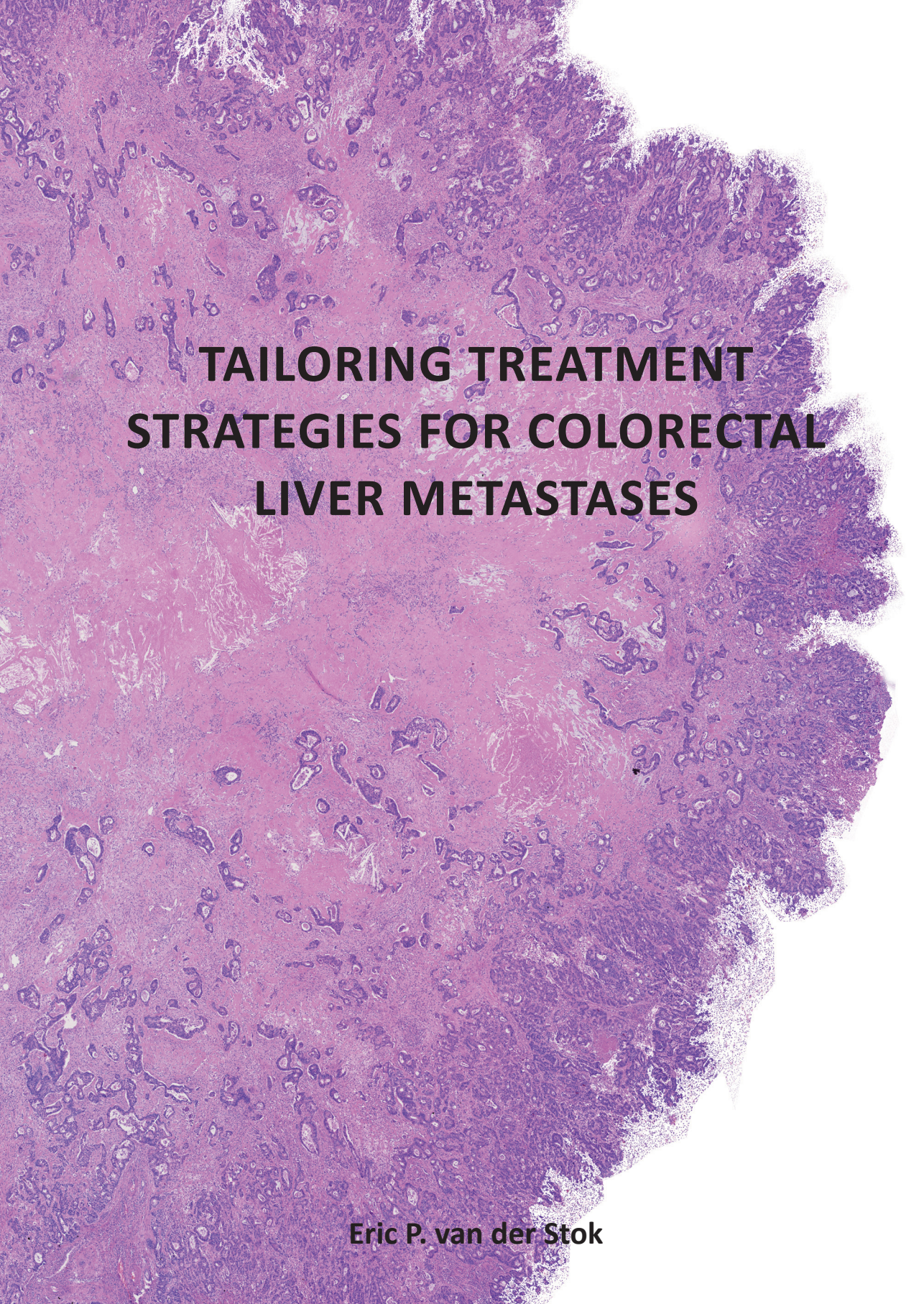


TAILORING TREATMENT STRATEGIES FOR COLORECTAL LIVER METASTASES

Eric P. van der Stok

TAILORING TREATMENT STRATEGIES FOR COLORECTAL LIVER METASTASES

Eric van der Stok

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

Financial support for the printing of this thesis was obtained from:

Chipsoft B.V.
CongressCompany
Erasmus MC Afdeling Heelkunde
Erasmus Universiteit Rotterdam
Raadsheeren
Medicidesk Rabobank Rotterdam
Servier Nederland Farma
Erbe Nederland B.V.

Research included in this thesis has been financially supported by:

KWF Kankerbestrijding

ISBN: 978-94-93019-63-8

Cover design and layout: Fenna Schaap || www.proefschriftmaken.nl
Printing: ProefschriftMaken || www.proefschriftMaken.nl

TAILORING TREATMENT STRATEGIES FOR COLORECTAL LIVER METASTASES

Maatwerk bij Behandelstrategieën voor Colorectale Levermetastasen

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag van de
rector magnificus

Prof. dr. R.C.M.E. Engels
En volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 21 september 2018 om 09:30 uur

Door

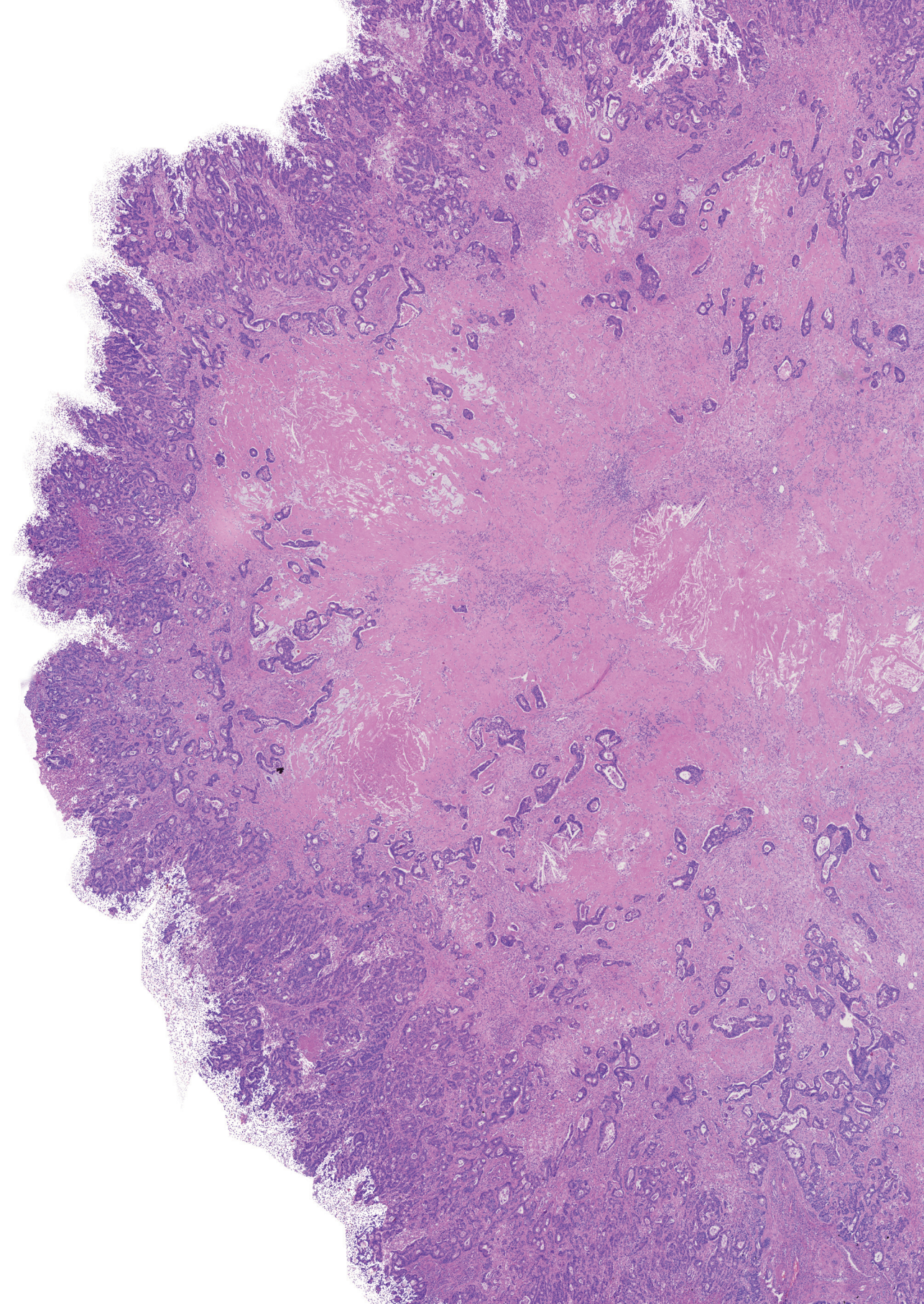
Eric Pieter van der Stok
geboren te Utrecht

PROMOTIECOMMISSIE

Promotor:	Prof. Dr. C. Verhoef
Overige leden:	Prof. Dr. J.J.B. van Lanschot Prof. Dr. S. Sleijfer Prof. Dr. J.H.W. De Wilt
Copromotor:	Dr. D.J. Grünhagen

Erasmus University Rotterdam

CONTENTS



PART i

Introduction, Aim and Outline of this Thesis

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of cancer worldwide, and the 2nd most lethal of all cancers [1]. Approximately 1.2 million new cases are diagnosed each year and more than 600,000 annual deaths are estimated to occur worldwide [1]. Around 50% of patients with CRC present with localized disease (stage I-II), about 25% with locoregional advanced-stage disease (stage III), and the remainder with metastases in distant organs (stage IV) [2-4]. Approximately 30% of patients with stage I-III disease develop recurrent disease after initial treatment [5, 6]; among patients with stage IV CRC, up to 65% have relapsed disease after treatment with curative intent [7-13]. Thirty years ago, stage IV colorectal cancers were answered with nihilism in terms of curative treatment options [14]. Today, indications for local, curative treatment are ever expanding. Liver resection is considered to be the optimal treatment for isolated colorectal liver metastases (CRLM) with 5-year survival rates up to 60% in selected patients [15, 16]. Until recently, only 10-20% of patients were considered suitable for attempted curative resection [15, 17]. Due to improvements in surgical technique, the acceptance of smaller resection margins, the introduction of more effective systemic chemotherapy, the use of portal vein embolization (VPE), radio frequency ablation (RFA) and stereotactic body radiation (STBR) more patients become eligible for liver surgery. Importantly, not all patients with colorectal liver metastases benefit as much from surgery and/or systemic therapies, which emphasizes the need for "tailored" treatment strategies.

Traditionally, a tailor is a person who makes, repairs, or alters clothing, professionally. The term "tailor" took on its modern sense in the 18th century [18]. "Tailoring" classically referred to a set of specific hand and machine sewing and pressing techniques that are unique to the construction of clothing. The term evolved, in the United Kingdom traditional tailoring is called "bespoke tailoring" [18]. The term "bespoke" in fashion is reserved for individually patterned clothing, in contrast with mass-manufactured "ready-to-wear". With the multifaceted clinical and molecular characteristics of colorectal cancer, the emergence of "individualized" or "tailored" therapies became apparent the past decade. "One-size fits nobody" may be as true in fashion as it is in cancer treatment.

This thesis describes studies aimed at optimizing and thus tailor treatment strategies for the individual patient with colorectal liver metastases. Aims were to:

- Conduct a multicenter randomized controlled trial to assess the effect of neo-adjuvant chemotherapy in high-risk resectable patients;
- Evaluate and improve logistics of multicenter randomized trials in the Netherlands in general;
- Identify and evaluate new biomarkers in order to improve patient selection for various treatment modalities (prognostic/predictive markers);
- Optimize patient referral for surgery and assessment of resectability for patients with CRLM;
- Assess and improve the value of surveillance after curative treatment for colorectal cancer;

PART I: MULTICENTER CLINICAL TRIALS ON COLORECTAL LIVER METASTASES IN THE NETHERLANDS

In patients with primarily resectable colorectal liver metastases, administration of systemic therapies is not standard of care in the Netherlands due to limited evidence supporting such a strategy. Until today it remains unknown whether chemotherapy in these patients impact overall survival. In 2013, the EORTC 40983 randomized trial (EPOC trial) published its mature results, which showed that 12 courses of perioperative chemotherapy only impact disease-free survival and not overall survival after resection of CRLM [19, 20]. Due to strict inclusion criteria this landmark study mainly included patients with a relatively low oncological risk profile. Consequently, patients with a high-risk profile who might benefit the most from chemotherapy may be underrepresented in these studies. Oncological risk is captured in various clinical risk scores, generally expressing tumor load. The CRS according to Fong et al. is the most widely used and validated score, able to distinguish between high- and low-risk patients in terms of survival outcomes [21, 22]. **Chapter 1** describes the protocol and rationale for the randomized controlled CHARISMA trial, aimed at assessing the impact of neo-adjuvant chemotherapy in addition to resection of CRLM in high-risk patients, specifically.

For patients with multi-organ oligo-metastases of colorectal cancer, local treatment may lead to superior overall survival in selected patients, as compared to palliative systemic treatments [23-25]. For more advanced disease there seems to be no role for local treatment, and palliative systemic therapies are currently standard of care for this group of patients. There are no prospective data on efficacy of local treatment for patients with multi-organ colorectal cancer. **Chapter 2** reports on the safety and feasibility of the multicenter randomized ORCHESTRA trial. In this trial, patients with multi-organ metastases of CRC are randomized for palliative systemic treatment, versus palliative treatment with additional maximal tumor debulking.

In the field of research, one of the most powerful designs is the randomized controlled trial (RCT). RCTs have had an enormous influence on the evaluation of interventions since the first of its kind in the 1940s [26]. The configuration and management of trials have evolved significantly since, and RCTs have become the "gold standard" for the comparative assessment of therapies. Large multicenter trials involve substantial complexity as a consequence of regulatory guidelines, financial investment and administrative burdens, even without taking into account trial inclusion and follow-up time [27-36]. The feasibility of RCTs can be facilitated by standardized and sensible regulatory and logistical guidelines. In **chapter 3**, the current thesis depicts the complex logistic, administrative, legal, ethical and financial landscape associated with the initiation of 2 oncological multicenter randomized trials in the Netherlands.

PART II: PROGNOSTIC AND PREDICTIVE FACTORS AFTER SURGERY FOR COLORECTAL LIVER METASTASES

A substantial number of patients develop recurrent disease after liver surgery for CRLM, with an associated high mortality rate, underlining the need for prognostic biomarkers [7, 37, 38]. Such prognostic biomarkers may allow a more personalized treatment strategy (predictive biomarkers). In recent years, several clinicopathological prognostic variables in patients with CRLM have been identified predicting the risk of relapse or death after a metastasectomy [39]. These variables have been integrated in various clinical risk scores (CRS) [21, 39-42]. As mentioned earlier, the CRS according to Fong et al. is the most widely used and validated score, able to distinguish between high risk and low risk patients in terms of survival outcomes [21]. **In chapter 4** the predictive value of this CRS is assessed in relation to overall survival benefit from neo-adjuvant systemic chemotherapy, which formed the basis for the CHARISMA study protocol (**chapter 1** of this book). Part of Fong's CRS is the lymph node status of the primary colorectal tumor. As the CRS was established before adjuvant therapies for node positive colon cancer became standard of care, the prognostic value of primary nodal status was re-assessed in the era of adjuvant chemotherapy for stage III colon cancer [43] (**chapter 5**).

For patients with resectable CRLM, no biomarkers exist that impact clinical management. Classic clinical variables are far from perfect in predicting patient outcome. New staging systems (i.e. biomarkers) are urgently needed to optimize treatment [44, 45]. Unravelling biological properties characterising tumours may be pivotal to designing these individualised therapies, based on biological predictors of outcome rather than or in addition to clinical predictors. Various groups have established molecular subtypes in primary cancers with distinct biology, predictive and prognostic value [46-49] [50, 51]. Biological markers may improve patient selection for (neo-) adjuvant therapies in addition to surgical management or intensive surveillance schemes. The current thesis describes clinical, histopathological and genetic biomarker research. In **chapter 6**, the prognostic value of C-Reactive Protein (CRP) post-liver resection was assessed. **Chapter 7** describes a study aimed at identifying a prognostic biomarker at mRNA level. **Chapter 8** represents a protocol for reliably scoring three distinct histopathological growth patterns of CRLM with prognostic power. In **chapter 9**, the predictive capacity of these growth patterns is assessed in terms of overall survival impact of chemotherapy in addition to surgery.

PART III: SURGICAL MANAGEMENT OF COLORECTAL LIVER METASTASES

In patients with colorectal liver metastases, surgery offers superior survival outcomes and the only potential for cure [15-17, 25, 52]. Therefore surgery, if technically possible, is the gold standard treatment modality for patients with liver-only metastases although no randomized controlled trials have been conducted on the subject. Undertaking such a trial has been argued to be unethical by some in the field [14]. Thus, in **chapter 10** a case-matched analysis was performed in patients with liver-only CRC metastases, treated with surgery or systemic

therapy alone.

Based on current evidence on outcome after surgery for CRLM, all patients should be considered for resection. At present, metastasectomy is considered if there is an expected functional liver remnant of at least 20-30%, if liver resection is anatomically feasible in relation to vascular and biliary structures, and if no unresectable extrahepatic metastases are present. Despite the definition of resectability, the decision of CRLM being amenable for surgery varies even between dedicated hepatobilliary surgeons [53-55]. For various types of cancer (esophageal, gastric and lung), significant inter-hospital and interregional differences exist for application of curative (local) treatment [56-58]. In Sweden, this has already been established for CRLM, with significant variations in selection for liver surgery based on hospital type, region, gender, and age [59]. **Chapter 11** investigated potential Dutch inter-hospital and regional differences in utilization of surgery for CRLM, and assessment of resectability by specialist liver surgeons.

After curative treatment, 30% of patients with stage I–III and up to 65% of patients with stage IV CRC develop recurrent disease [5-13]. Historically, CRC patients are routinely offered surveillance in order to detect disease recurrence at an early, asymptomatic stage, with the intention of treating these recurrences with curative intent and improving survival. To date, controversy continues to surround the yield of any surveillance after curative treatment. There exists great variance in surveillance protocols between hospitals all around the world [60-63]. In **chapter 12** the need of surveillance for patients after resected CRLM and a prolonged disease free interval was assessed, aiming to identify subgroups that may be excluded from follow-up at some stage after surgery. **Chapter 13** sets out a rigorous review of literature on surveillance after curative treatment of stage I-IV CRC, and reflects on its utility.

PART IV: DISCUSSION AND FUTURE PERSPECTIVES, SUMMARY AND APPENDICES

The final **chapters 14, 15** and **16** of this thesis provide a general summary and a discussion with future perspectives in the field of management of colorectal liver metastases. In **chapter 17** the appendices are published, including a list op publications, acknowledgements and a section about the author.

REFERENCES

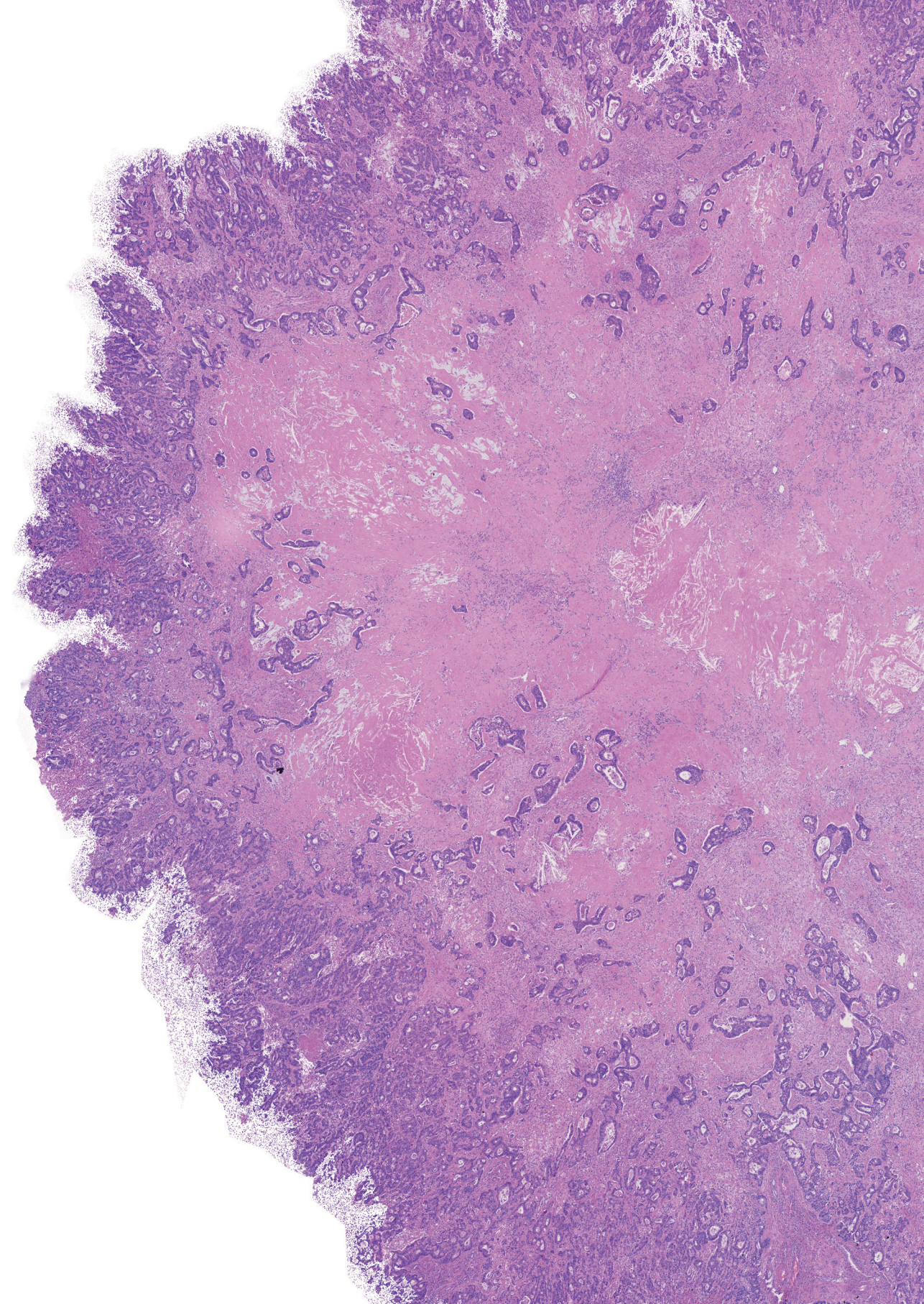
1. Torre, L.A., et al., *Global cancer statistics, 2012*. CA Cancer J Clin, 2015. **65**(2): p. 87-108.
2. Elferink, M.A., et al., *Marked improvements in survival of patients with rectal cancer in the Netherlands following changes in therapy, 1989-2006*. Eur J Cancer, 2010. **46**(8): p. 1421-9.
3. van der Pool, A.E., et al., *Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series*. Colorectal Dis, 2012. **14**(1): p. 56-61.
4. van Steenbergen, L.N., et al., *Improved survival of colon cancer due to improved treatment and detection: a nationwide population-based study in The Netherlands 1989-2006*. Ann Oncol, 2010. **21**(11): p. 2206-12.
5. Pita-Fernandez, S., et al., *Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis*. Ann Oncol, 2015. **26**(4): p. 644-56.
6. Rose, J., K.M. Augestad, and G.S. Cooper, *Colorectal cancer surveillance: what's new and what's next*. World J Gastroenterol, 2014. **20**(8): p. 1887-97.
7. de Jong, M.C., 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): p. 440-8.
8. Elias, D., et al., *Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: retrospective analysis of 523 patients from a multicentric French study*. J Clin Oncol, 2010. **28**(1): p. 63-8.
9. Glehen, O., et al., *Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study*. J Clin Oncol, 2004. **22**(16): p. 3284-92.
10. Jones, R.P., et al., *Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases*. Br J Surg, 2012. **99**(4): p. 477-86.
11. Pfannschmidt, J., H. Dienemann, and H. Hoffmann, *Surgical resection of pulmonary metastases from colorectal cancer: a systematic review of published series*. Ann Thorac Surg, 2007. **84**(1): p. 324-38.
12. Verwaal, V.J., et al., *Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer*. J Clin Oncol, 2003. **21**(20): p. 3737-43.
13. Warwick, R. and R. Page, *Resection of pulmonary metastases from colorectal carcinoma*. Eur J Surg Oncol, 2007. **33 Suppl 2**: p. S59-63.
14. Grunhagen, D., et al., *The history of adoption of hepatic resection for metastatic colorectal cancer: 1984-95*. Crit Rev Oncol Hematol, 2013. **86**(3): p. 222-31.
15. Kanas, G.P., et al., *Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors*. Clin Epidemiol, 2012. **4**: p. 283-301.
16. Rees, M., et al., *Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients*. Ann Surg, 2008. **247**(1): p. 125-35.
17. Elferink, M.A., et al., *Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands*. Int J Colorectal Dis, 2015. **30**(2): p. 205-12.

18. Bailey, N., *An Universal Etymological English Dictionary*. 1756.
19. Nordlinger, B., et al., *Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial*. Lancet, 2008. **371**(9617): p. 1007-16.
20. Nordlinger, 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**(12): p. 1208-15.
21. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases*. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
22. Sotiropoulos, G.C. and H. Lang, *Clinical scoring systems for predicting outcome after surgery for colorectal liver metastases: towards a better multidisciplinary approach*. Liver Int, 2009. **29**(1): p. 6-9.
23. Chua, T.C., et al., *Hepatectomy and resection of concomitant extrahepatic disease for colorectal liver metastases--a systematic review*. Eur J Cancer, 2012. **48**(12): p. 1757-65.
24. Leung, U., et al., *Colorectal Cancer Liver Metastases and Concurrent Extrahepatic Disease Treated With Resection*. Ann Surg, 2016.
25. Loupakis, F., et al., *Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer*. N Engl J Med, 2014. **371**(17): p. 1609-18.
26. *STREPTOMYCIN treatment of pulmonary tuberculosis*. Br Med J, 1948. **2**(4582): p. 769-82.
27. Kenter, M.J. and A.F. Cohen, *Re-engineering the European Union Clinical Trials Directive.* Lancet, 2012. **379**(9828): p. 1765-7.
28. Maskell, N.A., et al., *Variations in experience in obtaining local ethical approval for participation in a multi-centre study*. QJM, 2003. **96**(4): p. 305-7.
29. Ahmed, A.H. and K.G. Nicholson, *Delays and diversity in the practice of local research ethics committees*. J Med Ethics, 1996. **22**(5): p. 263-6.
30. Helfand, B.T., et al., *Variation in institutional review board responses to a standard protocol for a multicenter randomized, controlled surgical trial*. J Urol, 2009. **181**(6): p. 2674-9.
31. Rawlins, M., *A new era for UK clinical research?* Lancet, 2011. **377**(9761): p. 190-2.
32. Christian, M.C., et al., *A central institutional review board for multi-institutional trials*. N Engl J Med, 2002. **346**(18): p. 1405-8.
33. Duley, L., et al., *Specific barriers to the conduct of randomized trials*. Clin Trials, 2008. **5**(1): p. 40-8.
34. Yusuf, S., et al., *Sensible guidelines for the conduct of large randomized trials*. Clin Trials, 2008. **5**(1): p. 38-9.
35. Tully, J., et al., *The new system of review by multicentre research ethics committees: prospective study*. BMJ, 2000. **320**(7243): p. 1179-82.
36. Seiler, C.M., et al., *Assessment of the ethical review process for non-pharmacological multicentre studies in Germany on the basis of a randomised surgical trial*. J Med Ethics, 2007. **33**(2): p. 113-8.
37. D'Angelica, M., et al., *Effect on outcome of recurrence patterns after hepatectomy for colorectal metastases*. Ann Surg Oncol, 2011. **18**(4): p. 1096-103.
38. Butte, J.M., et al., *Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer:*

Potentially Curative Role of Salvage Repeat Resection. Ann Surg Oncol, 2015.

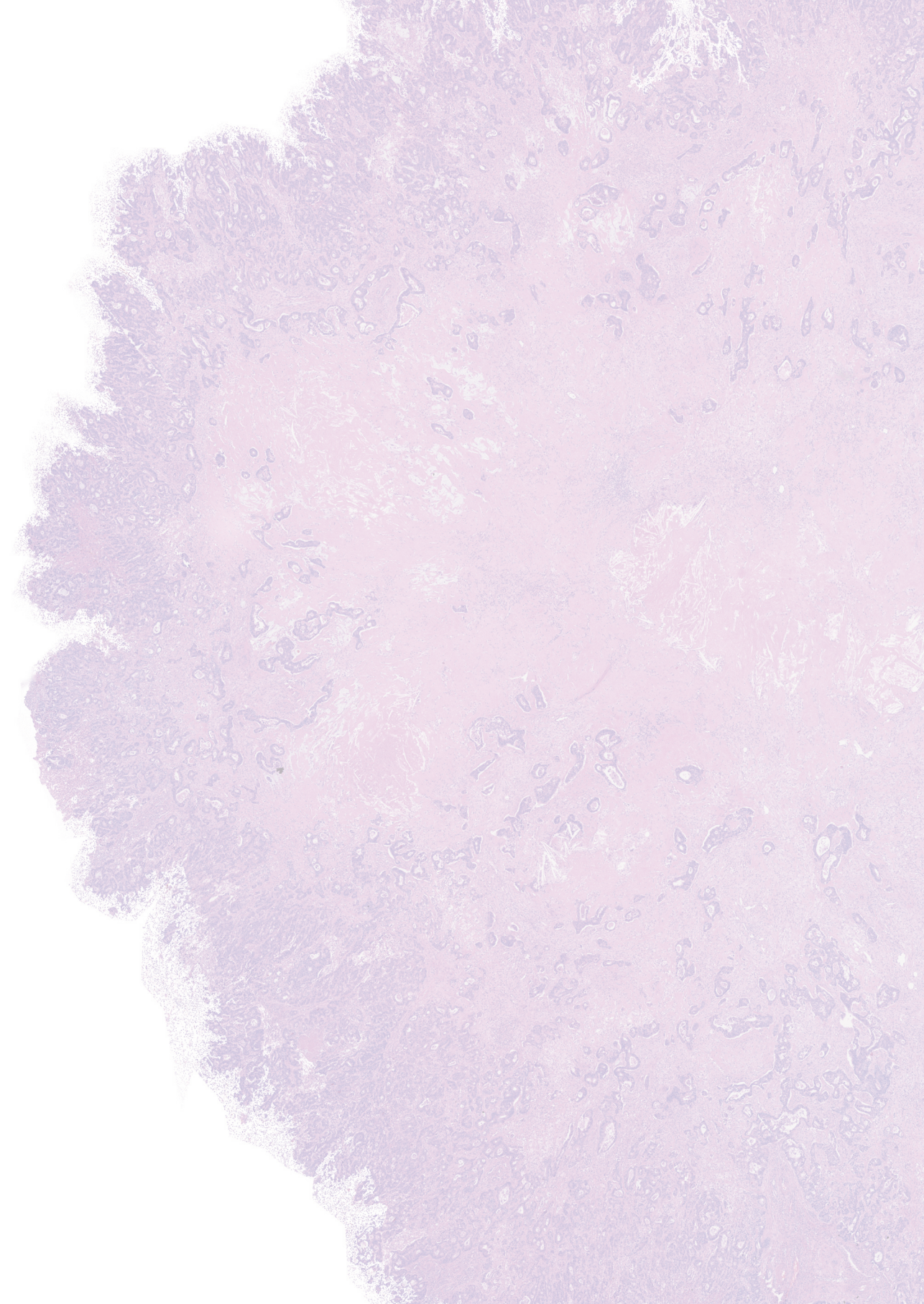
39. Matias, M., et al., *Prognostic Factors after Liver Resection for Colorectal Liver Metastasis*. Acta Med Port, 2015. **28**(3): p. 357-69.
40. Konopke, R., et al., *Prognostic factors and evaluation of a clinical score for predicting survival after resection of colorectal liver metastases*. Liver Int, 2009. **29**(1): p. 89-102.
41. Nagashima, I., et al., *A new scoring system to classify patients with colorectal liver metastases: proposal of criteria to select candidates for hepatic resection*. J Hepatobiliary Pancreat Surg, 2004. **11**(2): p. 79-83.
42. Nordlinger, B., et al., *Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie*. Cancer, 1996. **77**(7): p. 1254-62.
43. Andre, T., et al., *Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial*. J Clin Oncol, 2009. **27**(19): p. 3109-16.
44. Poston, G.J., *Staging of advanced colorectal cancer*. Surg Oncol Clin N Am, 2008. **17**(3): p. 503-17, viii.
45. Poston, G.J., et al., *Urgent need for a new staging system in advanced colorectal cancer*. J Clin Oncol, 2008. **26**(29): p. 4828-33.
46. Guinney, J., et al., *The consensus molecular subtypes of colorectal cancer*. Nat Med, 2015.
47. Paik, S., et al., *A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer*. N Engl J Med, 2004. **351**(27): p. 2817-26.
48. Hoshida, Y., et al., *Gene expression in fixed tissues and outcome in hepatocellular carcinoma*. N Engl J Med, 2008. **359**(19): p. 1995-2004.
49. Albain, K.S., et al., *Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial*. Lancet Oncol, 2010. **11**(1): p. 55-65.
50. Budinska, E., et al., *Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer*. J Pathol, 2013. **231**(1): p. 63-76.
51. Sadanandam, A., et al., *A colorectal cancer classification system that associates cellular phenotype and responses to therapy*. Nat Med, 2013. **19**(5): p. 619-25.
52. Heinemann, V., 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): p. 1065-75.
53. 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**(1): p. 38-47.
54. Jones, R.P., et al., *Effect of specialist decision-making on treatment strategies for colorectal liver metastases*. Br J Surg, 2012. **99**(9): p. 1263-9.
55. Young, A.L., et al., *Variation in referral practice for patients with colorectal cancer liver metastases*. Br J Surg, 2013. **100**(12): p. 1627-32.

56. Koeter, M., et al., *Hospital of diagnosis and probability to receive a curative treatment for oesophageal cancer*. Eur J Surg Oncol, 2014. **40**(10): p. 1338-45.
57. Siemerink, E.J., et al., *Effect of hospital characteristics on outcome of patients with gastric cancer: a population based study in North-East Netherlands*. Eur J Surg Oncol, 2010. **36**(5): p. 449-55.
58. Wouters, M.W., et al., *Variation in treatment and outcome in patients with non-small cell lung cancer by region, hospital type and volume in the Netherlands*. Eur J Surg Oncol, 2010. **36 Suppl 1**: p. S83-92.
59. Noren, A., H.G. Eriksson, and L.I. Olsson, *Selection for surgery and survival of synchronous colorectal liver metastases; a nationwide study*. Eur J Cancer, 2016. **53**: p. 105-14.
60. Labianca, R., et al., *Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up*. Ann Oncol, 2013. **24 Suppl 6**: p. vi64-72.
61. Meyerhardt, J.A., et al., *Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement*. J Clin Oncol, 2013. **31**(35): p. 4465-70.
62. Steele, S.R., et al., *Practice Guideline for the Surveillance of Patients After Curative Treatment of Colon and Rectal Cancer*. Dis Colon Rectum, 2015. **58**(8): p. 713-25.
63. Van Cutsem, E., et al., *Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up*. Ann Oncol, 2014. **25 Suppl 3**: p. iii1-9.



PART I

Multicenter Clinical Trials on Colorectal Liver Metastases in the Netherlands



CHAPTER 1

Neo-adjuvant chemotherapy followed by surgery versus surgery alone in high-risk patients with resectable colorectal liver metastases. The CHARISMA randomized multicenter clinical trial

E.P. van der Stok*, N. Ayez*
J.H. de Wilt
S.A. Radema
R. van Hillegersberg
R.M. Roumen
G. Vreugdenhil
P.J. Tanis
C.J. Punt
C.H. Dejong
R.L. Jansen
H.M. Verheul
K.P. de Jong
G. Hospers
J.M. Klaase
M.C. Legdeur
E. van Meerten
F. Eskens
N. van der Meer
B. van der Holt
C. Verhoef
D.J. Grünhagen

* Both authors contributed equally

BMC Cancer. 2015 Mar 26;15:180

ABSTRACT

BACKGROUND

Efforts to improve the outcome of liver surgery by combining curative resection with chemotherapy have failed to demonstrate definite overall survival benefit. This may partly be due to the fact that these studies often involve strict inclusion criteria. Consequently, patients with a high-risk profile as characterized by Fong's Clinical Risk Score (CRS) are often underrepresented in these studies. Conceptually, this group of patients might benefit the most from chemotherapy. The present study evaluates the impact of neo-adjuvant chemotherapy in high-risk patients with primary resectable colorectal liver metastases, without extrahepatic disease. Our hypothesis is that adding neo-adjuvant chemotherapy to surgery will provide an improvement in overall survival (OS) in patients with a high-risk profile.

METHODS/DESIGN

CHARISMA is a multicenter, randomized, phase III clinical trial. Patients will be randomized to either surgery alone (standard treatment, arm A) or to 6 cycles of neo-adjuvant oxaliplatin-based chemotherapy, followed by surgery (arm B). Patients must be ≥ 18 years of age with liver metastases of histologically confirmed primary colorectal carcinoma. Patients with extrahepatic metastases are excluded. Liver metastases must be deemed primarily resectable. Only patients with a CRS of 3-5 are eligible. The primary study endpoint is OS. Secondary endpoints are progression free survival (PFS), quality of life, morbidity of resection, treatment response on neo-adjuvant chemotherapy, and whether CEA levels can predict treatment response.

DISCUSSION

CHARISMA is a multicenter, randomized, phase III clinical trial that will provide an answer to the question if adding neo-adjuvant chemotherapy to surgery will improve OS in a well-defined high-risk patient group with colorectal liver metastases.

TRIAL REGISTRATION

The CHARISMA is registered at European Union Clinical Trials Register (EudraCT), number: 2013-004952-39.

BACKGROUND

COLORECTAL LIVER METASTASES: SURGICAL TREATMENT

Colorectal cancer (CRC) is one of the leading causes of cancer death. It is in the top 3 most commonly diagnosed cancers, with over 1.2 million new cases and over 600,000 deaths estimated to have occurred in 2008 worldwide [1]. In approximately 20% of patients distant metastases are present at time of diagnosis [2]. The liver is the most common metastatic site. Approximately 50% of patients with early-stage disease will eventually develop colorectal liver metastases (CRLM) [3, 4].

When metastases of CRC patients are restricted to the liver, possible curative treatment can be obtained by surgical resection. Complete surgical resection of CRLM improves 5-year survival rates to around 35-60% in selected patients [5-8]. However in only 10-20% of patients surgical resection of CRLM is feasible. Although surgery for CRLM provides the only potential for cure, cancer relapse is a common phenomenon, with a recurrence rate of up to 50% in the first 2 years after surgery [9].

CHEMOTHERAPY FOR COLORECTAL LIVER METASTASES

Initially, systemic treatment with 5-fluoruracil based regimens was standard of care in CRLM, improving OS from 6 to 10-12 months. The development of chemotherapeutic agents such as oxaliplatin and irinotecan has subsequently improved OS to a median of up to 24 months. Sequential treatment with all available cytotoxic agents, as well as the introduction of Epidermal Growth Factor receptor (EGFR) and Vascular Endothelial Growth Factor (VEGF) binding monoclonal antibodies have further increased overall survival [10-13].

The high relapse rate after curative resection of CRLM, and the efficacy of modern systemic treatment in the metastatic setting, have prompted investigators to perform numerous studies to evaluate the potential role of systemic chemotherapy combined with liver resection. The purpose of both adjuvant and neo-adjuvant chemotherapy is to treat microscopic disease that is not addressed by surgery. This microscopic disease may be promoting the high relapse rate that is observed after liver surgery [9]. Notably, current literature suggests that timing of additional chemotherapy (adjuvant vs. neo-adjuvant) seems to have no influence on outcome [14]. The role of perioperative chemotherapy in case of resectable CRLM was established in a randomized controlled trial [15]. In the mature OS analysis of this trial there was no significant effect on OS after a median follow up of 7 years [16].

STRATIFICATION BY CLINICAL RISK SCORE

In the past, several clinical risk scores for the outcome of patients with CRLM have been published [7, 17-25]. In 1999, Fong et al. described the most widely used CRS [19]. This prognostic scoring system has been verified by independent investigators [26]. Several authors have proposed the concept of stratification by CRS in relation to the effects of a multimodal treatment strategy on OS. These authors suggest that patients with a high-

risk score have a worse prognosis and might therefore benefit more from chemotherapy compared to patients with a low risk score [27-29].
These findings have prompted others and ourselves to retrospectively evaluate data on patients who have undergone liver resection for CRLM in the last decade with and without chemotherapy, stratified by CRS according to the Fong-criteria [30, 31].
As described earlier, efforts to improve outcome of liver surgery by combining the resection with chemotherapy have failed to demonstrate definite OS benefit. This may partly be due to the fact that these studies often involve strict study protocol inclusion criteria. Consequently, patients with a high clinical risk score - which might benefit the most from chemotherapy - are often underrepresented in these studies. Since genuine survival benefit has not yet been demonstrated, could this low impact of chemotherapy on survival then be explained by the *relatively* low risk profile of the patients included in these trials?

STUDY AIM AND HYPOTHESIS
The CHARISMA randomized clinical trial will evaluate the effect on OS of neo-adjuvant chemotherapy in patients with primary resectable CRLM and a CRS (Fong) of 3-5, thereby bearing a poor prognosis. The primary aim of this study is to compare OS in patients with resectable liver metastases randomized for treatment with chemotherapy, consisting of capecitabine and oxaliplatin (XELOX), followed by surgery versus surgery alone.
We hypothesize that neo-adjuvant chemotherapy will provide an improvement in OS in this high-risk patient group. Secondary endpoints in this study will be progression free survival (PFS), quality of life as assessed by QLQ-30 and MFI questionnaires, response to chemotherapy, morbidity of surgery and resection rate, and whether carcinoembryonic antigen (CEA) can predict for treatment response, PFS, and OS.

METHODS/DESIGN

Patients with CRLM and a high CRS will be evaluated for inclusion by the local multidisciplinary team meeting. In this meeting, at least two surgeons with expertise in liver surgery should be present. In case of doubt, the imaging can be sent to a central expert panel. Patients are eligible for randomization if, in the opinion of a local expert panel, radical resection of the CRLM (R0-resection) is feasible.
Patients will be randomized 1:1 to either (figure 1):

- Arm A:
  Surgery of the liver metastases
- Arm B:
  Neo-adjuvant oxaliplatin-based chemotherapy followed by surgery of the liver metastases

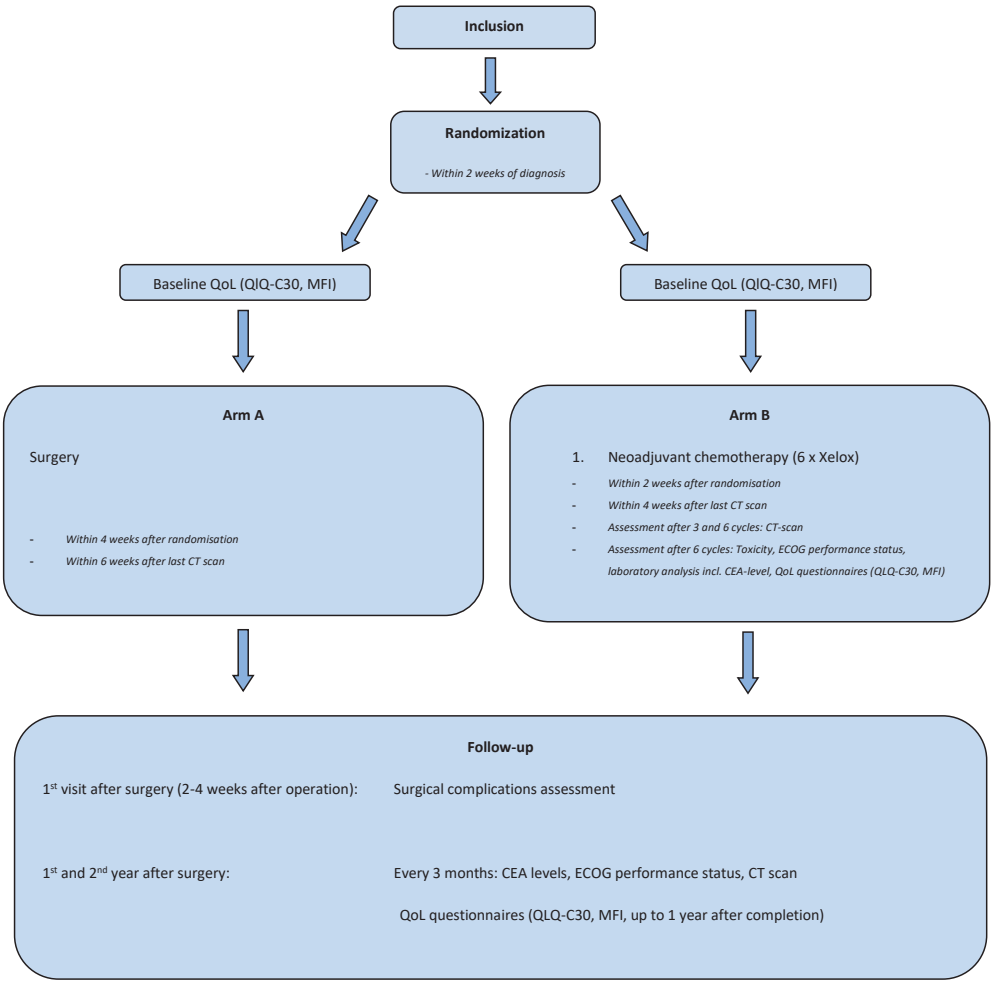


Figure 1. Study flowchart

STUDY POPULATION

INCLUSION CRITERIA

Age ≥ 18 years, ECOG performance status 0-1. Histologically confirmed primary colorectal carcinoma. Radiological confirmed and primary resectable CRLM. CRS of 3-5 (Fong). Adequate bone marrow, liver and renal functions.

Before any study related procedure will be pursued, written informed consent must be given according to ICH/GCP and national/local regulations.

EXCLUSION CRITERIA

Adjuvant chemotherapy for colorectal carcinoma given < 6 months prior to detection of the liver metastases. Prior non-colorectal malignancies, except for basal or squamous cell carcinoma of the skin, or patients with carcinoma in situ of the cervix. Extrahepatic colorectal metastases. Locally advanced rectal cancer in situ requiring long-course pre-operative chemoradiotherapy. Major surgical procedures < 4 weeks prior to randomization. Pregnancy. History of psychiatric disability. Clinically significant cardiovascular disease. Uncontrolled hypertension. Lack of physical integrity of the upper gastro-intestinal tract, malabsorption syndrome, or inability to take oral medication. Known peripheral neuropathy. Organ allografts requiring immunosuppressive therapy. Serious, non-healing wound, ulcer, or bone fracture. Current or recent use of full-dose oral anticoagulants or thrombolytic agents for therapeutic purposes. Chronic treatment with corticosteroids. Serious intercurrent infections. Current or recent treatment with another investigational drug or participation in another investigational study. Psychological, familial, sociological or geographical conditions hampering compliance to the study protocol and follow-up schedule.

ASSESSMENT OF OPERABILITY

All patients have to be screened by their treating surgeon for fitness to undergo liver surgery. In case of doubt, formal anesthetic assessment is mandatory prior to randomization.

ASSESSMENT OF RESECTABILITY

Prior to resection of the CRLM, an expert panel must review imaging of patients enrolled in this study in order to determine resectability. Resectability is defined as the possibility to achieve R0 resection. The liver remnant should comprise a portal vein, a hepatic artery, and a bile duct, one of the three main hepatic veins. The liver remnant should have sufficient liver function and 2 segments free of metastases at the time of resection.

If these prerequisites cannot be met, radiofrequency ablation (RFA) is allowed to obtain resectability. However, RFA may only be used in combination with liver resection if the number of lesions to be treated with RFA does not exceed 3 and the largest diameter of these lesions is less than 3 cm.

THERAPEUTIC REGIMEN OF PATIENTS ARM A

Patients should preferably be randomized within 2 weeks of the definitive diagnosis of CRLM. Patients allocated to Arm A should have their surgery within 4 weeks after randomization

and within 6 weeks after the last CT scan. Adjuvant chemotherapy after R0 resection is not allowed. Protocol therapy ends following the liver resection.

THERAPEUTIC REGIMEN OF PATIENTS ARM B

Patients in Arm B will receive 6 cycles of XELOX. Oxaliplatin will be administered in a 130 mg/m^2 dose, Capecitabine in a 1000 mg/m^2 dose. Patients should preferably be randomized within 2 weeks of the definitive diagnosis of CRLM. Patients allocated to Arm B should start neo-adjuvant chemotherapy within 2 weeks after randomization and within 4 weeks after the last CT scan. Treatment evaluation will occur after the 3rd and 6th chemotherapy cycle. In the case of progressive disease (PD) after the 3rd cycle, a resectability check will take place. If patients remained resectable, they will be planned for surgery within 4-6 weeks after completion of the 4th cycle. If patients are assessed to be irresectable, they will go off study protocol.
After the last day of chemotherapy exposure, resection should take place at least 4 weeks, but at maximum 6 weeks later. Treatment evaluation can take place according to local hospital procedures, but should at least consist of a CT scan of the thorax/abdomen and CEA level. Adjuvant chemotherapy after R0 resection is not allowed. Protocol therapy ends following the liver resection.

ENDPOINT

PRIMARY ENDPOINT
Primary endpoint of the study will be OS, calculated from the date of randomization to the date of death of the patient, from any cause. Patients still alive at the date of last contact will be censored.

SECONDARY ENDPOINTS
PFS will be defined from the date of randomization to the first event defined as local/distant recurrence or progression or death from any cause.

CRITERIA OF EVALUATION

Progressive or recurrent disease can be detected by imaging modalities (e.g. CT scan). A rise in serum tumor marker (e.g. CEA) is insufficient. In case of doubt, histological biopsy can provide definitive proof of progression/recurrence. Response to neo-adjuvant chemotherapy will be evaluated by CT scan using RECIST 1.1 criteria [32]. To evaluate the well being of patients the European Organization for Research and Treatment of Cancer Quality of Life

questionnaire (EORTC QoL) will be used. The EORTC QLQ-C30 is generally used to assess QoL of cancer patients; additionally the Multifactorial Fatigue Index (MFI) will be used. Toxicity will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Surgical complications will be defined according to the standard classification of surgical complications [33]. Postoperative mortality will be defined as any death during hospitalization or within 30 days from surgery. Complication and post-operative mortality rates will be securely monitored and documented.

STATISTICAL CONSIDERATIONS

SAMPLE SIZE AND ACCRUAL

On the basis of retrospective data, we expect the hazard ratio (HR) for arm B to be 0.60. For the detection of a HR of 0.60 for the chemotherapy arm and with an expected 5-year OS of 25% in arm A, with two-sided significance level $\alpha = 0.05$ and power $1 - \beta = 0.8$, 126 deaths have to be reported before the final analysis will take place. This number of events is expected to be reached after the recruitment of 224 patients with an average accrual rate of 56 patients per year, and an additional follow up of 2 years. A HR = 0.60 corresponds to an increase of 5-year OS of 43% in arm B.

RANDOMIZATION

Eligible patients should be registered after written informed consent and before start of treatment (based on inclusion/exclusion criteria). Patients will be randomized for surgery versus neo-adjuvant chemotherapy followed by surgery in a 1:1 design. During randomization patients will be stratified by center, CRS score and status of primary tumor (still in situ vs. resected) with a minimization procedure, ensuring balance within each stratum and overall balance.

