisch Centrum (AMC) Amsterdam. In oktober 2011 behaalde zij haar artsexamen. Aansluitend begon zij als art-assistent op de afdeling Interne Geneeskunde van Tergooi ziekenhuizen te Hilversum en Blaricum. Naast haar werkzaamheden als arts, was zij betrokken bij de implementatie van een ziekenhuisbreed protocol voor de diagnostiek en behandeling van *S. Aureus* bacteriemie (SAB) en deed zij onderzoek naar de uitkomsten voor en na invoer van dit protocol. In Januari 2014 startte zij haar opleiding tot internist (opleiders prof. dr. Geerlings, prof. dr. Prins, dr. Willems) en was daarbij tot 2016 werkzaam in Tergooi Ziekenhuizen (opleiders dr. S. Lobatto en dr. P.J. de Vries). Vanaf 2016 zette zij haar opleiding voort in het AMC. In 2017 begon zij aan haar differentiatie tot medisch oncoloog (opleiders dr. Westermann en dr. Tromp) en startte zij gelijktijdig haar promotie onderzoek onder begeleiding van prof. dr. C.J.A. Punt en prof. dr. T. van Gulik, vanaf 2019 opgevolgd door dr. R.J. Swijnenburg. Haar promotieonderzoek richtte zich op patiënten met colorectale levermetastasen met als doel de behandeling verder te individualiseren en optimaliseren. Ten tijde van haar promotieonderzoek was zij studie coördinator van de CAIRO5 studie; een multicenter fase 3 studie en begeleidde zij het landelijke lever expert panel van de Dutch Colorectal Cancer Group (DCCG). In april 2022 volbracht zij haar opleiding tot internist-oncoloog en vanaf mei 2022 is zij werkzaam op de afdeling MDL-oncologie van het Antoni van Leeuwenhoek te Amsterdam als internist-oncoloog met focus op gastro-enterologische oncologie. Zij woont samen met Frank en zoontje Seb (‘21) in Amsterdam-Oost.