STATISTICAL ANALYSIS PLAN

The main analysis addressing the primary endpoint is planned after 126 events. No interim analysis is planned.

ETHICS

The study has ethical approval from the Erasmus MC medical-ethical committee. The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

DISCUSSION

Currently, multimodal treatment is not incorporated in the standard of care for primary resectable colorectal liver metastases. To date, no definite evidence exists favoring administration of (neo) adjuvant chemotherapy in CRLM in addition to surgery. Considering the retrospective observations that pre-selection of patients by clinical prognostic characteristics may define a patient population expected to benefit from chemotherapy, CRS stratification provides the base for this randomized controlled trial.

Preceding studies of peri-operative chemotherapy combined with liver surgery often engaged strict study protocol inclusion criteria. Consequently, patients with a high CRS - which might benefit the most from chemotherapy - are often underrepresented in these studies. Possibly, this low impact of chemotherapy on survival could be explained by the *relatively* low risk profile of the patients included in these trials. Recently, two reports on patients with relatively low risk for recurrence have been published. Adam et al. performed an analysis of the LiverMetSurvey database on patients with solitary, metachronous, primarily resectable metastases. These patients have particularly favorable tumor biology and a low CRS. The authors concluded that these patients do not benefit from preoperative chemotherapy [34]. A recent systematic review of the literature by Lehmann et al. concludes that routine use of neo-adjuvant chemotherapy for patients with clearly resectable lesions limited to the liver is not recommended due to a lack of benefit on survival [35].

As mentioned before, several authors have proposed the concept of stratification by CRS with regard to the effects of systemic therapy. Tomlinson et al. demonstrated on actual 10-year survivors of liver surgery for CRLM that patients with a low CRS had a cure rate of 21% and that patients with a high CRS had a cure rate of 10% [27]. They suggest that this finding may be used to identify patients who might benefit from neo-adjuvant chemotherapy [27]. In a large, non-randomized study by Parks et al., adjuvant therapy did seem to improve OS [28]. In this study, patients with a high CRS had more benefit from adjuvant therapy than patients with a low CRS, again suggesting a role for CRS when considering chemotherapy.

These reports have stimulated others and our own unit to retrospectively evaluate data on patients that underwent liver resection for CRLM in the last decade with and without chemotherapy, stratified by CRS according to the Fong-criteria [19]. Rahbari et al. have evaluated the role of adjuvant chemotherapy in a cohort of 316 patients, of whom 43% were high-risk according to the "Memorial Sloan-Kettering Cancer Center CRS" (CRS>2). They found that adjuvant chemotherapy had a profound impact on OS in the high-risk population (HR=0.40), whereas in low-risk patients HR=0.90 [31]. In a recent manuscript by Hirokawa et al. similar results are described with de use of adjuvant chemotherapy [36]. In our population of patients that underwent resection for CRLM in Rotterdam (N=365), we have focused on neo-adjuvant chemotherapy. In this study, a pronounced improvement in OS was found in high-risk patients receiving neo-adjuvant chemotherapy versus no chemotherapy (median 67 months vs. 33 months, HR=0.55 [95% CI 0.35-0.84], p=0.006). This difference was absent

in the low-risk group (median 65 months vs. 56 months, HR=0.89 [95% CI 0.57-1.40], p=0.62) [30]. Notably, these studies were retrospective and non-randomized. The sample size calculation of the present study is based on these retrospective data.

In a recent editorial by Jarnagin et al. it is suggested that future trials should strongly consider stratification by some scoring system [29], given the results of the retrospective studies as mentioned above. Our study will evaluate patients with resectable CRLM without extra hepatic disease and a CRS of 3-5 thereby bearing a poor prognosis. The primary aim of this study is to compare OS rates of patients with resectable liver metastases randomized for treatment with chemotherapy consisting of capecitabine and oxaliplatin (XELOX) followed by surgery, versus surgery alone. We hypothesize that adding neo-adjuvant chemotherapy to surgical resection of CRLM will provide an improvement in OS in patients with a high-risk profile. As secondary objectives we will study PFS, quality of life, treatment response on neoadjuvant chemotherapy, morbidity of surgery and resection rate, and whether CEA can predict for treatment response, PFS, and OS.

LIST OF ABBREVIATIONS

CEA	=	Carcinoembryonic antigen
CRC	=	Colorectal cancer
CRLM	=	Colorectal liver metastases
CRS	=	Clinical risk score
ECOG	=	Eastern cooperative oncology group
OS	=	Overall survival
PFS	=	Progression free survival
RCT	=	Randomized controlled trial
RFA	=	Radiofrequency ablation
XELOX	=	Chemotherapy consisting of capecitabine and oxaliplatin

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

DG, the principal investigator on the CHARISMA trial, is extensively involved with the CHARISMA study concept and design. CV, head of department, sponsor, co-principal investigator, and DG are involved in supervising the study; critically revising the study protocol manuscript. NA, ES, co-investigators on the CHARISMA trial, are involved in drafting and critically revising the study protocol manuscript; provide administrative and technical support. NvdM, trial manager of the CHARISMA trial, was involved in the revision of the protocol. BvdH, trial statistician, was involved in the study design and protocol revision. JW, SR, RH, RR, GV, PT, CP, CD, RJ, HV, KJ, GH, JK, ML, EM, FS, are members of the writing committee. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

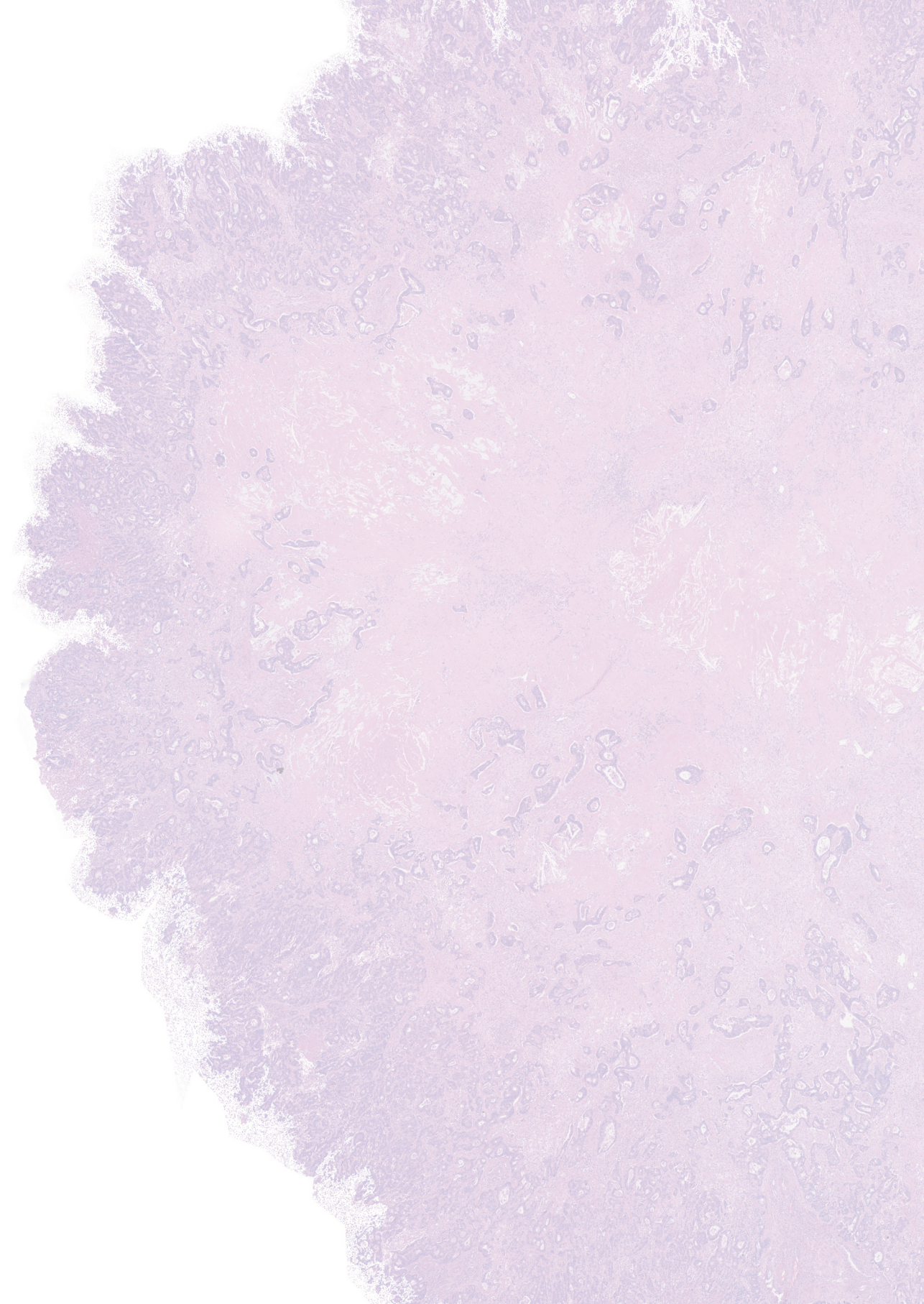
The outline of this trial was developed at the EORTC course Methods in Clinical Cancer Research, Flims, Switzerland, June 2012. This study is funded by the Dutch Cancer Society.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: Global cancer statistics. CA Cancer J Clin 2011, 61(2):69-90.
2. van der Pool AE, Damhuis RA, Ijzermans JN, de Wilt JH, Eggermont AM, Kranse R, Verhoef C: Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series. Colorectal Dis 2010, 14(1):56-61.
3. Bengmark S, Hafstrom L: The natural history of primary and secondary malignant tumors of the liver. I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy. Cancer 1969, 23(1):198-202.
4. van der Pool AE, Lalmahomed ZS, de Wilt JH, Eggermont AM, Ijzermans JM, Verhoef C: Local treatment for recurrent colorectal hepatic metastases after partial hepatectomy. J Gastrointest Surg 2009, 13(5):890-895.
5. Dols LF, Verhoef C, Eskens FA, Schouten O, Nonner J, Hop WC, Mendez Romero A, de Man RA, van der Linden E, Dwarkasing RS et al: [Improvement of 5 year survival rate after liver resection for colorectal metastases between 1984-2006]. Ned Tijdschr Geneeskd 2009, 153(11):490-495.
6. Primrose JN: Surgery for colorectal liver metastases. Br J Cancer 2010, 102(9):1313-1318.
7. 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(1):125-135.
8. van der Pool AE, de Wilt JH, Lalmahomed ZS, Eggermont AM, Ijzermans JN, Verhoef C: Optimizing the outcome of surgery in patients with rectal cancer and synchronous liver metastases. Br J Surg 2010, 97(3):383-390.
9. Petrelli NJ: Perioperative or adjuvant therapy for resectable colorectal hepatic metastases. J Clin Oncol 2008, 26(30):4862-4863.
10. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004, 350(23):2335-2342.
11. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F 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.
12. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pinter T, Lim R, Bodoky G et al: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009, 360(14):1408-1417.
13. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J et al: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol 2010, 28(31):4697-4705.

14. Araujo R, Gonen M, Allen P, Blumgart L, Dematteo R, Fong Y, Kemeny N, Jarnagin W, D'Angelica M: Comparison Between Perioperative and Postoperative Chemotherapy After Potentially Curative Hepatic Resection for Metastatic Colorectal Cancer. Ann Surg Oncol 2013.
15. 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(9617):1007-1016.
16. 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(12):1208-1215.
17. Minagawa M, Yamamoto J, Kosuge T, Matsuyama Y, Miyagawa S, Makuuchi M: Simplified staging system for predicting the prognosis of patients with resectable liver metastasis: development and validation. Arch Surg 2007, 142(3):269-276; discussion 277.
18. 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 Francaise de Chirurgie. Cancer 1996, 77(7):1254-1262.
19. 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.
20. 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(3):291-299.
21. Nagashima I, Takada T, Matsuda K, Adachi M, Nagawa H, Muto T, Okinaga K: A new scoring system to classify patients with colorectal liver metastases: proposal of criteria to select candidates for hepatic resection. J Hepatobiliary Pancreat Surg 2004, 11(2):79-83.
22. Konopke R, Kersting S, Distler M, Dietrich J, Gastmeier J, Heller A, Kulisch E, Saeger HD: Prognostic factors and evaluation of a clinical score for predicting survival after resection of colorectal liver metastases. Liver Int 2009, 29(1):89-102.
23. Schindl M, Wigmore SJ, Currie EJ, Laengle F, Garden OJ: Prognostic scoring in colorectal cancer liver metastases: development and validation. Arch Surg 2005, 140(2):183-189.
24. Lise M, Bacchetti S, Da Pian P, Nitti D, Pilati P: Patterns of recurrence after resection of colorectal liver metastases: prediction by models of outcome analysis. World J Surg 2001, 25(5):638-644.
25. Ueno H, Mochizuki H, Hatsuse K, Hase K, Yamamoto T: Indicators for treatment strategies of colorectal liver metastases. Ann Surg 2000, 231(1):59-66.
26. Mala T, Bohler G, Mathisen O, Bergan A, Soreide O: Hepatic resection for colorectal metastases: can preoperative scoring predict patient outcome? World J Surg 2002, 26(11):1348-1353.
27. 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.

28. Parks R, Gonen M, Kemeny N, Jarnagin W, D'Angelica M, DeMatteo R, Garden OJ, Blumgart LH, Fong Y: Adjuvant chemotherapy improves survival after resection of hepatic colorectal metastases: analysis of data from two continents. J Am Coll Surg 2007, 204(5):753-761; discussion 761-753.
29. Jarnagin WR, D'Angelica M: Systemic Therapy for Patients with Resectable Hepatic Colorectal Metastases: Improving Patient Selection. Ann Surg Oncol 2013.
30. Ayez N, Grünhagen DJ, de Jonge J, Ijzermans J, Eggermont AM, Verhoef C: The Role of Neoadjuvant Chemotherapy in Patients with Resectable Colorectal Liver Metastases. In: SSO (abstr 87). Orlando, Fl, USA; 2011.
31. Rahbari NN, Reissfelder C, Schulze-Bergkamen H, Jager D, Buchler 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.
32. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009, 45(2):228-247.
33. 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.
34. Adam R, Bhangui P, Poston G, Mirza D, Nuzzo G, Barroso E, Ijzermans J, Hubert C, Ruers T, Capussotti L et al: Is perioperative chemotherapy useful for solitary, metachronous, colorectal liver metastases? Ann Surg 2010, 252(5):774-787.
35. Lehmann K, Rickenbacher A, Weber A, Pestalozzi BC, Clavien PA: Chemotherapy before liver resection of colorectal metastases: friend or foe? Ann Surg 2012, 255(2):237-247.
36. 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.



CHAPTER 2

Safety and feasibility of additional tumor debulking to first line palliative chemotherapy for patients with multi-organ metastatic colorectal cancer in the multicenter randomized fase III ORCHESTRA trial

E.P. van der Stok*, E.C. Gootjes*
D.J. Grünhagen
J.W.A. Burger
T.E. Buffart
M.P. Tol
M.R. Meijerink
A.J. ten Tije
E. van Meerten
P.M. van de Ven
J. Nuyttens
C.J. Haasbeek
J.H. de Wilt
H.M.W. Verheul
C. Verhoef

* Both authors contributed equally

Adapted from ASCO abstract: Journal of Clinical Oncology 2016; 34 (04), DOI: 10.1200/jco.2016.34.4_suppl.tps788

ABSTRACT

BACKGROUND

For selected patients with oligometastatic colorectal cancer (mCRC), local treatment of metastases is standard of care based on retrospective reports showing long-term survival rates. Local treatment of metastases is technically feasible in an increasing number of patients with multi-organ mCRC. It is unknown if patients with extensive disease (multi-organ) will benefit from tumor debulking when added to first line palliative chemotherapy. The ORCHESTRA trial (NCT01792934) was designed to prospectively evaluate overall survival (OS) benefit from tumor debulking in patients with multi-organ mCRC.

METHODS

Patients with multi-organ mCRC were eligible if >80% tumor debulking was deemed feasible by resection, radiotherapy and/or thermal ablative therapy. All patients received oxaliplatin based chemotherapy ± bevacizumab. In case of stable disease or response at first evaluation (9 weeks), patients were randomized to continuation of chemotherapy or tumor debulking followed by chemotherapy. If patient withdrawal after randomization was <10%, the study was deemed feasible. Study continuation was based on the interim report on safety and feasibility after inclusion of 100 (of 478) patients.

RESULTS

Patients were randomized to the standard (N = 43) or intervention arm (N = 45). No patients withdrew after randomization. In 13.3% of patients debulking was not performed due to progressive disease (N = 5) or death (N = 1) prior to local treatment. Two patients had no lesions left to treat, 37 patients underwent tumor debulking. In 15 locally treated patients (40%) 21 serious adverse events related to debulking were reported. Postoperative 90-day mortality was 2.7% (N = 1). Chemotherapy was resumed in 89% of patients, median chemotherapy-free interval was 12.5 weeks (6-34) and 79% completed (the equivalent of) 8 cycles CAP(OX).

CONCLUSIONS

Tumor debulking is feasible and safe and does not prohibit administration of palliative chemotherapy in the majority of patients with multi-organ mCRC. The ORCHESTRA trial will continue accrual to determine whether tumor debulking will lead to > 6 months OS benefit while maintaining quality of life.

BACKGROUND

In the current multidisciplinary approach of stage IV metastatic colorectal cancer (mCRC), local treatment of oligometastases is increasingly performed. Large series of selected patients with liver-only metastases treated with complete surgical resection suggest that this approach improves 5-year survival rates to around 30-60%, and offer the only potential for cure [1-4]. Application of techniques such as radiofrequency or microwave ablation (RFA, MWA) and stereotactic ablative radiotherapy (SABR) potentially increases feasibility of local treatment of metastases.

For selected patients with oligometastatic colorectal cancer (mCRC), local treatment of metastases is standard of care based on retrospective reports showing long-term survival rates [5, 6]. Treatment options with curative intent are generally not available for patients with extensive hepatic and/or extrahepatic mCRC. These patients primarily receive palliative systemic treatment consisting of combination chemotherapy with agents targeting VEGF or EGFR. So far, reports on the benefit of local treatment for multi-organ metastases of CRC have major limitations, including being small, non-randomized, single-center and retrospective. It is unknown if patients with extensive disease will benefit from tumor debulking when added to first line palliative systemic therapy. In ovarian cancer, irradical but maximal resection of tumor lesions induces an overall survival benefit [7]. As a consequence, some clinicians suggest that maximal tumor debulking for metastatic disease may also benefit colorectal cancer patients [8], although there exists no evidence for such management. The benefit from local treatment of multi-organ metastases for these patients should be evaluated prospectively. Published retrospective reports were hampered by selection bias [5, 6]. The current manuscript reports the safety and feasibility of the ORCHESTRA trial (NCT01792934), a multicenter randomized trial, designed to prospectively evaluate overall survival (OS) benefit from tumor debulking by resection, radiotherapy and/or thermal ablative therapy in patients with multi-organ mCRC based on the first 100 included patients. The ORCHESTRA trial incorporates both systemic and local therapy in the experimental arm and combines local treatment modalities to pursue maximal tumor debulking. The trial aims to improve overall survival of patients with multi-organ mCRC by maximal tumor debulking after induction chemotherapy by at least six months.

The current report focused on feasibility of patient accrual, randomization, withdrawal after randomization, safety of the local treatment procedures in this patient population and the ability to administer adequate palliative systemic treatment in the intervention arm, being the current evidence based treatment regimen [9].

METHODS

The 'ORCHESTRA' trial is a randomized multicenter clinical trial for patients with multi-organ mCRC, comparing the combination of chemotherapy and maximal tumor debulking versus chemotherapy alone.

Written informed consent was obtained from all patients included. Patients were 18 years or older and had an indication for first line palliative systemic therapy for mCRC. They all had an ECOG performance status of 0-2 and adequate bone marrow, liver and renal function.

Patients with extensive multi-organ mCRC were eligible, as specified in table 1 below. Tumor debulking by a combination of resection, radiotherapy or thermal ablative therapy was deemed feasible by a multidisciplinary team, including a specialist in surgical oncology, radiotherapy, radiology and medical oncology.

Table 1: Main eligibility criteria

ORCHESTRA Eligible patients
Patients with CRC metastases in ≥ 2 different organs if at least
>1 extrahepatic metastases or
>5 hepatic metastases not located to one lobe or
either a positive para-aortal lymph nodes or celiac lymph nodes or adrenal
metastases or pleural carcinomatosis or peritoneal carcinomatosis
NB The primary tumor is excluded as metastatic site
Feasible radical tumor debulking. Incomplete tumor debulking is allowed only if at least 80% of metastases can be treated.

Patients who underwent prior local treatment were not excluded. Prior (adjuvant) systemic therapy should have been completed more than 6 months at diagnosis of extrahepatic metastatic disease. Comprehensive in and exclusion criteria are available at clinicaltrials.gov. Clinical data on medical history, tumor characteristics and treatment were entered in a web based electronic case record form, prospectively.

All patients received 5-FU/oxaliplatin based systemic therapy ± bevacizumab at physician discretion. Systemic therapy consisted of orally administered capecitabine 1000 mg/m^2 twice a day for two weeks and oxaliplatin 130 mg/m^2 intravenous (CAPOX) on day 1 in a 3-week cycle or comparable intravenous regimen consisting of oxaliplatin 85 mg/m^2 on day 1 and 400 mg/ m^2 LV followed by 400 mg/m2 5-FU bolus and 2400 mg/m^2 continuous infusion over 46 hours (modified FOLFOX6) of each 2-week cycle. Bevacizumab was added at physician discretion as intravenous infusion over 30-90 minutes on day 1 (in CAPOX regimen a 7.5 mg/kg 3-weekly, in FOLFOX regimen biweekly 5 mg/kg). The FOLFOX regimen could be combined with biweekly bevacizumab, 5 mg/kg). First evaluation of response was scheduled after 3 cycles of CAPOX(B) or 4 cycles of FOLFOX(B) by CT scan of thorax and abdomen. Response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST

1.1) [10]. Follow up scans were done at least every 3 months.

In case of stable disease or response, patients were randomized to continuation of systemic therapy (standard arm A) or tumor debulking (intervention arm B) followed by systemic therapy.

Patients who were randomized in the intervention arm, with stable disease at evaluation, first continued systemic therapy (3 x CAPOX or 4 x FOLFOX ± bevacizumab) followed by debulking if disease remained stable. Bevacizumab was omitted the chemotherapy cycle prior to tumor debulking. A definitive local treatment plan was determined by a multidisciplinary team based on the most recent evaluation scan. Patients were referred to a hospital with adequate local treatment expertise as appropriate.

Adverse events (AE) were documented according to Common Terminology Criteria for Adverse Events (CTCAE version 4.03) and documented to be related to systemic therapy, local therapy or not related. If related to systemic therapy only AE's > grade 2 were entered in the eCRF. All AE's related to local treatment were documented and furthermore graded according to Clavien Dindo [11]. Serious Adverse Events (SAE) are adverse events that resulted in death, were life-threatening, required inpatient hospitalization or caused prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity, or required intervention to prevent permanent impairment or damage. SAE's were reported to the competent authority within 7 days. Safety reports were drawn up and evaluated by an independent Data Safety Monitoring Board after inclusion of 25, 50 and 100 patients. Study continuation was based on the interim report on safety and feasibility after inclusion of 100 (of 478) patients.

A total of 478 patients are anticipated to be included to randomize 382 patient and meet the primary endpoint of an overall survival benefit of > 6 months (power 80%, type I error rate 5%). The study was deemed feasible if less than 10% of patients would withdraw from the study after randomization, after 20% of patients have been randomized (N=76).

The primary endpoint of the current randomized trial is overall survival counting from the date of study inclusion to the date of death. Secondary endpoints include progression free survival and quality of life, as well as evaluation of potential biomarkers such as CEA, MiRNA, (phospho)proteomics, Platelet derived RNA and genetic profiles.

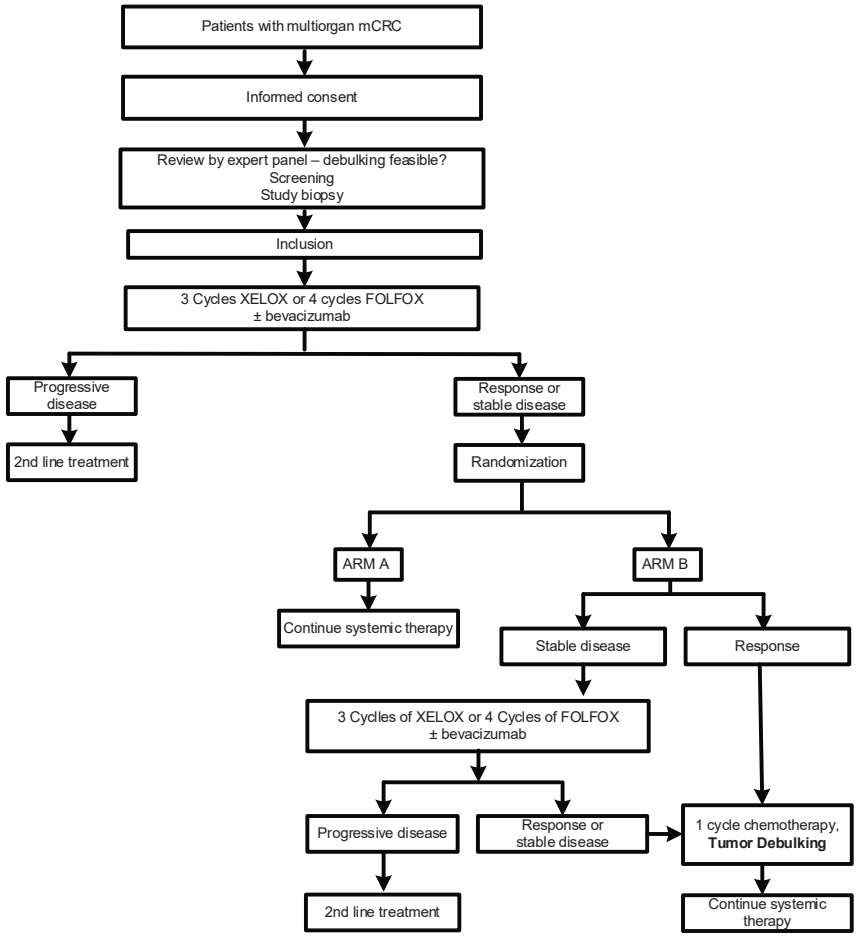


Figure 1. Study protocol flow chart

RESULTS

Between May 2013 and May 2015 the first 100 patients were included in 16 of 32 participating Dutch hospitals that are part of the Dutch colorectal cancer group (DCCG). During the first year, 6 centers were open and they included 5 patients, increasing to 30 centers in 2016 including 60 patients in one year. Currently over 200 patients are included (figure 2).

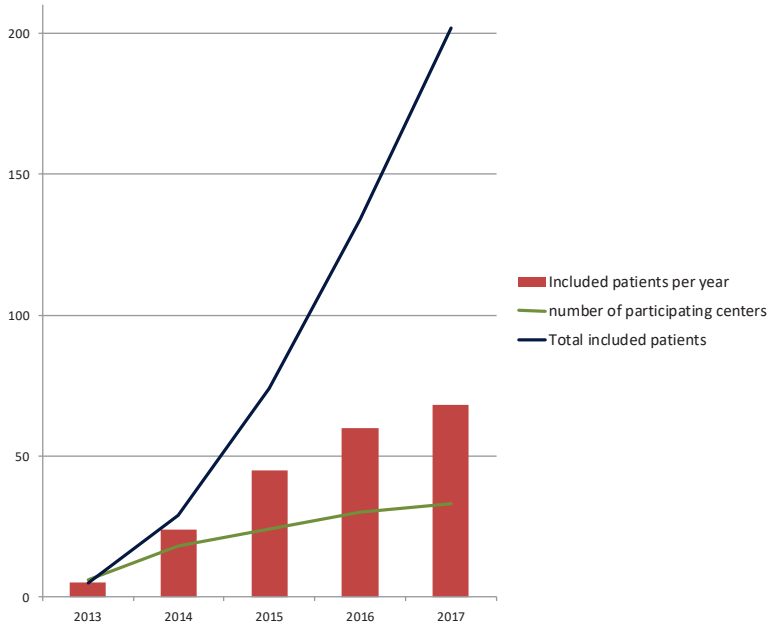


Figure 2. Included patients

Patients had a median age of 65 (range 30-78) years and 67% were male. Seventy-one percent had a primary tumor (either resected or in situ) located on the left side and approximately two-third of the population presented with synchronous metastatic disease. In 72 patients the primary tumor was previously resected and 34 patients also had prior local treatment of metastases.
In 35 patients more than 2 organs were involved in metastatic disease (up to 5 organs involved). Patients had a median of 6 metastatic lesions ranging from 2 to 18 lesions or diffuse disease. Twenty-six patients had less than 5 lesions, 43 patients had 5-10 lesions, and 31 patients had more than 10 or diffuse (peritoneal) disease. There were no significant differences in baseline characteristics between arm A and arm B prior to start of chemotherapy including baseline CEA and LDH values (see table 2). Liver metastases were present in 81 patients, 50 patients had lung metastasis, and 57 had distant lymph node metastases (from which 34 were located at poor prognostic site) [5]. Peritoneal disease was present in 33 patients, and respectively 7, 5 and 3 patients had bone, adrenal gland or skin/subcutaneous metastases.
All but one patients were treated with CAPOX (± bevacizumab (62%)), one patient was treated with FOLFOX, 3 more patients switched to FOLFOX during the course of the study due to capecitabine toxicity.

Table 2: Baseline characteristics

Parameter	Total (N = 100)	Arm A (%) (N = 43)	Arm B (%) (N = 45)	P value
Gender - Male	67	29 (67%)	31 (69%)	0.88
Age <65	51	25 (58%)	21 (47%)	0.28
Synchronous metastases	63	30 (70%)	26 (58%)	0.24
Left sided primary tumor	71	26 (60%)	35 (78)	0.08
Primary in situ	28	29 (67%)	33 (73)	0.55
Number of metastases				0.89
<5	26	11 (26%)	10 (22)	
5-10	43	18 (42%)	21 (47)	
>10 or diffuse	31	14 (33%)	14 (31)	
Number of organs involved				0.35
2	65	29 (67%)	26 (58%)	
>2	35	14	19	
CEA >5 ug/l	78	31 (79%)	37 (84%)	0.18
LDH normal level	77	37 (86%)	34 (65%)	0.28
Prior tumor treatments				
Prior (neo-) adjuvant chemo	19	34 (79%)	35 (78%)	0.88
Prior chemoradiation	14	4 (9%)	8 (17%)	0.25
Previous local treatment	34	26 (60%)	33 (73%)	0.20
Systemic therapy				
Chemotherapy				0.30
CAPOX	97	42 (98%)	45 (100%)	
FOLFOX	1	1	0	
Bevacizumab	62	25 (58%)	30 (67%)	0.41
Completed equivalent of 8 cycles CAP(OX)		30 (70%)	29 (64%)	0.65
Number of cycles CAPOX		11 (median) range 3-36	9 (median) range 4-31	0.42
Response at first evaluation				0.59
CR	1	0 (0%)	1 (2%)	
PR	41	21 (48%)	20 (44%)	
SD	46	22 (51%)	24 (53%)	
PD	3	-	-	

Prior to randomization, two patients went off study due to toxicity of systemic treatment, two patients died and five patients withdrew consent prior to starting or during the first cycles systemic therapy (no reason specified). Three patients had progressive disease and were not randomized per protocol. Eighty-eight patients were randomized to the standard (N=43) or intervention arm (N=45). No one withdrew after randomization (see figure 3).

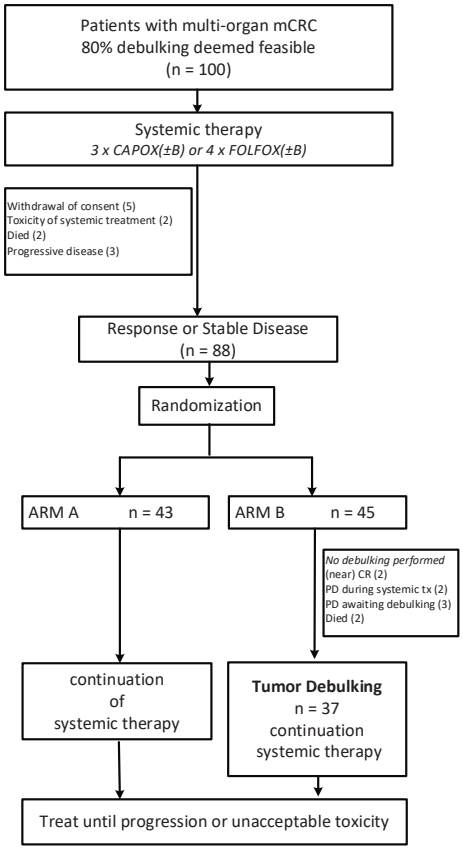


Figure 3. Simplified flowchart/consort diagram

ARM B - DEBULKING

In 13.3% of 45 patients randomized for local treatment, debulking was not performed due to progressive disease (N=5) or death (N=1) prior to local treatment (figure 2). Two patients, who had stable disease at randomization, progressed during the following courses of systemic therapy and debulking was not performed as per protocol. Two other patients showed progressive disease awaiting local treatment (one patient with a newly diagnosed brain metastasis, one patient with progressive liver metastases rendering debulking unfeasible). In one patient extensive peritoneal disease became apparent per-operatively, for which debulking was not feasibly, and the operation was terminated without treatment of any metastases. One patient died in a motor vehicle accident just prior to local treatment. On imaging prior to debulking, two patients had near complete response, with lesions too small to treat after systemic therapy.

In 37 of 45 allocated patients, debulking was performed. In 38% of patients debulking was performed with one single modality, the other patients required combined modalities. Eleven percent was treated by three modalities (surgery, RFA and radiotherapy). Four patients

required a second elective admission for second stage debulking. In 31 patients, debulking of >80 % of metastatic lesions was realized. These results are summarized in table 3.

Table 3: Debulking

Arm B	**N = 45**
ASA classification	Median 2 (range 1-3)
Debulking performed	N = 37 (82%)
More than 80% treated	N = 31 (69%)
Number of modalities	Median 2 (range 1-3) 1 N = 14 2 N = 19 3 N = 4
Total in-hospital local treatment days	Median 14 (range 1-50)
Systemic therapy resumed after local treatment	N = 33 (89%)
≥8 cycles capox or 12 folfox administered	N = 26 (78.8%)
Chemotherapy free interval in weeks	Median 12.5 (range 6-34)

ARM B – TREATMENT IMPACT

The total duration of hospital admission in days was median 9 days (range 1-50). This included elective hospital admission, unplanned readmissions (in 7 patients), radiotherapy sessions and/or percutaneous RFA sessions. In 7 (16%) patients a colostomy was created as part of the debulking procedure. In 5 patients with liver metastases hemihepatectomy was needed as part of debulking. Radiotherapy was administered in an outpatient setting. Patients had a median of 6 radiotherapy sessions (range 2-27).

A total of 77 severe adverse events (SAE's) were reported in all 100 patients, from which 32 occurred prior to randomization, 17 in arm A and 28 in arm B. In arm A, 6 SAE's were related to systemic therapy and 11 were not related to treatment. Of the SAE's in arm B, 1 was related to systemic therapy, and 21 were related to local treatment (in 15 patients = 40%, see tables 5A). The SAE's included infections, hepatobiliary and respiratory disorders and 16% did not resolve in 30 days (N=6).

In the intervention arm, 32 adverse events (AE's) related to debulking were reported (in 19 patients). Eleven of these AE's were ≥ grade 3 according to Clavien Dindo (see table 5B). Postoperative 90-day mortality was 2.7% (N = 1; hepatic failure).

Table 5A: Serious adverse events related to tumor debulking procedures	
Serious adverse events (According to CTCAE 4.03)	**N = 21 (%)**
Cardiac disorders	1 (5%)
Gastrointestinal disorders	3 (14.3)
Hepatobiliary disorders	5 (23.8)
Infections and infestations	5 (23.8)
Procedural complications	2 (9.5)
Renal and urinary disorders	1 (4.8)
Respiratory, thoracic and mediastinal disorders	4 (19)

Table 5B: Adverse events of debulking procedures ≥ grade 3 according to Clavien Dindo	
Clavien Dindo Grade	**Complication**
Grade 3	Presacral abscess
	Urinary anastomotic leak
	Wound aces
	Pleural effusion
	Colonic perforation
	Abdominal sepsis
	Biliary anastomotic leak/duct leak (3x)
Grade 4	Ileus
Grade 5	Liver failure

Chemotherapy could be resumed in 89% of patients in arm B. The four patients who did not resume chemotherapy all had stable disease at randomization and therefore completed 7 cycles of CAPOX prior to debulking (see figure 1). One patient could not restart due to complications of debulking, and one due to progressive disease. In the other 2 patients, the treating physician did not restart because the patients had no evaluable disease left and no symptoms to palliate after debulking had taken place. Altogether, 83% of patients who underwent debulking completed (the equivalent of) 8 cycles of CAP(OX). In general, 70% of patients in arm A and 64% of patients in arm B (p = 0.65) completed the equivalent of 8 cycles of CAP(OX), and there was no difference in median number of cycles administered between study arms (see table 1). The median chemotherapy-free interval was 12.5 weeks (range 6-34) after completion of the last pre-local treatment cycle of systemic therapy. The chemotherapy-free interval between the last debulking event and restart was median 5 weeks (range 1-24).

DISCUSSION

The current report demonstrated that it is feasible and safe to prospectively include and randomize patients with metastasized colorectal cancer (mCRC) for palliative systemic treatment and extensive tumor debulking in addition to palliative systemic treatment. Setting up an oncological trial of this kind and enrolling patients in 32 different hospitals in the Netherlands is a major challenge. Data in this manuscript show that at initiation patient inclusion was characterized by a "start up phase", with low patient accrual. This probably reflects initial unfamiliarity with the logistic implications of the trial protocol and the challenge of getting multidisciplinary consensus on feasibility of tumor debulking. Obtaining study approval in participating hospitals requires different procedures within each individual institute in the Netherlands [12], also causing a delay in inclusion. Commitment and close collaboration of specialists in multidisciplinary teams was needed in all participating centers. As shown, the ORCHESTRA protocol is currently implemented in 32 general and academic hospitals. If the ORCHESTRA trial has clinical implications in the future, the current study objectified its feasibility in various centers.

Although some patients withdrew from study participation, no one withdrew after randomization due to potential dissatisfaction with the treatment arm they were randomized to. Three patients developed disease progression in the interval between chemotherapy and resection (6.7%), and were excluded from local treatment. The incidence was lower in our study as compared to scarce reports in literature (6.7% versus 25% respectively) [13].

It remains challenging to plan all needed local treatment modalities in such a way that patients have sufficient time to recover without delaying systemic treatment. To prevent tumor progression and poor oncological outcome we aimed for a short chemotherapy-free interval. The interval reported in the current study was 12.5 weeks after the last cycle, and 5 weeks after last debulking modality. Literature on the impact of prolonged chemotherapy-free intervals on overall survival is scarce [14]. For patients undergoing two-stage hepatectomy for colorectal liver metastases, the interval between pre- and postoperative chemotherapy was median 18.7 weeks [14]. The interval between surgery and resuming chemotherapy within 10 weeks seemed to have positive predictive value on survival. In comparison, the current protocol seems safe.

Although the chemotherapy-free interval was 12.5 weeks in the intervention arm, there was no significant difference in the total amount of cycles of systemic therapy administered between the study arms. A comparable proportion of patients completed at least 8 cycles of capox(±B) (or the equivalent in FOLFOX) in both treatment arms.

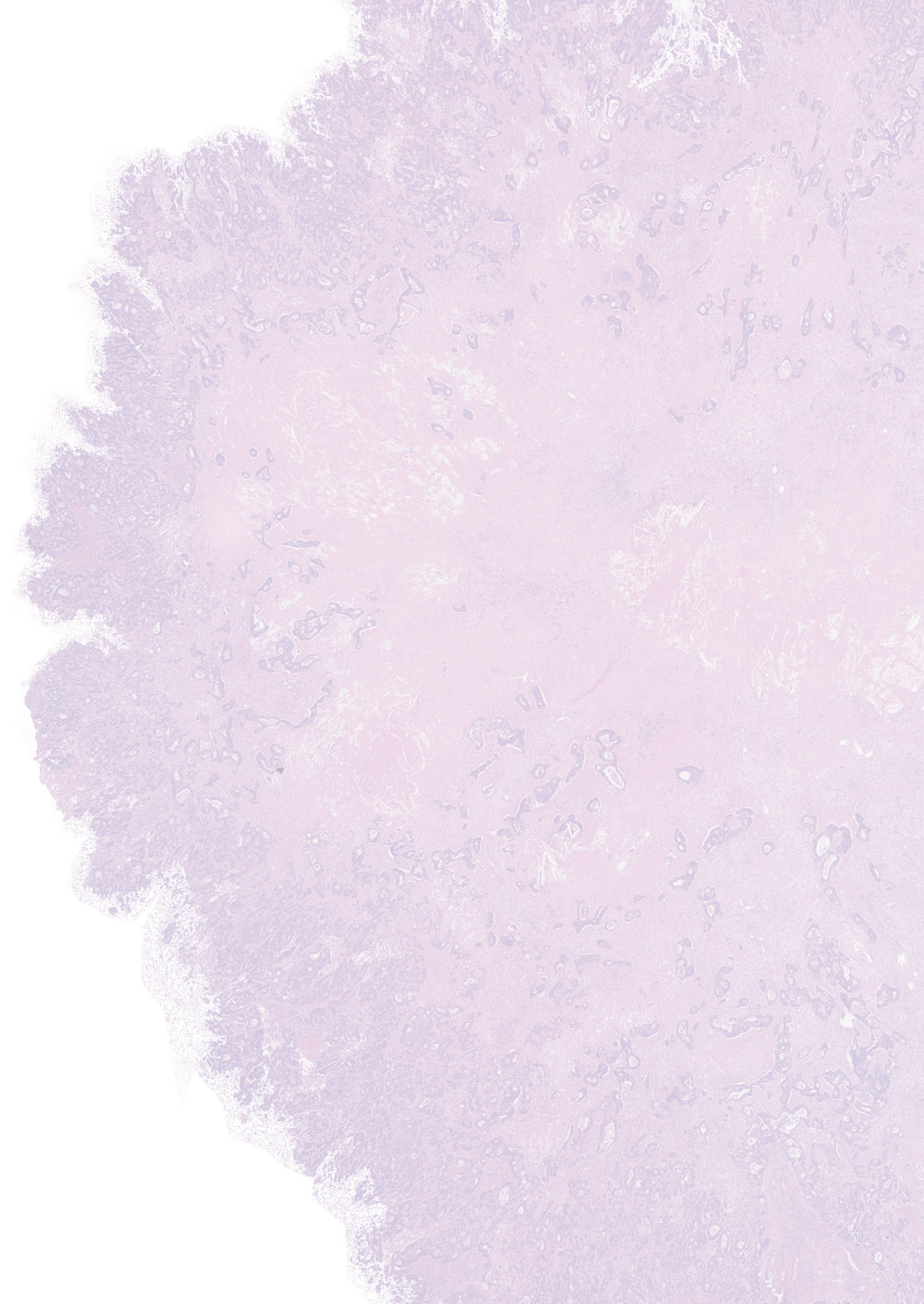
Another important factor in terms of safety and feasibility is the actual process of tumor debulking. This is an intensive process, with important treatment burden for the patients involved. Inevitably, serious adverse events occurred, with hospitalization up to 50 days for an individual patient. The morbidity and mortality of local treatments performed (2.7%) compares to surgical literature [15]. Patients randomized in arm B that underwent debulking

after 7 cycles of systemic therapy, due to stable disease at first evaluation did not have more SAE's related to the procedures despite having had more chemotherapy.

The current data show that tumor debulking is feasible and safe and does not prohibit administration of palliative systemic therapy in the majority of patients with mCRC. This study addresses a topical issue in everyday practice of multidisciplinary colorectal cancer care with a study design compatible with current treatment options, enabling the results to be readily implemented in treatment practice. Besides the reported adverse events, collected data on quality of life will then contribute largely to the decision whether it is worthwhile to offer this treatment strategy to patients with multi organ metastatic CRC. The ORCHESTRA trial will continue accrual to determine whether the primary aim of >6 months overall survival benefit of additional tumor debulking will be met.

REFERENCES

1. Kanas, G.P., et al., *Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors.* Clin Epidemiol, 2012. **4**: p. 283-301.
2. Pfannschmidt, J., H. Dienemann, and H. Hoffmann, *Surgical resection of pulmonary metastases from colorectal cancer: a systematic review of published series.* Ann Thorac Surg, 2007. **84**(1): p. 324-38.
3. Rees, M., et al., *Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients.* Ann Surg, 2008. **247**(1): p. 125-35.
4. Warwick, R. and R. Page, *Resection of pulmonary metastases from colorectal carcinoma*. Eur J Surg Oncol, 2007. **33 Suppl 2**: p. S59-63.
5. Chua, T.C., et al., *Hepatectomy and resection of concomitant extrahepatic disease for colorectal liver metastases--a systematic review.* Eur J Cancer, 2012. **48**(12): p. 1757-65.
6. Leung, U., et al., *Colorectal Cancer Liver Metastases and Concurrent Extrahepatic Disease Treated With Resection*. Ann Surg, 2016.
7. Tangjitgamol, S., et al., *Management of endometrial cancer in Asia: consensus statement from the Asian Oncology Summit 2009*. Lancet Oncol, 2009. **10**(11): p. 1119-27.
8. Primrose, J., T. Treasure, and F. Fiorentino, *Lung metastasectomy in colorectal cancer: is this surgery effective in prolonging life?* Respirology, 2010. **15**(5): p. 742-6.
9. Loupakis, F., et al., *Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer.* N Engl J Med, 2014. **371**(17): p. 1609-18.
10. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)*. Eur J Cancer, 2009. **45**(2): p. 228-47.
11. Dindo, D., N. Demartines, and P.A. Clavien, *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): p. 205-13.
12. van der Stok, E.P., et al., *[Local approval procedures act as a brake on RCTs]*. Ned Tijdschr Geneeskd, 2016. **160**(0): p. D148.
13. Vigano, L., et al., *Progression of Colorectal Liver Metastases from the End of Chemotherapy to Resection: A New Contraindication to Surgery?* Ann Surg Oncol, 2018. **25**(6): p. 1676-1685.
14. Kambakamba, P., et al., *Short Chemotherapy-Free Interval Improves Oncological Outcome in Patients Undergoing Two-Stage Hepatectomy for Colorectal Liver Metastases*. Ann Surg Oncol, 2016. **23**(12): p. 3915-3923.
15. Kingham, T.P., et al., *Hepatic parenchymal preservation surgery: decreasing morbidity and mortality rates in 4,152 resections for malignancy.* J Am Coll Surg, 2015. **220**(4): p. 471-9.



CHAPTER 3

Local approval procedures act as a brake on RCTs

E.P. van der Stok*, J. Huiskens*
B. Hemmes
D.J. Grünhagen
T.M. van Gulik
C.J.A. Punt
C. Verhoef

* Both authors contributed equally

Translated and adapted from: Nederlands Tijdschrift voor Geneeskunde 2016;160(0):D148

ABSTRACT

BACKGROUND

Large multicentre randomised controlled trials (RCTs) in the Netherlands are increasingly being impeded by major differences between local approval procedures. However, no national agenda exists as yet to improve this situation. The existence of major local differences in processing time and documentation required has been reported previously but little is known about the costs incurred and whether or not specific certifications and research contracts are mandatory. The current study evaluated these aspects of local procedures for obtaining approval of two oncological multicentre RCTs.

METHODS

All local procedures for obtaining approval of two randomised clinical trials were evaluated: the CAIRO5 and CHARISMA trials initiated by the Dutch Colorectal Cancer Group (DCCG). We objectified time between approval by the Medical Ethics Review Committee (METC) and final approval by the Board of Directors (RvB), the type and number of documents needed, and costs charged.

RESULTS

The median time interval between the approval by the central Medical Ethics Committee for participation of a local centre and the final approval for local feasibility by the Board of Directors of a participating centre was 90 days (range 4-312). The number of documents required for the procedure per centre ranged from 6-20. The charged costs by participating centres for the procedure ranged from €0-€1750, and amounted to €8575 for all procedures combined. The majority of the centres charged no costs.

CONCLUSION

The procedures for obtaining approval for local feasibility of participating centres in multicentre clinical trials in the Netherlands demonstrate a large variety in duration, content and costs. This is an obstacle for conducting clinical research efficiently and cost-effectively. Uniform regulations are urgently needed.

INTRODUCTION

Prospective randomised trials are considered the best instrument to test the effectiveness of medical interventions and are therefore at the core of 'evidence-based' healthcare. Novel treatment modalities are currently emerging at increased frequency, which results in a great demand for these types of trials [1]. Randomised trials typically involve a large number of patients, and therefore the participation of multiple centres. In 2014, 759 multicentre studies were assessed by a medical ethical committee (MEC) in The Netherlands, of which 43% were investigator-initiated studies [2]. The initiation and conduct of these multicentre studies require a significant investment of time and financial investment. This is an obstacle for investigator-initiated research, which unlike pharmaceutical industry-driven research typically has no structural financial and staff support.

Central medical ethical approval and subsequent local approval of the participating centres for feasibility are required before a trial can be initiated. The increasing complexity and diversity of the procedure to obtain approval for local feasibility in participating centres causes delay and increases costs of initiating trials [3-8]. This hampers the conduct of clinical research in The Netherlands [2, 9]. However, objective, raw data regarding these issues are scarce [3, 4, 6].

The so-called 'Richtlijn Externe Toetsing' (RET; Guideline for External Assessment) is the Dutch guideline in which the protocol is described for the procedure to obtain approval for local feasibility by a local centre. The guideline is prepared by the Central Committee on Research Involving Human Subjects of the Netherlands (CCMO) [10]. The scope of this directive is limited in terms of its absolute, mandatory influence on local levels (individual research institutions). The RET is endorsed by the Boards of Directors of University Medical Centres (UMC) and the Cooperating Top Clinical Medical Teaching Hospitals (Samenwerkende Topklinische opleidingsZiekenhuizen, STZ). These hospitals have expressed their endorsement of the RET by signing the so called 'Institution Statement' [11, 12]. The RET and the Institution Statement contain the following mandatory aspects: 1) to provide a signed "research statement" by the local researchers, on behalf of supporting facilities in a participating centre for assessment by the central Medical Ethical Committee (MEC) [13]; 2) an insurance for subjects of each participating centre; and 3) substantive scientific/medical ethical assessment of the study protocol and patient information leaflet may only be done by the central MEC and not by individual participating centres. Other aspects of the local procedures at the level of a participating centre such as the duration, charges and content of the process are not regulated by the CCMO (and the CCMO is not allowed to do so). The Institution Statement only stipulates in general that local centres should have efficiently organized their applications for participation in multicentre trials [11].

The Netherlands ranks internationally among the countries with the highest scientific output, in terms of volume and quality [14, 15]. One of the most important parameters that determine the success rate of clinical research is the speed by which logistic procedures that are required

for study initiation are completed. This is especially true for pharmaceutical industry-initiated clinical research, in which pharmaceutical companies monitor the procedural and financial parameters of individual countries and centres [9]. In such reports the procedure required for obtaining approval for local feasibility is mentioned as a cause of declining attractiveness at an international level to carry out multicentre clinical trials in The Netherlands [9]. The feasibility of multicentre research can be facilitated by standardization of this procedure. Data from The Netherlands and other countries specifically indicate a delay caused by the local feasibility procedures, confirming that this procedure is complex and difficult to optimize in terms of efficiency and costs [14, 16-24].
In the current study we have evaluated the procedure for obtaining approval for local feasibility of the participating centres of two investigator-initiated, multicentre randomised trials of the Dutch Colorectal Cancer Group (DCCG): CHARISMA and CAIRO5 [25, 26]. The purpose of our evaluation is to provide real-life data on this issue and thereby to contribute to the development of a more efficient, less costly and uniform procedure.

METHODS

The CHARISMA and CAIRO5 trial evaluate treatment strategies for patients with colorectal liver metastases [25, 26]. The CHARISMA trial was reviewed by the MEC of the Erasmus Medical Centre, Rotterdam, The Netherlands, and the CAIRO5 trial by the MEC of the Academic Medical Centre, Amsterdam, The Netherlands.

THE PROCEDURE

Figure 1 provides an overview of the full procedure for medical ethical approval and approval for local feasibility in participating centres. The time period that was involved in obtaining local approval in participating centres was evaluated based on four periods:

A. The time period starting from the date of central medical ethical approval of the MEC to include a new participating centre in the trial up to the date at which official approval was obtained (local feasibility) from the local board of directors of each participating centre.
B. The time period between the date of central approval of the MEC to include a participating centre in the trial and the date of submission of the complete file to the board of directors of each participating centre.
C. The time period between the date of submission of the complete file to the board of directors of each participating centre and the date of local approval to start the trial.
D. The time period between the date of approval of the board of directors of each participating centre and the date of written confirmation by the coordinating research team that the trial was open for inclusion.

The procedure for obtaining local approval in each centre was also evaluated according to the following:

- ☐ The number and type of documents required for the local procedures that were requested per centre. We specifically kept track of the obligation to deliver a Clinical Trial Agreement (CTA) and a "Good Clinical Practice (GCP)" certificate or "Basic Rules and Organization for Clinical researchers (BROK)" from the local principal investigator;
- ☐ Any fees charged by the local MEC/trial bureau for the procedure of obtaining local feasibility approval.

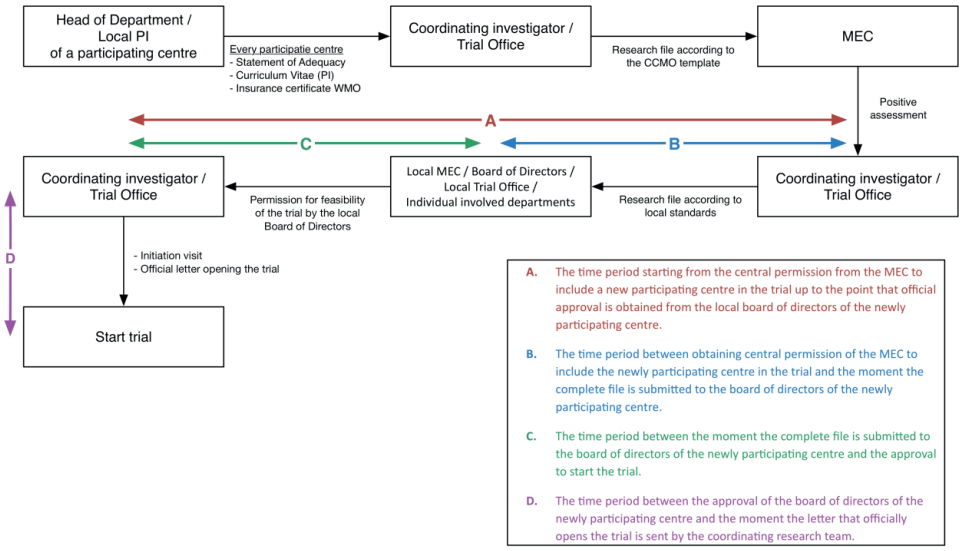


Figure 1: Overview of the procedure for obtaining approval to start a multicentre clinical trial in a participating hospital

RESULTS

At the time of this analysis the CHARISMA trial was open in 9 centres and the CAIRO5 trial in 19 centres.

TIME PERIODS

The median and range of time periods A, B, C and D are displayed in table 1. For primary medical ethical review, an initial maximum of 60 weeks is allowed for the Medical Ethical Committee to assess a study protocol [27]. Therefore, a 60-day time period was chosen as a reference in our analyses. The median time to obtain approval of local feasibility from central medical ethical approval (period A) was 90 days, with a range from 4-312 days. Time period A took more than 60 days in 68% of all procedures. Figure 2 displays the variation in time period A between the

CHARISMA and CAIRO5 trials in centres that were open for both trials. Time period B had the most impact on total duration of approval (time period A). This period took more than 60 days in 46% of all procedures. Time period C took more than 60 days in only 21% of procedures.

Table 1: The median and range of time periods A, B, C and D (days) of all procedures in both trials and of CAIRO5 and CHARISMA trial, respectively.

Time period	Days	
	Median	Range
A:		
All procedures	90	4-312
CAIRO5	136	4-312
CHARISMA	63	32-217
B:		
All procedures	64	2-308
CAIRO5	91	2-308
CHARISMA	60	15-116
C:		
All procedures	21	3-178
CAIRO5	21,5	3-178
CHARISMA	17	3-315
D:		
All procedures	68	3-351
CAIRO5	113	3-351
CHARISMA	41	9-78

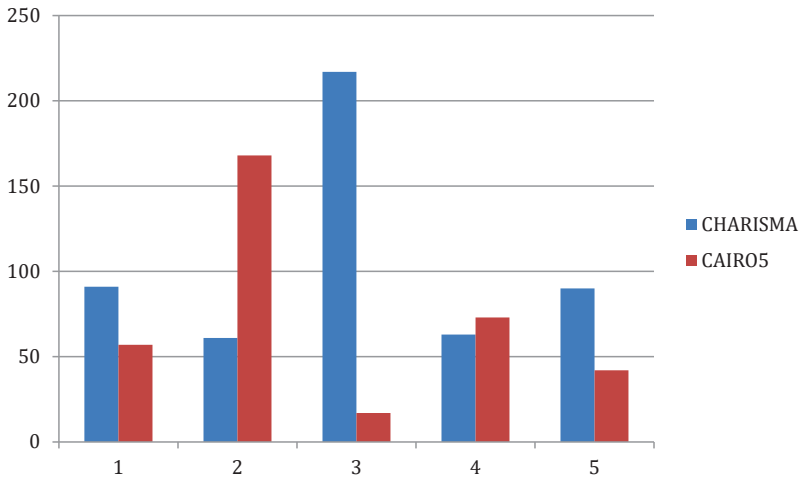


Figure 2: The variation in time period A in 5 centres open for both the CHARISMA and CAIRO5 trial. X-axis: centres, Y-axis: time period in days.

DOCUMENTS

The median number of documents that were requested per centre for the procedure was 10 (range 6-20) and did not differ between CHARISMA and CAIRO5 (range 7-16 and 6-20, respectively).

A CTA was mandatory in 69% of the centres, and 65% of the centres requested a certificate of good clinical practice (GCP). Notification in the curriculum vitae of the local investigator that the GCP certificate was obtained was often considered as sufficient.

In case the local committee demanded that any standard file had to be adjusted it predominantly concerned the CTA and/or the patient information leaflet. The type of modifications of CTA that were requested differed greatly among centres. Also, local centres often required additional documents, such as an estimate of radiological diagnostics or laboratory tests that may or may not take place in the context of the trial. Documents that are required by supportive departments could cause delay of the procedure. Figure 3 displays the different types of documents that were involved in all procedures.

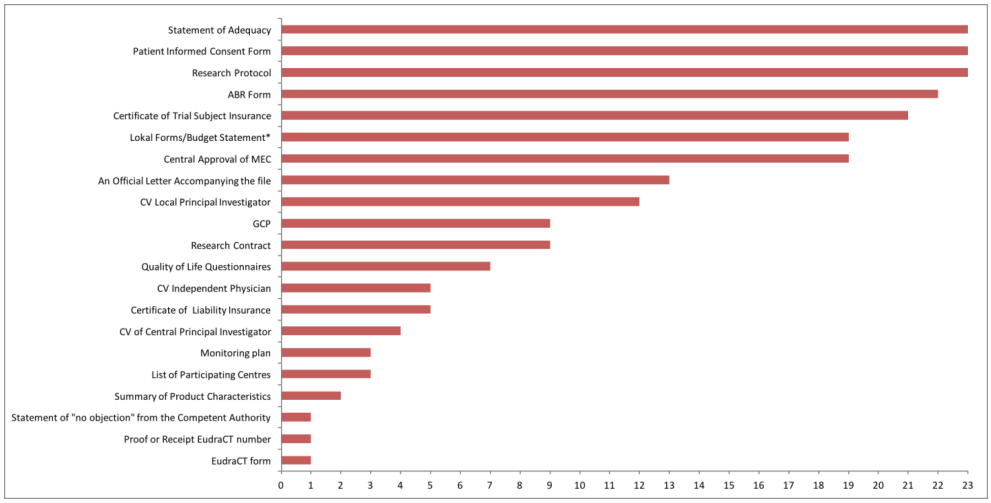


Figure 3: Overview of types of documents that participating centres requested for their local approval procedures. X-axis: number of centres, Y-axis: document type.

** local budget form or other local forms designed by participating centres; Procedures were analysed in a total of 23 centres.*

FEES

The average fee charged for the procedure of obtaining local feasibility was €318, with a range of €0-1,750. 62% of the centres charged no costs for the procedure.

For CAIRO5 the mean fees were €226 with a range of €0-1,750, the total fees were €4,075. For CHARISMA the mean fee was €500 with a range of € 0-1,750, the total fees were € 4,500. For all analysed procedures, an unanticipated budget of €8,575 was needed.

DISCUSSION

The current analysis demonstrates that the procedure for obtaining approval of local feasibility in participating centres to multicentre trials greatly varies in duration, content and financial burden. In 2012 a new guideline for external validation (RET 2012) was implemented in the Netherlands, and this guideline is operational since the 1st of March 2012 [10]. The foremost modification in the guideline was the abolition of the "local feasibility statement", which implied that a medical-ethical reassessment of the content of the trial protocol by the local centre should no longer be performed. The track for obtaining approval of local feasibility for multicentre research has been re-evaluated by research groups and by the CCMO after implementation of the new guideline in 2012 [3, 6, 7]. The median time lapse between obtaining approval by the central MEC and obtaining approval by the board of directors of the local participating centre was 50 days for centres who adhered to the new guideline and 118 days for those who did not [3]. In our study the median time lapse of this procedure was 90 days, with a range up to 312 days.

Results from an evaluation by the CCMO showed that within 60 days, 50% of the centrally approved studies were opened for inclusion of patients in a participating centre [6, 27, 28]. In our study this percentage is 32%. In contrast to central medical ethical reviews of clinical research, the procedure for local approval has no time limit.

The time period between the date of submission of the complete file to the board of directors of a participating centre and the date of local approval to start the trial is relatively short. An explanation for this phenomenon is the introduction of the RET 2012 (abolishment of a double review). From a researcher's perspective however, the delay now occurs between the date of central approval of the MEC to include a participating centre in the trial and the date of submission of the complete file to the board of directors of a participating centre. In other words: it is challenging to submit a complete file by local standards, due to variation in local standards. This period cannot be influenced by the RET 2012 or the CCMO, due to autonomy of individual participating hospitals. Our study demonstrates that the period of time of the total procedure has not been shortened. The CCMO also suggested this in their review [6, 28].

Lastly, we observed a delay between the date of final local approval and the date of dispatch of the letter by which the trial is officially open for accrual. This is the responsibility of the research teams and not of the participating centres or local medical ethical committees, and is not part of the RET 2012.

THE PROCEDURE

In 2012 the "research statement" replaced the local feasibility statement. The research statement has to be signed by the head of department/healthcare group manager/local researcher on behalf of the participating centre [13]. However, departments that are not directly involved in the primary care of study patients, i.e. diagnostic departments such as radiology, clinical laboratory and pathology, are usually not involved in an early stage of the local approval procedure. This often results in a delay at a later stage when these departments

are confronted with study procedures that involve their collaboration. The use of a uniform procedure involving all departments that is initiated as soon as a local centre shows interest and before the research statement is signed, may prevent unnecessary delays.

DOCUMENTS

We observed a large variation among centres in the number and characteristics of documents that were requested for local approval. There is no national standard research file in The Netherlands for this procedure such as exists for the procedure of central approval by the medical ethics committee.

Handling of the CTA is causing an important delay in the procedure. This was also recognized in the evaluation by the CCMO [6, 28]. Of note, not all centres require a CTA. The CTA is a frequent cause of time-consuming correspondence between the legal departments of the initiating organization/centre and the local centres. In The Netherlands a CTA between the initiating party and participating centres is not mandatory by law, and there is no uniformity regarding the content of CTA. Several CTA templates are available, however these have been drafted by different authorities and show substantial disparities (CCMO [29], STZ-Nefarma-ACRON-NKI [30]). We observed significant differences among participating centres in the items that their legal departments requested to modify within a CTA.

Because a GCP certificate is legally not mandatory for local investigators, these were not always available [31]. Where legislation is not available or multi-interpretable the Dutch Federation of University Medical Centres (NFU) academic hospitals together contribute to its development. In case of the GCP certificate the NFU urges to make this mandatory [32]. Currently, the GCP certificate is a cause for delay. A clear statement on this issue in the CCMO guideline would facilitate this process.

FINANCIAL COMPENSATION

Despite the signing of the 'Institution Statement' by 8 academic hospitals and 27 Cooperating Top Clinical Medical Teaching Hospitals [12], 38% of the hospitals charged widely varying fees for the procedure of local approval. The Institution Statement only mentions that participation in multicentre research should be organized and supported as efficiently as possible, and the issue of charges is not mentioned. The observed variation in fees is not logical and undesirable, especially for investigator-initiated studies that usually have a limited budget. We support a procedure that is free of charges or a procedure with transparent and uniform costs, taking the nature of the study into account (investigator- versus pharmaceutical industry-initiated).

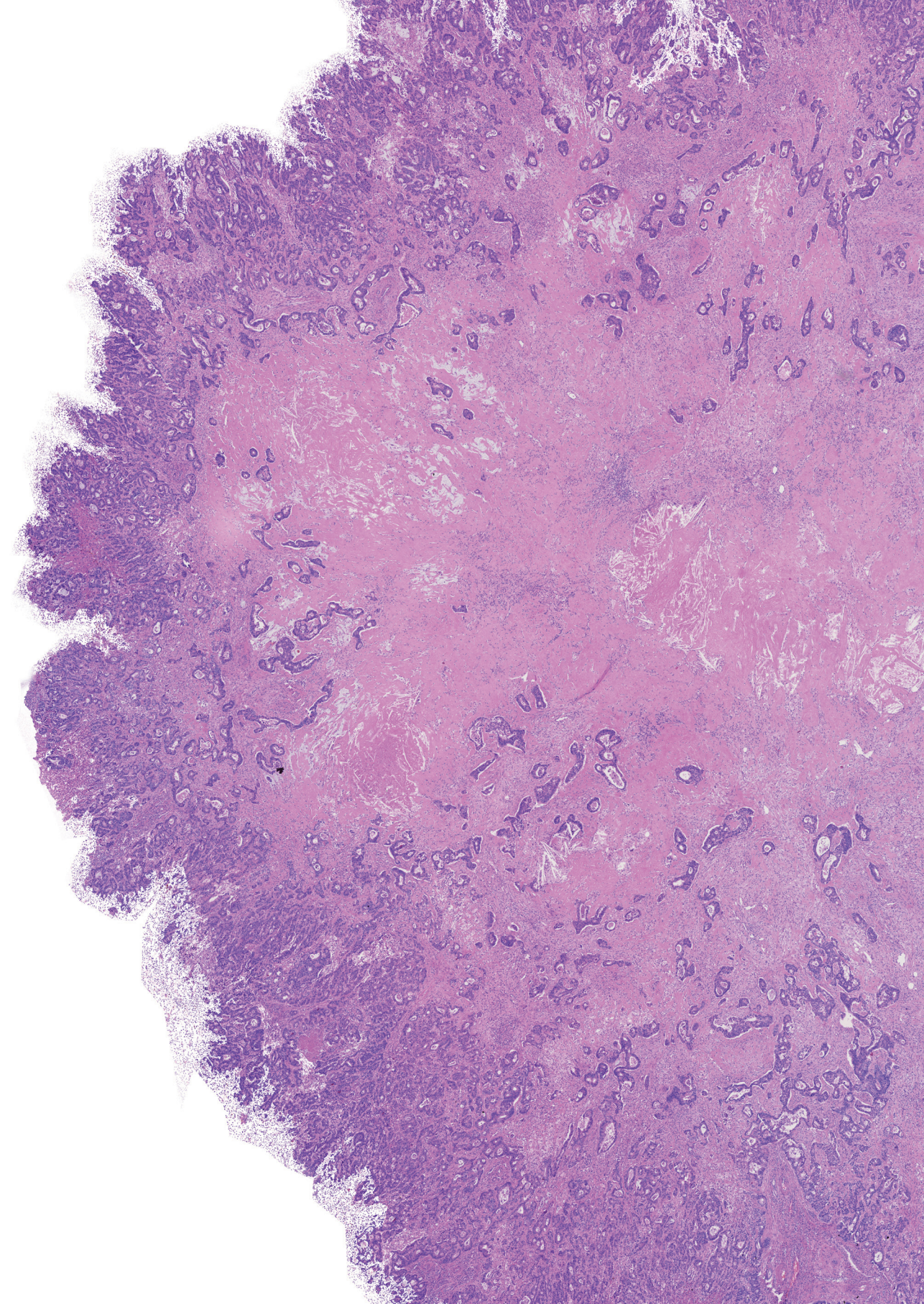
CONCLUSION

Great variation exists in the procedures for obtaining approval for local feasibility of multicentre research in terms of time, content and costs. These variations are unpredictable and form a serious obstacle in conducting scientific clinical research in The Netherlands. Delay in the process of initiation of studies decrease the chance of successful accrual of patients and thereby endanger their successful completion. This is not acceptable from the perspective of patients, researchers and funding bodies. This process is not within the scope of the RET 2012. Consensus on simplification of the procedure is urgently needed. Collaboration with all stakeholders on further standardization, centralization and digitalization of the procedure would be of great value. Currently three Dutch cancer research groups, the Dutch Colorectal Cancer Group (DCCG), Breast Cancer Research Group (BOOG), Hematology Oncology Research group (HOVON), in collaboration with the Dutch Comprehensive Cancer Centre (IKNL) and Dutch Cancer Foundation (KWF) are initiating the a national platform (Dutch Oncology Research Platform, DORP) which aims among other issues to coordinate and create uniformity in logistical procedures in investigator-initiated clinical cancer research in The Netherlands.

REFERENCES

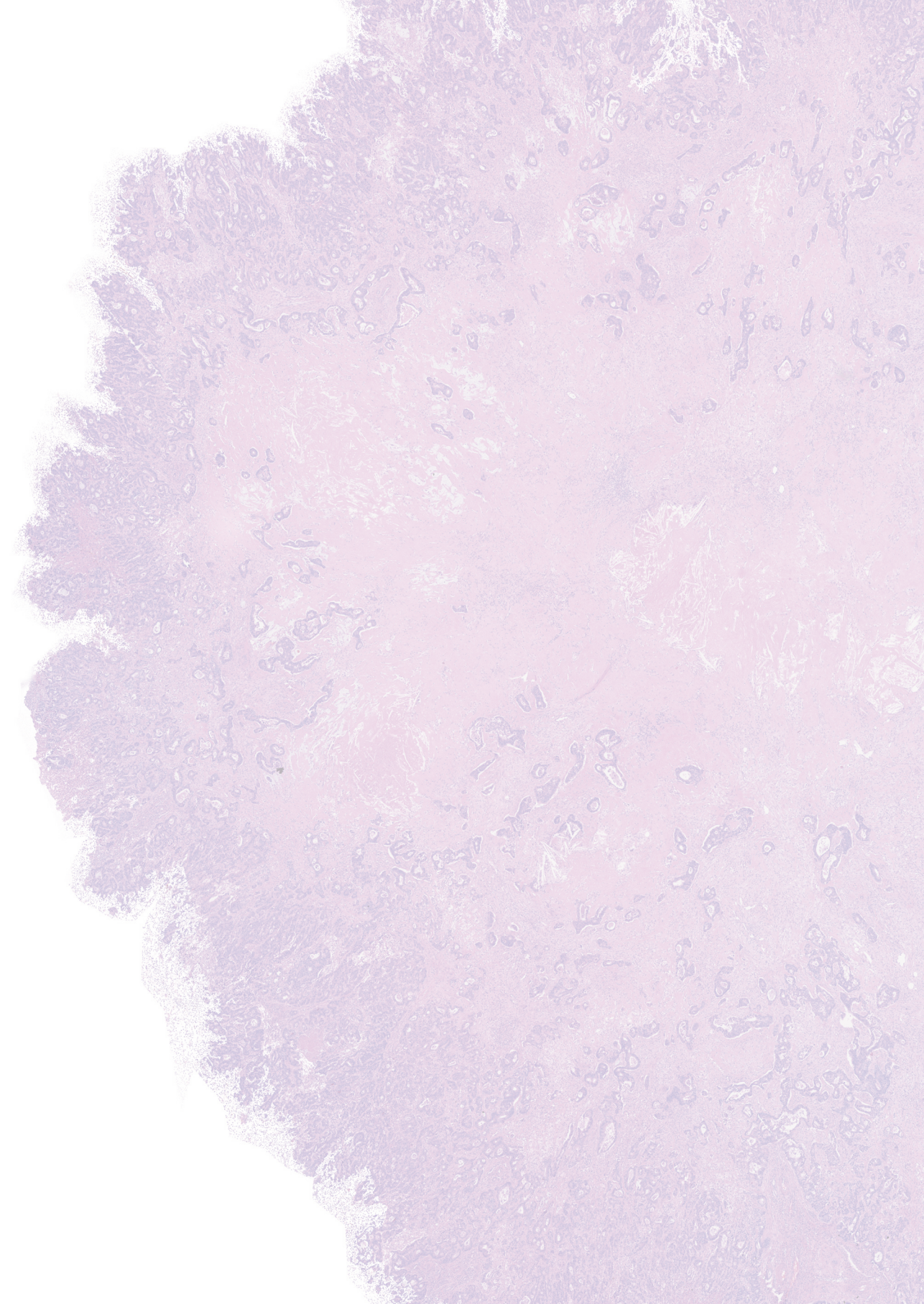
1. ClinicalTrials.gov, Trends, Charts, and Maps. http://clinicaltrials.gov/ct2/resources/trends, 2015.
2. (CCMO), C.C.M.O., Jaarverslag 2014. http://www.ccmo.nl/attachments/files/ccmo-jaarverslag-2014.pdf, 2015.
3. Oude Rengerink, K., M. Kruijt, and B.W. Mol, [Medical-ethical review of multicenter studies can be obtained faster: new directive leaves room for improvement]
   Medisch-ethische toetsing van multicentrisch onderzoek kan sneller: nieuwe richtlijn biedt ruimte voor verbetering. Ned Tijdschr Geneeskd, 2013. **157**(42): p. A6302.
4. van Santvoort, H.C., M.G. Besselink, and H.G. Gooszen, [Obtaining medical ethical approval for a multicentre, randomised study: prospective evaluation of a ponderous process] Het verkrijgen van medisch-ethische goedkeuring voor een multicentrische, gerandomiseerde trial: prospectieve evaluatie van een moeizaam proces. Ned Tijdschr Geneeskd, 2008. **152**(38): p. 2077-83.
5. Evers, J.L.H., Onnodig traag verlopende medisch-ethische toestemming voor multicentrische klinische trials. Nederlands Tijdschrijft voor Geneeskunde 2008. **152**(38): p. 2062-2064.
6. Heringa, J.W., M.D. Al, and G.H. Koeter, [Review of multicentre clinical trials is simplified; more trial centres are approved in the first assessment round]
   Toetsing multicentrisch onderzoek is vereenvoudigd. Ned Tijdschr Geneeskd, 2014. **158**: p. A7558.
7. Vissenberg R., H.W., Oudijk M., van Golde R., Evaluatieonderzoek verzuipt in regelgeving. Medisch Contact, 2013(45): p. 2354-2356.
8. E.A. Ooms, A.C.A., C.W. Burger, Mensgebonden onderzoek traag op gang. Medisch Contact, 2006(19): p. 803-805.
9. Nederland, n.-v.i.g., Klinisch geneesmiddelen onderzoek in Nederland. www.nefarma.nl/stream/com-nctd-monitor-2012, 2012.
10. (CCMO), C.C.M.O., CCMO-richtlijn Externe Toetsting 2012. http://www.ccmo.nl/attachments/files/ccmo-richtlijn-externe-toetsing-geldig-vanaf-1-7-2015-stcrt-2015-17447.pdf, 2015.
11. (CCMO), C.C.M.O., Instellingsverklaring. http://www.ccmo.nl/attachments/files/instellingsverklaring-medisch-wetenschappelijk-onderzoek-17122012.pdf, 2015.
12. (CCMO), C.C.M.O., Instellingslijst. http://www.ccmo.nl/attachments/files/lijst-umc-en-stz-instellingsverklaringen-17122012.pdf, 2015.
13. (CCMO), C.C.M.O., Onderzoeksverklaring. http://www.ccmo.nl/nl/erkende-metc-s-en-multicenteronderzoek, 2015.
14. Kenter, M.J. and A.F. Cohen, Re-engineering the European Union Clinical Trials Directive. Lancet, 2012. **379**(9828): p. 1765-7.
15. Ahmed Ali, U., P.C. van der Sluis, Y. Issa, et al., Trends in worldwide volume and methodological quality of surgical randomized controlled trials. Ann Surg, 2013. **258**(2): p. 199-207.
16. Maskell, N.A., E.L. Jones, R.J. Davies, and B.M.M.s. committee, Variations in experience in obtaining local ethical approval for participation in a multi-centre study. QJM, 2003. **96**(4): p. 305-7.

17. Ahmed, A.H. and K.G. Nicholson, Delays and diversity in the practice of local research ethics committees. J Med Ethics, 1996. **22**(5): p. 263-6.
18. Helfand, B.T., A.K. Mongiu, C.G. Roehrborn, et al., Variation in institutional review board responses to a standard protocol for a multicenter randomized, controlled surgical trial. J Urol, 2009. **181**(6): p. 2674-9.
19. Rawlins, M., A new era for UK clinical research? Lancet, 2011. **377**(9761): p. 190-2.
20. Christian, M.C., J.L. Goldberg, J. Killen, et al., A central institutional review board for multi-institutional trials. N Engl J Med, 2002. **346**(18): p. 1405-8.
21. Duley, L., K. Antman, J. Arena, et al., Specific barriers to the conduct of randomized trials. Clin Trials, 2008. **5**(1): p. 40-8.
22. Yusuf, S., J. Bosch, P.J. Devereaux, et al., Sensible guidelines for the conduct of large randomized trials. Clin Trials, 2008. **5**(1): p. 38-9.
23. Tully, J., N. Ninis, R. Booy, and R. Viner, The new system of review by multicentre research ethics committees: prospective study. BMJ, 2000. **320**(7243): p. 1179-82.
24. Seiler, C.M., P. Kellmeyer, P. Kienle, M.W. Buchler, H.P. Knaebel, and I.S. Group, Assessment of the ethical review process for non-pharmacological multicentre studies in Germany on the basis of a randomised surgical trial. J Med Ethics, 2007. **33**(2): p. 113-8.
25. Huiskens, J., T.M. van Gulik, K.P. van Lienden, 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**: p. 365.
26. Ayez, N., E.P. van der Stok, H. de Wilt, 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**: p. 180.
27. CCMO, Beoordelingstermijnen Toetsingscommissie. http://www.ccmo.nl/nl/beoordelingstermijnen-toetsingscommissie, 2016.
28. (CCMO), C.C.M.O., Evaluatie RET 2012. http://www.ccmo.nl/attachments/files/ccmo-rapport-evaluatie-ret2012-dd-15-mei-2014.pdf, 2015.
29. Onderzoek, C.C.M., Onderzoekscontract http://www.ccmo.nl/attachments/files/ccmo-template-clinical-trial-agreement-engels-v15jul13.doc, 2015.
30. opleidingsZiekenhuizen, S.T., Onderzoekscontract. http://www.stz.nl/download/7a4445f2e0990207d5eb5cfb13d66c1a1875.pdf, 2015.
31. Ministerie van Volksgezondheid, W.e.S., Wet medisch-wetenschappelijk onderzoek met mensen. http://wetten.overheid.nl/BWBR0009408/geldigheidsdatum_04-01-2016#Paragraaf5, 1998.
32. Centra, N.F.v.U.M., Kwaliteitsborging mensgebonden onderzoek 2.0 http://www.nfu.nl/img/pdf/NFU-12.6053_Kwaliteitsborging_mensgebonden_onderzoek_2.0.pdf.



PART II

Prognostic and Predictive Factors After Surgery for Colorectal Liver Metastases



CHAPTER 4

The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: clinical risk score as possible discriminator

N. Ayez
E.P. van der Stok
D.J. Grünhagen
J. Rothbarth
E. van Meerten
A.M. Eggermont
C. Verhoef

European Journal of Surgical Oncology. 2015 Jul;41(7):859-67

ABSTRACT

AIM

The combination of surgery and chemotherapy (CTx) is increasingly accepted as an effective treatment for patients with colorectal liver metastases (CRLM). However, controversy exists whether all patients with resectable CRLM benefit from perioperative CTx. We investigated the impact on overall survival (OS) by neo-adjuvant CTx in patients with resectable CRLM, stratified by the clinical risk score (CRS) described by Fong et al.

METHODS

Patients who underwent surgery for CRLM between January 2000 and December 2009 were included. We compared OS of patients with and without neo-adjuvant CTx stratified by the CRS. The CRS includes five prognosticators and defines two risk groups: low CRS (0–2) and high CRS (3–5).

RESULTS

363 patients (64% male) were included, median age 63 years (IQR 57–70). Prior to resection, 219 patients had a low CRS (neo-adjuvant CTx: N=65) and 144 patients had a high CRS (neo-adjuvant CTx: N=88). Median follow-up was 47 months (IQR 25–82). In the low CRS group, there was no significant difference in median OS between patients with and without CTx (65 months (95% CI 39–91) vs. 54 months (95% CI 44–64), P = 0.31). In the high CRS group, there was a significant difference in OS between patients with and without CTx (46 months (95% CI 24–68) vs. 33 month (95% CI 29–37), P=0.004).

CONCLUSION

In our series, patients with a high CRS benefit from neo-adjuvant CTx. In patients with a low risk profile, neo-adjuvant CTx might not be beneficial.

INTRODUCTION

Colorectal carcinoma is one of the leading causes of cancer death world-wide, mostly as a consequence of metastatic disease [1]. Administration of combined chemotherapy regimens improves survival rates of patients with colorectal metastases (CRLM) [2-4]. If metastases are confined to the liver, surgical resection is the most effective therapy, providing the only potential for cure [5, 6]. However, cancer relapse after curative resection of CRLM is a common phenomenon, with recurrence rates up to 50% in the first 2 years [7]. In an attempt to reduce these recurrence rates, the combination of liver resection with systemic therapy, either pre-, peri- or postoperatively, is increasingly researched. Multiple studies have investigated the impact of adjuvant chemotherapy in addition to surgery for CRLM, but have failed to show survival benefit [8, 9]. Recently, the mature results of the landmark EORTC 40983 trial, studying the impact of perioperative chemotherapy, were published showing no overall survival benefit for patients in the chemotherapy group [10, 11]. Therefore, the exact role of systemic therapy in combination with resection for CRLM remains unclear. Nonetheless, some reports recommend to treat the majority of patients with CRLM with neo-adjuvant chemotherapy [12].

In order to predict the likelihood of tumour recurrence and survival after resection of CRLM, several Clinical Risk Scores (CRS) have been developed [5, 13-18]. The most widely used and validated CRS has been described by Fong et al. in 1999 [15]. In this publication, 5 independently prognostic clinical variables for survival after surgery for CRLM are identified. Furthermore, 2 risk groups (high/low) are characterized: patients with a high risk profile have significantly worse overall survival rates as compared to patients with a low risk profile. Although all CRLM may well be regarded as "high risk", this CRS may explain, at least in part, the relative lack of efficacy of systemic therapy when combined with surgery in the metastasized setting. It is not uncommon in other types of malignancies (e.g. breast, primary colon) to reserve the use of adjuvant chemotherapy to those patients with the most advanced disease (highest risk profile). The present retrospective study aimed to evaluate overall survival outcome in patients with and without neo-adjuvant chemotherapy, stratified by their clinical risk profile as described by Fong.

PATIENTS AND METHODS

Between January 2000 and December 2009, all consecutive patients who underwent liver resection for CRLM were analysed. Patients were assessed by Fong's CRS and excluded from the analysis if they had missing data to calculate the CRS and/or extrahepatic disease. Calculation of the CRS was based on clinical data at diagnosis of CRLM. The clinical prognosticators in Fong's CRS were: (1) node positive status of primary tumour (pathological), (2) disease-free interval from the primary to discovery of the liver metastases < 12 months, (3) number of

metastases > 1 (radiological), (4) size of the largest metastases > 5 cm (radiological) and (5) preoperative CEA level > 200 ng/ml [15]. Each criterion is assigned one point. The prognostic value of this scoring system has been verified by independent research groups [19, 20]. We identified two risk groups: low risk (CRS 0-2) and high risk (CRS 3-5), in concordance with the original study. The rationale for dividing patients in two risk groups was to evaluate whether the CRS may play a role in explaining the relative lack of efficacy of chemotherapy when combined with surgery in the metastasized setting.

CHEMOTHERAPY

Erasmus MC Cancer Institute is a tertiary referral hospital for patients with CRLM. In our treatment protocol, perioperative chemotherapy is not standard of care for patients with CRLM. All patients in this study were assessed by a dedicated liver surgeon in a multidisciplinary meeting with respect to resectability before potential administration of chemotherapy. Patients in our hospital received neo-adjuvant chemotherapy in case of multiple (≥ 4), synchronous metastases. However, a large proportion of patients in this study received neo-adjuvant chemotherapy in the referring hospital, after which patients are transferred to our unit for liver surgery. The reason for administering one type of chemotherapy over another was based on local treatment protocols. All patients received a combination of 5-fluorouracil (5-FU)/Capecitabine and Oxaliplatin or Irinotecan, with or without Bevacizumab. The response to neo-adjuvant systemic therapy was assessed after two or three cycles by CT scan (according to RECIST [21]) and carcinoembryonic antigen levels. Further treatment strategy was determined on basis of the tumour response and extent of the disease. When the liver metastases were resectable, a laparotomy was planned at least three weeks after the last course of neo-adjuvant chemotherapy. Bevacizumab had to be excluded from the last course of chemotherapy to ensure an interval of at least six weeks. All patients included in this study had resectable CRLM; resectability of liver metastases was assessed by a liver surgeon at diagnosis. None of the patients received standard adjuvant systemic therapy after liver surgery.

Patients were included after 2000, as from then on modern chemotherapy and biologicals were available. In our unit, the definition of resectability has not changed since 2000 (i.e. possibility of an R0 resection, the feasibility of securing vascular in- and outflow as well as biliary drainage to the remaining segments, and a future liver remnant of at least 20-30%)

FOLLOW-UP

Follow-up after resection of CRLM consisted of clinical examination and measurement of CEA every 3 months. Abdominal imaging (ultrasound, CT-thorax-abdomen) was performed at 3, 6, 9 and 12 months in the first year, every 6 months the second year and once per year thereafter. If recurrent disease occurred, palliative or curative treatment strategies were considered by the multidisciplinary team.

OUTCOME

Overall survival (OS) was defined as the interval in months between resection of CRLM and death, or the date of last follow-up. Disease-free survival (DFS) was defined as the interval in months between resection of CRLM and recurrence, death without recurrence, or date of last follow-up without recurrence.

STATISTICS

Descriptive values are expressed as median (interquartile range (IQR)). Variables were compared by means of chi-square analysis or Fischer's exact test (depending on the sample size) or with the independent Student's t test or Mann-Whitney U test when appropriate. Survival analysis was performed by the Kaplan-Meier method. Comparison between survival curves was made by log-rank tests. For the multivariate analysis only parameters with a p value < 0.10 in the univariate model were entered in the Cox regression model. The SPSS statistical package (version 21.0, Chicago, IL, USA) was used for statistical analysis; a p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Between January 2000 and December 2009, 442 patients underwent liver resection for CRLM. Of these, 77 patients (17%) were excluded due to extrahepatic disease and/or missing data for calculation of the CRS. 2 patients were lost to follow-up, leaving 363 patients eligible for analysis (42 patients extrahepatic disease, 33 patients missing data and 2 patients with both, 2 lost to follow-up). Neo-adjuvant chemotherapy was given in 153 (42%) patients. 51 patients received adjuvant chemotherapy for primary colorectal cancer (30 patients in the low CRS group and 21 patients in the high CRS group, P=0.093). 15 of these patients received further neo-adjuvant chemotherapy for liver metastases.

Neo-adjuvant capecitabine monotherapy was administered in 5 patients and 5-FU/LV monotherapy was administered in 3 patients. The majority of patients receiving neo-adjuvant chemotherapy received either oxaliplatin-based chemotherapy (88%) or irinotecan-based chemotherapy (7%). Sixty-seven patients received concomitant bevacizumab (44%) mostly in combination with oxaliplatin (88%). The median number of chemotherapy cycles for all patients was 5 (IQR 4-6). The median number of chemotherapy cycles was also 5 (IQR 4-6) in patients with a low risk profile and 6 in patients with a high CRS (IQR 4-7). Patient characteristics are displayed in tables 1, 2 and 3. Eighty-four patients (23%) had an R1 resection. The numbers of R1 resections in patients with and without neo-adjuvant chemotherapy were comparable: 28% vs. 20% (P=0.09), respectively. An R1 resection occurred more often in patients with a high CRS than in patients with a low CRS (38% versus 14%, P<0.001). The median follow-up of patients in this study was 47 months (IQR 25-82). Five patients (1.6%) died postoperatively.

Table 1: Characteristics of patients with a low CRS by neo-adjuvant chemotherapy treatment (CTx)

		Without CTx (N=154)		Without CTx (N=65)			All patients (N=219)	
Variables		**value**	**% or IQR**	**value**	**% or IQR**	***P value***	**value**	**% or IQR**
Male		95	62%	47	72%	0,133	142	
Age	median	66	59-72	63	58-70	0,09	65	59-71
Primary tumour								
Rectal cancer		70	46%	36	55%	0,179	106	48%
pT-stage								
pT3		107	70%	40	62%	0,253	147	67%
pT4		9	6%	7	11%	0,201	16	7%
Positive lymph node (p)		61	40%	46	71%	0,145	80	37%
CEA µg/L								
	median	15	6-49	16	6-47	0,726	16	6-48
	mean	48		37			45	
Liver metastases								
Synchronous (≤ 3mo)		27	18%	40	62%	< 0,001	67	31%
Synchronous (≤ 12mo)		59	38%	50	77%	< 0,001	109	50%
Diameter (cm) (r)	median	3,2	2,4-4,4	2,6	2-3,8	0,016	3	2-4
Number of metastases (r)								
	median	1	1-2	2	1-4	< 0,001	1	1-2
Bilobar		22	14%	27	42%	< 0,001	49	22%
R1 resection (p)		21	14%	10	16%	0,743	31	14%

***p** = as objectified by pathologist, r = as objectified by radiologist*

Table 2: Characteristics of patients with a high CRS by neo-adjuvant chemotherapy treatment (CTx)

		Without CTx (N=56)		Without CTx (N=88)			All patients (N=144)	
Variables		**value**	**% or IQR**	**value**	**% or IQR**	***P value***	**value**	**% or IQR**
Male		36	64%	54	61%	0,724	90	63%
Age	median	61	57-69	61	56-68	0,397	61	56-68
Primary tumour								
Rectal cancer		26	46%	25	28%	0,028	51	35%
pT-stage								
pT3		43	77%	73	83%	0,362	116	81%
pT4		6	11%	7	8%	0,573	13	9%
Positive lymph node (p)		49	88%	68	77%		117	81%
CEA µg/L								
	median	26	8-70	55	8-229	0,246	34	8-202
	mean	104		274			208	
Liver metastases								
Synchronous (≤ 3mo)		27	48%	72	82%	< 0,001	99	69%
Synchronous (≤ 12mo)		46	82%	81	92%	0,073	127	88%
Diameter (cm) (r)	median	3,5	2-5,5	4,5	3-6	0,033	4	2,5-5,8
Number of metastases (r)								
	median	3	2-3	4	2-5	0,004	3	2
Bilobar		31	55%	55	63%	0,394	86	60%
R1 resection (p)		21	38%	32	38%	0,949	53	38%

***p** = as objectified by pathologist, r = as objectified by radiologist*

Table 3: Characteristics of patients by CRS

		CRS low (N=219)		CRS high (N=144)			All patients (N=363)	
Variables		**value**	**% or IQR**	**value**	**% or IQR**	***P value***	**value**	**% or IQR**
Male		142	65%	90	63%	0,65	232	64%
Age	median	65	59-71	61	56-68	0,001	63	57-70
Primary tumour								
Rectal cancer		106	48%	51	35%	0,015	157	43%
pT-stage								
pT3		147	67%	116	81%	0,005	263	73%
pT4		16	7%	13	9%	0,554	29	8%
Positive lymph node (p)		80	37%	117	81%	< 0,001	197	54%
CEA µg/L								
	median	16	6-48	34	8-203	< 0,001	19	6-69
	mean	45		208			110	
Liver metastases								
Synchronous (≤ 3mo)		67	31%	99	69%	< 0,001	166	46%
Synchronous (≤ 12mo)		109	50%	127	88%	< 0,001	236	65%
Diameter (cm) (r)	median	3	2-4	4	3-6	< 0,001	3,4	2,2-5
Number of metastases (r)								
	median	1	1-2	3	2-4	< 0,001	2	1-3
Bilobar		49	22%	86	2-4	< 0,001	135	37%
R1 resection (p)		31	14%	53	38%	< 0,001	84	23%
Chemotherapy								
Yes		65	30%	88	61%	< 0,001	153	42%
Response								
	CR	5	8%	3	3%		8	5%
	PR	40	64%	61	69%		101	67%
	SD	18	29%	22	25%		40	27%
	PD	0	0	1	1%		1	1%
No		154	70%	56	39%		210	48%

p *= as objectified by pathologist,* ***r*** *= as objectified by radiologist*

DISEASE FREE SURVIVAL AND RECURRENCE

For patients with neo-adjuvant chemotherapy the median DFS was 12 months (95% confidence interval (CI) 9-15) and for patients without chemotherapy it was 13 months (95% CI 10-16, P=0.83). In patients with a low CRS there was no difference in the median DFS between patients with and without chemotherapy (13 months, 95% CI 9-17) versus 16 months (95% CI 10-22, P=0.96). The 5-year DFS was 34% versus 27% respectively. In patients with a high CRS there was a significant difference in median DFS between patients with and without chemotherapy (11 months, 95% CI 7-15) versus 9 months (95% CI 8-10, P=0.02). The 5-year DFS was 24% versus 9% respectively (Figure 1).

During follow-up, 266 patients (73%) developed a recurrence. Local treatment was performed in 122 patients (47%) (surgery, radiofrequency ablation, stereotactic radiotherapy), 112 patients (43%) had palliative chemotherapy and 29 patients (11%) received neither chemotherapy nor local treatment. Patients treated with neo-adjuvant chemotherapy for initial CRLM developed less extrahepatic recurrences (versus hepatic or local) than patients treated without CTx (extrahepatic recurrence in patients treated with and without CTx: low CRS 46% vs. 67%, P=0,032 and high CRS 40% vs. 71%, P=0,001, respectively). In line with these findings, more patients with CTx for initial CRLM were treated with curative intent for recurrent disease, again in both risk groups. However, this difference did only reach

significance in the high CRS group (curative treatment for recurrent disease in patients treated with and without CTx, respectively: low CRS 45% vs. 59%, P=0,109, high CRS 22% vs. 43%, P=0,015).

OVERALL SURVIVAL

For patients with neo-adjuvant chemotherapy the median OS was 57 months (95% CI 40-74) and for patients without chemotherapy it was 45 months (95% CI 38-52), P=0.08. In patients with a low CRS there was no difference in the median OS between patients with and without chemotherapy (65 months, 95% CI 39-91) versus 54 months (95% CI 44-64, HR 0.83, P=0.31). The 5-year OS was 52% versus 46% respectively. In patients with a high CRS the median OS was significantly higher in patients who received chemotherapy compared with those who did not: 46 months (95% CI 24-68), versus 33 months (95% CI 29-37, HR 0.57, P=0.004). The 5-year OS was 46% versus 20% respectively (Figure 1).

Excluding all patients with a R1 resection yielded comparable results. In patients with a low CRS there was no difference in DFS and OS between patients treated with or without neo-adjuvant chemotherapy, respectively: DFS 14 (95% CI 8-20) vs. 17 months (95% CI 11-23, P=0,880), OS 78 (95% CI 51-105) vs. 57 (95% CI 42-72, HR 0.76, P=0,21). In patients with a high CRS there was a significant difference in DFS and OS between patients treated with or without neo-adjuvant chemotherapy, respectively: DFS 13 (95% CI 6-20) vs. 8 months (95% CI 6-10, P=0,003), OS 68 (95% CI 38-98) vs. 34 months (95% CI 31-37, HR 0.47, P=0,004).

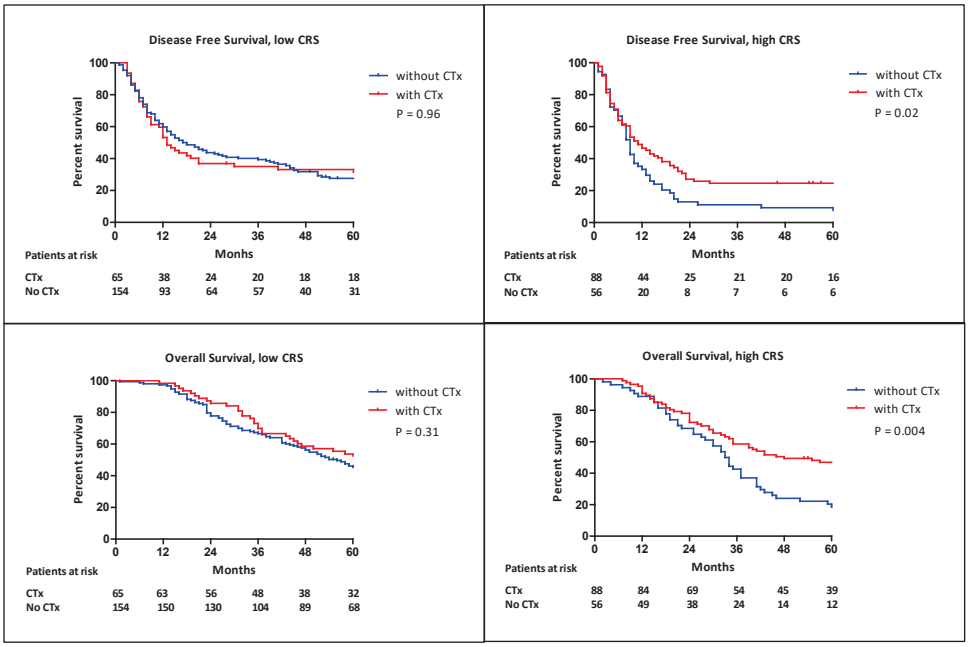


Figure 1. Low and high CRS in patients with and without neo-adjuvant chemotherapy (CTx): disease free survival and overall survival

UNIVARIATE AND MULTIVARIATE ANALYSES

In patients with a low CRS, the univariate analysis showed that 2 factors were prognostic for OS: T-stage primary tumour (T4) and positive resection margin (R1). In multivariate analysis these factors remained prognostic for OS (Table 4). In patients with a high CRS, 2 factors were prognostic for OS in univariate analysis: primary tumour location (colon vs. rectum) and administration of neo-adjuvant chemotherapy. In multivariate analysis, only administration of neo-adjuvant chemotherapy remained of significant influence on overall survival (Table 4). Excluding patients with a R1 resection from the uni- and multivariate analysis generated comparable results.

Table 4: Univariate and multivariate analysis, overall survival

		CRS low (N=219)			CRS high (N=144)		
Variables		**Median Survival (95% CI)**	**Univariate HR (95%CI) P-value***	**Multivariate HR (95% CI)**	**Median Survival (95% CI)**	**Univariate HR (95% CI) P-value***	**Multivariate HR (95% CI)**
Gender							
	Male	63 (49-77)	0,934 (0,666-1,310)	-	41 (33-49)	0,961 (0,648-1,424)	-
	Female	49 (36-62)	P=0,692		34 (29-39)	P=0,841	
Age							
	< 65	59 (47-71)	1,131 (0,816-1,566)	-	37 (31-43)	1,170 (0,786-1,742)	-
	> 65	54 (38-70)	P=0,460		35 (25-45)	P=0,438	
Primary tumour							
	Rectum	57 (42-72)	1,026 (0,742-1,421)	-	34 (28-40)	1,436 (0,969-2,130)	1,340 (0,9-1,995)
	Colon	58 (44-72)	P=0,875		43 (27-59)	0,072	P=0,149
T-stage							
	T4	32 (10-54)	2,447 (1,452-4,124)	2,670 (1,575-4,526)	41 (27-55)	1,134 (0,590-2,179)	-
	T 1-3	59 (49-69)	P=0,001	P < 0,001	37 (31-43)	0,706	
Lymph node							
	Positive	51 (43-59)	1,279 (0,919-1,779)	-	35 (30-40)	1,429 (0,849-2,406)	-
	Negative	65 (51-79)	P=0,279		57 (15-99)	P=0,179	
Liver metastases							
Disease free interval							
	< 12 months	54 (43-65)	0,768 (0,554-1,063)	-	37 (31-43)	1,045 (0,595-1,836)	-
	> 12 months	63 (40-86)	P=0,112		37 (28-46)	P=0,878	
Largest size metastases (cm)							
	> 5	59 (38-80)	1,123 (0,744-1,696)	-	35 (22-48)	1,244 (0,844-1,831)	-
	< 5	56 (45-67)	P=0,581		39 (33-45)	P=0,270	
Number metastases							
	> 1	58 (37-79)	1,087 (0,776-1,524)	-	32 (12-52)	1,214 (0,632-2,331)	-
	1	57 (45-69)	P=0,627		37 (31-43)	P=0,561	
CEA level µg/L							
	> 200	32 (16-48)	1,606 (0,657-3,924)	-	43 (14-72)	0,916 (0,590-1,420)	-
	< 200	58 (48-68)	P=0,299		35 (29-41)	P=0,916	
Tumour distribution							
	Bilobar	48 (21-75)	1,235 (0,847-1,8)	-	35 (29-41)	1,253 (0,845-1,858)	-
	Unilobar	58 (45-71)	P=0,272		41 (27-55)	P=0,263	
Resection margin							
	R1	43 (22-64)	1,927 (1,256-2,956)	1,837 (1,187-2,842)	41 (31-51)	1,259 (0,848-1,868)	-
	R0	61 (47-75)	P=0,003	P=0,006	34 (27-41)	P=0,259	
Chemotherapy							
	Yes	65 (39-91)	0,825 (0,570-1,195)	-	46 (24-68)	0,572 (0,390-0,841)	0,594 (0,403-0,876)
	No	54 (44-64)	P=0,309		33 (29-37)	P=0,004	P=0,009

DISCUSSION

This is the first study to demonstrate (retrospectively) that patients with primary resectable CRLM and a high clinical risk profile gain significant overall survival benefit when adding neo-adjuvant chemotherapy to resection for metastases. All patients in this study have a potential follow-up of at least 5 years, as can be seen by the few censored cases in the Kaplan Meier curves.

In the last decade, the development of modern chemotherapeutic agents and biologicals has significantly improved OS in patients with CRLM [2-4, 22-29]. The success of systemic therapy in the palliative setting has prompted studies that evaluated the role of chemotherapy in combination with liver resection [9-11, 30]. However, these studies regularly involve strict inclusion criteria. Consequently, patients with a high risk profile - who might benefit the most from chemotherapy – may be underrepresented in these studies. Since genuine survival benefit of multimodal therapies has not yet been demonstrated, could the insignificant impact of chemotherapy on overall survival then be explained by the relatively low risk profile of patients in these trials?

The present role of perioperative chemotherapy for primary resectable metastases was established in a large randomized controlled trial, which compared perioperative chemotherapy with surgery alone [10]. The mature overall survival data of this trial were recently published; although perioperative chemotherapy improved DFS, no increase in overall survival could be objectified after a median follow-up of 8,5 years [11]. This may be explained by the fact that at present, recurrences can be adequately treated by means of systemic and/or local therapies, as the authors suggest. Additionally, this trial was not powered upfront to detect differences in overall survival, as explained by the authors. Alternatively, the lack of impact on overall survival might be a result of the fact that patients eligible for randomization in the EORTC 40983 trial had a relatively low risk profile. Although the study population of the current study is comparable to the population in the EORTC trial in general (age 63 vs. 63; male 64% vs. 66%; rectal primary 43% vs. 42%, T3-T4 stage 73%-8% vs. 70%-13%; positive LN 54% vs. 56%; synchronous 46% vs. 35%; median number of lesions 2 vs. 1; median diameter 3 cm vs. the sum of largest diameter 50 mm -33 mm after CTx, respectively [10]), uncertainty exists about the exact number of high risk patients represented in EORTC 40983.

Several authors have advocated the concept of stratification by CRS. Tomlinson et al. demonstrated in actual 10-year survivors of liver surgery for CRLM that patients with a low CRS had a cure rate of 21% versus 10% in patients with a high CRS. They suggest this finding may be used to identify patients who might benefit from neo-adjuvant chemotherapy [31]. In a large, non-randomized study by Parks et al., adjuvant therapy did seem to improve OS after resection of CRLM [32]. In their study, patients with a high CRS had more benefit from adjuvant therapy than patients with a low CRS. Subsequently, Rahbari et al. performed a similar analysis as in our current study, however, with a different chemotherapy sequence [33].

Instead of neo-adjuvant chemotherapy, they analysed the effect of adjuvant chemotherapy in addition to resection of colorectal liver metastases and stratified patients according to Fong's CRS. The outcome has a striking similarity to our results. In patients with a high CRS, adjuvant chemotherapy was associated with a marked survival advantage, whereas it was of no benefit in patients with a low CRS. Additionally, Adam et al. performed an analysis of the LiverMetSurvey database on patients with solitary, metachronous, primarily resectable metastases. These patients have favourable tumour biology and a low CRS. The authors concluded that these patients do not benefit from preoperative chemotherapy [34]. Finally, Sorby et al. demonstrated in an exploratory retrospective analysis of the EORTC 40983 study that CEA was the strongest baseline predictive factor for the benefit of perioperative FOLFOX [35]. They conclude that moderately and highly elevated CEA serum levels were both predictive for the benefit of perioperative chemotherapy; an obvious explanation would be that elevated CEA is a surrogate for more advanced disease [35]. Again these results suggest a role for CRS's when considering chemotherapy in addition to surgery for CRLM, as delineated in a recently published editorial [36]. Undoubtedly other more sophisticated markers may better stratify patients with regard to outcome. However, cellular, proteomic or genomic markers are not readily available.

This study might be biased due to its non-randomized, retrospective, single-centre design. However, patients were included from a prospective database and differences in terms of the main characteristics did not influence OS. In our current treatment protocol perioperative chemotherapy is not considered to be standard of care for patients with CRLM. We consider patients for neo-adjuvant chemotherapy in case of initially difficult/irresectable liver metastases (ill location) or multiple (≥4) synchronous metastases. This implies that within the high CRS group, baseline characteristics differed between patients treated with and without neo-adjuvant chemotherapy on basis of number of metastases and time to development of metastases (see table 2). Due to our treatment protocol we selected patients with relatively unfavorable tumor biology for chemotherapy, even within the high risk group. However, this proves the significant overall survival benefit in patients with a high risk profile treated with CTx is even more striking. Within the low CRS group on the other hand, patients treated with or without chemotherapy had comparable survival rates. Again, patients within this group who received neo-adjuvant chemotherapy had significantly worse tumour characteristics (see table 1), again (at least partially) generated by our local selection criteria for multimodal therapy. Theoretically, these patients might have had poorer survival rates without neo-adjuvant chemotherapy. Therefore, this study at best suggests that neo-adjuvant chemotherapy will not improve survival in the low CRS group. Other factors might contribute to the OS benefit we find in high risk patients receiving neo-adjuvant CTx. Recurrent disease was hepatic in most cases for patients treated with neo-adjuvant CTx, in both risk groups (as compared to extra-hepatic). More patients receiving neo-adjuvant CTx for initial CRLM were treated with curative intent for recurrent disease; this difference only reached significance in the high risk group. Whether the location of recurrent disease and possibility to treat

recurrent disease with curative intent is a consequence of neo-adjuvant CTx for initial CRLM remains unknown. Additional prospective studies are needed to explore the exact role of multimodal therapies in patients with a high risk profile.

Since only patients coming to resection were included in this study, there is a potential bias of patients who have progressed under chemotherapy and were therefore not considered surgical candidates. This failure to comply with intention to treat principles is inherent to a retrospective study. However, progression under chemotherapy treatment is rare - 7% in the EORTC 40983 trial [10, 11] – with the availability of effective chemotherapeutics. Therefore we suggest that this phenomenon did not have a major impact on our conclusion. Finally, patients with a high risk profile might not have received chemotherapy for other reasons than pure oncologic risk, such as age and comorbidity.

The findings of this study have prompted a multicentre randomized controlled trial, investigating the effects of neo-adjuvant chemotherapy in high risk patients with resectable colorectal liver metastasis as compared to surgery alone (EudraCT number: 2013-004952-39).

CONCLUSION

In this study we demonstrate that stratifying patients with resectable CRLM according to their clinical risk profile, as described by Fong et al, could provide a useful tool for selecting patients who are most likely to obtain survival benefit from neo-adjuvant chemotherapy. Although the indication for neo-adjuvant chemotherapy may not solely be based on overall survival benefit, we believe it should be included in the decision making process.

CONFLICT OF INTEREST

None

ACKNOWLEDGEMENTS

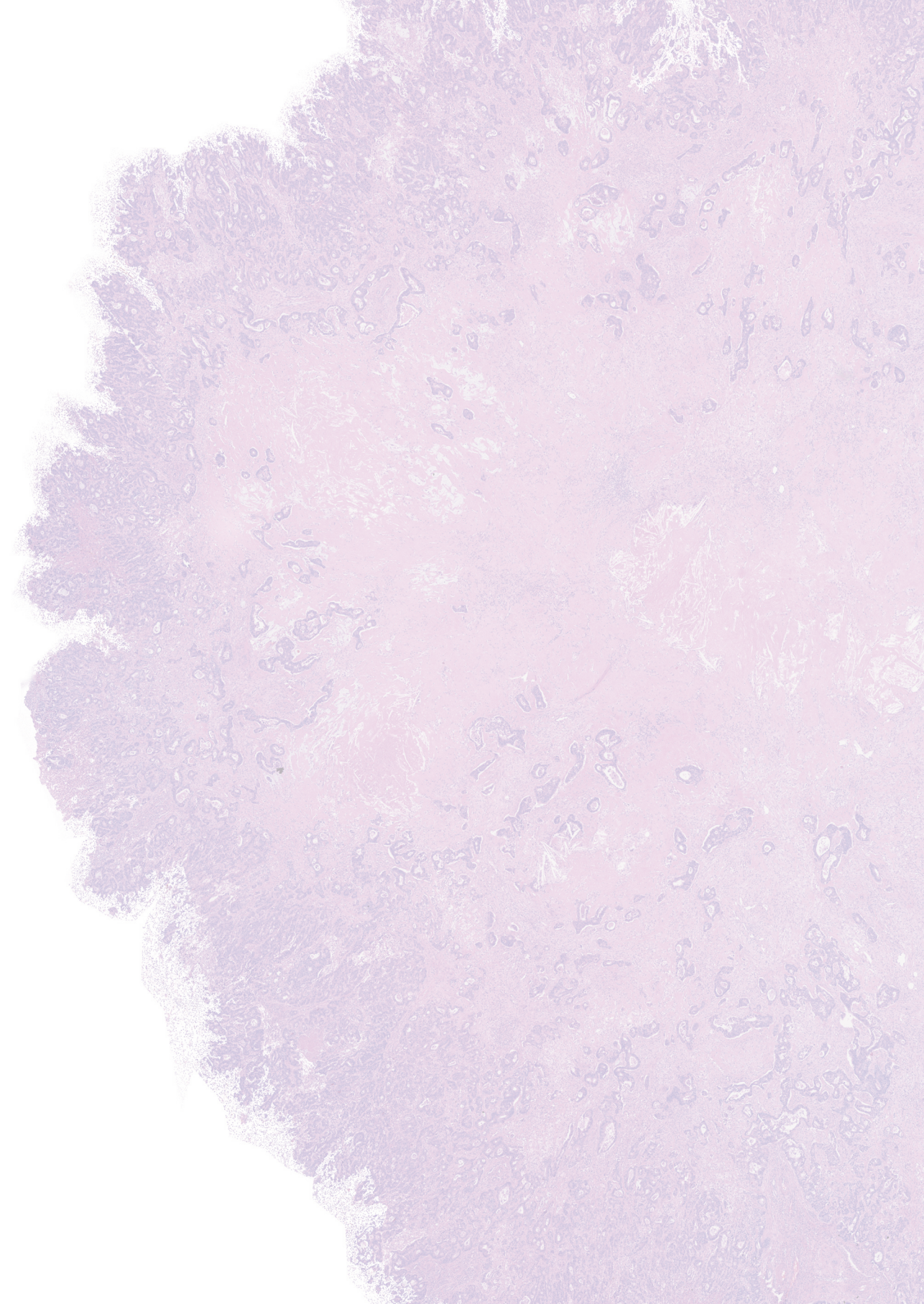
None

REFERENCES

1. Jemal, A., F. Bray, M.M. Center, J. Ferlay, E. Ward, and D. Forman, Global cancer statistics. CA Cancer J Clin, 2011. **61**(2): p. 69-90.
2. de Gramont, A., A. Figer, M. Seymour, et al., Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol, 2000. **18**(16): p. 2938-47.
3. Douillard, J.Y., D. Cunningham, A.D. Roth, et al., Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet, 2000. **355**(9209): p. 1041-7.
4. Saltz, L.B., J.V. Cox, C. Blanke, et al., Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med, 2000. **343**(13): p. 905-14.
5. Rees, M., P.P. Tekkis, F.K. Welsh, T. O'Rourke, and T.G. John, Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. Ann Surg, 2008. **247**(1): p. 125-35.
6. van der Pool, A.E., J.H. de Wilt, Z.S. Lalmahomed, A.M. Eggermont, J.N. Ijzermans, and C. Verhoef, Optimizing the outcome of surgery in patients with rectal cancer and synchronous liver metastases. Br J Surg, 2010. **97**(3): p. 383-90.
7. Petrelli, N.J., Perioperative or adjuvant therapy for resectable colorectal hepatic metastases. J Clin Oncol, 2008. **26**(30): p. 4862-3.
8. Mitry, E., A.L. Fields, H. Bleiberg, et al., Adjuvant chemotherapy after potentially curative resection of metastases from colorectal cancer: a pooled analysis of two randomized trials. J Clin Oncol, 2008. **26**(30): p. 4906-11.
9. Portier, G., D. Elias, O. Bouche, et al., Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial. J Clin Oncol, 2006. **24**(31): p. 4976-82.
10. Nordlinger, B., H. Sorbye, B. Glimelius, 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**(9617): p. 1007-16.
11. Nordlinger, B., H. Sorbye, B. Glimelius, 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): p. 1208-15.
12. Benoist, S. and B. Nordlinger, The role of preoperative chemotherapy in patients with resectable colorectal liver metastases. Ann Surg Oncol, 2009. **16**(9): p. 2385-90.
13. Minagawa, M., J. Yamamoto, T. Kosuge, Y. Matsuyama, S. Miyagawa, and M. Makuuchi, Simplified staging system for predicting the prognosis of patients with resectable liver metastasis: development and validation. Arch Surg, 2007. **142**(3): p. 269-76; discussion 277.
14. Nordlinger, B., M. Guiguet, J.C. Vaillant, et al., Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. Cancer, 1996. **77**(7): p. 1254-62.

15. Fong, Y., J. Fortner, R.L. Sun, M.F. Brennan, and L.H. Blumgart, Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
16. Iwatsuki, S., I. Dvorchik, J.R. Madariaga, et al., Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. J Am Coll Surg, 1999. **189**(3): p. 291-9.
17. Nagashima, I., T. Takada, K. Matsuda, et al., A new scoring system to classify patients with colorectal liver metastases: proposal of criteria to select candidates for hepatic resection. J Hepatobiliary Pancreat Surg, 2004. **11**(2): p. 79-83.
18. Konopke, R., S. Kersting, M. Distler, et al., Prognostic factors and evaluation of a clinical score for predicting survival after resection of colorectal liver metastases. Liver Int, 2009. **29**(1): p. 89-102.
19. Mala, T., G. Bohler, O. Mathisen, A. Bergan, and O. Soreide, Hepatic resection for colorectal metastases: can preoperative scoring predict patient outcome? World J Surg, 2002. **26**(11): p. 1348-53.
20. Reissfelder, C., N.N. Rahbari, M. Koch, et al., Validation of prognostic scoring systems for patients undergoing resection of colorectal cancer liver metastases. Ann Surg Oncol, 2009. **16**(12): p. 3279-88.
21. Eisenhauer, E.A., P. Therasse, J. Bogaerts, et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer, 2009. **45**(2): p. 228-47.
22. Tournigand, C., T. Andre, E. Achille, et al., FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol, 2004. **22**(2): p. 229-37.
23. Seymour, M.T., T.S. Maughan, J.A. Ledermann, et al., Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet, 2007. **370**(9582): p. 143-52.
24. Koopman, M., N.F. Antonini, J. Douma, et al., Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet, 2007. **370**(9582): p. 135-42.
25. Hurwitz, H., L. Fehrenbacher, W. Novotny, et al., Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med, 2004. **350**(23): p. 2335-42.
26. Saltz, L.B., S. Clarke, E. Diaz-Rubio, 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): p. 2013-9.
27. Van Cutsem, E., C.H. Kohne, E. Hitre, et al., Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med, 2009. **360**(14): p. 1408-17.
28. Douillard, J.Y., S. Siena, J. Cassidy, et al., Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol, 2010. **28**(31): p. 4697-705.
29. Douillard, J.Y., S. Siena, J. Cassidy, 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): p. 1346-55.

30. O'Connell, M.J., M.A. Adson, A.J. Schutt, J. Rubin, C.G. Moertel, and D.M. Ilstrup, Clinical trial of adjuvant chemotherapy after surgical resection of colorectal cancer metastatic to the liver. Mayo Clin Proc, 1985. **60**(8): p. 517-20.
31. Tomlinson, J.S., W.R. Jarnagin, R.P. DeMatteo, et al., Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol, 2007. **25**(29): p. 4575-80.
32. Parks, R., M. Gonen, N. Kemeny, et al., Adjuvant chemotherapy improves survival after resection of hepatic colorectal metastases: analysis of data from two continents. J Am Coll Surg, 2007. **204**(5): p. 753-61; discussion 761-3.
33. Rahbari, N.N., C. Reissfelder, H. Schulze-Bergkamen, 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**: p. 174.
34. Adam, R., P. Bhangui, G. Poston, et al., Is perioperative chemotherapy useful for solitary, metachronous, colorectal liver metastases? Ann Surg, 2010. **252**(5): p. 774-87.
35. Sorbye, H., M. Mauer, T. Gruenberger, et al., Predictive factors for the benefit of perioperative FOLFOX for resectable liver metastasis in colorectal cancer patients (EORTC Intergroup Trial 40983). Ann Surg, 2012. **255**(3): p. 534-9.
36. Jarnagin, W.R. and M. D'Angelica, Systemic therapy for patients with resectable hepatic colorectal metastases: improving patient selection. Ann Surg Oncol, 2014. **21**(1): p. 11-2.



CHAPTER 5

The prognostic value of the primary tumor's nodal status after surgery for colorectal liver metastases in the era of effective systemic therapy

E.P. van der Stok
D.J. Grünhagen
W.J. Alberda
M. Reitsma
J. Rothbarth
C. Verhoef

Digestive Surgery. 2015;32(3):208-16

ABSTRACT

BACKGROUND

Nodal status of primary colorectal cancer is of prognostic value for survival after resection for colorectal liver metastases (CRLM). However, the past decade effective adjuvant chemotherapy for lymph node positive primary colon cancer was introduced. This study evaluated the prognostic value of primary lymph node status in patients with resectable metachronous CRLM in the era of effective systemic therapy.

METHODS

Between January 2000 and December 2011, all consecutive patients undergoing curative liver resection for colorectal liver metastases (CRLM) were retrospectively analyzed. Overall survival (OS) was analyzed by localization of the primary tumor (colon vs. rectum) and by lymph node status (positive vs. negative) of the primary tumor.

RESULTS

286 patients with metachronous CRLM's were selected. 5-year OS was similar for colon and rectal primaries (42% and 40%, P=0.62). Lymph node positivity was only a prognostic factor in rectal primaries (N + 32% vs. N0 49% P=0.04) and not in colon primaries (N+ 42% vs. N0 41% P=0.99). In multivariate analysis, these results were confirmed.

CONCLUSION

The current study demonstrates that nodal status of primary colon malignancies does not have prognostic value in patients undergoing resection for metachronous CRLM. A possible explanation might be the administration of effective adjuvant chemotherapy in node positive colon cancer.

INTRODUCTION

Approximately half of patients suffering from colorectal cancer will develop metastases, with the liver being prone to distant disease progression. In approximately 20 percent of patients with colorectal malignancies, hepatic metastases are found at time of diagnosis, while another 30 percent of patients develop metachronous disease [1, 2]. Currently, surgical resection of colorectal liver metastases (CRLM) is the most effective therapy, providing the only potential for cure. With the contemporary surgical management of CRLM, patients experience 40-60 percent 5-year overall survival (OS) [3-7]. However, the management of colorectal cancer and CRLM continues to evolve, with modern multimodality therapies improving outcome.

Over time, multiple clinical risk scores (CRS) were developed to determine the outcome after surgery for CRLM [8-11]. Fong's CRS is the most widely used and validated [8]. In this CRS, five prognostic factors for OS are identified: node positive primary tumor, disease free interval <12 months, CEA levels >200µg/L, number of metastases >1 and size of metastases >5 cm. Initially, this CRS was designed to select patients who may benefit from CRLM resection. In addition, these criteria proved to be useful for stratifying patients in clinical trials. In most CRS's, lymph node positivity of the primary tumor is of prognostic value after surgery for CRLM [8, 9, 11].

In the era of effective systemic for colorectal cancer, traditional clinical risk scores as such may no longer provide a reliable prognostic tool. In the past two decades treatment strategies and surgical techniques for primary colorectal cancer and CRLM has altered significantly. Regarding primary colorectal tumors, two major developments have reshaped outcome of colorectal cancer patients: 1) the introduction of total mesorectal excision with or without neo-adjuvant (chemo-) radiation in rectal cancer [12-14]; and 2) the introduction of effective adjuvant chemotherapy in colon cancer [15]. These developments might influence the prognostic value of primary-tumor-related clinical variables specifically, such as lymph node status. In the Netherlands, adjuvant chemotherapy is reserved for lymph node positive primary colon malignancies, in contrast to lymph node positive rectal cancer where no adjuvant therapy is administered. Thus, localization of the primary tumor may indirectly influence the prognostic value of lymph node status. The purpose of this study is to evaluate specifically the prognostic value of nodal status in primary colorectal tumors in the modern era of adjuvant systemic chemotherapy.

PATIENTS AND METHODS

Between January 2000 and December 2011, all consecutive patients undergoing resection of CRLM in Erasmus MC Cancer Institute, a tertiary referral center, were retrospectively analyzed. Only patients with metachronous CRLM were selected. In our database, metachronous

disease is characterized by metastases occurring >3 months after diagnosis of primary tumor. As we aimed for a study population of patients in which we could test for the possible impact of adjuvant chemotherapy on node positive primaries, we needed to exclude all synchronous metastases.

Patient characteristics were collected retrospectively from a prospectively maintained database. Data in our database comprises: gender, age, primary tumor site, primary tumor pathological and lymph node stage (pTN), carcinoembryonic antigen (CEA), interval between resection of primary tumor and detection of liver metastases, location, distribution, size and number of liver metastases. Furthermore, type of liver surgery, radicality of surgery (R1, R0), extrahepatic disease, adjuvant treatment of primary tumor, neo-adjuvant chemotherapy for CRLM, and neo-adjuvant (chemo) radiotherapy for primary rectal malignancies were listed.

In our center, patients with locally advanced rectal cancer have been treated with long course scheme neo-adjuvant radiotherapy: 45–50 Gy (in fractions of 1.8–2 Gy) with or without chemotherapy (capecitabine 825 mg/m2 twice a day only on radiotherapy days). For intermediate-risk rectal cancer, short course neo adjuvant radiotherapy is administered: 25 Gy (in fractions of 5 Gy). On basis of high age and/or comorbidities, short course pre-operative radiotherapy was considered.

Most resections of primary tumors were performed in referring hospitals, as ours is a tertiary referral center for liver surgery. Lymph node harvesting during colorectal surgery is, as in many other countries, standardized by Dutch guidelines. In the yearly Dutch Surgical Colorectal Audit (DSCA), nodal harvesting is delineated as a quality indicator of colorectal cancer care. According to the Dutch Institute for Clinical Auditing, at least 10 lymph nodes were analyzed by the pathologist in > 91% of patients that underwent surgery for colon cancer nationwide [16]. Although resections of primary tumors in our patient cohort generally occurred outside our institution, lymph node harvesting was performed in a standardized process resulting in a consistent number of nodes analyzed by a pathologist.

Liver resections were performed by a single or two-stage procedure and included wedge resections, segmental resections, hemihepatectomies and radiofrequency ablations (RFA). Hepatic parenchymal resection was performed with an ultrasonic surgical aspirator and a monopolar coagulator. R0-resections were defined by the absence of microscopic tumor invasion of the resection margins, and R1-resections were defined by the presence or microscopic tumor invasion of the resection margins.

During follow-up, patients visited the outpatient clinic every 3 months in the first 2 years after CRLM resection for clinical examination and CEA-determination. Thereafter, patients visited the outpatient clinic every 6 months and were discharged from follow up after 5 years. Abdominal imaging (ultrasound, CT of thorax and abdomen) was performed biannually during the first 3 years and thereafter annually. If disease recurred, a decision on whether to initiate chemotherapy treatment (again) or to perform a second resection was made by the multidisciplinary team.

Overall survival (OS) was defined as the interval in months between resection of the first

CRLM and death, or the date of last follow-up.

STATISTICAL ANALYSIS

Descriptive values are expressed as median (interquartile range (IQR)). Categorical data are presented as numbers (percentage frequencies). Differences between subgroups were compared by the usage of chi-square tests (χ^2) or Fisher's exact tests as appropriate. Continuous data with a skewed distribution are displayed as medians and compared with the Mann-Whitney U test. Overall survival was calculated by Kaplan-Meier curves and log-rank tests were used to examine any differences in survival rates. The prognostic value of variables was calculated by Cox's proportional hazard models. For the multivariate analysis only parameters with a P-value less than 0.40 in the univariate model were entered in the Cox regression model. P-values less than 0.05 were considered significant. All statistical analysis was performed by using SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Between January 2000 and December 2011 a total of 623 patients underwent CRLM resection with curative intent, of which 293 patients (47%) presented with metachronous CRLM. In 7 patients primary lymph node status was unknown, leaving 286 patients eligible for analysis. The median follow up was 36 months (IQR 21-57). Primary tumor localization was colon in 165 patients and rectum in 121 patients. A total of 145 patients (51%) had a positive lymph node status of the primary tumor.

The baseline patient and tumor characteristics of both primary colon and rectal malignancies are outlined in table 1 and 2. In primary colon malignancies, patient- and tumor characteristics of patients with liver metastases from a lymph node positive versus lymph node negative tumor differed on basis of administration of adjuvant chemotherapy (90% vs. 4%, P<0.001) and Fong's clinical risk profile (high risk: 38% vs. 8%, P<0.001), respectively. Characteristic of patients with liver metastases from a primary node positive vs. node negative rectal malignancy differed on basis of tumor stage (T3/T4: 87% vs. 53%, P<0.001) adjuvant systemic therapy for primary (13% vs. 2%, P=0.02), neo-adjuvant systemic therapy for CRLM (N+ 29% vs. N0 14%, P=0.05) and again Fong's risk profile (high risk 31% vs. 12%, P=0,01), respectively.

Table 1: Patient characteristics by primary tumor location							
Variable	**Colon (N=165)**		**Rectum (N=121)**		**P-value**	**All patients (N=286)**	
	Value	**% or IQR**	**Value**	**% or IQR**	**p**	**Value**	**% or IQR**
Male	97	59%	77	64%	0,407	174	61%
Age, median	65	58-73	64	60-69	0,782	64	59-72
Primary tumor							
T3/T4	140	86%	85	0,71	0,002	225	80%
Lymph node positive	82	50%	63	52%	0,692	145	51%
Adjuvant CTx	72	45%	9	0,07	<0,001	81	29%
Liver metastases							
DFI < 12 months	57	35%	48	40%	0,374	105	37%
Number metastases > 1	83	50%	53	44%	0,306	136	48%
Diameter metastases > 5	43	26%	31	26%	0,918	74	26%
CEA > 200 µg/L	9	6%	13	11%	0,156	22	9%
Bilobair distribution	52	32%	38	32%	0,984	90	32%
Fong 3-5	37	23%	26	22%	0,801	63	23%
Neo-adjuvant CTx	46	28%	26	22%	0,219	72	25%

ADJUVANT CHEMOTHERAPY IN LYMPH NODE POSITIVE COLON CANCER

Adjuvant chemotherapy (primary) was administered to patients with stage III colon carcinoma according to Dutch national guidelines. From 82 node positive primary colon cancers, 69 patients (90%) were treated with adjuvant chemotherapy. 13 patients with lymph node positive colon cancer (16%) did not receive adjuvant chemotherapy. In 5 cases reasons for not administering adjuvant chemotherapy were unknown. In the remaining 8 cases reasons were: refusal (n=2), complications of surgery (n=2), age (n=1), palliative chemotherapy due to irradical resection (n=1), operation primary in 1988 prior to guideline initiation (n=1), chronic use of immunosuppressant (n=1). Chemotherapeutic regimens consisted mainly of 5FU/LV/Xeloda with or without oxaliplatin. Median number of adjuvant cycles for lymph node positive colon carcinoma was 7 (IQR 6-11).

NEO-ADJUVANT CHEMO- (RADIO-) THERAPY FOR LOCALLY ADVANCED RECTAL CANCER

Of all patients with CRLM from a primary rectal malignancy, 30% (N=33) received long course (chemo-) radiotherapy (CtRtx). There was no difference in administration of long course CtRtx between node positive versus node negative rectal primaries (N+: 19%, N0: 32%, P=0.74). In node positive rectal primaries, median survival did not differ between patients undergoing long course CtRtx (30 months, 95% CI 20-40) vs short CtRtx or no CtRtx (43 months, 95% CI 25-61, P=0,53). In all patients with CRLM from rectal primaries that received long course CtRtx, no median survival difference was found between node positive and node negative patients (N+: 30 months, 95% CI 20-40 vs. N0: median not reached at 5 years, P=0,325).

NEO-ADJUVANT CHEMOTHERAPY FOR CRLM

In our current protocol, perioperative chemotherapy is not considered as standard treatment for patients with CRLM. A large proportion of patients in this study received neo-adjuvant chemotherapy in the referring hospital. The reason for administering one type of chemotherapy over another was based on local treatment protocols. All patients received a combination of 5-fluorouracil (5-FU)/Capecitabine and Oxaliplatin or Irinotecan, with or without Bevacizumab.

Neo-adjuvant systemic therapy for CRLM was administered in 25% of all patients. There was no difference in engagement of neo-adjuvant chemotherapy for CRLM between primary colon and rectal malignancies (28% vs. 22%, P=0.22).

OVERALL AND DISEASE-FREE SURVIVAL

The median survival of all patients after resection of CRLM was 45 months (95% CI 39-51) with an estimated 5-year overall survival of 41%. The estimated median survival of patients with primary colon cancer was 47 months (95% CI 41-53) versus 43 months (95% CI 36-50) in primary rectal cancer. The estimated 5-year overall survival of colon cancer patients was 42% and rectal cancer patients was 40%. This did not differ significantly (P=0.62) (Figure 1). The estimated median disease-free survival of patients with primary colon cancer was 16 months (95% CI 11-21) and did not differ significantly from the median disease-free survival of patients with rectal cancer (13 months, 95% CI 7-19, P=0.36).

Table 2: Patient characteristics by primary tumor's nodal status							
Variable	**Colon N0 (N=83)**		**Colon N+ (N=82)**		**P-value**	**All patients (N=165)**	
	Value	**% or IQR**	**Value**	**% or IQR**	**p**	**Value**	**% or IQR**
Male	53	64%	44	54%	0,183	97	59%
Age, median	66	59-74	64	57-71	0,48	65	58-73
Primary tumor							
T3/T4	67	83%	73	89%	0,247	140	86%
Adjuvant CTx	3	0,04	69	0,9	< 0,001	72	45%
Liver metastases							
DFI < 12 months	26	31%	31	38%	0,381	57	35%
Number metastases > 1	42	51%	41	50%	0,938	83	50%
Diameter metastases > 5	23	28%	20	25%	0,627	43	26%
CEA > 200 µg/L	5	7%	4	6%	0,681	9	6%
Bilobair distribution	29	35%	23	28%	0,341	52	32%
Fong 3-5	6	0,08	31	0,38	< 0,001	32	22%
Neo-adjuvant CTx	28	34%	18	22%	0,091	46	28%
Variable	**Rectum N0 (N=58)**		**Rectum N+ (N=63)**		**P-value**	**All patients (N=121)**	
	Value	**% or IQR**	**Value**	**% or IQR**	**p**	**Value**	**% or IQR**
Male	38	66%	39	62	0,68	77	64
Age, median	65		63		0,536	64	60-69
Primary tumor							
T3/T4	31	0,53	54	0,87	< 0,001	85	71%
Neo-adjuvant RTx, long course	17	32%	16	29%	0,74	33	30%
Adjuvant CTx	1	0,02	8	0,13	0,022	9	7%
Liver metastases							
DFI < 12 months	24	41%	24	38%	0,712	48	40%
Number metastases > 1	24	41%	29	47%	0,552	53	44%
Diameter metastases > 5	16	28%	15	24%	0,671	31	26%
CEA > 200 µg/L	8	15%	5	9%	0,308	13	11%
Bilobair distribution	16	28%	22	35%	0,385	38	32%
Fong 3-5	7	0,12	19	0,31	0,012	26	22%
Neo-adjuvant CTx	8	0,14	18	0,29	0,048	26	22%

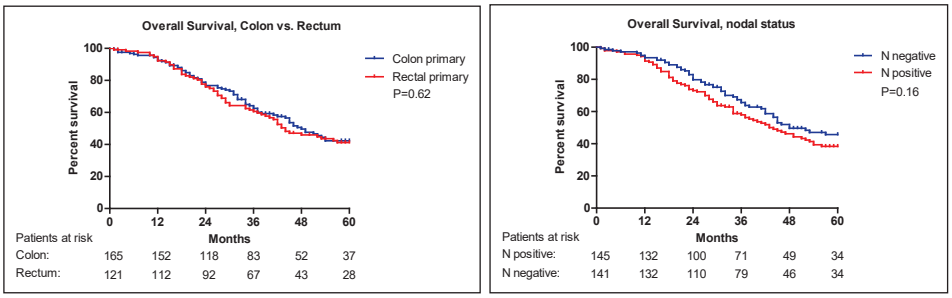


Figure 1. Overall survival in all patients by primary tumor location and nodal status

OVERALL SURVIVAL AND DISEASE-FREE SURVIVAL BY LYMPH NODE STATUS

For all patients, the estimated 5-year overall survival was 38% in lymph node positive patients versus 45% in lymph node negative patients (P=0.16) (figure 1). In patients with metastasis from a primary colon malignancy, the estimated 5-year overall survival was 42% in lymph node positive patients versus 41% in lymph node negative patients (P=0.991) (figure 2). In patients with liver metastases form a lymph node positive rectal tumor; the 5-year survival was 32% versus 49% in node negative rectal cancer (P=0.04) (figure 2).

The estimated median disease-free survival for lymph node positive colon cancer (19 months 95% CI 11-27) was similar to lymph node negative colon cancer (16 months 95% CI 13-19, P=0.54) (figure 2). In node positive rectal cancer estimated median disease-free survival was 9 months (95%CI 6-12) versus 14 (95% CI 6-22) in node negative rectal cancer (P=0.05) (figure 2).

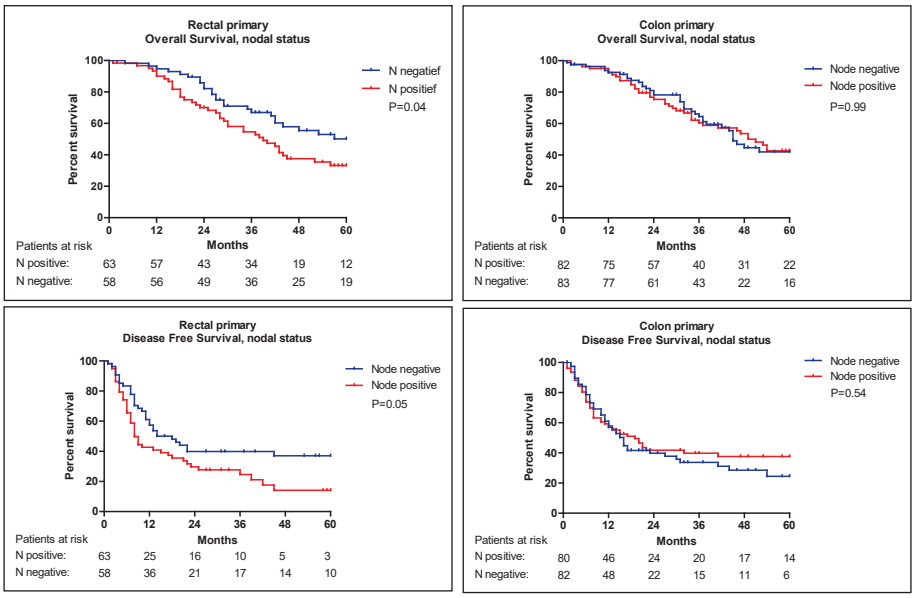


Figure 2. Nodal status in patients with CRLM from colon primary and rectal primary malignancies: disease free survival and overall survival

PROGNOSTIC VALUE OF INDIVIDUAL PARAMETERS IN FONG'S CRS

None of Fong's individual variables, other than nodal status of primary tumor, proved to be of prognostic value in the cohort of patients included in this study. However, adding up the points to Fong's CRS, differentiating between patients with a high and low risk profile, confirmed its prognostic value: 5-year OS in low risk patients (CRS 0-2) 43% vs. 33% in high-risk patients (CRS 3-5), P=0.03, (figure 3).

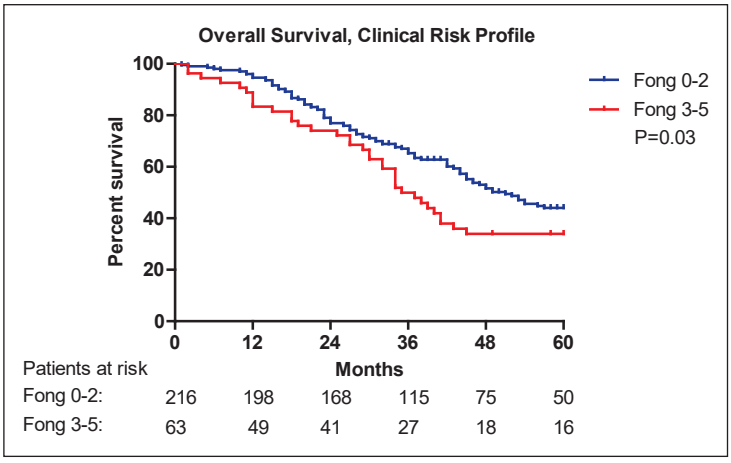


Figure 3. High and low clinical risk profile as described by Fong's CRS: overall survival

UNIVARIATE AND MULTIVARIATE ANALYSES

In patients with liver metastases from a primary colon malignancy, the univariate analysis in relation to OS showed 7 factors with a P-value of less than 0.40 (table 3). Adjuvant CTx highly correlated with node positivity (Pearson product-moment correlation coefficient = 0,864) and could not be entered in the multivariate analysis. From the 6 variables in multivariate analysis, only age was prognostic for OS (Table 3).

In patients with liver metastases from a primary rectal malignancy, the univariate analysis in relation to OS showed 5 factors with a P-value of less than 0.40. In multivariate analysis only the primary tumor's nodal status was prognostic for OS: HR 1,942 (95% CI 1,105-3,412; P=0,021) (Table 3).

Table 3: Univariate analysis, overall survival

		Colon primary (N=165)			Rectal primary (N=121)		
Variable		**Median Survival (95% CI)**	**Univariate HR (95%CI) P-value***	**Multivariate HR (95% CI)**	**Median Survival (95% CI)**	**Univariate HR (95% CI) P-value***	**Multivariate HR (95% CI)**
Gender							
	Male	45 (38-52)	1,175 (0,747-1,846)	-	53 (35-71)	0,822 (0,505-1,340)	-
	Female	51 (43-59)	P=0,486		42 (35-49)	P=0,433	
Age (mean)		-	1,017 (0,997-1,038) P=0,103	1,022 (1,002-1,042) P=0,033	-	0,999 (0,973-1,026) P=0,962	-
Primary tumor							
T-stage	T 0-2	NR	0,516 (0,248-1,074)	0,816 (0,443-1,505)	NR	0,773 (0,444-1,344)	0,915 (0,498-1,168)
	T 3-4	45 (35-55)	P=0,077	P=0,516	42 (35-48)	P=0,362	P=0,774
Lymph node	Positive	49 (38-60)	0,998 (0,641-1,553)	0,761 (0,466-1,243)	38 (26-50)	1,662 (1,014-2,724)	1,942 (1,105-3,412
	Negative	45 (39-51)	P=0,992	P=0,275	NR	P=0,040	P=0,021
Adjuvant CTx	Yes	37 (23-51)	1,458 (0,934-2,276)	-	56 (0-138)	1,110 (0,446-2,766)	-
	No	46 (39-53)	0,097		43 (36-50)	P=0,823	
Neo-adjuvant	Yes	-	-	-	39 (25-53)	1,314 (0,778-2,219)	1,359 (0,768-2,404)
long CtRtx	No	-	-	-	52 (38-66)	P=0,307	P=0,292
Liver Metastases							
Disease free interval							
	< 12 months	45 (35-60)	0,992 (0,623-1,580)	-	40 (32-48)	1,204 (0,740-1,959)	-
	> 12 months	48 (36-60)	P=0,975		45 (30-60)	P=0,454	
Largest size metastases (cm)							
	> 5	37 (21-53)	1,238 (0,766-2,001)	1,169 (0,735-1,861)	36 (21-51)	1,333 (0,781-2,275)	1,572 (0,860-2,873)
	< 5	49 (43-55)	P=0,384	P=0,509	44 (31-57)	P=0,292	P=0,142
Number metastases							
	> 1	44 (36-52)	1,210 (0,776-1,885)	-	40 (27-53)	1,219 (0,747-1,990)	-
	1	NR	P=0,400		45 (35-55)	P=0,428	
CEA level µg/L							
	> 200	32 (12-52)	1,053 (0,424-2,614)	-	38 (33-43)	1,309 (0,644-2,661)	-
	< 200	47 (41-53)	P=0,911		45 (33-57)	P=0,456	
Tumour distribution							
	Bilobar	38 (29-47)	1,289 (0,812-1,046)	1,244 (0,745-2,077)	39 (27-51)	1,146 (0,682-1,926)	-
	Unilobar	51 (44-58)	P=0,281	P=0,404	45 (36-54)	P=0,606	
Clinical Risk Score							
	3-5	34 (30-38)	1,545 (0,950-2,511)	1,527 (0,878-2,653)	38 (25-51)	1,425 (0,819-2,480)	1,070 (0,573-1,997)
	0-2	51 (44-58	P=0,079	P=0,134	48 (35-61)	P=0,210	P=0,901
Chemotherapy							
	Yes	NR	0,680 (0,398-1,164)	0,663 (0,386-1,138)	40 (30-50)	1,098 (0,617-0,953)	-
	No	46 (36-56)	P=0,160	P=0,136	44 (32-56)	P=0,752	

NR *Not Reached,* ***CEA*** *Carcinoembryonic Antigen, Univariate p value < 0.40 included in Multivariate analysis with a maximum of 1 variable per 10 event*

DISCUSSION

In this study, we specifically evaluated the prognostic value of primary tumor nodal status for survival after resection of CRLM. In different CRS's, independent variables for survival after resection for CRLM have been established, however, before the current era of effective systemic therapy. The most widely used and validated CRS is Fong's (1999). The relevance of CRS's has already been questioned with respect to peri-operative chemotherapy for CRLM [17]. Ayez et al. showed that neo-adjuvant chemotherapy for CRLM downstaged tumor size and CEA levels, which in turn changes the CRS. Correspondingly, adjuvant systemic therapy for lymph node positive primary colorectal cancer might specifically affect the prognostic importance of the lymph node status. In this study, patients who underwent surgery for CRLM, and of whom the primary tumor was treated with effective systemic therapy, were analyzed. We showed that Fong's CRS (high versus low risk) by itself still proved to be of prognostic value. However, lymph node status in patients with liver metastasis from a primary colon malignancy lost its prognostic value for OS, in contrast to lymph node status in patients with metastases of a rectal malignancy. This finding may be explained by the standard administration of adjuvant chemotherapy in lymph node positive primary colon cancer, in contrast to rectal cancer where no adjuvant treatment is adopted in the Netherlands.

Our findings are supported by a recently published study of Thomay et al. [18], which aimed to determine the prognostic value of the number of regional lymph node metastases in patients with stage IV colorectal cancer. The number of regional lymph node metastases correlated with survival among patients undergoing resection of CRLM, but lost prognostic significance in the subset of patients who underwent hepatectomy with peri-operative oxaliplatin- or irinotecan- based chemotherapy. As in our study, systemic treatment possibly devaluates the prognostic capacity of primary tumor lymph node status for survival after surgery for CRLM. The adjuvant treatment discrepancy between node positive colon and node positive rectal primaries in the Netherlands provided two different patient cohorts with liver metastases, in which the effect of modern multimodal therapy on the prognostic value of lymph nodes could be assessed. Our results suggest that modern chemotherapy in colon cancer nullifies the negative prognostic effect of lymph node status in these patients specifically. This difference cannot be explained by lower survival rates on basis of primary tumor localization: 5-year survival rates by primary tumor in our cohort are 42% for colon and 40% for rectal cancer (P=0.62), in concordance with literature [19]. An alternative explanation for the difference in prognostic value of lymph node status between colon and rectal primary might be the effect of "stage migration" in rectal cancer, caused by neo-adjuvant (chemo-) radiotherapy. Long course (chemo-) radiotherapy leads to tumor downstaging and less positive lymph nodes [12, 13, 20]. Therefore, patients with positive lymph nodes after (chemo-) radiotherapy might have a more aggressive tumor biology than lymph node negative patients, which may result in a survival difference. However, in a subgroup analysis of lymph node positive rectal cancer patients, there was no survival difference between

patients who received "long course" neoadjuvant (chemo-) radiotherapy versus "short course" radiotherapy (5x5Gy) followed by surgery with a short interval or no neoadjuvant radiation at all. Moreover, the administration of long course (chemo-) radiotherapy, short course radiotherapy or no neoadjuvant treatment did not differ significantly between lymph node positive and node negative rectal cancer patients. Thus, the potential effect of stage migration by neo-adjuvant (chemo-) radiotherapy in rectal cancer patients seems limited.

Fong's study was conducted in a cohort of patients undergoing surgery for CRLM in a different era (1984-1998). In both colon and rectal cancer, (neo-) adjuvant therapies were developed over the years. The demonstration that postoperative adjuvant treatment with Fluorocouracil and Levamisole reduced mortality rate by 33 percent among patients with stage III colon cancer [21] prompted multiple trials which initially established Fluorouracil plus Leucovorin (FL) as the standard adjuvant treatment for stage III (lymph node positive) colon cancer [22-27]. Subsequently, the MOSAIC trial [15, 28] confirmed additional survival of 20% was obtained when adding Oxaliplatin to the chemotherapy regimen. Parallel to these developments, total mesorectal excision (TME) in rectal cancer was established which resulted in a drastic decrease in local recurrence rate. Neoadjuvant (chemo-) radiotherapy further decreased local recurrence [8, 29, 30]. In contrast to colon cancer, the effectiveness of adjuvant chemotherapy after rectal surgery is still under debate. Therefore, as mentioned before, no adjuvant chemotherapy is administered for lymph node positive rectal cancer in the Netherlands. Notably, in the literature, despite the difference in adjuvant treatment between colon and rectal cancer, 5 year survival is similar: 58% vs. 59% respectively, for all stages and ages [19].

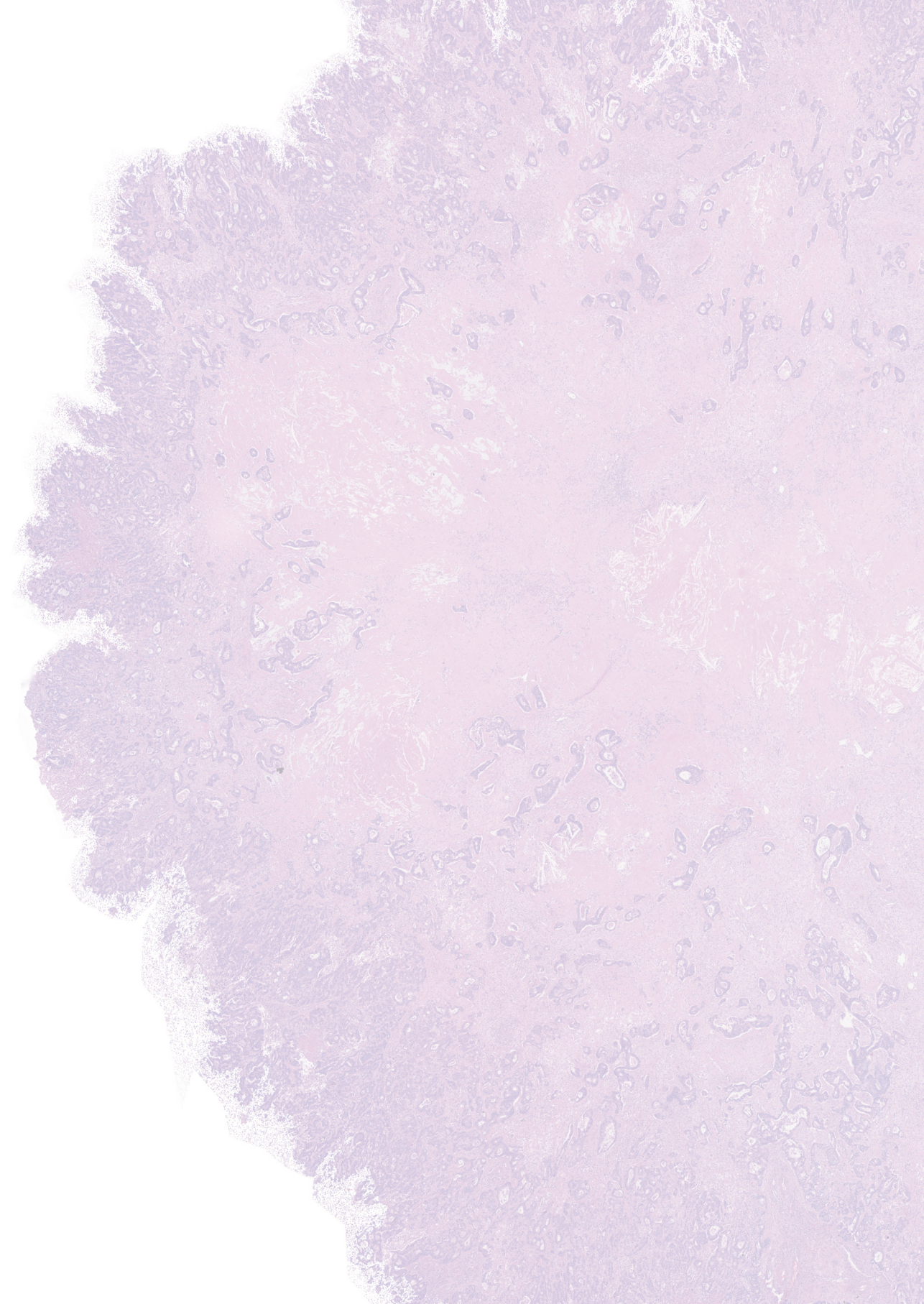
Due to the retrospective nature of this analysis, this study has drawbacks. Only a selected group of patients was considered eligible for CRLM resection and different adjuvant chemotherapeutic regiments were used. Additionally, CRS's might have lost their clinical value, as nowadays surgical resection will take place irrespective of patients risk classification. Nonetheless, CRS's are still useful in clinical research, specifically for stratification of patients in prospective trials.

In conclusion, in patients who underwent resection of CRLM, lymph node status of the primary tumor was not of prognostic value in patients with primary colon cancer. Interestingly, in primary rectal cancer lymph node status was a prognostic factor. This difference may be caused by the administration of effective adjuvant chemotherapy in node positive colon cancer.

REFERENCES

1. Bengmark, S. and L. Hafstrom, The natural history of primary and secondary malignant tumors of the liver. I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy. Cancer, 1969. **23**(1): p. 198-202.
2. van der Pool, A.E., et al., Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series. Colorectal Dis, 2012. **14**(1): p. 56-61.
3. Dols, L.F., et al., [Improvement of 5 year survival rate after liver resection for colorectal metastases between 1984-2006] Verbetering in 5-jaarsoverleving na leverresectie voor colorectale metastasen tussen 1984-2006. Ned Tijdschr Geneeskd, 2009. **153**(11): p. 490-5.
4. Primrose, J.N., Surgery for colorectal liver metastases. Br J Cancer, 2010. **102**(9): p. 1313-8.
5. Rees, M., et al., Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. Ann Surg, 2008. **247**(1): p. 125-35.
6. van der Pool, A.E., et al., Optimizing the outcome of surgery in patients with rectal cancer and synchronous liver metastases. Br J Surg, 2010. **97**(3): p. 383-90.
7. Ayez, N., et al., Long-term results of the "liver first" approach in patients with locally advanced rectal cancer and synchronous liver metastases. Dis Colon Rectum, 2013. **56**(3): p. 281-7.
8. Fong, Y., et al., Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
9. Nordlinger, B., et al., Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. Cancer, 1996. **77**(7): p. 1254-62.
10. Iwatsuki, S., et al., Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. J Am Coll Surg, 1999. **189**(3): p. 291-9.
11. Nagashima, I., et al., A new scoring system to classify patients with colorectal liver metastases: proposal of criteria to select candidates for hepatic resection. J Hepatobiliary Pancreat Surg, 2004. **11**(2): p. 79-83.
12. Bosset, J.F., et al., Chemotherapy with preoperative radiotherapy in rectal cancer. N Engl J Med, 2006. **355**(11): p. 1114-23.
13. Gerard, J.P., et al., Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. J Clin Oncol, 2006. **24**(28): p. 4620-5.
14. Kapiteijn, E., et al., Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med, 2001. **345**(9): p. 638-46.
15. Andre, T., et al., Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol, 2009. **27**(19): p. 3109-16.
16. Dutch Institute for Clinical Auditing, Dutch Surgical Colorectal Audit.
17. Ayez, N., 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): p. 2757-63.

18. Thomay, A.A., et al., Modern chemotherapy mitigates adverse prognostic effect of regional nodal metastases in stage IV colorectal cancer. J Gastrointest Surg, 2014. **18**(1): p. 69-74.
19. Lemmens, V., et al., Trends in colorectal cancer in the south of the Netherlands 1975-2007: rectal cancer survival levels with colon cancer survival. Acta Oncol, 2010. **49**(6): p. 784-96.
20. Gerard, A., et al., Preoperative radiotherapy as adjuvant treatment in rectal cancer. Final results of a randomized study of the European Organization for Research and Treatment of Cancer (EORTC). Ann Surg, 1988. **208**(5): p. 606-14.
21. Moertel, C.G., et al., Levamisole and Fluorouracil for Adjuvant Therapy of Resected Colon-Carcinoma. New England Journal of Medicine, 1990. **322**(6): p. 352-358.
22. Wolmark, N., et al., The benefit of leucovorin-modulated fluorouracil as postoperative adjuvant therapy for primary colon cancer: results from National Surgical Adjuvant Breast and Bowel Project protocol C-03. J Clin Oncol, 1993. **11**(10): p. 1879-87.
23. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. Lancet, 1995. **345**(8955): p. 939-44.
24. O'Connell, M.J., et al., Controlled trial of fluorouracil and low-dose leucovorin given for 6 months as postoperative adjuvant therapy for colon cancer. J Clin Oncol, 1997. **15**(1): p. 246-50.
25. Zaniboni, A., Adjuvant chemotherapy in colorectal cancer with high-dose leucovorin and fluorouracil: impact on disease-free survival and overall survival. J Clin Oncol, 1997. **15**(6): p. 2432-41.
26. O'Connell, M.J., et al., Prospectively randomized trial of postoperative adjuvant chemotherapy in patients with high-risk colon cancer. J Clin Oncol, 1998. **16**(1): p. 295-300.
27. Wolmark, N., et al., Clinical trial to assess the relative efficacy of fluorouracil and leucovorin, fluorouracil and levamisole, and fluorouracil, leucovorin, and levamisole in patients with Dukes' B and C carcinoma of the colon: results from National Surgical Adjuvant Breast and Bowel Project C-04. J Clin Oncol, 1999. **17**(11): p. 3553-9.
28. Andre, T., et al., Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med, 2004. **350**(23): p. 2343-51.
29. Pahlman, L. and B. Glimelius, Radiotherapy additional to surgery in the management of primary rectal carcinoma. Acta Chir Scand, 1990. **156**(6-7): p. 475-85.
30. Frykholm, G.J., B. Glimelius, and L. Pahlman, Preoperative or postoperative irradiation in adenocarcinoma of the rectum: final treatment results of a randomized trial and an evaluation of late secondary effects. Dis Colon Rectum, 1993. **36**(6): p. 564-72.



CHAPTER 6

The prognostic value of post-operative serum C-reactive protein level for survival after surgery for colorectal liver metastases

E.P. van der Stok
D.J. Grünhagen
J. Rothbarth
C. Verhoef

Acta Chirurgica Belgica. 2015 Sep-Oct;115(5):348-55

ABSTRACT

BACKGROUND

Increasing emphasis is put on the concept that inflammation is a key player in tumor progression. In the tumor microenvironment, inflammatory cells mediate tumor growth. Elevated C-reactive protein (CRP) levels are identified as being representative of a systemic inflammatory response. Therefore, studies have successfully linked peri-operative CRP levels to survival after surgery for primary colorectal cancer. The aim of this study was to investigate the prognostic value of the post-operative systemic inflammatory response as represented by serum CRP levels after resection of colorectal liver metastases (CRLM).

METHODS

Between January 2004 and December 2012, all patients who underwent resection for CRLM were analyzed. The total post-operative acute inflammatory response was objectified by the area under the curve (AUC, trapezium rule). Peak CRP concentrations were determined. The impact of peak CRP values and total CRP response on disease free survival (DFS) and overall survival (OS) was analyzed; patients were stratified by clinical risk score and/or administration of neo-adjuvant chemotherapy.

RESULTS

The final study population consisted of 403 patients. The OS of patients with a high CRP response (AUC, upper quartile) was equal to patients with intermediate (AUC, middle quartiles) or low (AUC, lower quartile) responses. Similarly, total post-operative CRP response did not impact survival when stratifying patients for CRS and/or administration of neo-adjuvant chemotherapy. Peak CRP concentrations did not impact survival accordantly.

CONCLUSION

Total post-operative inflammatory response, as evidenced by CRP serum levels, had no prognostic value for survival after surgery for CRLM.

ABBREVIATIONS

AUC:	Area Under the Curve
CEA:	CarcinoEmbryonic Antigen
CRP:	C-Reactive Protein
CRLM:	ColoRectal Liver Metastases
CRS:	Clinical Risk Score
CTx:	Chemotherapy
DFS:	Disease Free Survival
OS:	Overall Survival

INTRODUCTION

Over the past decades, the concept that inflammation is fundamental for tumor development has been extended. Various studies describe the tumor microenvironment as a crucial partaker in the neoplastic process, where inflammatory cells nurture proliferation, survival and migration of tumor cells [1-3]. Given the link between inflammation and cancer, multiple studies investigated the importance of a systemic inflammatory response evidenced by elevated CRP levels in patients with advanced cancers [4-6], as well as patients undergoing resection of primary colorectal cancer. Several studies showed that a perioperative elevation in CRP, either at baseline or postoperatively, correlated with poor survival after resection of primary colorectal malignancies [7-12]. However other studies report conflicting evidence on the prognostic value of CRP [13], and little is known about the prognostic value of CRP serum levels in liver-only metastatic colorectal disease specifically. Elevated CRP levels at baseline (pre-operative) might be indicative of a chronic state of low-grade systemic inflammation nurturing the tumor cell microenvironment and therefore impacting overall survival [1, 14, 15]. Alternatively it is suggested that post-operative changes in growth factor levels induced by resection of CRLM, or of other origin, might increase pathological cellular proliferation of occult micro metastases in the liver [16-18]. Some reports suggest that post-operative CRP levels play a role in prognostication after surgery for different types of malignancies. In our hospital, no pre-operative CRP levels are objectified standardly. However, post-operative CRP levels could be analyzed retrospectively, and therefore the aim of this study was to evaluate the effect of a post-operative acute inflammatory response, as represented by CRP levels, on survival after resection of CRLM. As part of the analysis, patients were stratified by clinical risk profile [19] and/or administration of neo-adjuvant chemotherapy.

PATIENTS AND METHODS

PATIENT SELECTION, BLOOD PROCESSING

Between January 2004 and December 2012, all consecutive patients who underwent liver resection for CRLM were analyzed from a prospectively maintained database. Patients were assessed by availability of post-operative CRP levels, and excluded from the analysis if they had missing data to calculate a representative immune response. Since 2004, CRP levels were determined on a daily basis during the post-operative course. The total post-operative acute inflammatory response was objectified by the area under the curve (AUC, trapezium rule). The AUC calculation was based on 4 different post-operative time points between day 1 and 7. In addition, post-operative peak serum CRP concentrations between days 1-7 were objectified. The impact of the total response and peak CRP concentrations on disease free survival (DFS) and overall survival (OS) was analyzed. Patients were stratified by clinical risk score and/or administration of neo-adjuvant chemotherapy. Differences in DFS and OS were compared between high (upper quartile), middle (middle quartiles) and low responders

(lower quartile). In our hospital, blood samples were taken between 0700 a.m. and 0800 a.m. daily, which rules out diurnal variations within this cohort. CRP serum levels were measured on a Cobas c701 modular analyzer by particle enhanced immunoturbidimetric assay.
Patient characteristics were collected retrospectively from a prospectively maintained database. Data in our database comprises: gender, age, primary tumor site, primary tumor pathological and lymph node stage (pTN), carcinoembryonic antigen (CEA), interval between resection of primary tumor and detection of liver metastases, location, distribution, size and number of liver metastases. Furthermore, type of liver surgery, radicality of surgery (R1, R0), extrahepatic disease, adjuvant treatment of primary tumor, neo-adjuvant chemotherapy for CRLM, and neo-adjuvant (chemo) radiotherapy for primary rectal malignancies were listed. No patients received adjuvant chemotherapy for CRLM.

SURGICAL METHODS

Liver resections were performed by a single or two-stage procedure and included wedge resections, segmental resections, hemihepatectomies and radiofrequency ablations (RFA). In case of a two-stage resection, vena porta embolization was performed between the first and second stage of resection. Hepatic parenchymal resection was performed with an ultrasonic surgical aspirator and a monopolar coagulator. R0-resections were defined by the absence of microscopic tumor invasion of the resection margins, and R1-resections were defined by the presence or microscopic tumor invasion of the resection margins.

FOLLOW-UP

During follow-up, patients visited the outpatient clinic every 3 months in the first 2 years after CRLM resection for clinical examination and CEA-determination. Thereafter, patients visited the outpatient clinic every 6 months and were discharged from follow up after 5 years. Abdominal imaging (CT of thorax and abdomen) was performed biannually during the first 3 years and thereafter annually. If disease recurred, a decision on whether to initiate chemotherapy treatment (again) or to perform a second resection was made by the multidisciplinary team.

OUTCOME

Overall survival (OS) was defined as the interval in months between resection of CRLM and death, or the date of last follow-up. Disease-free survival (DFS) was defined as the interval in months between resection of CRLM and recurrence, death without recurrence, or date of last follow-up without recurrence.

STATISTICS

Descriptive values are expressed as median (interquartile range (IQR)). Variables were compared by means of chi-square analysis or Fischer's exact test (depending on the sample size) or with the independent Student's t test or Mann-Whitney U test when appropriate.

Survival analysis was performed by the Kaplan-Meier method. Comparison between survival curves was made by log-rank tests. The prognostic value of variables was calculated by Cox's proportional hazard models. For the multivariate analysis, known prognostic factors and parameters with a P-value less than 0.10 in the univariate model were entered in the Cox regression model.

As mentioned before, the total inflammatory response as represented by post-operative CRP levels was objectified by calculating the area under the curve, trapezium rule. This enables us to estimate the total exposure to CRP as a function of time, in a standardized time-frame, over a standardized number of time points. The SPSS statistical package (version 21.0, Chicago, IL, USA) was used for statistical analysis; a p-value of ≤0.05 was considered statistically significant.

RESULTS

CLINICOPATHOLOGICAL CHARACTERISTICS

632 patients were analyzed. 22 patients underwent an unfinished 2-staged liver resection due to progression of disease, 18 patients had an incomplete "liver-first" approach for synchronous disease for the same reason. These patients were excluded from analysis. From the remaining 592 patients, 189 were excluded from the analysis due to missing data to calculate the AUC (missing or too few CRP levels). These missing values were a consequence of either a short hospital stay (<4 days) or, erroneously, at certain time points no serum was collected. The final study population consisted of 403 patients. Median follow up of all patients was 28 months (17-51). 55% of patients (N=223) received neo-adjuvant chemotherapy for CRLM. 46% of patients (N=182) had a high clinical risk profile, as defined by Fong's clinical risk score for liver metastases [19]. Patients' clinicopathological characteristics are depicted for CRP as represented by the AUC (table 1a) and for CRP as represented by peak concentrations above or below 100 mg/L (table 1b). Post-operative complications according to the Clavien-Dindo classification did not differ between patients with a high (AUC upper quartile) and low (AUC lower quartile) CRP serum response (table 1a). Median post-operative hospital stay was 7 days (5-8) for patients with a high serum CRP response versus 7 days (6-10) for patients with a low CRP serum response (P=0,054).

Table 1a: Clinicopathological characteristics of patients by CRP serum response (AUC, lower versus upper quartile).

Variables		CRP serum response (AUC) Lower Quartile (N=103)		CRP serum response (AUC) Upper Quartile (N=100)			All patients (N=403) (incl. middle Quartiles)	
		Value	% or IQR	Value	% or IQR	P-value	Value	% or IQR
Male		62	60%	76	76%	0,016	262	65%
Age	Median	65	59-70	63	58-72	0,761	64	58-71
Primary tumor								
Rectal cancer		45	44%	46	46%	0,741	165	41%
T3/T4		83	81%	70	73%	0,156	316	81%
Positive lymph node		61	60%	58	60%	0,999	235	60%
Liver metastases								
CEA	Median	21	7,36-65	11	5,85-49,25	0,084	19,10	6,70-65,00
Synchronous (≤ 3mo)		53	52%	59	59%	0,280	224	56%
Diameter (cm)	Median	3,25	2,2-5,4	3	2-4	0,830	3	2,0-4,7
Number of metastases	Median	2	1-4	2	1-3	0,408	2	1-4
Bilobar distribution		32	31%	37	37%	0,372	168	42%
Neo-adjuvant Ctx		62	60%	41	41%	0,006	223	55%
R1 resection		19	19%	18	18%	0,935	81	21%
Extrahepatic disease		11	11%	10	10%	0,874	42	10%
Clinical risk score 3-5		47	47%	44	47%	0,914	182	46%
Days of hospitalization	Median	7	6-10	7	5-8	0,054	7	5-8
Clavien-Dindo	Grade 1	8	8%	9	9%	0,463	36	9%
	Grade 2	14	14%	15	16%		53	14%
	Grade 3	9	9%	7	7%		24	6%
	Grade 4	1	1%	1	1%		3	1%
	Grade 5	0	0%	3	3%		5	1%

***CEA** = Carcinoembryonic Antigen; **CRP** = C-Reactive Protein; **AUC** = Area Under the Curve*

Table 1b: Clinicopathological characteristics of patients by peak CRP concentration (<100 mg/L versus >100 mg/mL).

Variables		Peak CRP <100 mg/L (N=68)		Peak CRP >100 mg/L (N=335)			All patients (N=403)	
		Value	% or IQR	Value	% or IQR	P-value	Value	% or IQR
Male		37	54%	225	67%	0,044	262	65%
Age	Median	64	59-70	64	58-71	0,874	64	58-71
Primary tumor								
Rectal cancer		33	49%	132	40%	0,169	165	41%
T3/T4		54	81%	262	81%	0,922	316	81%
Positive lymph node		42	62%	193	60%	0,695	235	60%
Liver metastases								
CEA	Median	22,5	8,51-79,5	18,44	6,16-64,5	0,546	19,10	6,70-65,00
Synchronous (≤ 3mo)		34	50%	190	57%	0,309	224	56%
Diameter (cm)	Median	3,8	2,3-6,08	3,1	2,0-4,7	0,094	3	2,0-4,7
Number of metastases	Median	2	1-4	2	1-4	0,548	2	1-4
Bilobar distribution		20	30%	148	44%	0,024	168	42%
Neo-adjuvant Ctx		44	65%	179	53%	0,088	223	55%
R1 resection		13	19%	68	21%	0,806	81	21%
Extrahepatic disease		10	15%	32	10%	0,205	42	10%
Clinical risk score 3-5		34	51%	148	47%	0,560	182	46%
Days of hospitalization	Median	8	6-10	7	5-8	0,009	7	5-8
Clavien-Dindo	Grade 1	6	9%	30	9%	0,516	36	9%
	Grade 2	7	11%	46	14%		53	14%
	Grade 3	6	9%	18	6%		24	6%
	Grade 4	1	2%	2	1%		3	1%
	Grade 5	0	0%	5	2%		5	1%

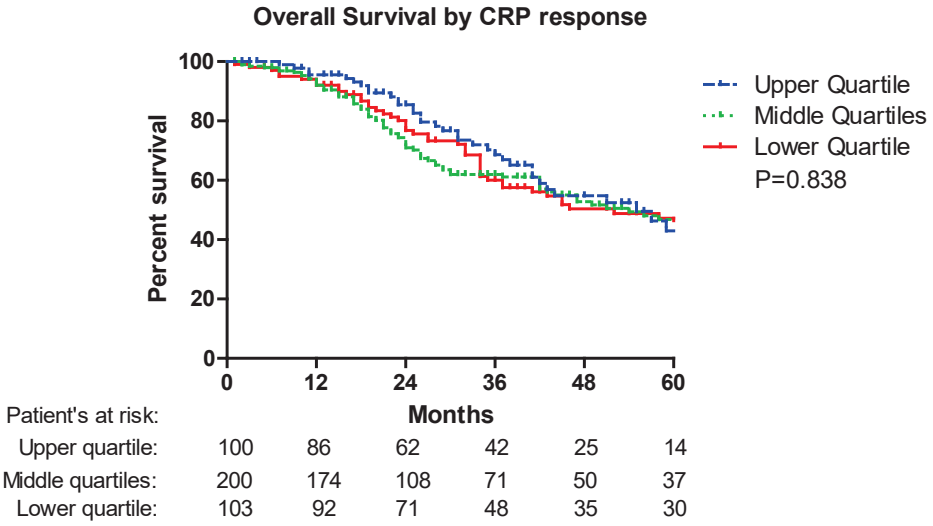
SURVIVAL AND TOTAL SERUM RESPONSE

The overall survival of patients with a high serum response of CRP (AUC, upper quartile) was equal to patients with intermediate (AUC, middle quartiles) or low (AUC, lower quartile) serum responses. Median OS was 55 (95% Confidence Interval (CI) 37-73), 54 (95% CI 26-82) and 52 months (95%CI 32-72) respectively (P=0,838 figure 1). Correspondingly, DFS was 9 (95% CI 5-13), 12 (95% CI 9-15) and 13 months (95% CI 7-19) for high-, middle-, and lower-inflammatory response, respectively (P=0,368, figure 1).

Overall survival did not differ between patients with a CRP concentration of >100 mg/L between days 1-7 post operatively versus patients without CRP levels <100 mg/L. Median OS for CRP >100 mg/L was 57 months (95%CI 38-76) versus 37 months in patients with CRP <100 mg/L, (95%CI 29-45), P=0,274. Univariate analysis of the prognostic value of peak CRP concentration, presented as quartiles and as a continuous variable, showed no significant impact on OS (table 2).

Multivariate analysis was performed including known prognostic clinicopathologic variables for survival after surgery for CRLM. In this analysis, CRP was added to the model by 4 definitions. 1) CRP concentrations as defined by the AUC presented as quartiles; 2) CRP concentrations as defined by the AUC as a continuous variable; 3) CRP peak values between

days 1 and 7 post operatively; 4) CRP peak value as a continuous variable. In all 4 analyses, CRP did not impact OS significantly (table 2).



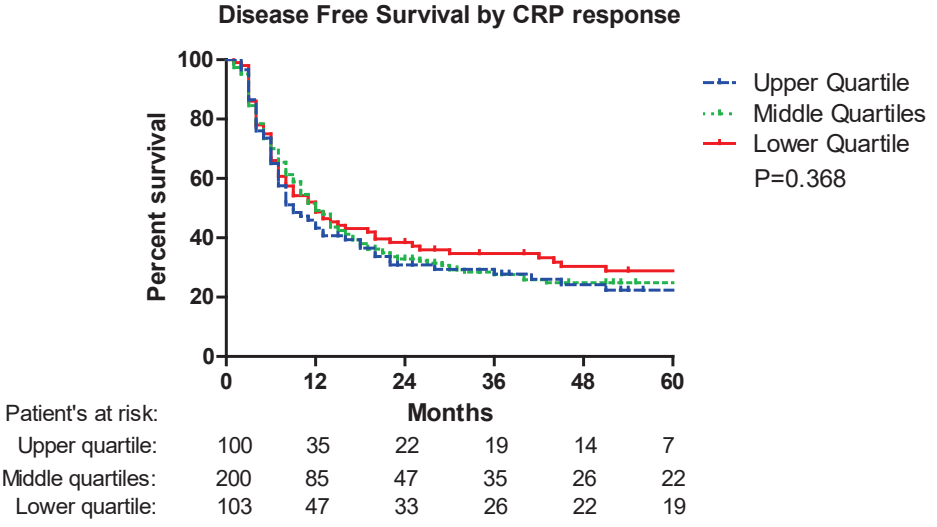


Figure 1. Overall survival and disease free survival of patients after surgery for colorectal liver metastases stratified by post-operative C-reactive protein serum response. The response was calculated by the area under the curve (trapezium rule) from CRP serum levels on 4 different time points between day 1 and day 7 post-operatively. Survival for patients in the upper, two middle, and lower quartiles of response was compared.

Table 2: Multivariate analysis of known prognostic factors. All variables have been included in the multivariate analysis. Multivariate analysis was repeated 4 times: with 1) CRP AUC as quartiles, 2) CRP AUC as continuous variable, 3) CRP peak value as quartiles, 4) CRP peak value as continuous variable

Variable			Univariate HR (95%CI) P-value	Multivariate HR (95% CI) P-value
Gender		Male	0,886 (0,653-1,203) P=0,438	-
Primary tumour		Rectum	1,135 (0,843-1,529) P=0,404	-
T-stage		T 3-4	1,493 (0,976-2,284) 0,065	-
Lymph node		Positive	1,619 (1,178-2,225) 0,003	-
Liver Metastases Disease free interval		< 12 months	0,967 (0,702-1,332) 0,836	-
Largest size metastases (cm)		> 5	1,363 (0,968-1,921) 0,076	-
Number metastases		> 1	1,117 (0,818-1,524) 0,488	-
CEA level µg/L		> 200	1,167 (0,722-1,886) 0,529	-
Tumour distribution		Bilobar	1,249 (0,926-1,685) 0,145	-
Resection margin		R1	1,438 (1,018-2,032) 0,039	-
Chemotherapy		Yes	0,979 (0,727-1,318) 0,889	-
1)	CRP: AUC Quartiles	Lower	1,000 0,840	1,000 0,879
		Middle (2)	1,063 (0,751-1,503) 0,732	1,104 (0,737-1,654) 0,630
		Upper	0,951 (0,627-1,444) 0,815	0,102 (0,679-1,790) 0,694
2)	CRP: AUC (continuous)		0,999 (0,998-1,000) 0,245	1,000 (0,998-1,001) 0,808
3)	CRP: peak value quartiles	Lower	1,000 0,555	1,000 0,428
		Middle (2)	0,829 (0,583-1,178) 0,294	0,842 (0,564-1,257) 0,400
		Upper	0,937 (0,625-1,403) 0,751	1,094 (0,681-1,755) 0,711
4)	CRP: peak value (continuous)		0,999 (0,997-1,001) 0,415	1,000 (0,998-1,003) 0,917

CRP *C-Reactive Protein;* ***CEA*** *Carcinoembryonic Antigen;* ***CRS*** *Clinical Risk Score;* ***AUC*** *Area Under the Curve;*

TOTAL IMMUNE RESPONSE: STRATIFICATION BY CLINICAL RISK PROFILE

The clinical risk score described by Fong did correlate with overall survival: median OS for CRS 0-2 was 59 months (95% CI 40-78) versus 37 months (95% CI 29-45) in patients with a CRS 3-5 (P=0,004). The prognostic value of CRP levels was stratified for patients' clinical risk score. Median OS (months) of patients with a high risk profile (CRS 3-5) that underwent resection was 41 (95% CI 32-50) for patients with a high inflammatory response versus 37 (95% CI 24-50) in patients with a low inflammatory response (P=0,562). Median OS (months) of patients with a low risk profile (CRS 0-2) that underwent resection was 59 (95% CI 44-74) for patients with a high inflammatory response versus 71 (95% CI 37-105) in patients with a low inflammatory response (P=0,981).

In patients with a CRS of 3-5 with CRP concentration of >100 mg/L, median OS was 59 months (95% CI 39-79) versus 45 months (95% CI 12-78) in patients with CRP <100 mg/L (P=0,664). In patients with a CRS of 0-2, a CRP concentration of >100 mg/L resulted in a median OS of 38 months (95% CI 10-66) versus 35 months (95% CI 25-45, P=0,333).

TOTAL IMMUNE RESPONSE: STRATIFICATION BY NEO-ADJUVANT CHEMOTHERAPY

The prognostic value of CRP levels was stratified for use of neo-adjuvant chemotherapy. Median OS (months) of patients that underwent resection without neo-adjuvant chemotherapy was 51 (95% CI 29-73) for patients with a high inflammatory response versus 58 (95% CI 14-102) in patients with a low inflammatory response (P=0,348). Median OS (months) of patients that underwent resection with neo-adjuvant chemotherapy was 55 (95% CI 24-68) for patients with a high inflammatory response versus 43 (95% CI 14-72) in patients with a low inflammatory response (P=0,243).

All analyses were repeated using a CRP concentration cut-off of 100 mg/L. Only in patients receiving neo-adjuvant chemotherapy did CRP impact OS. Concentrations of CRP <100 mg/L had a protective effect on survival. With CRP <100 mg/L, OS was 78 months (95% CI 57-99) versus 35 months (95% CI 24-46, P=0,043). In a multivariate analysis with known prognostic factors this protective impact was insignificant (HR 0,645 95% CI 0,391-1,064; P=0,086).

TOTAL IMMUNE RESPONSE: STRATIFICATION BY CLINICAL RISK PROFILE AND NEO-ADJUVANT CHEMOTHERAPY

In patients with a high clinical risk score (3-5), baseline characteristics between patients with a high CRP response versus a low CRP response differed on basis of administration of neo-adjuvant chemotherapy: high CRP serum response 60% CTx (N=26), vs. low CRP response 85% CTx (N=40), (P=0,005).

The prognostic value of CRP levels was stratified for both the use of neo-adjuvant chemotherapy as well as patient's clinical risk profile. In concordance with results illustrated earlier in the total study population, there was no survival difference between patients with a high or low total CRP serum response (AUC, upper vs. middle vs. lower quartile), stratified by risk profile and by administration of neo-adjuvant chemotherapy.

All analyses were repeated using a CRP concentration cut-off of 100 mg/L. Only in high-risk patients receiving neo-adjuvant chemotherapy did CRP impact OS. Concentrations of CRP <100 mg/L had a protective effect on survival. With CRP <100 mg/L, OS was 85 months (95% CI 27-143) versus 35 months (95% CI 15-55, P=0,034). In a multivariate analysis with known prognostic factors this protective impact was insignificant (HR 0,644 95% CI 0,344-1,206; P=0,169).

DISCUSSION

In this study, total post-operative inflammatory response as evidenced by CRP serum levels (AUC, trapezium rule) did not prove to be of prognostic value for survival after surgery for CRLM. The inflammatory response represented by peak CRP levels had no significant impact on OS either. No prognostic value was found for CRP levels stratified by patient's clinical risk score and/or administration of neo-adjuvant chemotherapy. This is the first study to stratify the prognostic value of post-operative CRP levels for administration of neo-adjuvant CTx and patients' clinical risk profile.

Tumor progression is a complex process that depends on both the intrinsic properties of the tumor as well as the interaction with its microenvironment. Within this microenvironment, inflammatory cells play an important role in promoting tumor growth through complex biological mechanisms [1-3]. It is only for the past two decades that these complex interactions are being recognized and understood. CRP is a classic acute phase reactant identified in 1930 [20], representative of a systemic inflammatory response. Given the link between inflammation and cancer, multiple studies have been conducted to evaluate the relationship between elevated serum levels of CRP and tumor stage or survival after resection of primary colorectal malignancies [7-12]. These studies analyzed either CRP alone or CRP as part of the Glasgow Prognostic Score, which includes serum albumin in addition to CRP [21]. Few studies objectified the prognostic value of pre-operative CRP levels as a component of the GPS for survival after resection of CRLM [22-24]. The limited studies that have investigated the relation between CRP alone and survival after surgery for CRLM, evaluated both pre-operative and/or post-operative CRP levels [25-28]. Wong et al. and Hamilton et al. found a pre-operative elevated serum level to be predictive of poor outcome after surgery for colorectal liver metastases (CRLM) [25, 28], and a study of de Jong et al. showed that the magnitude of post-operative immune response, as represented by systemic CRP levels, correlated with overall survival [26]. Overall, without substantive critical appraisal made, limited studies agree that for CRLM a high baseline CRP or a high peri-/post-operative response is indicative of poor prognosis.

This study not only analyzed the potential effect on survival after resection in general, it also stratified for administration of neo-adjuvant chemotherapy and/or patients' clinical risk score. No evidence was found suggesting that CRP levels do impact survival in any of these subgroups. The current analysis found no overall- or disease free survival disadvantage for

patients with an augmented post-operative immune response in general, which suggest the potential impact of CRP on survival after resection of CRLM is unlikely. In a review of literature [27], the prognostic value of CRP serum levels on cancer specific survival is already questioned. Although more patients undergoing resection without neo-adjuvant chemotherapy had an elevated immune response, this did not result in poorer survival outcomes. In fact: overall survival of patients with a high clinical risk score treated without neo-adjuvant chemotherapy and a high CRP response did not differ from low risk patients treated with neo-adjuvant chemotherapy. If there would be any influence of a post-operative acute systemic inflammatory response on occult disease, as measured by CRP levels, it expectedly should have been measured in these subgroups. Our results support the hypothesis that an abrupt, non-specific post-operative inflammatory response does not drive occult tumor cell proliferation.

Conceptually there is a difference between pre- and post-operative evidence of systemic inflammation. Pre-operative elevated CRP levels could be a surrogate for a chronic state of low-grade inflammation, reflecting the microenvironment and metastatic potential of the tumor [1, 14, 15]. Alternatively, this response could be nonspecific secondary to concurrent infections, tumor necrosis or local tissue damage. Evidently, tissue damage plays a major role in the post-operative inflammatory response. However, if occult micro-metastases are exposed to such an augmented post-operative inflammatory response, this could enhance pathologic tumor proliferation. In animal experiments, enhanced tumor growth during liver regeneration has been described and could be explained by local or systemic changes in growth factors [16, 29-32].

Naturally, this study has drawbacks. It might be biased by its retrospective, single-center nature. Also, there were insufficient data on CRP levels to be able to calculate an inflammatory response uniformly in all patients over a time period of more than 4 days. As mentioned before, this is a consequence of early discharge or missed serum collection (missing values). The patient group included might have had greater resections as compared to patients discharged early in the post-operative course. Still, in all included patients, the immune response was structurally objectified in a corresponding time frame by using the AUC trapezoid method. A hypothetical drawback of the AUC-method might be that significant peak CRP concentrations might not be accounted for. The AUC of a patient with a significant rise of CRP rapidly returning to normal might be similar to that of a patient whose CRP lingers around a lower value. Thus, the prognostic impact of peak CRP levels and CRP levels above a threshold of >100 mg/L was analyzed. Further, CRP was determined regardless of the post-operative course. The post-operative course might influence CRP levels. Even if this would have been the case, it is hypothesized that a post-operative exposure of occult disease to an elevated inflammatory response triggers tumor proliferation, irrespective of the origin of that inflammatory response [16-18].

To our knowledge, this is the largest patient cohort assessing post-operative CRP levels with respect to survival after surgery for CRLM and the first study stratifying for known risk factors

and administration of neo-adjuvant chemotherapy. We hypothesize that abrupt, post-operatively augmented CRP levels do not induce tumor proliferation, nor alter the tumor microenvironment of occult residual disease. Further research should focus on pre-operative markers for systemic inflammation. One may question the concept of a general, quantitative measurement of systemic inflammation (CRP levels) representing an enhanced state of inflammation at tumor site. Although other reports have objectified a relation between a general inflammatory response and oncological outcome, future research should focus on a more qualitative measurement of the inflammatory response in the tumor microenvironment in relation to survival. The characterization of such a specific immune infiltrate is of greater scientific and clinical interest, with respect to prognostication and development of tailor-made systemic therapies.

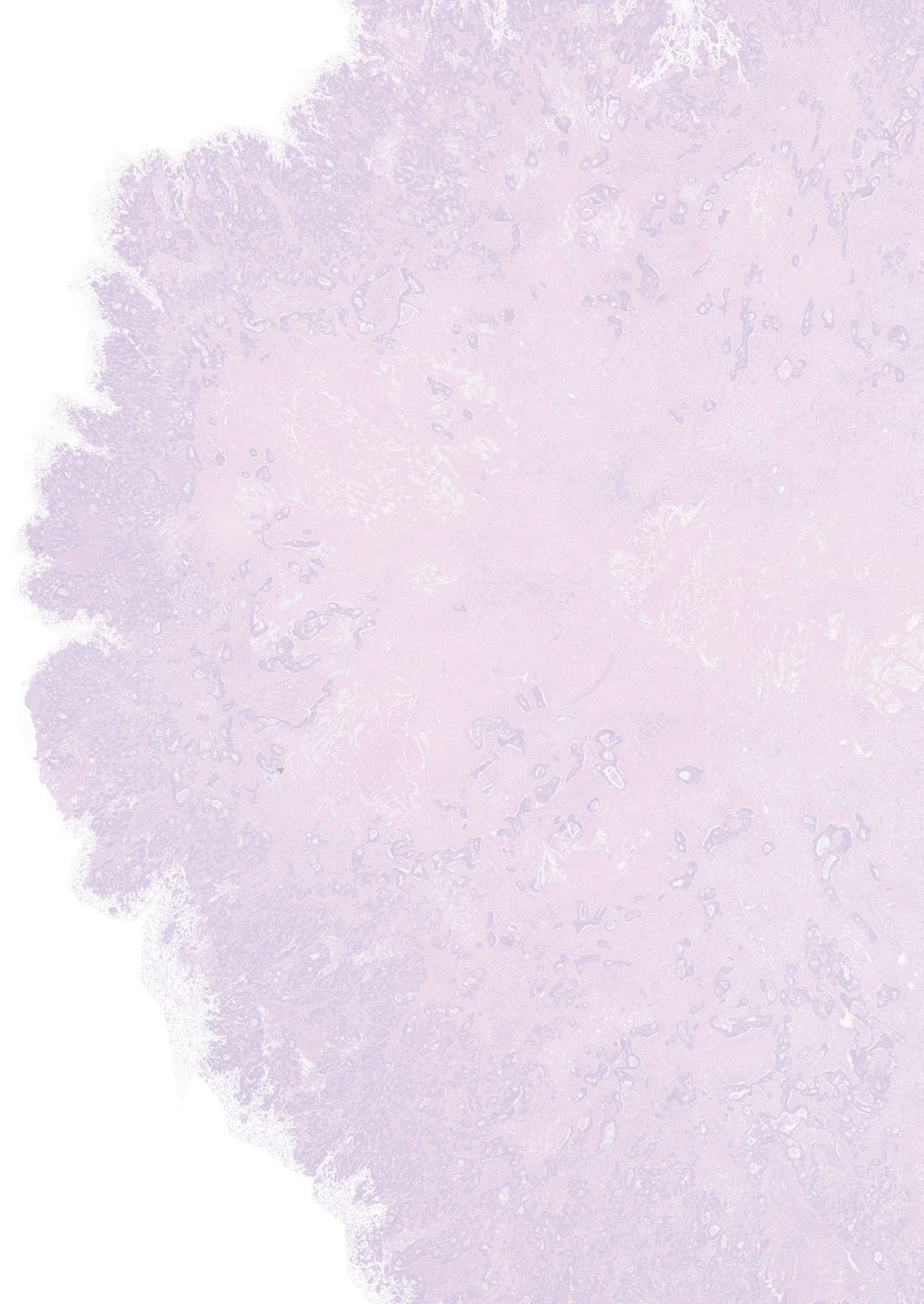
To conclude, in this study post-operative CRP levels did not prove to be of prognostic value for survival after surgery for CRLM.

REFERENCES

1. Coussens, L.M. and Z. Werb, *Inflammation and cancer*. Nature, 2002. **420**(6917): p. 860-7.
2. Elinav, E., et al., *Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms*. Nat Rev Cancer, 2013. **13**(11): p. 759-71.
3. Grivennikov, S.I., F.R. Greten, and M. Karin, *Immunity, inflammation, and cancer*. Cell, 2010. **140**(6): p. 883-99.
4. Jagdev, S.P., et al., *Improving the accuracy of pre-operative survival prediction in renal cell carcinoma with C-reactive protein*. Br J Cancer, 2010. **103**(11): p. 1649-56.
5. Roxburgh, C.S. and D.C. McMillan, *Role of systemic inflammatory response in predicting survival in patients with primary operable cancer*. Future Oncol, 2010. **6**(1): p. 149-63.
6. Nozoe, T., H. Saeki, and K. Sugimachi, *Significance of preoperative elevation of serum C-reactive protein as an indicator of prognosis in esophageal carcinoma*. Am J Surg, 2001. **182**(2): p. 197-201.
7. Nozoe, T., et al., *Significance of preoperative elevation of serum C-reactive protein as an indicator for prognosis in colorectal cancer*. Am J Surg, 1998. **176**(4): p. 335-8.
8. McMillan, D.C., K. Canna, and C.S. McArdle, *Systemic inflammatory response predicts survival following curative resection of colorectal cancer.* Br J Surg, 2003. **90**(2): p. 215-9.
9. McMillan, D.C., et al., *A prospective study of tumor recurrence and the acute-phase response after apparently curative colorectal cancer surgery*. Am J Surg, 1995. **170**(4): p. 319-22.
10. Koike, Y., et al., *Preoperative C-reactive protein as a prognostic and therapeutic marker for colorectal cancer*. J Surg Oncol, 2008. **98**(7): p. 540-4.
11. Erlinger, T.P., et al., *C-reactive protein and the risk of incident colorectal cancer*. JAMA, 2004. **291**(5): p. 585-90.
12. Il'yasova, D., et al., *Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort*. Cancer Epidemiol Biomarkers Prev, 2005. **14**(10): p. 2413-8.
13. Tsilidis, K.K., et al., *C-reactive protein and colorectal cancer risk: a systematic review of prospective studies*. Int J Cancer, 2008. **123**(5): p. 1133-40.
14. Philip, M., D.A. Rowley, and H. Schreiber, *Inflammation as a tumor promoter in cancer induction*. Semin Cancer Biol, 2004. **14**(6): p. 433-9.
15. Heikkila, K., S. Ebrahim, and D.A. Lawlor, *A systematic review of the association between circulating concentrations of C reactive protein and cancer*. J Epidemiol Community Health, 2007. **61**(9): p. 824-33.
16. Stolz, D.B., et al., *Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat*. Cancer Res, 1999. **59**(16): p. 3954-60.
17. Radinsky, R., *Modulation of tumor cell gene expression and phenotype by the organ-specific metastatic environment*. Cancer Metastasis Rev, 1995. **14**(4): p. 323-38.
18. de Jong, K.P., et al., *Effect of partial liver resection on tumour growth*. J Hepatol, 1996. **25**(1): p. 109-21.
19. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases.* Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
20. Tillett, W.S. and T. Francis, *Serological Reactions in Pneumonia with a Non-Protein Somatic*

Fraction of Pneumococcus. J Exp Med, 1930. **52**(4): p. 561-71.

21. McMillan, D.C., et al., *Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer*. Int J Colorectal Dis, 2007. **22**(8): p. 881-6.
22. Kobayashi, T., et al., *Elevated C-reactive protein and hypoalbuminemia measured before resection of colorectal liver metastases predict postoperative survival*. Dig Surg, 2010. **27**(4): p. 285-90.
23. Nakagawa, K., et al., *The modified Glasgow prognostic score as a predictor of survival after hepatectomy for colorectal liver metastases*. Ann Surg Oncol, 2014. **21**(5): p. 1711-8.
24. Ishizuka, M., et al., *Systemic inflammatory response predicts postoperative outcome in patients with liver metastases from colorectal cancer*. J Surg Oncol, 2009. **100**(1): p. 38-42.
25. Hamilton, T.D., et al., *Identification of prognostic inflammatory factors in colorectal liver metastases*. BMC Cancer, 2014. **14**: p. 542.
26. de Jong, K.P., et al., *Portal and systemic serum growth factor and acute-phase response after laparotomy or partial hepatectomy in patients with colorectal liver metastases: a prognostic role for C-reactive protein and hepatocyte growth factor*. Scand J Gastroenterol, 2004. **39**(11): p. 1141-8.
27. Morris-Stiff, G., D. Gomez, and K.R. Prasad, *C-reactive protein in liver cancer surgery*. Eur J Surg Oncol, 2008. **34**(7): p. 727-9.
28. Wong, V.K., et al., *C-reactive protein as a predictor of prognosis following curative resection for colorectal liver metastases*. Br J Cancer, 2007. **96**(2): p. 222-5.
29. Picardo, A., et al., *Partial hepatectomy accelerates local tumor growth: potential roles of local cytokine activation*. Surgery, 1998. **124**(1): p. 57-64.
30. Asaga, T., et al., *The enhancement of tumor growth after partial hepatectomy and the effect of sera obtained from hepatectomized rats on tumor cell growth*. Jpn J Surg, 1991. **21**(6): p. 669-75.
31. de Jong, K.P., et al., *The effect of partial hepatectomy on tumor growth in rats: in vivo and in vitro studies*. Hepatology, 1995. **22**(4 Pt 1): p. 1263-72.
32. Mizutani, J., et al., *Promotion of hepatic metastases by liver resection in the rat*. Br J Cancer, 1992. **65**(6): p. 794-7.



CHAPTER 7

mRNA expression profiles of colorectal liver metastases as a novel biomarker for early recurrence after partial hepatectomy

E.P. van der Stok
M. Smid
A.M. Sieuwerts
P.B. Vermeulen
S. Sleijfer
N. Ayez
D.J. Grünhagen
J.W.M. Martens
C. Verhoef

Molecular Oncology. 2016 Dec;10(10):1542-1550

ABSTRACT

BACKGROUND

Identification of specific risk groups for recurrence after surgery for isolated colorectal liver metastases (CRLM) remains challenging due to the heterogeneity of the disease. Classical clinicopathologic parameters have limited prognostic value. The aim of this study was to identify a gene expression signature measured in CRLM discriminating early from late recurrence after partial hepatectomy.

METHODS

CRLM from two patient groups were collected: I) with recurrent disease ≤12 months after surgery (N=33), and II) without recurrences and disease free for ≥36 months (N=30). The patients were clinically homogeneous; all had a low clinical risk score (0-2) and did not receive (neo-) adjuvant chemotherapy. Total RNA was hybridised to Illumina arrays, and processed for analysis. A leave-one-out cross validation (LOOCV) analysis was performed to identify a prognostic gene expression signature.

RESULTS

LOOCV yielded an 11-gene profile with prognostic value in relation to recurrent disease ≤12 months after partial hepatectomy. This signature had a sensitivity of 81.8%, with a specificity of 66.7% for predicting recurrences (≤12 months) versus no recurrences for at least 36 months after surgery (X2 $P<0.0001$).

CONCLUSION

The current study yielded an 11-gene signature at mRNA level in CRLM discriminating early from late or no relapse after partial hepatectomy.

INTRODUCTION

Colorectal cancer is one of the most commonly diagnosed cancers worldwide [1]. Approximately 15-25% of patients with colorectal cancer (CRC) present with synchronous liver metastases and another 20% have a metachronous disease development [2]. For patients presenting with isolated liver metastases, partial hepatectomy is the only potentially curative treatment option. Reported 5-year survival rates are 40-60% [3-5]. A substantial number of patients develop recurrent disease after liver surgery, underlining the need for prognostic biomarkers [6-8]. Such prognostic biomarkers may allow a more personalised treatment strategy. In recent years, several clinicopathological prognostic variables in patients with isolated colorectal liver metastases (CRLM) have been identified predicting the risk of relapse after a metastasectomy [9]. These variables have been integrated in various clinical risk scores (CRS) [9-13]. The CRS according to Fong et al. is the most widely used and validated score, able to distinguish between high risk and low risk patients in terms of survival outcomes [10]. This score is composed of 5 prognostic variables: positive lymph node status of the primary tumour, diagnosis of liver metastases within 12 months after resection of primary tumour, serum CEA ≥200ng/ml, >1 liver metastases, a metastasis of >5cm diameter. Each variable accounts for 1 point. Patients with 0-2 points are categorised as low risk, patients with 3-5 points as high risk. Still, outcomes after surgery remain heterogeneous: low risk patients may develop early recurrences - approximately 50% of patients with a low CRS develop metastases within 12 months after surgery - while high risk patients may remain disease free [14, 15]. Unravelling the biological properties characterising tumours may be pivotal to designing individualised therapies, based on biological predictors of outcome rather than or in addition to clinical predictors. Various groups have established molecular subtypes in primary cancers with distinct biology, predictive and prognostic value [16-19] [20, 21]. Biological markers may improve patient selection for (neo-) adjuvant therapies in addition to surgical management or intensive surveillance schemes.

The ability to analyse tumours at DNA-, RNA-, and protein- level promises to revolutionize our understanding of the malignant disease process, and hopefully this will herald new (superior) biomarkers. The aim of the current study was to identify a prognostic gene signature at mRNA level in patients with a low CRS, effective in identifying patients at high risk of early recurrence after surgery for CRLM.

METHODS

PATIENTS AND TREATMENT

Erasmus MC Cancer Institute is a tertiary referral centre for liver surgery. In the current retrospective study, patient characteristics were collected from a prospectively maintained database. All patients undergoing resection for CRLM are prospectively entered into an

institutional database. This database includes standard clinicopathological variables. Patients selected for the current study had a low risk profile (Fong's clinical risk score 0-2 [10]) and did not receive treatment with (neo-) adjuvant chemotherapy for the resectable CRLM in line with the Dutch guidelines that do not support routine administration of chemotherapy/ biologicals in the case of primary resectable colorectal liver-only metastases. Patients were further selected according to the following criteria: I) patients with recurrent disease within 12 months after hepatectomy, and II) patients without recurrent disease and a disease free survival of at least 36 months after hepatectomy. Thus, "two extremes" were selected in terms of recurrent disease. All resections were performed between 2000 and 2009. Hepatic parenchymal resection was performed with an ultrasonic surgical aspirator and a monopolar coagulator. R0-resections were defined by the absence of microscopic tumour invasion of the resection margins, and R1-resections were defined by the presence or microscopic tumour invasion of the resection margins [22].

During follow-up, patients visited the outpatient clinic every 4 months in the first 2 years after CRLM resection for clinical examination and CEA-determination. Thereafter, patients visited the outpatient clinic every 6 months and were discharged from follow up after 5 years. Abdominal imaging (CT of thorax and abdomen) was performed twice a year during the first 3 years and thereafter annually. If disease recurred, a decision on whether to initiate chemotherapy treatment or to perform local therapy was made by a multidisciplinary team. Disease free survival (DFS) was defined as the interval in months between resection of CRLM and recurrence.

TISSUE COLLECTION AND ASSESSMENT

After resection of CRLM, tumour tissue is standardly fixed on formalin and embedded in paraffin in the department of pathology according to standard protocols, and stored. For the current study, tumour samples (N=80) of CRLM were retrieved from the selected patient groups. In the case a patient had more than one metastasis, there were no additional selection criteria in terms of which tumour to analyse. The formalin fixed, paraffin embedded (FFPE) samples were evaluated by a pathologist for colon tumour cell content: only specimens with at least 30% tumour cells in the tissue block were included (N=63). The final study population consisted of 33 samples for group I with disease recurrence within 12 months and 30 samples for group II without disease recurrence and a DFS of 36 months.

The established tumour growth patterns are assessed by a dedicated pathologist and at least one additional observer in all resected CRLM in Erasmus MC Cancer Institute [23, 24]. Three tumour growth patterns have been reported in literature, with a distinct growing pattern [23, 24]. These patterns consist of a pushing type, a replacing type and a desmoplastic type. Briefly, in the pushing type the metastasis has a displacing interaction with the normal liver parenchyma, and is separated from normal cells by a thin layer of reticulin fibres. The replacing type infiltrates the normal liver parenchyma. The desmoplastic type has a band of desmoplastic tissue that separates tumour cells from the liver parenchyma.

On a patient level, the growth patterns were classified by two methods for analysis in relation to outcomes. First, when a pattern was expressed in >75% of the CRLM the patient was classified as such. If no pattern was expressed in >75%, the growth pattern was classified as a "mixed type". Second, based on prognostic evidence reported in the literature, if any percentage of the pattern was a replacement type, the patient was classified as such [25-28]. Tumour differentiation and inflammation at the leading edge of the tumour were also objectified, for the current study specifically.

RNA EXTRACTION AND PURIFICATION

Depending on the size of the FFPE samples, total RNA was extracted from 3 to 6 x 20 µm sections. Following paraffin removal with xylene the high-pure RNA paraffin kit was used according the supplier's instructions (Roche, Mannheim, Germany). Following isolation, RNA was stored in RNase/DNase-free water at -80°C. Quality control was performed as previously described [29].

GENE EXPRESSION PROFILES

Illumina Whole Genome- cDNA-mediated Annealing, Selection, Extension and Ligation (WG-DASL) V4 assay is an array-based method for expression profiling of partially degraded RNA molecules such as those isolated from Formalin-Fixed Paraffin-Embedded samples. In the HumanHT-12 v4 BeadChip assay 29,285 annotated transcripts corresponding to 27,253 coding transcripts with well-established annotations are measured. The WG-DASL assay was performed according to the manufacturer's instructions. In summary, 1,000 ng total RNA was used from the 63 FFPE samples. 500 ng of total RNA from a pool of fresh frozen tumour RNA samples (I-scan control) was included in each individual hybridisation experiment of 11 to 23 samples to evaluate possible inter-assay differences (supplementary 1). Total RNA was converted to cDNA using biotinylated oligo-dT18 and random nonamer primers. The biotinylated cDNA was annealed to the DASL Assay Pool (DAP) probe groups, which contain oligonucleotides specifically designed to interrogate each target sequence of the transcript. The DAP was annealed to targeted cDNA during a 16 hours temperature gradient (70° to 37°C) incubation. Hybridisation of these oligonucleotides to the targeted cDNA site, followed by enzymatic extension and ligation was used to create a Polymerase Chain Reaction (PCR) template that was amplified with a set of universal PCR primers [30]. Cy3-coupled primers were used to facilitate the precipitation of the single stranded labelled products, which were hybridised to the whole genome HumanHT-12 v4 BeadChips containing 12 identical microarrays each. The microarrays were scanned using a confocal type imaging system with Cy3 (532 nm) laser illumination Illumina I-scan reader (N0262). Fluorescent intensities were read and images were extracted using software version 1.8.13.5. Each sequence type is represented by an average of 30 beads on the array.

Eight hybridisations did not meet our criteria of an average intensity signal of at least 500 prior to background correction and normalisation and were re-measured at an input of 2,000 ng total RNA.

DATA ANALYSIS

Scanned data were uploaded into GenomeStudio software version 2011.1 via the Whole Genome DASL gene expression module for further analysis. The average signal, detection P-value, Bead standard error and average beads were used to quantile normalise the data in the statistical language R (www.r-project.org) using the "lumi" package [31]. The expression raw data are available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/ entry nr.: GSE81423).

STATISTICS

A leave-one-out cross validation (LOOCV) was performed using Biometric Research Branch ArrayTools (BRB-ArrayTools, http://linus.nci.nih.gov/BRB-ArrayTools.html), starting with the top 25% most variable genes (N=7,101) in all samples as input. Samples were classified in two classes: recurrences ≤12 months (class 1) or no recurrences and a disease free survival ≥36 months (class 2). In each round of the LOOCV, genes with a univariate P-value <0.001 were selected to differentiate between class 1 and class 2 (patients with and without recurrent disease). The linear prediction rule was defined by the inner sum of the weights (Wi) and expression (Xi) of these significant genes. In the prediction model, a sample was classified to class 2 if the sum was greater than the established threshold ($\sum_i W_i X_i$ >threshold). From the available prediction algorithms, the "Support Vector Machine" (SVM) proved the most accurate classifier (75% correct classification, supplementary 2), resulting in an 11-gene signature (Table 3). Through this algorithm, each patient could be classified as "high risk" or "low risk" on basis of the identified expression profile (molecular risk).

Descriptive values are expressed as median (interquartile range (IQR)). Variables were compared by means of Chi-square analysis or Fischer's exact test (depending on the sample size) or with the independent Student's t test or Mann-Whitney U test when appropriate. The SPSS statistical package (version 21.0, Chicago, IL, USA) was used for statistical analysis; a two-sided P-value of ≤0.05 was considered statistically significant.

ETHICAL APPROVAL

Of all patients, an informed consent was available, to use residual tissue for research purposes. The data and tissue used in the current study was employed in an anonymous fashion. As prescribed by national regulations, the current study was not subject to the "Medical Research Involving Human Subjects Act".

RESULTS

PATIENTS

Clinicopathological features of both patient groups (with recurrences ≤12 months and without recurrences and a DFS ≥36 months) are outlined in table 1. The groups were

homogeneous in terms of clinicopathological characteristics, as expected since all patients were selected to have a low CRS according to Fong 9. There was no difference in tumour differentiation, histological growth pattern and inflammation (at the leading edge of the tumour). The respective molecular risk groups did not differ on basis of the assessed biological (pathological) characteristics.

GENES ASSOCIATED WITH EARLY RECURRENCE

Through a LOOCV analysis, an 11-gene profile was constructed capable of discriminating patients at high- from low risk of recurrence (Table 3 and supplementary 3). Clinicopathological features of patients by the identified molecular risk groups (low- and high-risk) are depicted in table 2. These groups differed on basis of location of primary tumour and, inherently, the administration of neo-adjuvant radiotherapy for primary CRC. Of the 37 patients with at high molecular risk, 27 developed recurrent disease within 12 months. This yielded a sensitivity of the signature of 81.8%, with a specificity of 66.7% (X2 $P<0.0001$, table 4a). From the group of patients with recurrences within 12 months, the subgroup of patients with hepatic recurrences was identified (N=17). All patients with hepatic recurrences were at high molecular risk based on the 11-gene signature, resulting in a 100% sensitivity and 56% specificity for hepatic recurrences specifically (X2 $P<0.0001$, table 4b).

In the KEGG Pathway Database (http://www.genome.jp/kegg/pathway.html) and Gene Ontology Consortium database (http://geneontology.org) the respective genes were searched and pathways in which they are known to be involved are depicted in supplementary 4 (KEGG) and supplementary 5 (Gene Ontology). Two genes, CLRN3 and KIAA0219, have not been described and not been registered in both databases.

Table 1: Clinicopathological characteristics of patients by recurrence

		DFS ≤12 Months (N=33)		DFS ≥36 + No Recurrence Months (N=30)			All patients (N=63)	
		Value	% / IQR	Value	% or IQR	P-value	Value	% / IQR
Male		19	58%	18	60%	0.845*	37	59%
Age	Median	67	58-71	63.5	58-72	0.895^	65	58-72
Primary tumour								
Location (right sided)		6	18%	4	13%	0.599*	10	16%
Rectal cancer		17	52%	12	40%	0.360*	29	46%
T stage 3/4		25	76%	23	77%	0.933*	48	76%
Positive lymph node (pN+)		17	52%	14	47%	0.701*	31	49%
Adjuvant CTx		8	24%	6	20%	0.686*	14	22%
Neo-adjuvant RTx		10	32%	6	20%	0.277*	16	26%
Liver metastases								
CEA > 200		2	6%	0	0%	0.164*	2	3%
Synchronous	DFI < 12	11	33%	9	30%	0.777*	20	32%
Diameter > 5 (cm)		6	18%	3	10%	0.354*	9	14%
Number of mets > 1		7	21%	6	20%	0.905*	13	21%
Bilobar		6	18%	4	13%	0.599*	10	16%
R1 resection		5	15%	1	3%	0.110*	6	10%
Growth pattern 1	Replacement	23	70%	16	53%	0.284*	39	62%
	Desmoplastic	3	9%	7	23%		10	16%
	Pushing	1	3%	0	0%		1	2%
	Mixed	6	18%	7	23%		13	21%
Growth pattern 2	Replacement (any)	28	85%	22	73%	0.259*	50	79%
Differentiation	Good	4	13%	2	7%	0.657*	6	10%
	Moderate/Good	3	10%	6	21%		9	15%
	Moderate	6	19%	5	18%		11	19%
	Poor/Moderate	11	36%	11	39%		22	37%
	Poor	7	23%	4	14%		11	19%
Inflammation	Increased	5	16%	5	18%	0.321*	10	17%
	Moderate/ Increased	3	10%	8	29%		11	19%
	Moderate	10	32%	8	29%		18	31%
	Decreased/ Moderate	6	19%	2	7%		8	14%
	Decreased	7	23%	5	18%		12	20%

*DFS=Disease Free Survival; pN+=pathological node positivity; CTx=Chemotherapy; RTx=Radiotherapy; CEA=Carcinoembryonic Antigen; R1=microscopic irradical; *= Pearson X2; ^=Mann-Whitney U test;*

Table 2: Clinicopathological characteristics of patients by molecular risk

		High Risk (N=37)		Low Risk (N=26)			All patients (N=63)	
		Value	% / IQR	Value	% or IQR	P-value	Value	% / IQR
Male		21	57%	16	62%	0.845*	37	59%
Age	Median	64	57-70	68	60-72	0.895^	65	58-72
Primary tumour								
Location (right sided)		4	11%	6	23%	0.599*	10	16%
Rectal cancer		21	57%	8	31%	0.360*	29	46%
T stage 3/4		30	81%	18	69%	0.933*	48	76%
Positive lymph node (pN+)		20	54%	11	42%	0.701*	31	49%
Adjuvant CTx		7	19%	7	27%	0.686*	14	22%
Neo-adjuvant RTx		13	37%	3	12%	0.277*	16	26%
Liver metastases								
CEA > 200		2	6%	0	0%	0.164*	2	3%
Synchronous	DFI < 12	12	32%	8	31%	0.777*	20	32%
Diameter > 5 (cm)		7	19%	2	8%	0.354*	9	14%
Number of mets > 1		7	19%	6	23%	0.905*	13	21%
Bilobar		5	14%	5	19%	0.599*	10	16%
R1 resection		5	14%	1	4%	0.110*	6	10%
Growth pattern 1	Replacement	27	73%	12	46%	0.284*	39	62%
	Desmoplastic	4	11%	6	23%		10	16%
	Pushing	1	3%	0	0%		1	2%
	Mixed	8	31%	5	14%		13	21%
Growth pattern 2	Replacement (any)	29	78%	21	81%	0.259*	50	79%
Differentiation	Good	4	11%	2	9%	0.657*	6	10%
	Moderate/Good	5	14%	4	17%		9	15%
	Moderate	6	17%	5	22%		11	19%
	Poor/Moderate	14	39%	8	35%		22	37%
	Poor	7	19%	4	17%		11	19%
Inflammation	Increased	5	14%	5	22%	0.321*	10	17%
	Moderate/ Increased	5	14%	6	26%		11	19%
	Moderate	11	31%	7	30%		18	31%
	Decreased/ Moderate	6	17%	2	9%		8	14%
	Decreased	9	25%	3	13%		12	20%

*DFS=Disease Free Survival; pN+=pathological node positivity; CTx=Chemotherapy; RTx=Radiotherapy; CEA=Carcinoembryonic Antigen; R1=microscopic irradical; *= Pearson X2; ^=Mann-Whitney U test;*

Table 3: The identified 11-gene signature

Nr.	Parametric P-value	Fold-change	Unique ID	Name
1	5.53e-05	0.55	ILMN_1786920	JARID1A
2	0.0003634	0.51	ILMN_1698404	ERN1
3	0.0004586	0.47	ILMN_1683082	RPUSD1
4	0.0004769	0.59	ILMN_2067408	CLRN3
5	0.0009447	0.53	ILMN_1668374	ITGB5
6	0.0007405	2.07	ILMN_1678061	CASS4
7	0.0006431	1.79	ILMN_1684183	RAD9A
8	0.0004545	1.8	ILMN_3238676	ULBP2
9	0.0002883	1.98	ILMN_2381758	G3BP2
10	0.0002758	2.03	ILMN_1783636	COX6A1
11	0.0002593	1.87	ILMN_1656042	KIAA0319

P-value, Relative fold change (DFS ≤12 months vs. no recurrence and DFS ≥36 months), ID , Names (annotations) of genes

Table 4a: Prognostic impact of the molecular risk profile for all site recurrences

	Molecular Risk		
True Recurrence	**Low**	**High**	**Total**
No	20	10	30
Yes	6	27	33
Total	26	37	63

Sensitivity	81.8%
Specificity	66.7%
PPV	73%
NPV	76.9%

Pearson X2: P<0.0001

PPV=Positive Predictive Value; NPV=Negative Predictive Value

Table 4b: Prognostic impact of the molecular risk profile for hepatic recurrences

	Molecular Risk		
True Recurrence	**Low**	**High**	**Total**
No	26	20	46
Yes	0	17	17
Total	26	37	63

Sensitivity	100%
Specificity	56%
PPV	46%
NPV	100%

Pearson X2: P<0.0001

PPV=Positive Predictive Value; NPV=Negative Predictive Value

DISCUSSION

The clinical and biological diversity of CRLM urges the need for prognostic biomarkers and tailor-made treatment strategies [15]. Despite improvement of therapies for liver-only stage IV CRC resulting in improved survival rates, knowledge on treatment response and risks of relapse or progression is still scarce. A substantial number of patients develop recurrent disease following resection of CRLM, underlining the need for prognostic factors [6-8]. More insights into biological tumour behaviour may result in better understanding of treatment failure and may yield biomarkers for risks of relapse or prediction of response to therapy. This could improve identification of patients who will or will not benefit from tailored treatment strategies, e.g. more intensified (neo-) adjuvant treatments for those with a high risk for relapse and potentially less intensified approaches for those with a low risk profile. Currently, prognostication and prediction in resectable CRLM is solely based on clinical parameters, with sub-optimal performance. As an exception, KRAS/BRAF mutation status may impact response to treatment and outcome in CRLM as in primary colorectal cancer [32] [33-37]. Nevertheless, both clinical and the latter mutational status fails to impact clinical management of CRLM [38].

In the present study, mRNA expression profiles in CRLM were objectified in low risk patients who underwent hepatectomy with curative intent, without (neo-) adjuvant chemotherapy. All patients were homogeneous in terms of clinical risk, as defined by current standards

9. Within this homogeneous group with respect to clinical risk, we were still able to select two opposite ends of the clinical spectrum: patients with recurrences within 12 months after surgery and patients without recurrences for at least 36 months post-surgery. Analysis of differential gene expression of CRLM of these 2 adverse patient groups resulted in the identification of an 11-gene expression profile, able to discriminate between patients with early versus late or no recurrences after partial hepatectomy.

The fact that we were still able to identify two extremes (in terms of time to recurrence) in a clinically homogeneous group confirms the shortcomings of classic clinical risk scoring. The selection of these specific groups provided the opportunity to find molecular differences involved in outcome in a cohort where clinical parameters are incapable to do so. As all patients were chemo naive, true prognostic impact (tumour biology) could be researched. Chemo-naivety ruled out potential influences of the systemic regimens on the RNA expression in the tumour samples. Comparable studies lack true focus on prognostics, since the majority of these patients underwent pre- or postoperative systemic treatment [39-41]. The current chemo naïve patient cohort is unique, and the molecular risk profile identified in the current study therefore promising.

There is a strong potential for gene expression based-biomarkers such as the one identified in the current study. The 11-gene signature may serve as a novel blueprint for individualised therapies; either in combination with or without the classic clinical risk scores. Identification of patients for neo-adjuvant (preoperative) therapy is certainly possible since prognostic gene expression profiles may be detected in liquid biopsies before surgery [42, 43]. Currently the clinical risk scores do not impact clinical management, although some retrospective reports have suggested they may be effective [44, 45] (this is prospectively investigated at present in the CHARISMA trial [46]). There may be a synergistic effect between the clinical risk score and the molecular score of the current study. As all patients developing liver recurrences in the current study were at high molecular risk, the 11-gene signature may also play a role in identifying patients that benefit from regional chemotherapy specifically (e.g. hepatic arterial infusion pump [47]). Therefore, after thorough validation, the current biomarker may be effective in selecting patient groups for various treatment strategies.

There was no clear link between the mRNA expression profiles and other previously identified pathological features in CRLM, such as the tumour growth patterns. As stated earlier, three types of CRLM growth patterns can be observed: a pushing type, a replacing type and a desmoplastic type [23, 24]. The clinical impact of these growth patterns is still under investigation as their pathological presence is widely recognised. The molecular risk groups of the current study may be associated with a corresponding distinctive phenotype, possibly in the form of any of the established growth patterns. If such apparent tumour phenotypes exist, one could hypothesise that obvious differences may be recognisable at molecular level accordingly. In the current study, there was a trend towards an association between the high molecular risk group and the replacing growth pattern. A replacing growth pattern has repeatedly been associated with worse outcomes as compared to the desmoplastic

growth pattern [28, 48, 49]. In the current study the association is argumentative. A possible explanation for the lack of significance may be that these growth patterns are a specific characteristic of the leading edge of tumours. The gene expression data from the tumour samples in the current study are not exclusively retrieved from tumour tissue present in the leading edge. Currently, gene expression profiles for each of the growth patterns are assessed in an on-going study through laser macro-dissection of representative parts of the tumour.

Some of the functional annotations of the 11 genes in the signature provided insight into underlying biological mechanisms involved in recurrence, yet no evident common pathways could be discerned (see supplementaries 4 and 5). JARID1A, one of the 11 genes, is part of the "KDM5 family" of histone demethylases removing tri- and di-methylation marks of lysine 4 of histone H3 at transcription start site in actively transcribed genes. We find JARID1A upregulated in patients with early recurrences in the current study which is in line with growing evidence for a causal role of this marker in relation to cancer progression [50]. ERN1 (endoplasmic reticulum to nucleus signalling 1) is an important endoplasmic reticulum (ER) stress sensor. ERN1 signalling is a pro-angiogenic mechanism 45 and since we found ERN1 increased in patients with early recurrences, angiogenesis may be a contributing factor. Natural killer group 2, member D ligand ULBP2 and Ras-GAP binding protein G3BP2 are two extrinsic stress induced proteins contributing to progression. ULBP2, whose expression is low in patients having an early recurrence and whose receptor is on the surface of natural killer (NK) cells and specific T-cells, implies immune modulation 46 in recurrence. G3BP2 is known to affect matrix stiffness as does RPUSD1 (RNA pseudouridylate synthase domain containing 1) by controlling lateral growth of collagen II fibrils. G3BP2 and RPUSD1, with decreased and increased expression in the current study respectively, suggest that extracellular remodelling may affect the occurrence of recurrences as well. Potentially connected to the latter we find integrin subunit beta 5 (ITGB5), which is overexpressed in higher stages of CRC 47 and which modulates adhesion phenomena, and CASS4 the less studied signalling scaffold of the CAS (Crk-associated substrate) family which affects motility. Expression of these genes was elevated (ITGB5) and decreased (CASS4) in patients with early recurrence in the current study implying a role for migration, invasion and possibly progenitor cell function 48 and inhibition of apoptosis in cancer recurrence as well. The barely studied KIAA0319L and transmembrane protein clarin 3 (CLRN3) as well as COX6A1, which is involved in oxidative phosphorylation, affect recurrence rate but for now we cannot connect these proteins mechanistically to disease progression. Finally, the RAD9A checkpoint protein is required for proper localization of topoisomerase II-binding protein 1 (TopBP1) regulating cell cycle checkpoints, DNA repair, telomere stability and apoptosis [51-53] thereby preserving genomic integrity in all types of DNA aberrations [51-53]. In the current study, RAD9A was relatively downregulated in patients with early recurrences suggesting loss of genomic integrity is another contributing factor to recurrence [52]. Overall, we can conclude that recurrence of metastatic colorectal cancer in the liver is influenced by multiple complementary factors.

Limitations of the current study are its retrospective nature, the selection bias in terms of

DFS and a relatively small sample size. Based on the current study, it is challenging to provide advice regarding treatment management for the patient group 36>DFS>12. The present molecular marker profile therefore needs extensive validation in a larger independent cohort. This cohort should consist of patients representing the complete (continuous) spectrum in relation to recurrent disease, and possibly (but not necessarily) with both high- and low clinical risk scores. The current setting with two extremes in terms of recurrences was chosen as a first step in establishing a prognostic signature. If any relevant expression profiles exist in relation to recurrent disease, they are most likely to be identified within these extremes. KRAS and BRAF status would have been informative in terms of assessment of baseline risk for relapse. It is a timely topic of interest in CRLM. These molecular entities were not available in the current cohort. Ideally, in a validation study for the current molecular biomarker, all known prognostic molecular factors should be assessed (including other established signatures) such that all respective molecular markers can be put into context [32-37, 39-41]. A general point of discussion related to this type of translational research is the impact of inter- and intra- tumour heterogeneity on the reproducibility of results. Multiple studies show that even within single tumours heterogeneity exists [54, 55]. Despite any consensus on what lesion to analyse (e.g.: the largest) or what area within a tumour (e.g.: leading edge or core), heterogeneity will affect the generated results. Interestingly, these features of heterogeneity are known to have prognostic associations by itself in resected colorectal liver metastases [56]. Future studies should possibly also address spatial and temporal tumour heterogeneity, in addition to identification of a new biomarker.

CONCLUSION

In summary, in the current study a prognostic signature was constructed with the mRNA expression profiles of tumour tissue from resected CRLM. The signature consists of 11 genes of which the expression-patterns were able to discriminate between patients with early recurrences (≤12 months) versus no recurrences (≥36 months) after partial hepatectomy. This biomarker requires validation in a larger cohort representative of the complete clinical spectrum in terms of relapse and treated without (neo-) adjuvant therapy, including any other established prognostic molecular markers.

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENTS

We thank Vanja de Weerd and Katharina Biermann for assistance with collecting the data and processing of specimen.

RESEARCH SUPPORT

We would like to thank Mr. Winkelman for making this study possible with a personal grant.

APPENDIX A

Supplementary data.

REFERENCES

1. Torre, L.A., et al., *Global cancer statistics, 2012*. CA Cancer J Clin, 2015. **65**(2): p. 87-108.
2. van der Pool, A.E., et al., *Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series*. Colorectal Dis, 2012. **14**(1): p. 56-61.
3. Dols, L.F., et al., *[Improvement of 5 year survival rate after liver resection for colorectal metastases between 1984-2006] Verbetering in 5-jaarsoverleving na leverresectie voor colorectale metastasen tussen 1984-2006*. Ned Tijdschr Geneeskd, 2009. **153**(11): p. 490-5.
4. Rees, M., et al., *Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients*. Ann Surg, 2008. **247**(1): p. 125-35.
5. Primrose, J.N., *Surgery for colorectal liver metastases*. Br J Cancer, 2010. **102**(9): p. 1313-8.
6. D'Angelica, M., et al., *Effect on outcome of recurrence patterns after hepatectomy for colorectal metastases*. Ann Surg Oncol, 2011. **18**(4): p. 1096-103.
7. Butte, J.M., et al., *Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection*. Ann Surg Oncol, 2015.
8. de Jong, M.C., 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): p. 440-8.
9. Matias, M., et al., *Prognostic Factors after Liver Resection for Colorectal Liver Metastasis.* Acta Med Port, 2015. **28**(3): p. 357-69.
10. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases*. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
11. Konopke, R., et al., *Prognostic factors and evaluation of a clinical score for predicting survival after resection of colorectal liver metastases.* Liver Int, 2009. **29**(1): p. 89-102.
12. Nagashima, I., et al., *A new scoring system to classify patients with colorectal liver metastases: proposal of criteria to select candidates for hepatic resection.* J Hepatobiliary Pancreat Surg, 2004. **11**(2): p. 79-83.
13. Nordlinger, B., et al., *Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie*. Cancer, 1996. **77**(7): p. 1254-62.
14. Poston, G.J., *Staging of advanced colorectal cancer.* Surg Oncol Clin N Am, 2008. **17**(3): p. 503-17, viii.
15. Poston, G.J., et al., *Urgent need for a new staging system in advanced colorectal cancer*. J Clin Oncol, 2008. **26**(29): p. 4828-33.
16. Guinney, J., et al., *The consensus molecular subtypes of colorectal cancer.* Nat Med, 2015.
17. Paik, S., et al., *A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer*. N Engl J Med, 2004. **351**(27): p. 2817-26.
18. Hoshida, Y., et al., *Gene expression in fixed tissues and outcome in hepatocellular carcinoma.* N Engl J Med, 2008. **359**(19): p. 1995-2004.

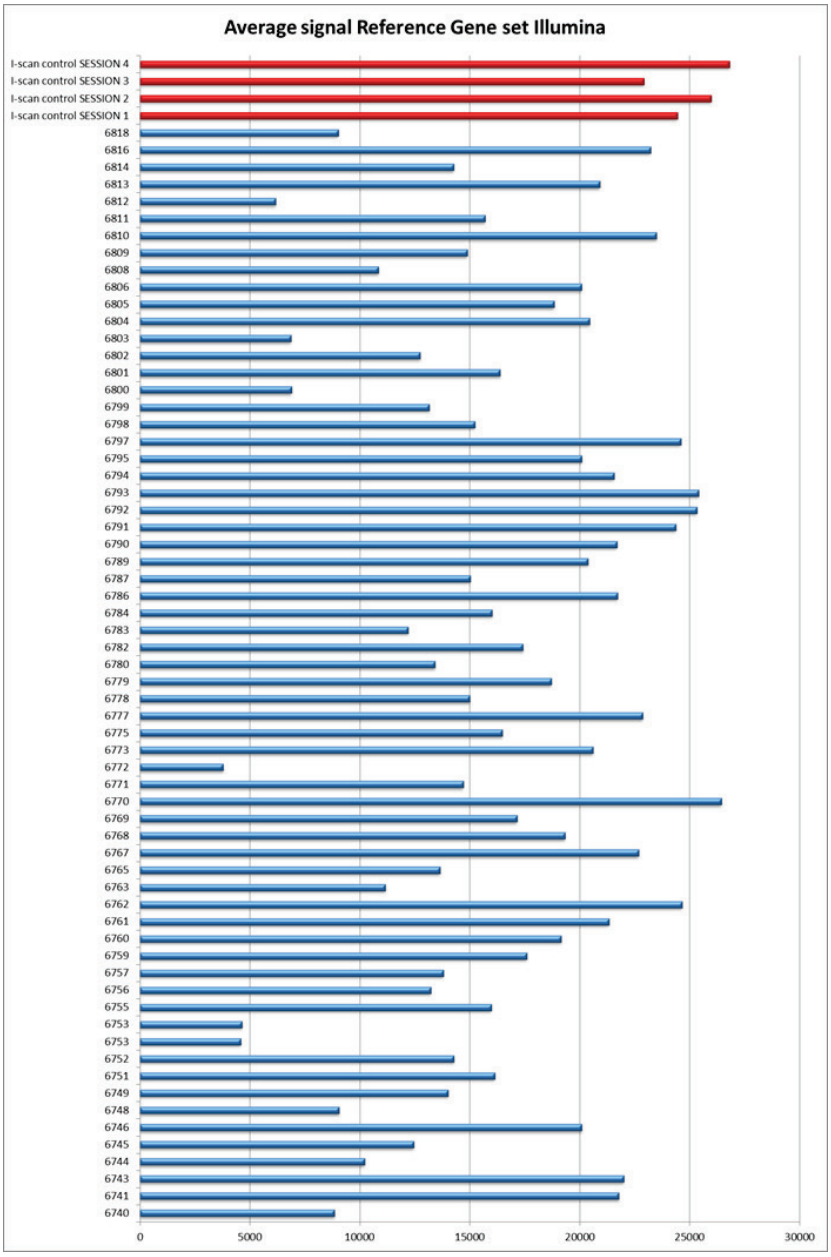
19. Albain, K.S., et al., *Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial*. Lancet Oncol, 2010. **11**(1): p. 55-65.
20. Budinska, E., et al., *Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer*. J Pathol, 2013. **231**(1): p. 63-76.
21. Sadanandam, A., et al., *A colorectal cancer classification system that associates cellular phenotype and responses to therapy*. Nat Med, 2013. **19**(5): p. 619-25.
22. Ayez, N., et al., *Outcome of microscopic incomplete resection (R1) of colorectal liver metastases in the era of neoadjuvant chemotherapy*. Ann Surg Oncol, 2012. **19**(5): p. 1618-27.
23. Vermeulen, P.B., et al., *Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia*. J Pathol, 2001. **195**(3): p. 336-42.
24. Van den Eynden, G.G., et al., *The multifaceted role of the microenvironment in liver metastasis: biology and clinical implications*. Cancer Res, 2013. **73**(7): p. 2031-43.
25. Van den Eynden, G.G., et al., *The histological growth pattern of colorectal cancer liver metastases has prognostic value*. Clin Exp Metastasis, 2012. **29**(6): p. 541-9.
26. Okano, K., et al., *Fibrous pseudocapsule of metastatic liver tumors from colorectal carcinoma. Clinicopathologic study of 152 first resection cases*. Cancer, 2000. **89**(2): p. 267-75.
27. Lunevicius, R., et al., *Clinicopathological significance of fibrotic capsule formation around liver metastasis from colorectal cancer*. J Cancer Res Clin Oncol, 2001. **127**(3): p. 193-9.
28. Eefsen, R.L., et al., *Growth pattern of colorectal liver metastasis as a marker of recurrence risk*. Clin Exp Metastasis, 2015. **32**(4): p. 369-81.
29. Mustafa, D.A., et al., *A Method to Correlate mRNA Expression Datasets Obtained from Fresh Frozen and Formalin-Fixed, Paraffin-Embedded Tissue Samples: A Matter of Thresholds*. PLoS One, 2015. **10**(12): p. e0144097.
30. Fan, J.B., et al., *A versatile assay for high-throughput gene expression profiling on universal array matrices*. Genome Res, 2004. **14**(5): p. 878-85.
31. Du, P., W.A. Kibbe, and S.M. Lin, *lumi: a pipeline for processing Illumina microarray*. Bioinformatics, 2008. **24**(13): p. 1547-8.
32. Passiglia, F., et al., *Can KRAS and BRAF mutations limit the benefit of liver resection in metastatic colorectal cancer patients? A systematic review and meta-analysis*. Crit Rev Oncol Hematol, 2016. **99**: p. 150-7.
33. Karagkounis, G., et al., *Incidence and prognostic impact of KRAS and BRAF mutation in patients undergoing liver surgery for colorectal metastases*. Cancer, 2013. **119**(23): p. 4137-44.
34. Lin, Q., et al., *Determinants of long-term outcome in patients undergoing simultaneous resection of synchronous colorectal liver metastases*. PLoS One, 2014. **9**(8): p. e105747.
35. Loes, I.M., et al., *Impact of KRAS, BRAF, PIK3CA, TP53 status and intraindividual mutation heterogeneity on outcome after liver resection for colorectal cancer metastases*. Int J Cancer, 2016. **139**(3): p. 647-56.
36. Margonis, G.A., et al., *Association Between Specific Mutations in KRAS Codon 12 and Colorectal Liver Metastasis*. JAMA Surg, 2015. **150**(8): p. 722-9.
37. Vauthey, J.N., et al., *RAS mutation status predicts survival and patterns of recurrence in patients*

undergoing hepatectomy for colorectal liver metastases. Ann Surg, 2013. **258**(4): p. 619-26; discussion 626-7.

38. Zakaria, S., et al., *Hepatic resection for colorectal metastases: value for risk scoring systems?* Ann Surg, 2007. **246**(2): p. 183-91.
39. Ito, H., et al., *Gene expression profiles accurately predict outcome following liver resection in patients with metastatic colorectal cancer.* PLoS One, 2013. **8**(12): p. e81680.
40. Snoeren, N., et al., *Exploring gene expression signatures for predicting disease free survival after resection of colorectal cancer liver metastases*. PLoS One, 2012. **7**(11): p. e49442.
41. Balachandran, V.P., et al., *A validated prognostic multigene expression assay for overall survival in resected colorectal cancer liver metastases*. Clin Cancer Res, 2016.
42. Mostert, B., et al., *mRNA expression profiles in circulating tumor cells of metastatic colorectal cancer patients*. Mol Oncol, 2015. **9**(4): p. 920-32.
43. Mostert, B., et al., *KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue.* Int J Cancer, 2013. **133**(1): p. 130-41.
44. Ayez, N., et al., *The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator*. Eur J Surg Oncol, 2015. **41**(7): p. 859-67.
45. Rahbari, N.N., 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**: p. 174.
46. Ayez, N., 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**: p. 180.
47. Kemeny, N., et al., *Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer.* N Engl J Med, 1999. **341**(27): p. 2039-48.
48. Nielsen, K., et al., *The morphological growth patterns of colorectal liver metastases are prognostic for overall survival.* Mod Pathol, 2014. **27**(12): p. 1641-8.
49. Pinheiro, R.S., et al., *Tumor growth pattern as predictor of colorectal liver metastasis recurrence*. Am J Surg, 2014. **207**(4): p. 493-8.
50. Rasmussen, P.B. and P. Staller, *The KDM5 family of histone demethylases as targets in oncology drug discovery*. Epigenomics, 2014. **6**(3): p. 277-86.
51. Greer Card, D.A., M.L. Sierant, and S. Davey, *Rad9A is required for G2 decatenation checkpoint and to prevent endoreduplication in response to topoisomerase II inhibition*. J Biol Chem, 2010. **285**(20): p. 15653-61.
52. Broustas, C.G. and H.B. Lieberman, *Contributions of Rad9 to tumorigenesis*. J Cell Biochem, 2012. **113**(3): p. 742-51.
53. Lieberman, H.B., et al., *The role of RAD9 in tumorigenesis*. J Mol Cell Biol, 2011. **3**(1): p. 39-43.
54. Marusyk, A., V. Almendro, and K. Polyak, *Intra-tumour heterogeneity: a looking glass for cancer?* Nat Rev Cancer, 2012. **12**(5): p. 323-34.
55. Tabassum, D.P. and K. Polyak, *Tumorigenesis: it takes a village.* Nat Rev Cancer, 2015. **15**(8): p. 473-83.
56. Sveen, A., 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): p. e1006225.

APPENDIX A

Supplementary data

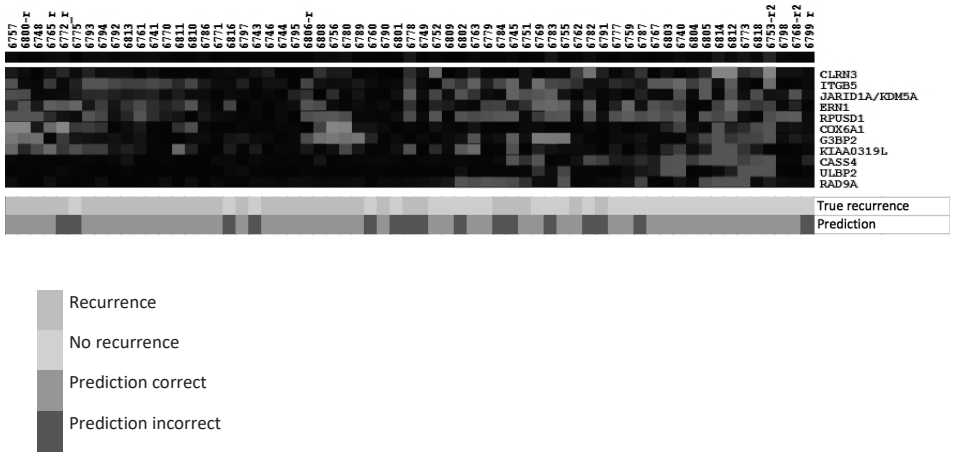


Supplementary 1. Average signal references. In red: I-scan control samples (inter-assay), in blue: tumour samples.

Supplementary 2: Raw data output from the "Leave-one-out-cross-validation" analysis

Sample	Array ID	Class label	Nr of genes in classifier	SVM Correct?	Sample	Array ID	Class label	Nr of genes in classifier	SVM Correct?
1	6812	No	9	YES	**33**	6744	Yes	12	YES
2	6740	No	12	YES	**34**	6746	Yes	12	YES
3	6803	No	10	YES	**35**	6748	Yes	10	YES
4	6743	No	13	NO	**36**	6749	Yes	11	NO
5	6752	No	11	YES	**37**	6751	Yes	12	YES
6	6759	No	11	YES	**38**	6761	Yes	10	YES
7	6760	No	13	NO	**39**	6762	Yes	12	YES
8	6767	No	10	YES	**40**	6770	Yes	11	YES
9	6777	No	10	YES	**41**	6771	Yes	9	YES
10	6755	No	12	YES	**42**	6784	Yes	14	NO
11	6769	No	10	YES	**43**	6789	Yes	12	YES
12	6773	No	11	YES	**44**	6790	Yes	9	YES
13	6779	No	10	YES	**45**	6791	Yes	12	NO
14	6782	No	11	NO	**46**	6792	Yes	9	YES
15	6783	No	12	NO	**47**	6793	Yes	9	YES
16	6787	No	12	NO	**48**	6745	Yes	15	NO
17	6799_r	No	15	NO	**49**	6756	Yes	11	YES
18	6801	No	16	NO	**50**	6757	Yes	17	YES
19	6804	No	9	YES	**51**	6765_r	Yes	12	YES
20	6805	No	11	YES	**52**	6778	Yes	16	NO
21	6763	No	12	YES	**53**	6780	Yes	12	YES
22	6798	No	13	YES	**54**	6786	Yes	10	YES
23	6802	No	10	NO	**55**	6808	Yes	12	YES
24	6809	No	13	YES	**56**	6794	Yes	11	YES
25	6814	No	10	YES	**57**	6811	Yes	11	YES
26	6818	No	10	YES	**58**	6795	Yes	11	YES
27	6775	No	15	NO	**59**	6797	Yes	11	YES
28	6816	No	15	NO	**60**	6810	Yes	11	YES
29	6768-r2	No	12	YES	**61**	6813	Yes	10	YES
30	6753-r2	No	10	YES	**62**	6806-r	Yes	11	YES
31	6772_r	Yes	13	NO	**63**	6800-r	Yes	12	YES
32	6741	Yes	11	YES					

Class label: No=no recurrence and DFS ≥36 months after hepatectomy, Yes=recurrence ≤12 months after hepatectomy; SVM=Support Vector Machine algorithm



Supplementary 3. Expression heatmap of the 11 genes (vertical), in all analysed samples (horizontal). True recurrence and the prediction outcome of the currently established algorithm are depicted under the heatmap.

Supplementary 4: Pathways in which the identified genes are described to be involved in from the "KEGG Pathway Database"

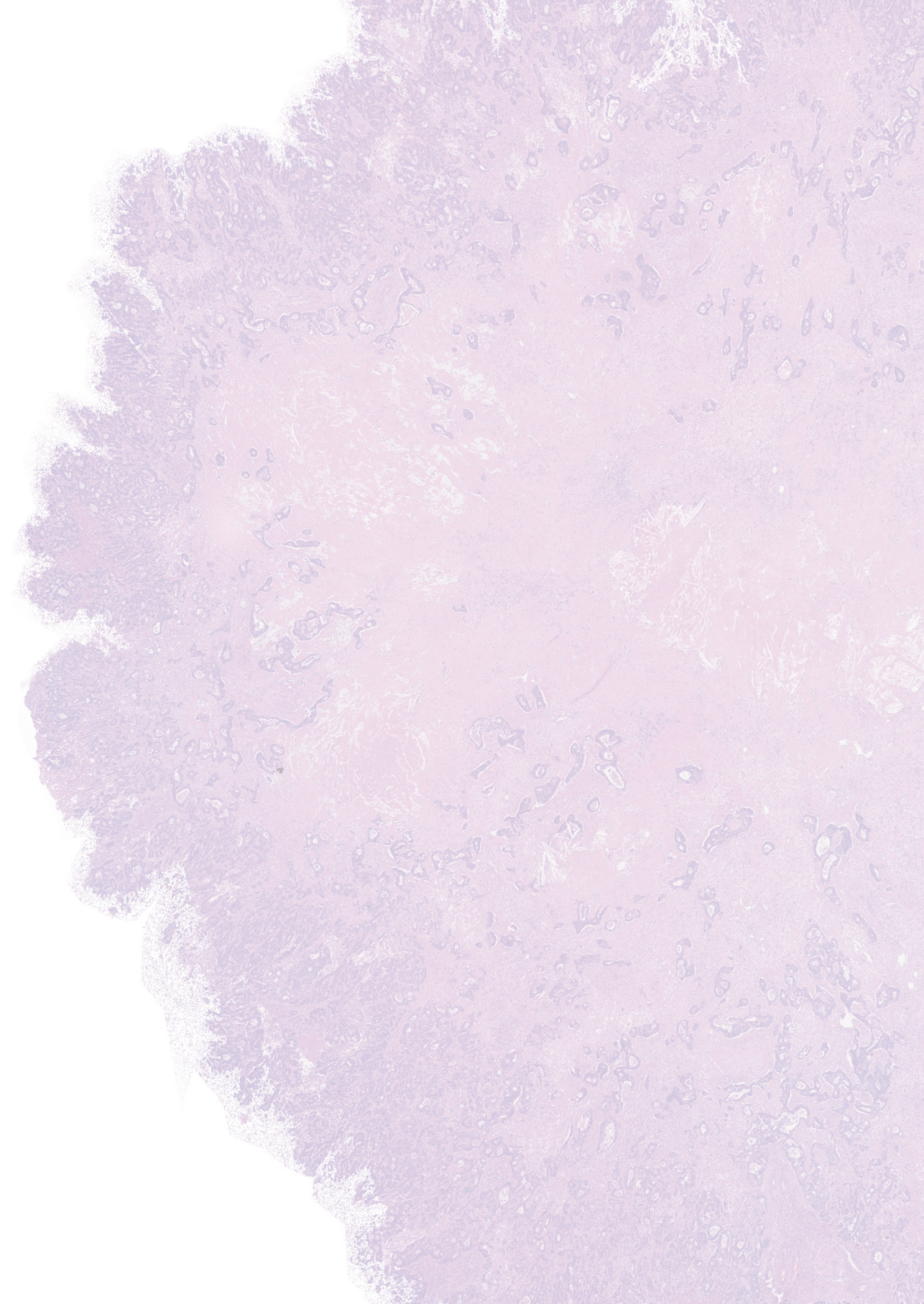
ID	Gene Name	KEGG_PATHWAY
COX6A1	Cytochrome c oxidase subunit 6A1	Oxidative phosphorylation
		Metabolic pathways
		Cardiac muscle contraction
		Non-alcoholic fatty liver disease (NAFLD)
		Alzheimer's disease
		Parkinson's disease
		Huntington's disease
		Protein processing in endoplasmic reticulum
		Non-alcoholic fatty liver disease (NAFLD)
		Alzheimer's disease
ITGB5	Integrin subunit beta 5	Phagosome
		PI3K-Akt signaling pathway
		Focal adhesion
		ECM-receptor interaction
		Regulation of actin cytoskeleton
		Proteoglycans in cancer
		Hypertrophic cardiomyopathy (HCM)
		Arrhythmogenic right ventricular cardiomyopathy (ARVC)
		Dilated cardiomyopathy
ULBP2	UL16 binding protein 2	Natural killer cell mediated cytotoxicity

Supplementary 5: Pathways in which the identified genes are described to be involved in from the "Gene Ontology Consortium"

ID	Gene Name	GOTERM_BP_DIRECT
CASS4	Cas scaffolding protein family member 4	Phosphorelay signal transduction system cell adhesion signal transduction by protein phosphorylation Cell adhesion
COX6A1	Cytochrome c oxidase subunit 6A1	Signal transduction by protein phosphorylation Generation of precursor metabolites and energy Transcription initiation from RNA polymerase II promoter Gene expression Respiratory electron transport chain Cellular metabolic process Small molecule metabolic process Hydrogen ion transmembrane transport
ERN1	Endoplasmic reticulum to nucleus signaling 1	Endothelial cell proliferation Transcription, DNA-templated Regulation of transcription, DNA-templated, mRNA cleavage mRNA catabolic process, Protein phosphorylation Activation of signaling protein activity involved in unfolded protein response Cell cycle arrest Activation of JUN kinase activity Regulation of macroautophagy Endoplasmic reticulum unfolded protein response Positive regulation of RNA splicing Response to endoplasmic reticulum stress Cellular response to vascular endothelial growth factor stimulus Peptidyl-serine autophosphorylation IRE1-mediated unfolded protein response Cellular protein metabolic process Protein autophosphorylation mRNA splicing, via endonucleolytic cleavage and ligation mRNA endonucleolytic cleavage involved in unfolded protein response Intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress Cellular response to glucose stimulus RNA phosphodiester bond hydrolysis, endonucleolytic Positive regulation of endoplasmic reticulum unfolded protein response

Supplementary 5: Pathways in which the identified genes are described to be involved in from the "Gene Ontology Consortium" (Continue)

ID	Gene Name	Insulin metabolic process
G3BP2	G3BP stress granule assembly factor 2	Peptidyl-serine trans-autophosphorylation Cytoplasmic sequestering of NF-kappaB Ras protein signal transduction mRNA transport
ITGB5	Integrin subunit beta 5	Antigen processing and presentation of peptide antigen via MHC class I Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent Muscle contraction Cell-matrix adhesion Transforming growth factor beta receptor signaling pathway Integrin-mediated signaling pathway Extracellular matrix organization Endodermal cell differentiation Antigen processing and presentation of exogenous peptide antigen via MHC class I Stress fiber assembly Viral entry into host cell Epithelial cell-cell adhesion
KDM5A	Lysine demethylase 5A	Negative regulation of transcription from RNA polymerase II promoter Chromatin organization, Transcription from RNA polymerase II promoter, Spermatogenesis Male gonad development Circadian regulation of gene expression Histone H3-K4 demethylation Positive regulation of transcription, DNA-templated Oxidation-reduction process Negative regulation of histone deacetylase activity
RAD9A	RAD9 checkpoint clamp component A	DNA replication checkpoint DNA damage checkpoint Double-strand break repair via homologous recombination DNA repair DNA replication Double-strand break repair Cellular response to DNA damage stimulus Intra-S DNA damage checkpoint Cellular response to ionizing radiation Nucleic acid phosphodiester bond hydrolysis Positive regulation of intrinsic apoptotic signaling pathway in response to DNA damage
RPUSD1	RNA pseudouridylate synthase domain 1	tRNA pseudouridine synthesis
ULBP2	UL16 binding protein 2	Antigen processing and presentation Natural killer cell activation Natural killer cell mediated cytotoxicity



CHAPTER 8

International consensus guidelines for scoring the histopathological growth patterns of liver metastasis

P.J. van Dam
E.P. van der Stok
L.A. Teuwen
G.G. Van den Eynden
M. Illemann
S. Frentzas
A.W. Majeed
R.L. Eefsen
R.R.J. Coebergh van den Braak
A. Lazaris
M.C. Fernandez
B. Galjart
O.D. Laerum
R. Rayes
D.J. Grünhagen
M. Van de Paer
Y. Sucaet
H. S. Mudhar
M. Schvimer
H. Nyström
M. Kockx
N.C. Bird
F. Vidal-Vanaclocha
P. Metrakos
E. Simoneau
C. Verhoef
L.Y. Dirix
S. Van Laere
Z.H. Gao
P. Brodt
A.R. Reynolds
P.B. Vermeulen

British Journal of Cancer. 2017 Nov 7;117(10):1427-1441

ABSTRACT

BACKGROUND

Liver metastases present with distinct histopathological growth patterns (HGPs), including the desmoplastic, pushing and replacement HGPs and two rare HGPs. The HGPs are defined owing to the distinct interface between the cancer cells and the adjacent normal liver parenchyma that is present in each pattern and can be scored from standard haematoxylin-and-eosin-stained (H&E) tissue sections. The current study provides consensus guidelines for scoring these HGPs.

METHODS

Guidelines for defining the HGPs were established by a large international team. To assess the validity of these guidelines, 12 independent observers scored a set of 159 liver metastases and inter-observer variability was measured. In an independent cohort of 374 patients with colorectal liver metastases (CRCLM), the impact of HGPs on overall survival after hepatectomy was determined.

RESULTS

Good-to-excellent correlations (intraclass correlation coefficient >0.5) with the gold standard were obtained for the assessment of the replacement HGP and desmoplastic HGP. Overall survival was significantly superior in the desmoplastic HGP subgroup compared with the replacement or pushing HGP subgroup (P=0.006).

CONCLUSIONS

The current guidelines allow for reproducible determination of liver metastasis HGPs. As HGPs impact overall survival after surgery for CRCLM, they may serve as a novel biomarker for individualised therapies.

INTRODUCTION

Despite many years of basic and clinical research aimed at curbing tumour growth, metastasis still remains the principle cause of death in the majority of solid tumours. The liver is a frequent site of metastasis for tumours originating from the gastrointestinal tract, pancreas, breast and lung; the liver also hosts metastases of renal cell carcinoma, melanoma and sarcoma [1, 2]. For example, ±80% of all metastases from colorectal cancer (CRC) occur in the liver [3]. Approximately 20-25% of patients with CRC present with liver metastases at the time of diagnosis with a further 20-25% of patients expected to develop liver metastases at a later date.

We and others have shown that the majority of metastases to the liver present in one of three common distinct histopathological growth patterns (HGPs), known as desmoplastic HGP, pushing HGP or replacement HGP, and two rare HGPs. These HGPs are distinguishable because the interface between the cancer cells of the metastasis and the surrounding normal liver is distinct in each growth pattern. Moreover, the HGPs are recognisable by light microscopy in standard haematoxylin-and-eosin (H&E)-stained tissue sections [2, 4, 5]. The distinct topography of cancer cells in each HGP predicts HGP-specific interactions with parenchymal (hepatocytes and cholangiocytes) and non-parenchymal cells (sinusoidal endothelial cells, stellate cells and immune cells) of the liver. However, despite these clear differences in the biology of these metastases, the molecular drivers of the distinct HGPs remain unknown. It is also currently unclear whether these distinct HGPs require different clinical management strategies.

An overview of previous reports where these HGPs have been studied is provided in Table 1. One of the most important observations made in these studies is that liver metastases with a replacement HGP do not rely on sprouting angiogenesis for a vascular supply but instead co-opt the sinusoidal vasculature of the liver [4-6]. This is inferred from the specific morphology of replacement- type liver metastases [4-10] and is consistent with the small endothelial cell proliferation fraction reported in these metastases [4, 11-13]. This co-option of sinusoidal blood vessels and peri-sinusoidal space (space of Disse) in the replacement HGP is in contrast to the desmoplastic HGP where extensive stromal remodelling and angiogenesis are observed [5, 6]. Desmoplastic liver metastases have an upregulated uPA-uPAR-PAI-1 proteolytic system and an elevated content of type I and type IV collagens [14-16].

Interestingly, there is some evidence that the HGP of CRC liver metastases may be predicted by the histology of the primary tumour. Primary CRC can be classified as having a pushing margin or an infiltrative margin as defined by Jass et al (1987) [17]. When liver metastases were classified as being 'encapsulated' (which probably corresponds to the desmoplastic HGP) or non-encapsulated (which probably corresponds to pushing HGP or replacement HGP), 69% of the primary CRCs with pushing margins, as defined by the Jass criteria (Jass et al, 1987), developed encapsulated liver metastases while only 17% of primary CRCs with an infiltrative margin developed encapsulated liver metastases [18]. The type of primary cancer

can also be predictive of the liver metastases HGP, as almost all breast cancer liver metastases adopt a replacement growth pattern, while liver metastases of CRC can present with any of the different HGPs described [4, 6].

Importantly, the HGPs of liver metastases were shown to have prognostic significance. Both Van den Eynden et al (2012) and Nielsen et al (2014) [12, 19] studied the impact of the HGPs on overall survival in patients with metastatic CRC. In both studies, the desmoplastic HGP represented superior overall survival. However, in these studies, the results regarding the relative incidence of the different HGPs and the prognostic values of the replacement and pushing HGPs were contradictory. These contradicting results may have been a consequence of differences in the treatment history of the patients in both studies or due to the low number of patient samples that were examined but were likely also due to differences in the methodology used to assess the HGPs. These disparities highlight the need to develop consensus guidelines for scoring the HGPs of liver metastases.

A second important reason to develop such guidelines is the emerging prognostic or predictive value of the HGPs. The clinical and biological diversity of, for instance, CRC liver metastases indeed urges the need for predictive biomarkers to facilitate tailor-made treatment strategies [20, 21]. Frentzas et al (2016) [6] demonstrated that CRC liver metastases with a replacement HGP respond poorly to bevacizumab treatment, likely because these tumours utilise vessel co-option instead of angiogenesis. By contrast, desmoplastic liver metastases, which are angiogenic, showed a better response to bevacizumab [6]. These data strongly suggest that HGPs can be used to guide the choice of treatment for individual patients with liver metastases.

Finally, guidelines for scoring the HGPs of liver metastases are important for future mechanistic studies that are aimed at elucidating the molecular drivers of each HGP. These studies will require the establishment of preclinical liver metastasis models that mimic the distinct growth patterns using established cell lines or patient-derived xenografts (PDXs). Studies using such models should lead to identification of novel targets to facilitate precision treatments for patients with liver metastases.

The aims of the current manuscript are therefore to (a) propose consensus guidelines for scoring the HGPs of liver metastases, (b) test the analytical validity of these guidelines, (c) validate the prognostic significance of the HGPs of liver metastasis, (d) speculate on the molecular mechanisms that may underlie the differences in the growth patterns, and (e) highlight future research directions for growth pattern research.

Table 1: overview of studies that have addressed the association of the growth patterns of liver metastases with clinical and pathological parameters

First author	Reference (in chronological order)	Methodology	Study population	Main results
Masson P	Les Tumeurs. In: Traité de Pathol et Thérapie appliquées par Sergent, Ribadeau-Dumas et Babonneix, 1923, Vol.27, Part II	Histology: H&E or comparable stain	case reports	Description of replacement, sinusoidal & desmoplastic growth patterns.
Hamperl H	Über die Gutartigkeit und Bösartigkeit von Geschwülsten. Verh Dtsch Ges Path 35, 1951, 29	Histology: silver stain	case reports	Description of replacement growth and sinusoidal growth
Elias H	Acta Hepato-Splenol 1962; 9: 357–386 Oncology (International Journal of Cancer Research and Treatment) 1964; 18(3): 210-224	Histology: H&E, silver stain	23 autopsy cases	- 4 growth patterns: expansive, invasive, destructive, replacing. - Description of the replacement of normal hepatocytes by tumour cells: 'normal liver cells join a tumour at its periphery' 'Carcinoma cells are located within liver plates together with structurally normal liver cells without signs of compression or lysis of the latter'
Nakashima T	Hum Pathol 1982; 13:563-568	Histology: H&E, silver stain Growth pattern assessment at the boundary between tumour and liver parenchyma	HCC, 24 autopsy liver specimens (no chemotherapy)	- 3 growth patterns: sinusoidal (25%), replacing (46%), encapsulated (29%) - Association of HGP with degree of differentiation (anaplastic, intermediate, well differentiated (respectively)) - Incorporation of some hepatocytes in the tumour in the sinusoidal and replacing growth pattern.
Terayama N	Jpn J Clin Oncol 1996; 26:24-29	Histology: H&E, silver stain. Growth pattern assessment at the boundary between tumour and liver parenchyma	Liver metastasis, 100 autopsy liver specimens (lung, pancreas, stomach, gallbladder/bile duct, colon cancer)	- 5 parenchymal growth patterns: sinusoidal, replacement, encapsulated, expansive, unclassified - growth pattern frequency dependent on cancer histiotype (portal growth (resembling lymphangitis carcinomatosa of the lung) in poorly differentiated cases) - higher frequency of replacement growth in small metastases
Terayama N	Hepatology 1996; 24:816-819	IHC for vWF, histochemistry with UEA-I 3D-morphometry after vascular casting	Same study population as above	- phenotypic changes of sinusoidal blood vessels in the adjacent liver parenchyma - in small metastases: sinusoidal blood vessels in continuity with blood vessels of liver metastases. - blood vessel density increased according to the size up to 3 mm in diameter, remaining stable over 3mm
Okano K	Cancer 2000; 89:267–75	Histology: H&E Presence of fibrous tissue between tumour and liver parenchyma: none, thin, thick (10 or more layers of collagen bundles)	Liver metastases of 152 patients with CRC; liver resection with curative intent (30%/70% synchronous/metachronous)	- no (pseudo)capsule (39%), thin (30%), thick (31%) - no association of HGP with size or histological differentiation of the metastases - thick capsule associated with intrabile ductal growth, no capsule associated with vascular invasion - thick capsule associated with better overall survival (multivariate analysis)

Table 1: overview of studies that have addressed the association of the growth patterns of liver metastases with clinical and pathological parameters (continue)

First author	Reference (in chronological order)	Methodology	Study population	Main results
Lunevicius R	J Cancer Res Clin Oncol 2001; 127:193-199 (comparable results in: - Ohlsson B et al., World J Surg 1998; 22:268-277 - Morino T et al., Arch Jpn Chir 1991; 60: 154-164)	Histology: H&E Non-capsule: tumour cells face the hepatic parenchyma directly (no fibrous capsule); Capsule: fibrous band of 0,5mm thickness or more Intermediate IHC for α-smooth muscle actin, desmin, vimentin, CD-8, CD-68, collagen Type I, MMP-1, MMP-2, and TIMP-1	Liver metastases of 69 patients with CRC	- Non-capsule (45%), intermediate (35%), capsule (20%) = 20% 'encapsulated' - Encapsulation more frequently around differentiated metastatic cancers - increased survival rates related to encapsulation (5 yr, not long-term)
Vermeulen PB	J Pathol 2001; 195: 336–342	Histology: H&E, silver stain Liver metastasis HGP (dominant HGP) IHC: double-staining for CD31/Ki67 and for CD34/ α- SMA IHC for caspase cleavage site of CK18	Liver metastases of 26 patients with CRC (chemonaive/elective surgery/one metastasis per patient)	- desmoplastic (42%), pushing (46%), replacement (12%) (dense, mild and no inflammatory infiltrate, respectively) - highest ECP and highest ECP/TCP in pushing HGP - higher fraction of immature microvessels in the desmoplastic versus the pushing GP
Stessels F	British Journal of Cancer 2004; 90, 1429 – 1436	Histology: H&E Liver metastasis HGP (according to Vermeulen et al. 2001) IHC for CAIX, CD68, fibrin, LYVE-1	Liver metastases of 45 patients with BC (28 necropsy-cases) and 28 patients with CRC (surgical resection specimens)	- HGP is cancer type-dependent. BC: desmoplastic (2%), pushing (2%), replacement (96%); CRC: desmoplastic (50%), pushing (18%), replacement (32%) - less CAIX, less fibrin and less macrophages in the BC metastases and in the replacement-type metastases of CRC - cooption of LYVE-1-positive sinusoidal blood vessels in replacement HGP
Allison KH	Arch Pathol Lab Med 2004; 128: 1418-1423	Histology: H&E	Case reports of occult breast cancer invasion of the liver (and review of literature).	Liver failure and death due to breast cancer metastasis in the liver with sinusoidal growth pattern.
Rajaganeshan R	British Journal of Cancer 2007; 96:1112 – 1117	Histology: H&E Invasive margin of primary CRC (according to Jass et al. 1987) Liver metastasis HGP (according to Lunevicius et al. 2001) IHC for CD31: computer-aided image analysis to determine MVD	Liver metastases of 55 patients with colorectal cancer; resection with curative intent (53%/47% synchronous/metachronous)	- Association between HGP of primary CRC and HGP of liver metastases (pushing and capsulated; infiltrative and noncapsulated, respectively) - Positive correlation of MVD at the tumour margin of primary CRC and matched liver metastases.
Illeman M	Int J Cancer 2009; 124, 1860–1870 Comment by Van den Eynden G et al. Int J Cancer 2009; 125; 1494-1495	Histology: silver stain Liver metastasis HGP (according to Vermeulen et al. 2001) Invasive margin of primary CRC (according to Jass-criteria) IHC: uPAR, PAI-1, LN5γ2 ISH: uPA	14 liver metastases and matched primary CRC (14 patients (1-5 metastases/patient)) (43%/57% synchronous/metachronous)	- No correlation between the degree of cancer cell budding in the CRC and the HGP of liver metastases - desmoplastic GP (43%), pushing (57%), replacement (0%) - up-regulation (as in all primary CRC) of uPA-PAI system in 5/6 desmoplastic <> 0/8 pushing metastases. - differences between activity of uPA-PAI system between primary breast carcinoma and liver metastases of breast cancer

Table 1: overview of studies that have addressed the association of the growth patterns of liver metastases with clinical and pathological parameters (continue)

First author	Reference (in chronological order)	Methodology	Study population	Main results
Nyström H	Anticancer Research 2012; 32: 5183-5192	Histology: H&E and chemical reticular staining; dominant pattern was used, largest metastasis was used if multiple metastases Picro-Sirius Red staining for type I and type III collagens IHC for type I and type IV collagens HGP according to Vermeulen PB et al. 2001	Liver metastases of 48 patients with colorectal cancer (fraction with neo-adjuvant treatment)	- desmoplastic (47%) and pushing (53%), no replacement HGP (0%) - higher levels of type I and type IV collagen in desmoplastic metastases - shorter overall survival in patients with pushing metastases - no prediction of HGP by analyzing the characteristics of the primary CRC - no association of size of the liver metastases and HGP
Van den Eynden G	Clin Exp Metastasis 2012; 29:541–549	Histology: H&E, silver stain. HGP assessment at the boundary between tumour and liver parenchyma: one GP if >75% of interface, mixed if GPs > 25% of interface IHC double-staining for CD34/Ki67 and staining for CAIX	Liver metastases of 205 patients with colorectal cancer; resections with curative intent (50%/50% synchronous /metachronous)	- 27,8% replacement; 15,6% pushing; 34,6% desmoplastic; 17,6% mixed (n=9:insufficient sampling of interface) - ECP (%) highest in pushing HGP, TCP (%) lowest in replacement HGP - higher ECP and TCP if CAIX-expression at interface - metastases with pushing HGP larger than desmoplastic/replacement HGP - at 2 year FU: lower survival fraction in the group with pushing component
Løvendahl Eefsen R	Journal of Oncology 2012; Article ID 907971, 12 pages	Histology: H&E, silver stain. HGP assessment at the boundary between tumour and liver parenchyma: one HGP if >75% of the visualised interface, mixed if GPs > 25% of the visualised interface (according to Vermeulen et al. 2001). IHC for CD31, uPAR IHC double-staining for CD34/Ki67	62 liver metastases obtained from 24 chemonaive patients with CRC (21 (88%) patients with synchronous metastases)	- 4 observers for HGP scoring: inter-observer kappa-values: 0.52-0.69 (due to 'mixed' versus 'uniform' pattern scoring differences) - desmoplastic (25.8%), pushing (33.9%), replacement (21%), mixed (19.3%). - 20/24 patients with same HGP in all metastases. - elevated expression of uPAR in desmoplastic and replacement HGP - ECP/TCP highest in the pushing HGP - no association of primary tumour characteristics (obtained from pathology report) and HGP
Simone C	Journal of Medical Case Reports 2012; 6: 402	Histology: H&E	Case reports of occult cancer invasion of the liver (and review of literature)	Liver failure and death due to cancer metastasis in the liver with sinusoidal growth pattern
Nielsen K	Mod Pathol 2014; 27: 1641-1648	Histology: H&E, silver stain (?) (according to Vermeulen et al. 2001; 75% cut-off for dominant growth pattern)	- 217 liver metastases obtained from 217 patients with CRC (treatment data not available) - 22 re-resections of 16 patients	- pushing (33%), desmoplastic (32%), replacement (11%), mixed growth pattern (24%) - desmoplastic metastases were significantly smaller. - replacement growth pattern related to shorter overall survival - in 13/22 recurrent metastases, a new growth pattern was found.
Pinheiro RS	Am J Surg 2014; 207: 493-498	Histology: H&E (according to criteria reported by Jass et al. for primary CRC: 'pushing' and 'infiltrative'	91 patients with CRC liver metastases (mean number of lesions of 2.9)	- infiltrative margins (resembling replacement growth) as independent risk factor for recurrence and inferior 5yr DFS rate

Table 1: overview of studies that have addressed the association of the growth patterns of liver metastases with clinical and pathological parameters (continue)

First author	Reference (in chronological order)	Methodology	Study population	Main results
Eefsen RL	Clin Exp Metastasis 2015; 32: 369-381	Histology: H&E, silver stain (according to Vermeulen et al. 2001; 75% cut-off for dominant growth pattern)	224 CRC liver metastases obtained from 224 patients (largest metastasis only)	- recurrence free survival longer if desmoplastic HGP then if replacement HGP (both for chemonaive patients and patients who received neo-adjuvant treatment)
Eefsen RL	Cancer Microenviron 2015; 8(2): 93-100	- Histology: H&E, silver stain (according to Vermeulen et al. 2001; 75% cut-off for dominant growth pattern) - IHC for uPAR, CD3, CD68	237 CRC liver metastases from 237 patients (selection of one metastasis per patient based on regression grade)	In untreated patients only: higher expression of uPAR in the desmoplastic HGP and higher number of macrophages (CD68) in the replacement HGP
Kuczynski EA	J Natl Cancer Inst 2016; 108 (8)	- Histology - contrast-enhanced ultrasound (vessel perfusion) - miRNA sequencing and qRT-PCR	Orthotopic human HCC model to study sorafenib resistance	Resistance based on a change of growth pattern with vessel co-option during treatment
Siriwardana PN	Medicine 2016; 95(8): e2924	Histology: H&E ('infiltrative' and 'encapsulated' HGP; 75% cut-off for dominant growth pattern)	30 patients with CRC liver metastases. No pre-surgical systemic treatment. One randomly selected metastasis per patient.	Longer overall survival if encapsulated liver metastasis.
Frentzas S	Nature Medicine 2016; 22: 1294-1302	Histology: H&E (according to the current guidelines)	152 CRC liver metastases from 79 patients	The HGPs predict response to bevacizumab-chemotherapy treatment and survival. Patients with liver metastases with a replacement growth pattern have a less favourable outcome.

METHODS

CONSTRUCTING THE GUIDELINES

The team of experts responsible for the proposed guidelines consists of members of the Liver Metastasis Research Network (www.lmrn.org) all of whom have had experience in the assessment of the HGPs of liver metastases. Based on many observations in previous studies on HGPs [2, 5, 6, 11-13, 22], and consequently multiple discussions at annual meetings of the LMRN, consensus was reached on a systematic approach to assessing the HGPs. A draft manuscript was produced by a writing committee and was circulated to all the co-authors. Final approval of the guidelines occurred during the annual meeting of the LMRN on 16-17 June 2016 in Umeå, Sweden.

AGREEMENT IN HGP SCORE BETWEEN DIFFERENT SAMPLES FROM THE SAME METASTASIS

The extent of agreement in HGP score between different samples from the same lesion was addressed in 50 liver metastases of CRC for which ≥4 formalin-fixed-paraffin embedded (FFPE) blocks were available for scoring. The samples used for this analysis were obtained from the Department of Surgical Oncology of the Erasmus MC Cancer Institute (Rotterdam, The Netherlands). The total number of blocks included in the analysis was 234; median number of blocks available per lesion was 4 (range = 4-16 blocks per lesion). H&E-stained sections from all 234 blocks were scored for HGP by EPvdS, RRJCvdB, BG and PBV according to the proposed guidelines (a consensus score was agreed upon during multiple sessions at a multihead microscope). Each block was then assigned to an HGP category: those scored as >50% desmoplastic were categorised as predominant desmoplastic HGP (n = 121 blocks), those scored as >50% pushing were categorised as predominant pushing HGP (n = 7 blocks), and those scored as >50% replacement were categorised as predominant replacement HGP (n = 98 blocks). In the case that no predominant HGP was found, the block was categorised as having a mixed HGP (n = 8 blocks). In order to determine the extent of agreement in HGP score between different blocks derived from the same lesion, for each of the 50 lesions we calculated the percentage of blocks that fell into the same category.

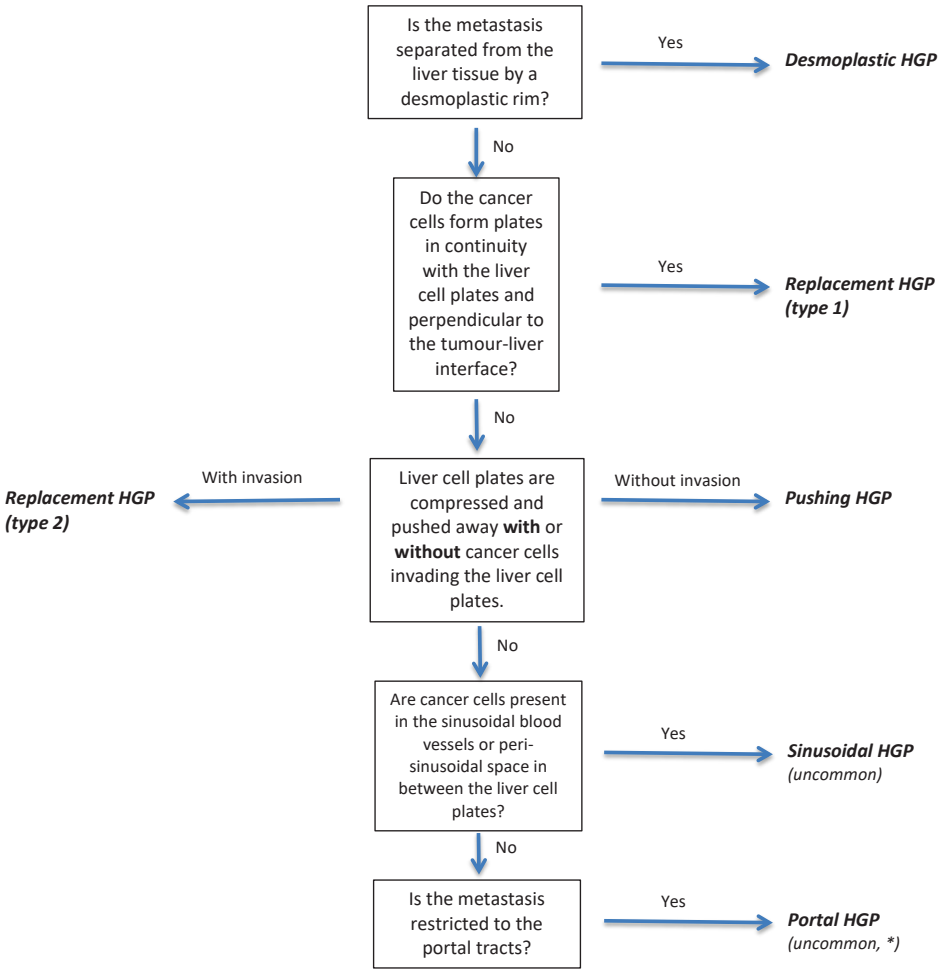
ANALYTICAL VALIDATION STUDY

For the analytical validation of the guidelines, representative H&E-stained sections from 159 FFPE sections of CRC (n = 129) and breast cancer (BC) (n = 30) liver metastases (obtained from patients undergoing routine resection) were retrieved from the archives of the pathology laboratory of the St Augustinus Hospital (GZA Hospitals), Wilrijk-Antwerp, Belgium. This set was then divided into a training set of 60 metastases and a validation set of 99 metastases by PBV and GGvdE. The sections were scanned on a 3DHISTECH (3DHISTECH Ltd., Budapest, Hungary) scanning device. Explanatory notes were provided for each of the images in the training set. These notes included the 'gold standard' HGP score, that is, the consensus HGP score as agreed by two pathologists (PBV and GGvdE) with >10 years of experience in scoring

the HGPs. The images and the explanatory notes were then uploaded to the 'Pathomation whole slide image viewer' (Pathomation BVBA, Antwerp, Belgium) on the website of the Liver Metastasis Research Network (www.lmrn.org). The decision tree and the guidelines (Figure 1 and Tables 2 and 3, respectively) were also published on this website.

Twelve participants volunteered to take part in the validation study. Of these 12 participants, only 4 had prior experience of scoring liver metastasis growth patterns, while the remaining 8 participants had no prior experience of scoring liver metastasis growth patterns. Of these 12 participants, only 3 were professionally trained pathologists, while the remaining 9 participants were scientists with either a biological sciences or medical background. Participants were given access to the data on the website of the Liver Metastasis Research Network. The participants were first asked to study the decision tree and the guidelines so that they could understand how HGPs are to be scored. They were then asked to examine the training set and the explanatory notes. Once these tasks were completed, they were then given access to the validation set of 99 liver metastases and asked to record their HGP scores for each of the cases. The participants assessed the HGPs of each metastasis (percentage of interface occupied by a growth pattern for all growth patterns present in >5% of the length of the interface) and submitted their results electronically within the Pathomation image viewer. The submitted results were then compared with the pre-established 'gold standard'.

Figure 1. Decision tree to assess the growth patterns of liver metastases based on the key histopathological characteristics.



* Exceptional non-parenchymal growth of liver metastases:

Table 2: Key histopathological characteristics of the growth patterns of liver metastases.
(* Treatment related inflammation could be present)

	Histology of interface	Desmoplastic	Pushing	Replacement (two types)	Sinusoidal	Portal
Obligatory criteria	*Architecture*	Desmoplastic rim between liver and metastatic tissue	Liver pushed aside by metastatic tissue	Hepatocytes are replaced by cancer cells	Cancer cells grow in the sinusoidal vessels or in the peri-sinusoidal space (Disse).	Proliferation of cancer cells within portal tracts and septa
	Mimicking of liver architecture	-	-	++	+	-
	Invasion around sinusoidal capillaries	-	-	+ (+ Disse space)	++	-
	Invasion of cancer cells in the liver cell plates with direct contact between hepatocytes and cancer cells	-	-	++	-	--/+
	Desmoplastic reaction	++	-	-	-	-
	Compression of liver cell plates	+	++	- /+	-	
	Contour	Sharp	Sharp	Irregular	Irregular	Sharp
Additional criteria	*Inflammatory infiltrate*	++	+/- *	- *	- *	+
	Glandular differentiation (adenocarcinoma)	Well-differentiated	Well-differentiated	Moderately to poorly differentiated	Poorly differentiated	Well to moderately differentiated
	Proliferation of bile ducts	+/-	-	-	-	-/+
Caution	*Portal tracts*	**Do not score as desmoplastic area**				
	Liver capsule	**Do not score HGP when near liver capsule**				

Table 3: standard method for histopathological growth pattern assessment of liver metastases

- The growth pattern is a histological parameter assessed by light microscopy imaging of haematoxylin-and-eosin sections of FFPE tissue of liver metastases.
- The histological growth patterns of liver metastases can be evaluated by a pathologist or by any other investigator trained by a pathologist. A training set of H&E images is available as supplementary data.
- The growth pattern is a characteristic of the tumor-liver interface. The center of the metastasis does not contribute to the classification of a growth pattern.
- A histochemical silver impregnation staining of the sections (e.g. Gordon-Sweet's reticulin staining) has added value to discern fibrosis/preservation of the supportive tissue architecture of the spaces of Disse and sinusoids.
- The three common growth patterns are: desmoplastic, pushing and replacement (two types)
- Two rare growth patterns are: sinusoidal and portal
- When more than one growth pattern is present in a metastasis: estimate the relative fraction of each growth pattern with a length of ≥5% of the total length of the interface (e.g. 80% desmoplastic/20% pushing; 95% replacement/5% pushing).
- In case of multiple metastases/patient: assess the growth pattern(s) in every individual liver metastasis and note the anatomical position.

- Caveats:
    - Portal tracts at the tumour-liver interface should not be evaluated as areas with a desmoplastic growth pattern.
    - Reactive ductular proliferation in the desmoplastic rim can simulate a replacement growth pattern.
    - Metastases with a replacement growth pattern usually have no or a very mild inflammatory infiltrate. Exceptionally, these metastases can have a dense infiltrate obscuring the interface. This should not be misinterpreted as desmoplastic growth.
    - Metastases adjacent to the liver capsule should be assessed with caution to avoid overestimation of desmoplastic growth.
    - Tissue cores from needle biopsy procedures cannot be used to assess the growth pattern of liver metastases.
    - If less than 20% of the expected interface is present in the tissue section, a disclaimer stating 'insufficient tumour-liver interface' should be added.
    - Delayed fixation (e.g. autopsy cases) or Radiofrequency Ablation (RFA) can impair the quality of the tissue so that reliable assessment of growth patterns is not possible.
    - If no viable tumour tissue is present in the metastasis, this should be mentioned (treatment effect: fibrosis, infarct-type necrosis, acellular mucin lakes)

CLINICAL VALIDATION STUDY

The clinical validity was assessed by a survival analysis. For this we used representative H&E-stained tissue sections of FFPE CRC liver metastases from 374 patients who underwent surgical resection at the Department of Surgical Oncology of the Erasmus MC Cancer Institute (Rotterdam, The Netherlands) between 2000 and 2015. The HGPs were determined by EPvdS, RRJCvdB, BG and PBV according to the proposed guidelines (for patient details, see Table 4). A consensus score was agreed upon during multiple sessions at a multihead microscope. Patients for whom >50% of the tumour-liver interface was identified as exhibiting one of the three HGP were allocated to a group labelled as predominantly of this HGP (i.e., >50% desmoplastic were categorised as predominant desmoplastic

HGP; >50% pushing were categorised as predominant pushing HGP; >50% replacement were categorised as predominant replacement HGP). In cases of multiple sections per metastasis or multiple liver metastases per patient, the mean percentage was used. Overall survival was

considered the time interval between the date of liver metastasis resection and the date of death or last follow-up.

OVERALL SURVIVAL ANALYSIS USING DIFFERENT HGP CUT-OFFS

Survival analyses were also performed using different cut points to define the predominant HGP. Owing to the low numbers of patients presenting with a predominant pushing HGP (only 3% of patients when using a cut-off of >50%), this analysis was limited to a comparison of replacement HGP patients with desmoplastic HGP patients. Cut points of >50%, >70%, >80%, >90% or 100% were used to define whether a tumour had a predominant replacement HGP or a predominant desmoplastic HGP.

STATISTICAL ANALYSIS

Descriptive values are expressed as median (interquartile range). Variables were compared by chi-square analysis, Fisher's exact test or with independent Student's t-test or Mann-Whitney U-test. The Kaplan-Meier method was used to generate survival estimates, which were compared by Log-rank test. Cox regression models were used to correct for potential confounders. Only parameters with a P-value <0.10 in the univariate model were entered in the multivariate Cox regression model. The SPSS statistical package (version 21.0, SPSS, Chicago, IL, USA) was used. A P-value ≤0.05 was considered statistically significant.

RESULTS

GUIDELINES FOR SCORING THE HGPS OF LIVER METASTASES

In these guidelines, three common (desmoplastic HGP, pushing HGP and replacement HGP) and two rare (sinusoidal HGP and portal HGP) growth patterns are described that can all be identified in H&E stained specimens of FFPE liver metastases. The key histopathological characteristics of the HGPs are summarised in Table 2. The consensus guidelines for scoring the HGPs are summarised in Table 3. In order to assist the observer in scoring the different HGPs, we have constructed a decision tree that can be easily followed to determine the growth pattern (Figure 1).

In the desmoplastic HGP, the cancer cells of the metastasis are separated from the liver tissue by a rim of desmoplastic tissue (Figures 2A-D). The metastasis does not mimic the liver architecture and there is no direct contact between cancer cells and hepatocytes. New blood vessels in the desmoplastic rim are formed by sprouting angiogenesis. There is often a dense lymphocytic infiltrate at the interface of the desmoplastic and liver tissue that can sometimes obscure the interface (Figure 2E). A proliferation of bile ducts, often called 'ductular reaction', can sometimes be seen surrounding the desmoplastic metastasis (Figure 2E). It is important to note that portal tracts that lie directly adjacent to a metastasis should not be confused with the desmoplastic tissue (Figure 2F). Also, areas directly underneath the liver capsule should not be confused with desmoplastic tissue.

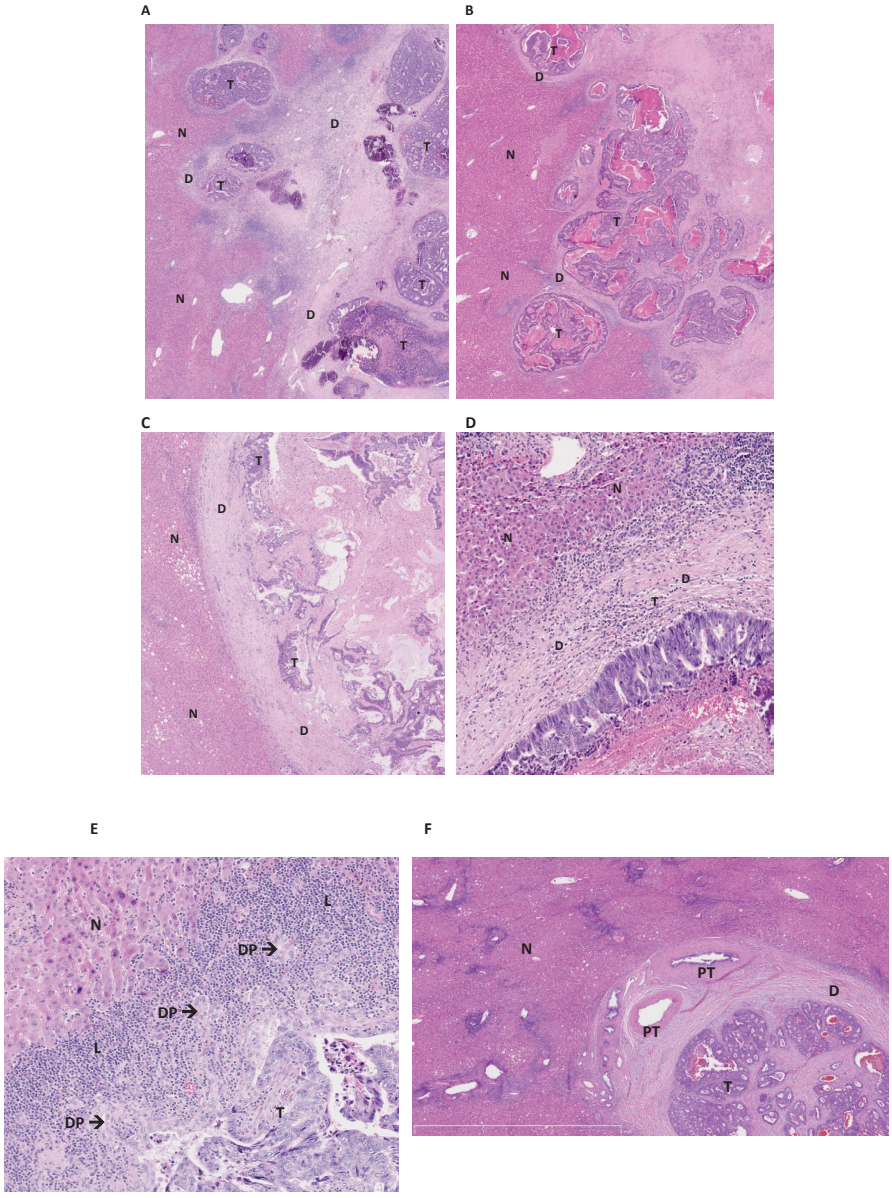


Figure 2. H&E images of the desmoplastic histopathological growth pattern. **(A–C)** Low magnification images of the desmoplastic histopathological growth pattern. **(D)** Higher magnification image of the desmoplastic histopathological growth pattern. **(E)** Desmoplastic histopathological growth pattern with ductular proliferation (also known as ductular reaction) and dense lymphocyte infiltrate. **(F)** Portal tracts at the tumour–liver interface. D, desmoplastic rim; DP, ductular proliferation; L, lymphocyte infiltrate; N, normal liver parenchyma; PT, portal tract; T, vital tumour tissue. Scale bar = 1000 mM **(A–C and F)**, 100 mM **(D and E)**.

In the pushing HGP, the liver cell plates that surround the metastasis are pushed away and are compressed (Figures 3A-C). There is no desmoplastic rim surrounding the metastasis but also no direct contact between cancer cells and hepatocytes within the liver cell plates. As in the desmoplastic HGP, the metastasis does not mimic the liver architecture.

In the replacement HGP, cancer cells form cell plates that are in continuity with the liver cell plates (Figures 4A-E). This permits the cancer cells to replace the hepatocytes within the liver cell plates and allows these metastases to co-opt the sinusoidal blood vessels at the tumour-liver interface, without inducing sprouting angiogenesis. There are two subtypes of the replacement HGP. In the first type (type 1) the liver cell plates used by the cancer cells are perpendicular to the tumour-liver interface (Figures 4B and C), and in the second type (type 2), the liver cell plates are pushed away while the cancer cells replace the hepatocytes (Figures 4D and E). The latter type of replacement HGP should not be confused with the pushing HGP (Figures 3A-C).

There are two rare HGPs of liver metastases, namely, the sinusoidal HGP and the portal HGP. In the sinusoidal HGP, the cancer cells are present as emboli within the lumens of the sinusoidal blood vessels and/or grow in the peri-sinusoidal space (Figure 5). As the cancer cells do not exit the blood vessels or enter the liver cell plates, there is no cell-cell contact between the cancer cells and the hepatocytes in this sinusoidal HGP. As in the replacement HGP, the sinusoidal blood vessels are co-opted as a means of vascularisation. In our experience, the sinusoidal HGP occurs in patients with rapidly progressing liver metastases and is therefore often encountered in autopsy specimens (Allison et al, 2004; Simone et al, 2012). In the portal HGP, the growth of cancer is restricted to the connective tissue areas of the portal tracts, liver septa and liver capsule. The portal HGP has been detected in animal models of liver metastases by us and only very infrequently in human BC or CRC liver metastases.

The current guidelines also address the fact that a liver metastasis may have more than one growth pattern. In order to collect all the available information for subsequent data analysis, the guidelines propose to estimate the relative fraction of each HGP that constitutes ≥5% of the total length of the interface. In the typical pathology archive, there may be either one tissue block available per liver lesion or multiple tissue blocks available per liver lesion. In the case where multiple blocks are available for a given lesion, we recommend that the HGP from each block is determined. The mean average HGP score should then be calculated to produce a single score for percentage of desmoplastic, percentage of pushing and percentage of replacement HGP for each lesion. In case of multiple liver metastases from a single patient, it is recommended that the HGPs of every individual single lesion be scored separately and the information saved together with the anatomical position of the respective lesion.

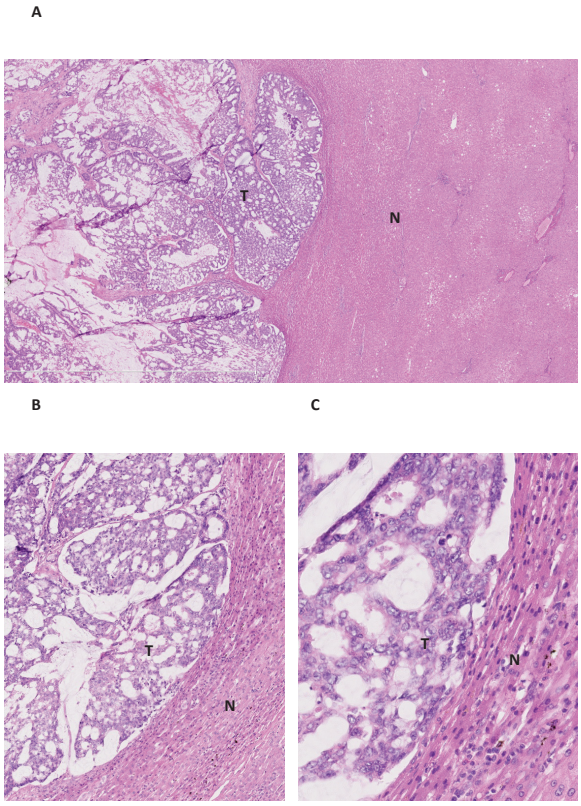


Figure 3. H&E images of the pushing histopathological growth pattern. **(A)** Low magnification image of the pushing histopathological growth pattern. **(B and C)** Higher magnification images of the pushing histopathological growth pattern. N, normal liver parenchyma; T, vital tumour tissue. Scale bar = 500 mM **(A)**, 100 mM **(B)**, 50 mM **(C)**.

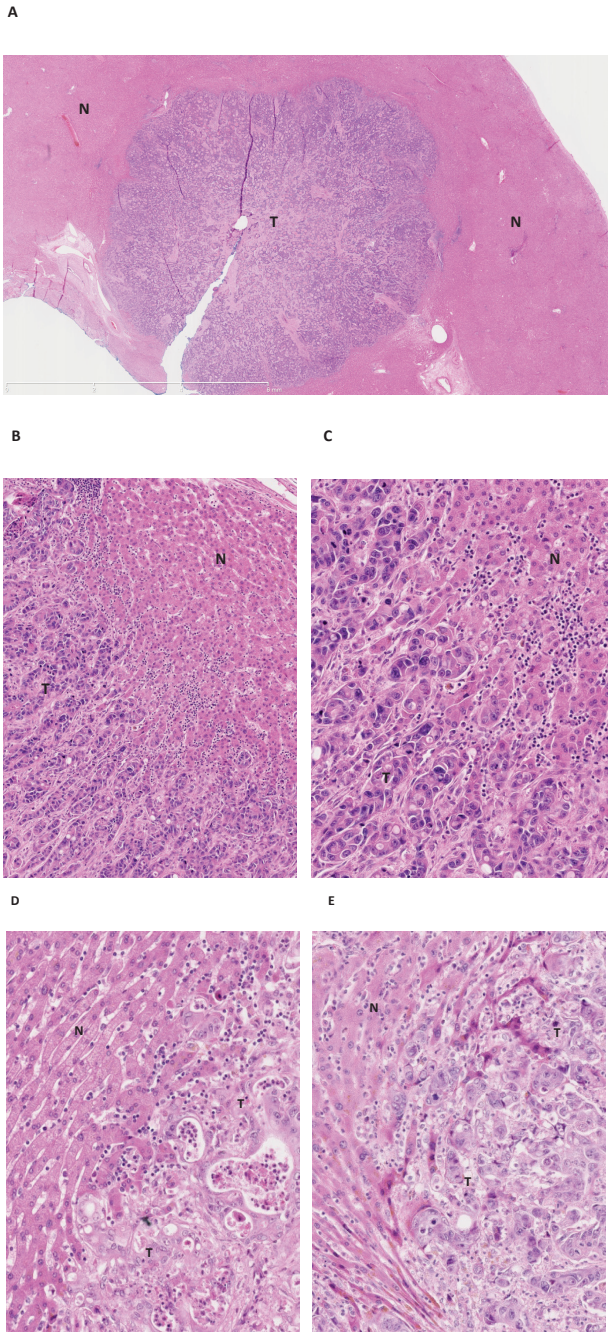


Figure 4. H&E images of the replacement histopathological growth pattern. **(A)** Low magnification image of the replacement histopathological growth pattern. **(B and C)** Higher magnification images of the type 1 replacement histopathological growth pattern. **(D and E)** Higher magnification images of the type 2 replacement histopathological growth pattern. N, normal liver parenchyma; T, vital tumour tissue. Scale bar = 2000 mM **(A)**, 100 mM **(B, D and E)**, 50mM **(C)**.

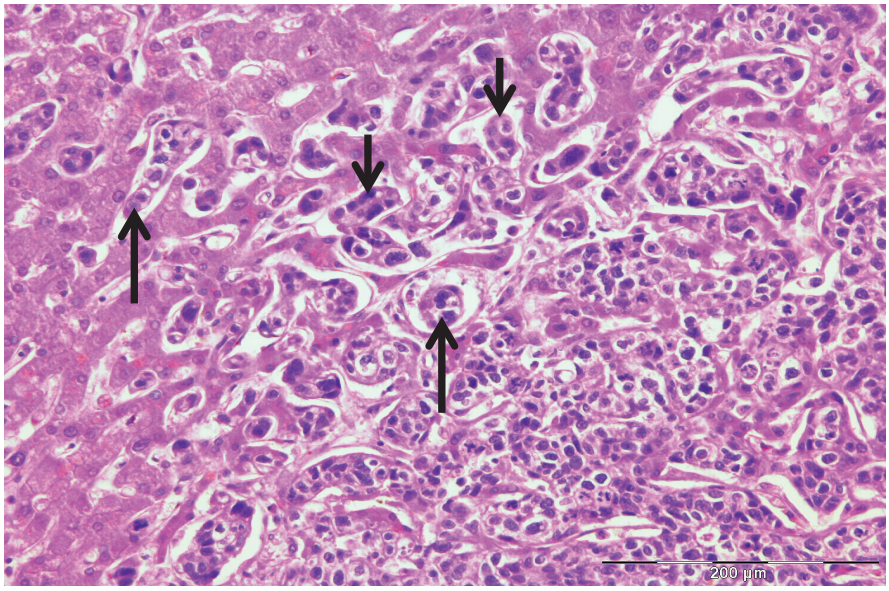


Figure 5. H&E image of the sinusoidal histopathological growth pattern. Arrowheads indicate tumour cell emboli present within the lumen of liver sinusoidal vessels. N, normal liver parenchyma. Scale bar = 100 mM.

IMPORTANT CAVEATS FOR SCORING THE HGPS OF LIVER METASTASES

In order to score the HGPs correctly, there are issues of concern that are related to the amount of available tumour-liver interface (where the HGPs are assessed), the quality of the tissue and the process of data collection. These points of concern are summarized in Table 3. First, tissue cores from needle biopsy procedures should not be used to assess the HGPs of liver metastases. The obvious reason is that the amount of interface present in a tissue core is minimal and insufficient to cover the possible heterogeneity of the HGPs within a single metastasis. Second, if <20% of the expected interface is present in the tissue section, a disclaimer stating 'insufficient tumour-liver interface' should be added. There are various circumstances that may cause <20% of the expected interface to be present in the tissue section. These include, but are not limited to, the following: (a) the presence of excessive damage to the tissue section or (b) when a considerable proportion of the tumour present in the section forms a border with the liver capsule rather than forming a border with the liver parenchyma. Third, if no viable tumour tissue is present in the metastasis, scoring the HGP is not possible. Often this is due to treatment before surgery that causes replacement of the cancerous tissue by areas of fibrosis, infarct-type necrosis or cell-free lakes of mucinous substance. Fourth, an adequate quality of the liver metastasis tissue is essential. Indeed, delayed fixation (e.g., autopsy cases) or radiofrequency ablation can impair the quality of the tissue so that reliable assessment of HGPs is not possible.

AGREEMENT IN HGP SCORE BETWEEN DIFFERENT SAMPLES FROM THE SAME METASTASIS

When considering the diagnostic utility of the HGPs, one point of concern is whether a single tissue section adequately captures the growth pattern of the entire lesion. In order to address this issue, we examined whether scoring the HGP from a single tissue sample is as accurate as scoring the HGP from multiple tissue samples. To do this, we examined an unselected set of 50 liver metastases for which multiple (≥4) tissue blocks were available. The median number of blocks available per lesion in this set was four (range = 4-16 blocks per lesion). A single tissue section from each block was scored for the HGP and then assigned to an HGP category: predominant desmoplastic HGP, predominant pushing HGP, predominant replacement HGP, or mixed HGP (according to strictly defined criteria, as detailed in the Methods section). We then assessed the degree to which blocks from the same lesion fell into the same category. We found that, for 82% of the lesions examined (41 out of 50 lesions), the HGP category was in complete agreement (100% agreement) across all blocks tested. Among the 9 other lesions examined, we found agreement between: 4 out of 5 blocks (80% agreement) for two lesions, 3 out of 4 blocks (75% agreement) for 4 lesions, 3 out of 5 blocks for 2 lesions (60% agreement), and 2 out of 4 blocks for 1 lesion (50% agreement). There were no cases with an agreement <50%.

ANALYTICAL VALIDATION OF THE GUIDELINES TO ASSESS THE HGPS OF LIVER METASTASIS

In order to assess the analytical validity of the current guidelines, a validation study was performed. In the first stage, 12 participants (a mixed group composed of 3 pathologists, 4 clinicians and 5 basic scientists) underwent a training exercise, in which they were provided with a copy of the guidelines contained herein and were then asked to score a training set of whole slide digital images of 60 liver metastases. Subsequently, they were provided with a validation set comprising 99 additional whole slide digital images. The scores of this validation set were then used to compare the participants' results with the gold standard.

The results of the validation study were tabulated with rows representing the different participants (n = 12) and columns indicating the percentage of interface occupied by a HGP in the set of 99 metastases. Using an intraclass correlation coefficient (ICC), the participants' scores were compared with the gold standard, (i.e., the consensus result of the pathologists GGvdE and PBV). This resulted in ICC coefficients for desmoplastic, pushing, replacement (type 1), replacement (type 2) and replacement (independent of type) HGP for each individual participant. ICC values >0.5 represent a good reproducibility and values >0.7 an excellent reproducibility. After colour coding and unsupervised hierarchical clustering of the ICC coefficients of each HGP for all participants, the heat map (Figure 6) shows that, for the majority of the participants, good-to-excellent correlations with the gold standard were obtained for replacement (independent of subtype), replacement (type 1) and desmoplastic HGP. This indicates that the key characteristics of the desmoplastic HGP and the replacement HGP were recognisable by most participants. The results also show that the participants found the pushing HGP and the type 2 replacement HGP more difficult to distinguish.

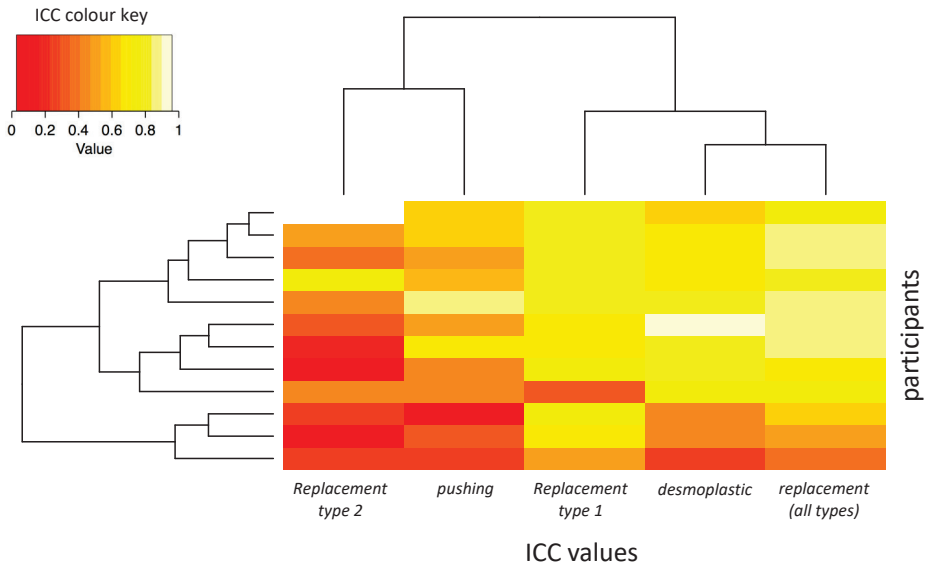


Figure 6. Heat-map of the unsupervised hierarchical clustering of the color-coded mean intra-class correlation coefficient of each growth pattern for all observers (n=12)

CLINICAL VALIDATION OF THE GUIDELINES TO ASSESS THE HGPS OF LIVER METASTASES: SURVIVAL ANALYSIS

In order to test the prognostic value of the growth patterns as scored according to our current guidelines, the HGPs were scored in CRC liver metastases resection specimens from a series of 374 patients (Table 4). Correlation of the HGPs with overall survival was then analysed. For the analysis of overall survival, the 374 patients were stratified into one of the three subgroups. Patients for whom >50% of the tumour-liver interface was identified as desmoplastic HGP were classified as predominant desmoplastic HGP, while patients for whom >50% of the tumour-liver interface was identified as pushing HGP or replacement HGP were classified as predominant pushing HGP or predominant replacement HGP, respectively. According to these criteria, the desmoplastic HGP was predominant in 183 (49%) patients, the replacement HGP in 177 (47%) patients and the pushing HGP in 10 (3%) patients. No dominant HGP could be found in the liver metastases of 4 patients (1%) and so these patients were excluded from the survival analyses.

Figure 7 shows the overall survival curves for each subgroup. The median time of follow-up was 34 months (95% CI: 17-61 months). The median overall survival for the desmoplastic subgroup was 64 months (95% CI: 51-77 months). For the replacement subgroup median overall survival was 36 months (95% CI: 30-42 months). Median overall survival was not reached for the pushing subgroup. Overall survival was significantly superior in the desmoplastic subgroup as compared with the replacement or pushing subgroup (P = 0.006, Figure 7).

According to Table 5, three parameters were significantly different between the distinct HGP

subgroups: lymph node status of the primary CRC, absence or presence of metastasis within 1 year after resection of the primary CRC, and systemic treatment prior resection of liver metastasis. The effects on overall survival of these parameters were tested in a univariate Cox regression model. This was also carried out for the clinical risk score (CRS) according to Fong because of its established prognostic value [23] (Table 6). In a multivariate analysis, both a poor CRS (3–5) and the presence of a predominant replacement HGP resulted in a significantly shorter overall survival with respective HR of 1.97 (95% CI: 1.47-2.65) and 1.73 (95% CI: 1.28-2.33).

We then repeated the overall survival analysis in order to determine whether using different cut-offs to define the predominant HGP would affect the relationship between the HGP and overall survival. Owing to the low numbers of patients presenting with a predominantly pushing HGP (only 3% of patients when using a cut-off of >50%), we limited our analysis to a comparison of overall survival between the replacement HGP patients versus the desmoplastic HGP patients. Cut points of >50%, >70%, >80%, >90% or 100% were used to define whether a tumour had a predominant replacement HGP or a predominant desmoplastic HGP (Table 7). The first thing that emerges from this analysis is that, predictably, the use of higher cut-offs incrementally reduces the number of patients eligible for inclusion in the analysis. For example, while 360 patients are eligible using a cut point of >50%, this drops to 291 eligible patients using a cut point of >70% and drops to 134 patients using a cut point of 100%. However, overall survival was significantly superior in the desmoplastic HGP subgroup, as compared with the replacement HGP subgroup, at all cut-offs utilised (both by Kaplan-Meier analysis and in multivariate analysis, Table 7).

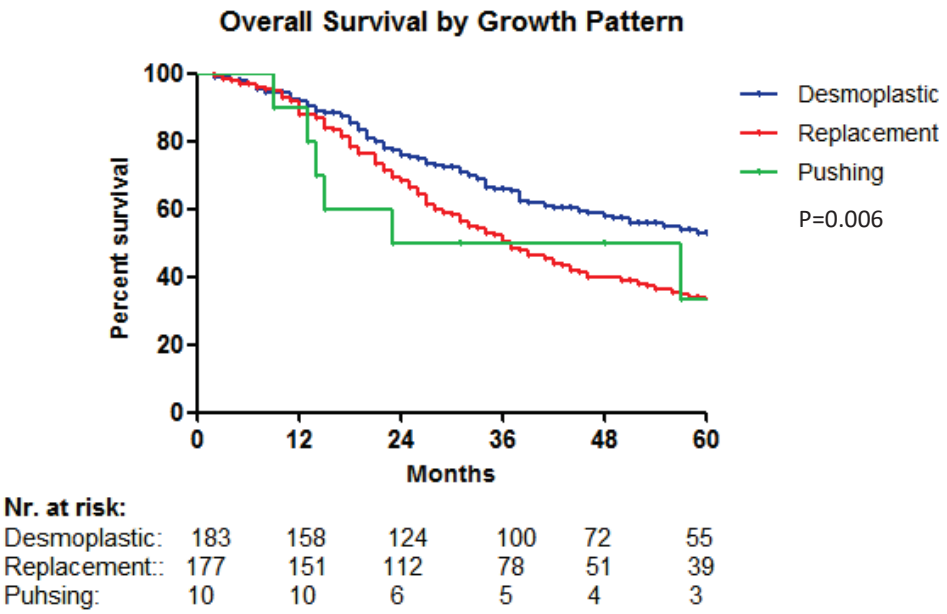


Figure 7. Kaplan-Meier curves depicting overall survival of patients with colorectal liver metastases, stratified by predominant (>50%) HGP

Table 5: Clinicopathological characteristics in the final cohort used for clinical validation, excluding 4 patients with a "mixed type"HGP

Variables		Desmoplastic (183)		Replacement (177)		Pushing (10)			All patients (N=370)	
		Value	% / IQR	Value	% or IQR	Value	% or IQR	P-value	Value	% / IQR
Male		120	66%	112	63%	6	60%	0.865	238	64%
Age	Median	63	56-71	64	59-70	63	58-73	0.591	63	57-70
Primary tumour										
Rectal cancer		79	43%	84	48%	4	40%	0.758	167	45%
T stage 3/4		130	78%	132	79%	8	80%	0.982	270	79%
Positive lymph node		90	55%	113	68%	6	60%	0.049	209	61%
Liver metastases										
CEA >200ng/ml		12	8%	11	8%	0	0%	0.892	23	8%
Synchronous (<1 year)		127	70%	102	58%	10	100%	0.004	239	65%
Diameter Largest >5cm		34	19%	30	17%	3	33%	0.484	67	19%
Number of metastases >1		122	67%	105	59%	4	44%	0.165	231	63%
Bilobar		85	46%	63	36%	4	40%	0.112	152	41%
Neo-adjuvant Ctx		110	60%	48	27%	5	50%	<0.001	163	44%
R1 resection		49	27%	48	28%	1	10%	0.473	98	27%
Extrahepatic disease		25	14%	18	10%	0	0%	0.298	43	12%
CRS 3-5		64	36%	68	40%	5	50%	0.548	137	38%

Abbreviations: CEA = carcinoembryonic antigen; CRS = clinical risk score; CTx = chemotherapy; HGP = histopathological growth pattern; IQR = interquartile range.

Table 6: Univariate and multivariate analysis (overall survival)

Univariate	HR (95%CI)	P-value	Multivariate	HR (95%CI)	P-value
Positive Lymph Node	1.267 (0.947-1.697)	0.111	Neo-adjuvant CTx	1.224 (0.893-1.678)	0.208
Synchronous (<1 year)	1.162 (0.878-1.537	0.294	CRS 3-5 (Fong)	1.971 (1.466-2.650)	<0.001
Neo-adjuvant CTx	1.280 (0.974-1.681)	0.076	Desmoplastic HGP	1	
CRS 3-5 (Fong)	2.005 (1.521-2.642)	<0.001	Replacement HGP	1.729 (1.283-2.332)	<0.001
Desmoplastic HGP	1		Pushing HGP	1.269 (0.553-2.908	0.574
Replacement HGP	1.556 (1.181-2.050)	0.019			
Pushing HGP	1.435 (0.627-3.283)	0.392			

Abbreviations: CI= confidence interval; CRS= clinical risk score; CTx= chemotherapy; HGP= histopathological growth pattern; HR= hazard ratio.

Table 7: Comparison of overall survival in patients with a predominant replacement HGP versus patients with a predominant desmoplastic HGP (using different % HGP cut-offs to determine the predominant HGP)

HGP Cut-off	Total pts in analysis	No. of pts predominant replacement	No. of pts predominant desmoplastic	Kaplan-Meier P-value	Hazart ratio from multivariate analysis
>50%	360	177	183	P=0.001	1.79 (95% CI: 1.31–2.43) P<0.001
>70%	291	139	152	P=0.009	1.72 (95% CI: 1.21–2.46) P=0.003
>80%	266	124	142	P=0.005	1.88 (95% CI: 1.29–2.73) P=0.001
>90%	233	108	125	P=0.003	2.13 (95% CI: 1.41–3.20) P=0.001
100%	134	51	83	P<0.001	2.89 (95% CI: 1.66–5.02) P=0.001

Abbreviations: pts = patients; CI = confidence interval; HGP = histopathological growth pattern.

DISCUSSION

The current manuscript describes the first guidelines for scoring the HGPs of liver metastases, based on an international consensus among experts in the field. Furthermore, we show that, by applying these guidelines after adequate training, a reproducible assessment of the HGPs in human specimens of liver metastases is possible. In addition, it is demonstrated that the HGPs scored according to these guidelines determine overall survival in patients with liver metastases of CRC.

Although the scoring can be performed reliably even by non-specialists and is reproducible between individuals as shown in our analytical validation study, some limitations were noted, among them the difficulty of distinguishing the pushing HGP from the type 2 replacement HGP. This distinction is important, given that the interaction of the cancer cells with the liver is completely different in the two HGPs. For example, the pushing growth pattern relies on sprouting angiogenesis for its vasculature while the replacement growth pattern co-opts the sinusoidal blood vessels of the liver [2, 5, 6].

In the current study, the HGPs scored according to the proposed guidelines predict outcome: patients with a predominant desmoplastic HGP have a significant overall survival advantage as compared with patients with a predominant replacement HGP or predominant pushing HGP. This is clearly in accordance with the studies in Table 1 that have addressed the prognostic value of the HGPs [6, 12, 16, 19, 22, 24-26]. However, there are some discrepancies in the reported impact of the replacement HGP and the pushing HGP on outcome (for details, see Table 1). These discrepancies may arise due to differences in the patient cohorts examined and/or due to differences in the way the HGPs were scored in different studies. For instance, the percentage of patients who received systemic treatment prior to the resection of liver metastases may influence the results, as suggested by the evidence that preoperative therapy may cause a conversion from a desmoplastic HGP to a replacement HGP [6, 27]. With regards to the methods in which the HGPs were scored, the cut-offs for classifying a metastasis as having a predominant HGP differ between different studies, ranging from 50% to 80% of the total length of the interface. Also, the distinction between the pushing HGP and the type 2 replacement HGP was not well defined in previous studies. Furthermore, all of these factors have impacted on the variability documented in the relative proportions of the different HGPs. Indeed, as an example, the pushing HGP was reported as the predominant HGP in ±7% of all samples in the study by Frentzas et al [6] and in ±3% of all samples in the survival study in this manuscript. However, in other studies, this fraction was as high as 16% [12], 33% [19], 34% [22] and even 50% [16]. These issues highlight the need for standardised and uniform criteria for HGP classification - a major aim of the present guidelines.

Although we show here that scoring the HGPs of liver metastases is reproducible, in order to use the HGPs as a biomarker for treatment decisions it will be necessary to develop accurate non-invasive surrogate markers for this histopathological parameter. For example, medical imaging of the liver, such as MRI or CT scans that are performed routinely in clinical

practice, might eventually be utilised as a surrogate method to determine the HGPs of liver metastasis. We note that Semelka et al [28] found that the presence of transient perilesional enhancement on the MRI image is correlated with the presence of a desmoplastic reaction around the rim of liver metastases. However, transient perilesional enhancement was not present on the MRI image for liver metastases that lacked a desmoplastic reaction [28]. It is therefore possible that transient perilesional enhancement is a potential surrogate marker that distinguishes desmoplastic HGP liver metastases from pushing/replacement HGP liver metastases on MRI scans of the liver. However, a prospective imaging study performed in a large series of chemotherapy-naïve patients is necessary in order to validate whether this imaging feature (or other imaging features) can be successfully used to predict the HGPs of liver metastasis. A study of this sort is currently ongoing in Sweden (Hanna Nyström, personal communication). Furthermore, by longitudinal assessment of the HGPs, through repeated imaging of the same patients, it will also be possible to appreciate the dynamic nature of the HGPs. This is relevant because several studies suggest that the HGP of a tumour can change. For instance, there is evidence that, after systemic treatment with an antiangiogenic agent, liver metastases can switch from an angiogenic desmoplastic HGP to the non-angiogenic replacement HGP [6, 27]. Furthermore, a change from an angiogenic growth pattern to a non-angiogenic growth pattern upon treatment with antiangiogenic therapy has also been reported in preclinical models of hepatocellular carcinoma [29], lung metastasis [30], glioblastoma (GBM) [31]; and brain metastasis [32].

There is currently limited understanding of the biological mechanisms that underlie the different HGPs. Moreover, it is unclear why some tumours elicit a desmoplastic and angiogenic response while others grow in a non-angiogenic manner and adopt the replacement growth pattern. HGPs are, however, a broader phenomenon not restricted to liver metastases and have also been described in primary lung cancer [33] and lung metastases [30, 34, 35], primary brain tumours and brain metastases [36-39], lymph node metastases [13, 40, 41] and skin metastases [42]. Common biological themes, based on the interplay between cancer cells and the organ microenvironment, may thus be responsible for the HGPs in different organs.

One working hypothesis to explain the biology of the different HGPs of liver metastases is that these HGPs recapitulate distinct reaction patterns of the liver to injury. Two reaction patterns to liver injury are known, liver fibrosis and liver regeneration, and they are characterised by specific cytokine profiles [43]. Fibrosis in the desmoplastic HGP may be mediated by the same biological mechanisms that drive liver fibrosis in response to injury. One hallmark of liver fibrosis is ductular reaction, which is a proliferation of activated cholangiocytes that form small nonfunctional bile ductular structures [44]. Indeed, ductular reaction is also present in the fibrotic rim of desmoplastic liver metastases (see Figure 2E). The replacement HGP, on the other hand, resembles liver regeneration, as cancer cells replace hepatocytes akin to the way that new hepatocytes replace older hepatocytes during liver regeneration [45, 46]. Soluble angiocrine factors (which are secreted by endothelial cells in sinusoidal blood

vessels) have a major role during liver fibrosis, liver regeneration and in liver development [43, 47-49]. However, the role of these angiocrine factors in liver metastasis growth patterns is yet to be elucidated and is currently under investigation.

Desmoplastic and pushing growth pattern tumours are associated with new vessel formation (via angiogenesis), while replacement growth pattern tumours are not dependent on angiogenesis and co-opt pre-existing sinusoidal blood vessels instead [2, 4-6]. Therefore, another significant question is: how are these different vascularization mechanisms co-ordinated in the liver? Although this is yet to be elucidated, insight may come from studies performed in GBM. Similar to the liver metastasis scenario, GBM tumours displaying either an angiogenic growth pattern or a vessel co-opting growth pattern have also been described [38]. Sakariassen et al used PDX models of GBM to study what drives these different growth patterns in the brain. They showed that, while angiogenic GBMs and vessel co-opting GBMs are genetically similar (as determined by array CGH), marked differences in gene expression occur between these growth patterns. Notably, vessel co-opting GBMs had upregulated expression of genes associated with foetal development and cell motility when compared with angiogenic tumours. In contrast, angiogenic GBMs showed higher expression of angiogenic regulators, such as VEGF and angiopoetin-2, when compared with vessel co-opting tumours [38]. These data suggest that tumour growth patterns are associated with differences in tumour gene expression, which may be drivers of the growth pattern. We are currently undertaking a transcriptomic analysis of human liver metastases to determine whether similar differences in gene expression can be found between liver metastases adopting different growth patterns (Van Laere et al, manuscript in preparation).

Other studies, performed using preclinical brain metastasis models, have shown that cancer cells adhere to pre-existing brain vessels during vessel co-option in the brain and that this may be mediated by distinct cell adhesion molecules expressed by cancer cells, such as β1-integrins or L1CAM [37, 39, 50, 51]. In the replacement growth pattern of liver metastases, our histopathological observations suggest that cancer cells also adhere to the sinusoidal blood vessels. Moreover, others have reported that 'adhesive co-option' of sinusoidal blood vessels by cancer cells, mediated by integrins, has a role in liver metastasis [52]. Taken together, these data suggest that the propensity for cancer cells to adopt a specific growth pattern may also involve important changes in the ability of cancer cells to adhere to pre-existing blood vessels.

In addition, the progression of cancer along pre-existing basement membranes (as observed in the replacement growth pattern) clearly resembles what pathologists recognise as in situ carcinoma, a presentation in which cancer cells respect the existing structure of the host organ. Although this is typically a feature that has been described to occur in primary tumours (i.e., occurring prior to invasion and subsequent metastasis), reversion of metastatic cancer to this in situ phase has indeed been documented in other sites, for instance, in lymph node metastases [53]. Therefore, the adoption of the replacement growth pattern may also represent a form of reversion to in situ tumour growth.

We find that, while approximately one-third of patients present with a 'pure' growth pattern (i.e., 100% desmoplastic, 100% pushing or 100% replacement HGP), approximately two-thirds of patients present with a mixed growth pattern. Unfortunately, the biological basis for this heterogeneity of growth pattern within the same patient remains unclear. However, future studies aimed at addressing the molecular mechanisms that underlie the growth patterns should provide insight as to the basis for this heterogeneity. As for the practical significance of a mixed growth pattern, it is apparent that, even when a mixture of growth patterns is present, the predominant growth pattern can still have a significant effect on patient outcome. For example, around two thirds of the patients included in our overall survival analysis (using the >50% cut point) presented with a mixture of growth patterns, and yet the predominant growth pattern still had a statistically significant effect on overall survival. There is also evidence that treatment with standard therapies can alter the growth pattern of liver metastases from desmoplastic to replacement [6, 27]. It is therefore possible that, in some cases, liver metastases with a mixed growth pattern represent those tumours that are in a state of transition from one growth pattern to another. If it is the case that standard therapies can indeed drive a shift in growth pattern from a good prognosis pattern (i.e., desmoplastic) to a bad prognosis pattern (i.e., replacement), then it may be necessary to derive therapeutic strategies that can either prevent or combat this adverse transition.

In conclusion, we provide clear and reproducible guidelines for scoring the HGPs of liver metastasis. The HGPs have a prognostic and predictive value for patients with liver metastatic CRC as demonstrated here and in other retrospective studies [6, 12, 16, 19, 22, 24-26]. Prospective studies based on large cohorts of patients, and preferably linked to clinical trials, are now needed to confirm the clinical value of the HGPs and to assess the value of medical imaging, or circulating molecular markers, as potential surrogate biomarkers for the HGPs. Moreover, further studies are now warranted to understand the molecular mechanisms that underlie the HGPs, because these may eventually lead to HGP-specific treatment strategies for liver metastases. This could pave the way for an improved selection strategy for the type of systemic treatment before and/or after liver surgery and for personalised risk-adapted follow-up strategies.

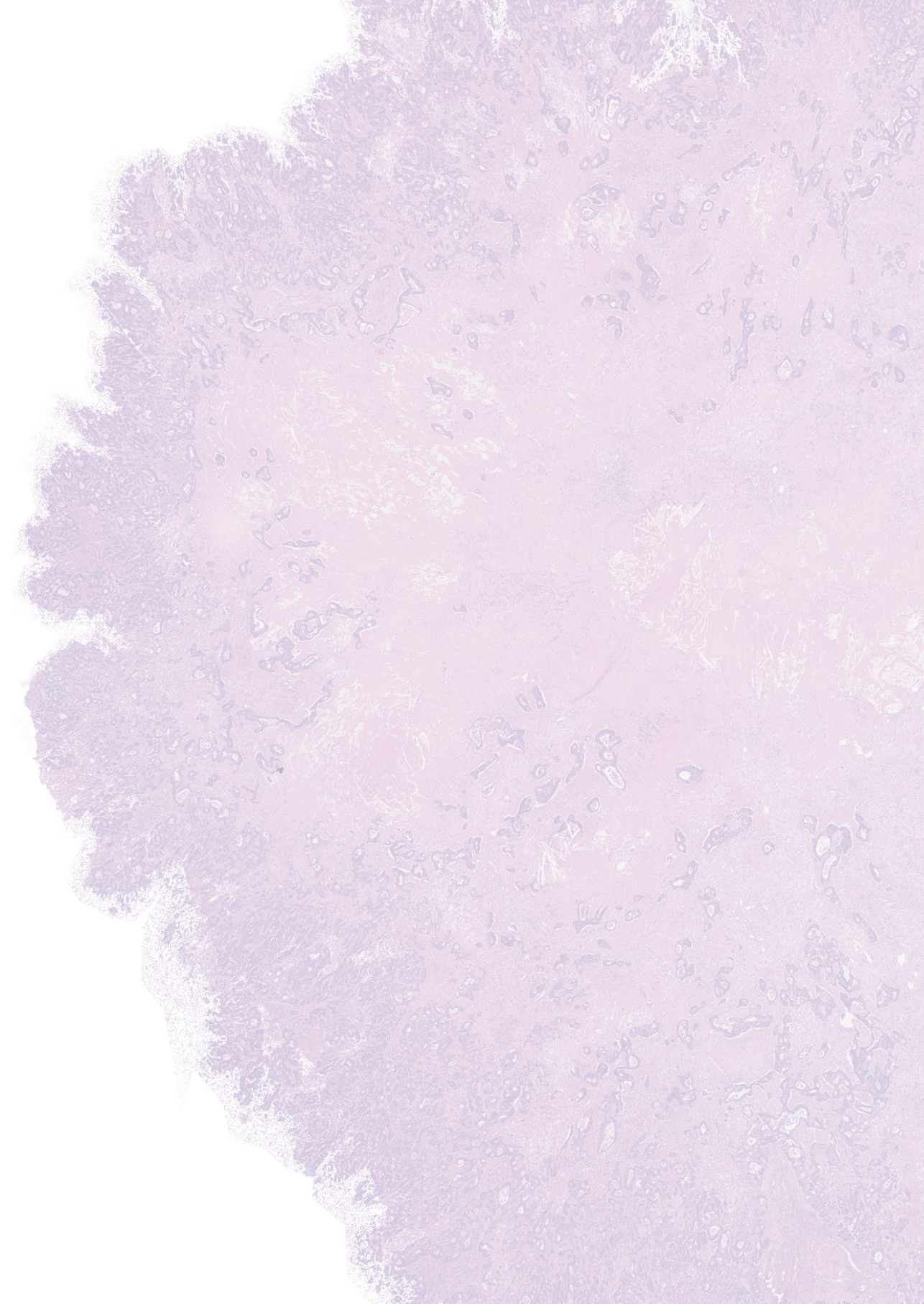
REFERENCES

1. Disibio, G. and S.W. French, *Metastatic patterns of cancers: results from a large autopsy study.* Arch Pathol Lab Med, 2008. **132**(6): p. 931-9.
2. Van den Eynden, G.G., et al., *The multifaceted role of the microenvironment in liver metastasis: biology and clinical implications*. Cancer Res, 2013. **73**(7): p. 2031-43.
3. Schmoll, H.J., et al., *ESMO Consensus Guidelines for management of patients with colon and rectal cancer*. a personalized approach to clinical decision making. Ann Oncol, 2012. **23**(10): p. 2479-516.
4. Stessels, F., et al., *Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia*. Br J Cancer, 2004. **90**(7): p. 1429-36.
5. Vermeulen, P.B., et al., *Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia*. J Pathol, 2001. **195**(3): p. 336-42.
6. Frentzas, S., et al., *Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases*. Nat Med, 2016. **22**(11): p. 1294-1302.
7. Elias, H., F. Bierring, and I. Grunnet, *Cellular Changes in the Vicinity of Metastatic Carcinoma, Observed by Light and Electron Microscopy*. Oncology, 1964. **18**: p. 210-24.
8. Elias H., S.J.C., Bouldin R.F., *Reaction of the normal liver parenchyma to metastatic carcinoma.* Acta Hepato-Splenologica, 1962. **9**(6): p. 357-386.
9. Hamperl, H., *Die Morphologie der Tumoren*. Lehrbuch Der Allgemeinen Pathologie Und Der Pathologischen Anatomie, 1956. **6**(3).
10. Masson, P., *Les Tumeurs*. Traité De Pathologie Et Thérapie Appliquées, 1923. **27**(2).
11. Eefsen, R.L., et al., *Histopathological growth pattern, proteolysis and angiogenesis in chemonaive patients resected for multiple colorectal liver metastases*. J Oncol, 2012. **2012**: p. 907971.
12. Van den Eynden, G.G., et al., *The histological growth pattern of colorectal cancer liver metastases has prognostic value*. Clin Exp Metastasis, 2012. **29**(6): p. 541-9.
13. Vermeulen, P.B., et al., *Lack of angiogenesis in lymph node metastases of carcinomas is growth pattern-dependent*. Histopathology, 2002. **40**(1): p. 105-7.
14. Eefsen, R.L., et al., *Inflammation and uPAR-Expression in Colorectal Liver Metastases in Relation to Growth Pattern and Neo-adjuvant Therapy*. Cancer Microenviron, 2015. **8**(2): p. 93-100.
15. Illemann, M., et al., *Two distinct expression patterns of urokinase, urokinase receptor and plasminogen activator inhibitor-1 in colon cancer liver metastases*. Int J Cancer, 2009. **124**(8): p. 1860-70.
16. Nystrom, H., et al., *Liver-metastatic potential of colorectal cancer is related to the stromal composition of the tumour*. Anticancer Res, 2012. **32**(12): p. 5183-91.
17. Jass, J.R., S.B. Love, and J.M. Northover, *A new prognostic classification of rectal cancer.* Lancet, 1987. **1**(8545): p. 1303-6.
18. Rajaganeshan, R., et al., *The influence of invasive growth pattern and microvessel density on prognosis in colorectal cancer and colorectal liver metastases*. Br J Cancer, 2007. **96**(7): p. 1112-7.
19. Nielsen, K., et al., *The morphological growth patterns of colorectal liver metastases are prognostic*

for overall survival. Mod Pathol, 2014. **27**(12): p. 1641-8.

20. Poston, G.J., *Staging of advanced colorectal cancer*. Surg Oncol Clin N Am, 2008. **17**(3): p. 503-17, viii.
21. Poston, G.J., et al., *Urgent need for a new staging system in advanced colorectal cancer*. J Clin Oncol, 2008. **26**(29): p. 4828-33.
22. Eefsen, R.L., et al., *Growth pattern of colorectal liver metastasis as a marker of recurrence risk*. Clin Exp Metastasis, 2015. **32**(4): p. 369-81.
23. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases*. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
24. Lunevicius, R., et al., *Clinicopathological significance of fibrotic capsule formation around liver metastasis from colorectal cancer*. J Cancer Res Clin Oncol, 2001. **127**(3): p. 193-9.
25. Okano, K., et al., *Fibrous pseudocapsule of metastatic liver tumors from colorectal carcinoma*. Clinicopathologic study of 152 first resection cases. Cancer, 2000. **89**(2): p. 267-75.
26. Pinheiro, R.S., et al., *Tumor growth pattern as predictor of colorectal liver metastasis recurrence*. Am J Surg, 2014. **207**(4): p. 493-8.
27. Mentha, G., et al., *Dangerous halo after neoadjuvant chemotherapy and two-step hepatectomy for colorectal liver metastases*. Br J Surg, 2009. **96**(1): p. 95-103.
28. Semelka, R.C., et al., *Perilesional enhancement of hepatic metastases: correlation between MR imaging and histopathologic findings-initial observations*. Radiology, 2000. **215**(1): p. 89-94.
29. Kuczynski, E.A., et al., *Co-option of Liver Vessels and Not Sprouting Angiogenesis Drives Acquired Sorafenib Resistance in Hepatocellular Carcinoma*. J Natl Cancer Inst, 2016. **108**(8).
30. Bridgeman, V.L., et al., *Vessel co-option is common in human lung metastases and mediates resistance to anti-angiogenic therapy in preclinical lung metastasis models*. J Pathol, 2016.
31. Rubenstein, J.L., et al., *Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption*. Neoplasia, 2000. **2**(4): p. 306-14.
32. Leenders, W.P., et al., *Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option*. Clin Cancer Res, 2004. **10**(18 Pt 1): p. 6222-30.
33. Pezzella, F., et al., *Non-small-cell lung carcinoma tumor growth without morphological evidence of neo-angiogenesis*. Am J Pathol, 1997. **151**(5): p. 1417-23.
34. Pezzella, F., et al., *Angiogenesis in primary lung cancer and lung secondaries*. Eur J Cancer, 1996. **32A**(14): p. 2494-500.
35. Szabo, V., et al., *Mechanism of tumour vascularization in experimental lung metastases*. J Pathol, 2015. **235**(3): p. 384-96.
36. Berghoff, A.S., et al., *Invasion patterns in brain metastases of solid cancers*. Neuro Oncol, 2013. **15**(12): p. 1664-72.
37. Bugyik, E., et al., *Lack of angiogenesis in experimental brain metastases.* J Neuropathol Exp Neurol, 2011. **70**(11): p. 979-91.
38. Sakariassen, P.O., et al., *Angiogenesis-independent tumor growth mediated by stem-like cancer cells*. Proc Natl Acad Sci U S A, 2006. **103**(44): p. 16466-71.

39. Valiente, M., et al., *Serpins promote cancer cell survival and vascular co-option in brain metastasis*. Cell, 2014. **156**(5): p. 1002-16.
40. Jeong, H.S., et al., *Investigation of the Lack of Angiogenesis in the Formation of Lymph Node Metastases*. J Natl Cancer Inst, 2015. **107**(9).
41. Naresh, K.N., A.Y. Nerurkar, and A.M. Borges, *Angiogenesis is redundant for tumour growth in lymph node metastases*. Histopathology, 2001. **38**(5): p. 466-70.
42. Colpaert, C.G., et al., *Cutaneous breast cancer deposits show distinct growth patterns with different degrees of angiogenesis, hypoxia and fibrin deposition*. Histopathology, 2003. **42**(6): p. 530-40.
43. Ding, B.S., et al., *Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis*. Nature, 2014. **505**(7481): p. 97-102.
44. Schuppan, D. and Y.O. Kim, *Evolving therapies for liver fibrosis*. J Clin Invest, 2013. **123**(5): p. 1887-901.
45. Dezso, K., et al., *Structural analysis of oval-cell-mediated liver regeneration in rats*. Hepatology, 2012. **56**(4): p. 1457-67.
46. Oertel, M., et al., *Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells*. Gastroenterology, 2006. **130**(2): p. 507-20; quiz 590.
47. Ding, B.S., et al., *Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration*. Nature, 2010. **468**(7321): p. 310-5.
48. Matsumoto, K., et al., *Liver organogenesis promoted by endothelial cells prior to vascular function*. Science, 2001. **294**(5542): p. 559-63.
49. Si-Tayeb, K., F.P. Lemaigre, and S.A. Duncan, *Organogenesis and development of the liver*. Dev Cell, 2010. **18**(2): p. 175-89.
50. Carbonell, W.S., et al., *The vascular basement membrane as "soil" in brain metastasis*. PLoS One, 2009. **4**(6): p. e5857.
51. Dome, B., J. Timar, and S. Paku, *A novel concept of glomeruloid body formation in experimental cerebral metastases*. J Neuropathol Exp Neurol, 2003. **62**(6): p. 655-61.
52. Kemperman, H., Y.M. Wijnands, and E. Roos, *alphaV Integrins on HT-29 colon carcinoma cells: adhesion to fibronectin is mediated solely by small amounts of alphaVbeta6, and alphaVbeta5 is codistributed with actin fibers*. Exp Cell Res, 1997. **234**(1): p. 156-64.
53. Barsky, S.H., et al., *'Revertant' DCIS in human axillary breast carcinoma metastases*. J Pathol, 1997. **183**(2): p. 188-94.



CHAPTER 9

Histopathological growth patterns as a guide for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases

E.P. van der Stok*, F.E. Buisman*
B. Galjart
J. Creasy
P.B. Vermeulen
E. Sadot
P.J. Allen
V.P. Balanchandran
W.R. Jarnagin
T.P. Kingham
D.J. Grünhagen
B. Groot Koerkamp
M.I. D'Angelica
C. Verhoef

* Both authors contributed equally

Submitted

ABSTRACT

BACKGROUND

Adjuvant systemic chemotherapy is widely administered in patients with colorectal liver metastases (CRLM). Histopathological growth patterns (HGPs) are an independent prognostic factor in patients with CRLM. This study evaluates whether HGPs can predict the effectiveness of adjuvant systemic chemotherapy in patients with resected CRLM.

METHODS

A multicenter cohort study, including patients from two centers, was conducted. Growth patterns were assessed according to the international concensus guidelines.

RESULTS

In total, 816 consecutive patients were included in the study. Patients with desmoplastic type HGP (dHGP) had a superior overall survival (OS) of 87 months compared to 51 months in patients with non-desmoplastic type HGP (non-dHGP), $p<0.001$. Adjuvant systemic chemotherapy was administered in 173 patients (21%). Patients receiving adjuvant systemic chemotherapy had a superior median OS of 79 months (95%CI 61-97 months) compared to 56 months for patients who did not receive adjuvant systemic chemotherapy ($p=0.02$). In patients with dHGP, OS did not improve with adjuvant systemic chemotherapy compared to resection only (adjusted Hazard Ratio (HR) 0.83, $p=0.60$). In patients with non-dHGP, OS did improve with adjuvant systemic chemotherapy compared to resection only (adjusted HR 0.66, $p=0.004$). In subgroup analysis, superior OS was observed only for patients with non-dHGP that did not receive preoperative chemotherapy (HR 0.51, $p<0.001$). No significant effect of adjuvant systemic chemotherapy was observed in patients after preoperative chemotherapy with either dHGP (HR 0.93, $p=0.84$) or non-dHGP (HR 0.93, $p=0.68$), or in patients with dHGP that were not pretreated (HR 2.50, $p=0.07$).

CONCLUSION

Patients with non-dHGP have improved OS with adjuvant systemic chemotherapy after resection of CRLM; patients with dHGP do not.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancer related death worldwide [1]. About 25% of patients with CRC are diagnosed with synchronous colorectal liver metastases (CRLM), and another 20% of patients develop metachronous metastases, predominantly in the liver [2-4]. The 10-year survival after surgery for CRLM is approximately 25% [5]. The survival remains poor due to development of recurrent disease in up to 80% of all patients.
In order to improve survival after resection of CRLM, several studies have evaluated the effect of (neo-)adjuvant treatments. Long-term follow-up of a phase 3 trial demonstrated no significant difference in overall survival (OS) for patients with and without perioperative systemic FOLFOX chemotherapy [6]. Some retrospective studies suggest that adjuvant systemic chemotherapy may only be effective in certain subgroups, such as patients with a high Clinical Risk Score (CRS) [7-9]. Better biomarkers are needed to improve patient selection for adjuvant systemic chemotherapy after hepatic resection of CRLM.
The histopathological growth pattern (HGP) is a new independent prognostic factor in patients with CRLM [10, 11]. Two main types of HGPs can be distinguished by a pathologist on microscopic examination (figure 1); a desmoplastic type (dHGP) and a non-desmoplastic type HGP (non-dHGP) [12]. In a previous study we found that 5-year OS was 78% in patients with dHGP versus 36% in months with non-dHGP ($p<0.001$) (submitted). The aim of the current study was to determine if HGPs can predict the effectiveness of adjuvant systemic chemotherapy after resection of CRLM.

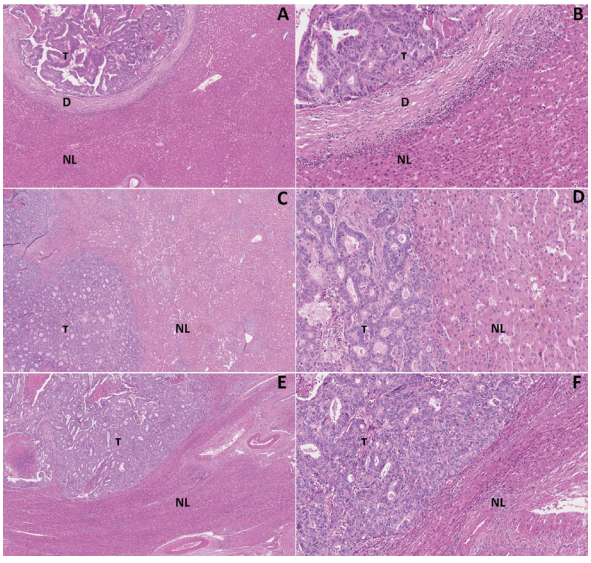


Figure 1. 1A-B: desmoplastic HGP low and high magnification; 1C-D: replacement HGP low and high magnification; 1E-F: pushing HGP low and high magnification. T: tumour; D: desmoplastic stroma; NL: normal liver parenchyma.

METHODS

PATIENTS

All consecutive patients who underwent a complete resection of CRLM from 2000-2012 at two large centers, Memorial Sloan Kettering Cancer Center (MSKCC, New York, United States) and Erasmus MC (Rotterdam, the Netherlands), were evaluated for inclusion. In MSKCC, most patients received systemic adjuvant and/or preoperative chemotherapy. In Erasmus MC, adjuvant systemic chemotherapy is not the standard of care after resection of CRLM, according to the Dutch guidelines. Preoperative systemic chemotherapy however is regularly administered in patients with synchronous and unresectable CRLM.

IN- AND EXCLUSION CRITERIA

Patients who did not undergo a complete resection for CRLM (i.e. not all lesions were resected or ablated), never had their primary tumour resected, or with extrahepatic disease before or at time of resection of CLRM were excluded. Patients who underwent ablative treatment without liver resection were also excluded. Patients receiving adjuvant Hepatic Arterial Infusion Pump (HAIP) chemotherapy were also excluded. Patients were also excluded if hematoxylin and eosin (H&E) stained tissue sections were not available or non-suitable for HGP evaluation. Non-suitable was defined as less than 20% tumor-liver interface, poor quality of the H&E tissue sections or when viable tumor tissue was absent [10].

Clinicopathological data and data on postoperative treatment were available from both prospectively maintained databases. Number and size of CRLM were derived from pathology reports. Any lesions treated with ablative therapies (Radio Frequency Ablation (RFA) or Microwave Ablation (MWA)) were added to the total number of CRLM treated. The Clinical Risk Score was calculated by assigning one point for the presence of each of the five components: nodal status of the primary tumour, disease-free interval between resection primary and diagnosis CRLM, number of CRLM, size largest CRLM, and serum carcinoembryonic antigen (CEA) level [9]. The CRS was subdivided into low risk (CRS 0-2) and high risk (CRS 3-5). A positive resection margin was defined as the presence of viable tumour at the resection margin. Preoperative chemotherapy was defined as any chemotherapy administered within 6 months before liver resection. Adjuvant chemotherapy was defined as any chemotherapy administered within 6 months after liver resection.

HGP CHARACTERIZATION

HGPs on H&E stained tissue sections were evaluated according to international guidelines [10]. In order to determine HGP type, all available H&E stained tissue sections off all available metastases were evaluated using light microscopy. The interface between tumour border and normal liver tissue was evaluated for the type of HGP and scored using percentages of each HGP type. Average HGP percentages were calculated per metastasis and patient (in case of multiple CRLM). Patients were classified in two groups: dHGP if all available slides

showed a 100% desmoplastic interface and non-dHGP if a replacement or pushing type HGP was found on one or more slides.

STATISTICAL ANALYSIS

Differences between groups in baseline characteristics were evaluated using the Chi-squared test for categorical variables and the Mann-Whitney U-test for continues variables. Median follow-up time was estimated using the reversed Kaplan-Meier method. No imputation of missing data was applied; complete case analysis for the regression analyses was performed. Survival was estimated by the Kaplan-Meier method and groups were compared using the log-rank test. OS was defined from the date of CRLM resection until the date of last follow-up or death. Uni- and multivariable analysis of OS was performed with Cox proportional hazard modeling. Results were reported as hazard ratios (HR) with 95% confidence intervals (CI). Subgroup analyses (Kaplan-Meier and univariable Cox regression) stratified for preoperative chemotherapy were performed, since previous studies showed a potential impact of preoperative chemotherapy on HGP type. A p-value of less than 0.05 was considered statistically significant. All analyses were carried out using SPSS (IBM Corp, version 21, Armonk, NY).

RESULTS

PATIENTS

At MSKCC, 1620 patients were evaluated for inclusion after surgery of CRLM, of whom 1248 patients (77.0%) received adjuvant chemotherapy. At Erasmus MC, 742 patients were evaluated for inclusion, of whom only 4 patients (0.5%) received adjuvant systemic chemotherapy. A total of 1546 patients (65%) were excluded; reasons for exclusion are listed in the flowchart of the study (figure 2). The most important reason for exclusion was missing H&E slides (n=621, 40%). The remaining 816 patients were included for analysis, of which 173 patients (21%) received adjuvant systemic chemotherapy, and 643 patients (89%) did not.

The baseline characteristics, comparing patients treated with and without adjuvant systemic chemotherapy, are displayed in table 1. Most patients treated with adjuvant systemic chemotherapy were from MSKCC (n=170, 98%), and patients without adjuvant systemic chemotherapy were mainly from the Erasmus MC (n=487, 76%).

A dHGP was found in 183 patients (22%) and non-dHGP in 633 patients (78%). The distribution of HGP's was similar among both adjuvant treatment groups (p= 0.65).

The median follow-up for the survivors was 76 months (Interquartile Range (IQR) 55-112 months). In total 453 patients (56%) died during follow-up. Patients receiving adjuvant systemic chemotherapy had a superior median OS of 79 months (95%CI 61-97 months) compared to 56 months (95%CI 49-63 months) for patients who did not receive adjuvant systemic chemotherapy (p=0.02, figure 3). Patients with dHGP had a superior survival (87 months, 95%CI not reached) compared to patients with non-dHGP (51 months 95%CI 45-57 months, p<0.001) (figure 4).

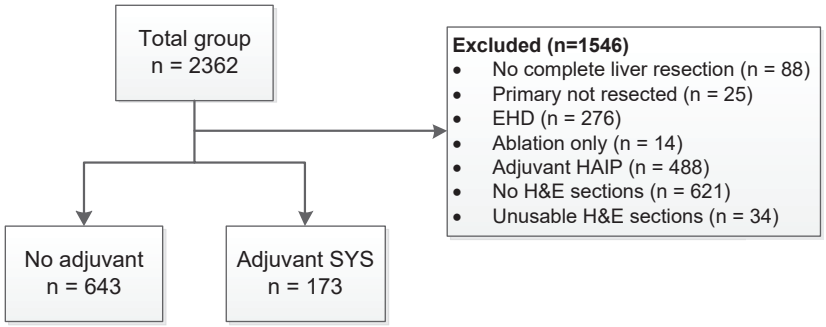


Figure 2. Study flowchart

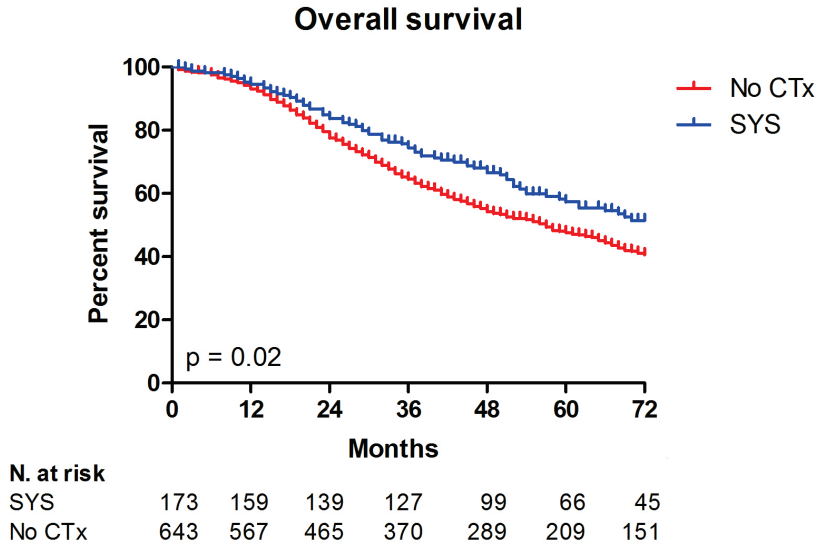


Figure 3. Kaplan-Meier curve for overall survival in patients with and without adjuvant systemic chemotherapy

Table 1: Overall patient characteristics

	All patients	No adjuvant CTx	Adjuvant CTx	P-value
Total	816 (100%)	643 (78.8%)	173 (21.2%)	-
Age (median, IQR)	64.0 (57.0-71.0)	64.0 (57.0-72.0)	64.0 (55.5-71.0)	0.43
Gender	90	4-312		
Male	136	4-312		
Female	63	32-217		
Nodal status primary tumour				0.17
N0	344 (42.5%)	263 (41.3%)	81 (47.1%)	
N+	465 (57.5%)	374 (58.7%)	91 (52.9%)	
Missing	7			
Disease free interval				0.08
< 12 months	528 (64.7%)	426 (66.3%)	102 (59.0%)	
≥ 12 months	288 (35.3%)	217 (33.7%)	71 (41.0%)	
Missing	-			
Number CRLM solitary tumour	376 (46.3%)	293 (45.9%)	83 (48.0%)	0.62
> 1 tumour	436 (53.7%)	346 (54.1%)	90 (52.0%)	
Missing	4			
Size largest tumour				0.22
≤ 5cm	643 (81.5%)	510 (82.4%)	133 (78.2%)	
> 5cm	146 (18.5%)	109 (17.6%)	37 (21.8%)	
Missing	27			
CEA				0.58
< 200	705 (91.7%)	552 (91.4%)	153 (92.7%)	
≥ 200	64 (8.3%)	52 (8.6%)	12 (7.3%)	
Missing	47			
Clinical risk score				0.28
Low	509 (65.5%)	395 (64.5%)	114 (69.1%)	
High	268 (34.5%)	217 (35.5%)	51 (30.9%)	
Missing	39			
Preoperative CTx				0.97
No	378 (46.4%)	298 (46.4%)	80 (46.2%)	
Yes	144 (17.6%)	113 (17.6%)	31 (17.9%)	
Missing	-			
Resection margin involved				0.06
No	702 (86.5%)	545 (85.3%)	157 (90.8%)	
Yes	110 (13.5%)	94 (14.7%)	16 (9.2%)	
Missing	4			
HGP				0.65
dHGP	183 (22.4%)	142 (22.1%)	41 (23.7%)	
Non-dHGP	633 (77.6%)	501 (77.9%)	132 (76.3%)	

* 31 patients (7.1%) received preoperative HAIP chemotherapy

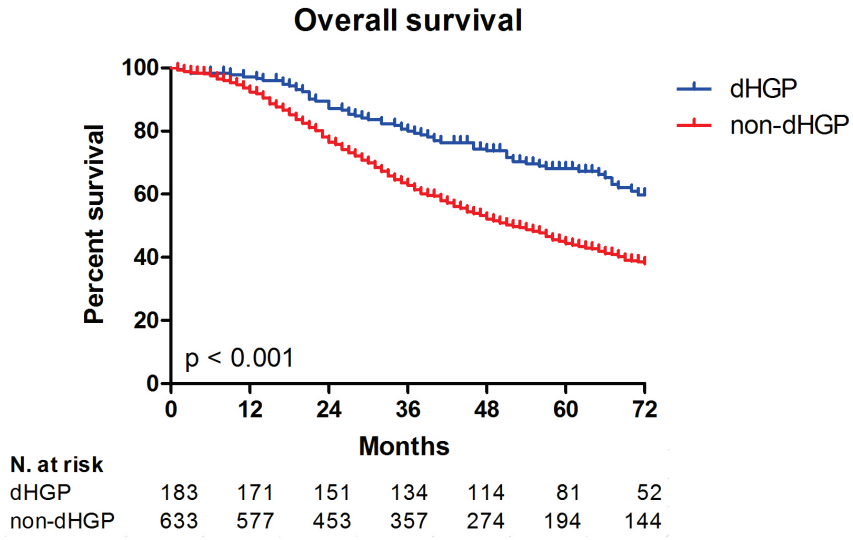


Figure 4. Kaplan-Meier curve for overall survival in patients with dHGP and non-dHGP

SURVIVAL OUTCOMES DHGP

The median OS in dHGP patients treated with adjuvant systemic chemotherapy was 79 months (95% CI 46-112), compared to 91 months (95% CI not reached) in patients that did not receive adjuvant systemic chemotherapy (p=0.33, figure 5a). In multivariable analysis (table 2), no association between OS and adjuvant systemic chemotherapy was found for patients with dHGP (adjusted HR 0.83, 95%CI 0.42-1.65, p=0.60).

Table 2: Uni- and multivariable Cox regression analysis for overall survival, dHGP

	Univariable			Multivariable		
Covariate	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.02	1.00-1.04	0.12	1.03	1.00-1.06	0.05
Node positive prim	1.06	0.66-1.72	0.80	1.25	0.71-2.21	0.44
DFI	1.01	1.00-1.02	0.14	1.01	1.00-1.02	0.16
Number CRLM	1.15	1.06-1.25	0.001	1.18	1.06-1.31	0.002
Diameter CRLM	1.04	0.96-1.11	0.34	1.02	0.94-1.11	0.60
CEA	1.00	1.00-1.00	0.19	1.00	1.00-1.00	0.40
R1 resection	1.49	0.68-3.26	0.32	1.22	0.49-3.06	0.6
Preoperative CTx	1.58	0.94-2.66	0.09	1.65	0.86-3.16	0.13
Adjuvant CTx	1.31	0.75-2.28	0.34	0.83	0.42-1.65	0.60

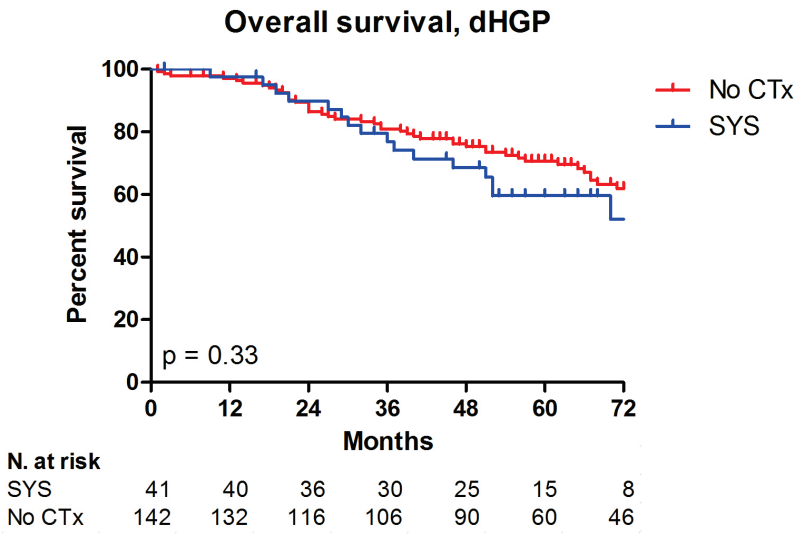


Figure 5a. Kaplan-Meier for overall survival in dHGP patients with and without adjuvant systemic chemotherapy

SURVIVAL OUTCOMES NON-DHGP

The median OS in non-dHGP patients treated with adjuvant systemic chemotherapy was 74 months (95%CI 53-96 months) compared to 45 months (95%CI 39-51 months) in patients not treated with adjuvant systemic chemotherapy (figure 5b, p=0.003). In multivariable analysis (table 3), adjuvant systemic chemotherapy remained significantly associated with improved survival (adjusted HR 0.66, 95%CI 0.50-0.88, p=0.004).

Table 3: Uni- and multivariable Cox regression analysis for overall survival, non-dHGP

	Univariable			Multivariable		
Covariate	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.02	1.01-1.03	0.001	1.02	1.01-1.03	<0.001
Node positive prim	1.39	1.13-1.71	0.002	1.48	1.18-1.86	0.001
DFI	1.00	0.99-1.00	0.39	1.00	0.99-1.01	0.72
Number CRLM	1.07	1.01-1.12	0.01	1.10	1.04-1.16	0.001
Diameter CRLM	1.05	1.02-1.08	0.002	1.07	1.03-1.11	<0.001
CEA	1.00	1.00-1.00	0.05	1.00	1.00-1.00	0.20
R1 resection	1.44	1.10-1.87	0.008	1.22	0.91-1.63	0.19
Preoperative CTx	1.08	0.88-1.32	0.47	0.99	0.78-1.25	0.91
Adjuvant CTx	0.68	0.52-0.88	0.004	0.66	0.50-0.88	0.004

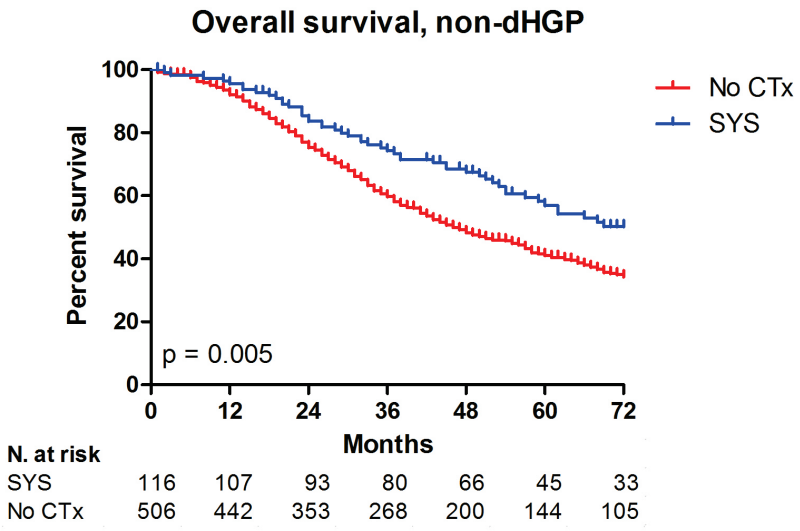


Figure 5b. Kaplan-Meier for OS in non-dHGP patients with and without adjuvant systemic chemotherapy

SUBGROUP ANALYSIS STRATIFIED BY PREOPERATIVE CHEMOTHERAPY

Subgroup analyses stratified for preoperative chemotherapy were performed (figure 6). No benefit of adjuvant systemic chemotherapy was found in patients with dHGP treated with (HR 0.93; 95%CI 0.48-1.83, p=0.84) or without (HR 2.50, 95%CI 0.94-6.62, p=0.07) preoperative chemotherapy. Also, no benefit of adjuvant systemic chemotherapy was found in patients with non-dHGP treated with preoperative chemotherapy (HR 0.93, 95%CI 0.64-1.34, p=0.68). Patients not pretreated with non-dHGP had superior survival outcomes with adjuvant systemic chemotherapy (HR 0.51, 95%CI 0.34-0.74, p<0.001).

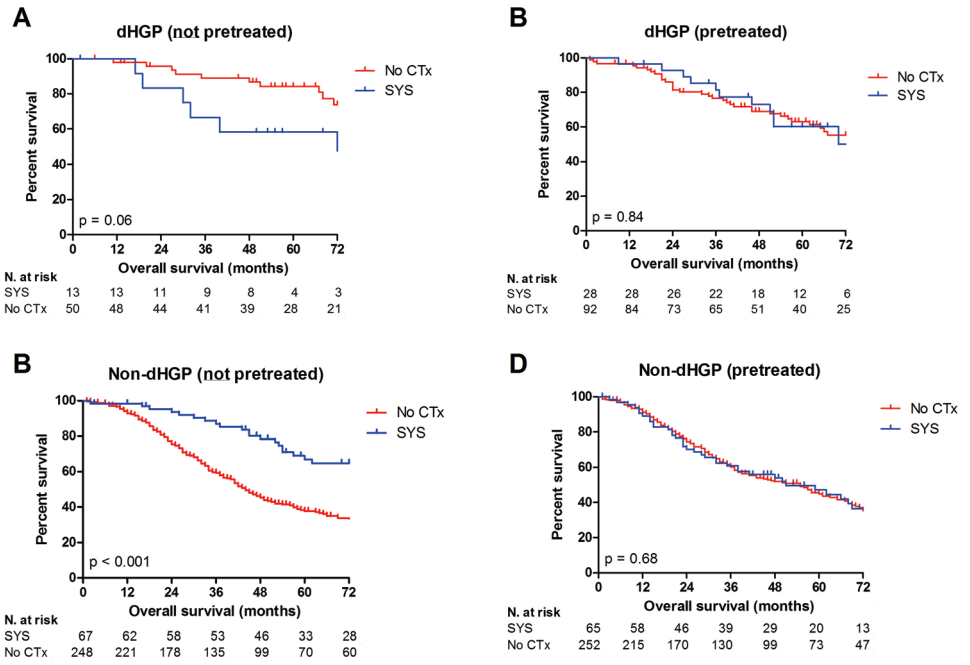


Figure 6. Kaplan-Meier for overall survival stratified for preoperative chemotherapy

DISCUSSION

We found that HGPs predict the effectiveness of adjuvant systemic chemotherapy. Adjuvant systemic chemotherapy seemed to be effective in non-dHGP patients, reflected by improved OS (adjusted HR 0.66, p=0.004). No effect of adjuvant chemotherapy on OS (adjusted HR 0.83, p=0.60) was observed in dHGP patients, suggesting that these patients may not benefit from adjuvant chemotherapy after resection of CRLM. Importantly, HGPs seemed to be only a predictive biomarker in patients that did not receive preoperative chemotherapy.

In order to determine the effectiveness of perioperative systemic chemotherapy, several studies have been performed. A large randomized trial evaluated the effectiveness of perioperative FOLFOX in patients with resectable CRLM (EORTC 40983). Although OS was not the primary endpoint of the study, no significant OS benefit was found after long-term follow-up. However, there are several non-randomized studies available indicating that subgroups of patients may benefit from additional treatment with chemotherapy. These studies suggest that (neo-) adjuvant systemic chemotherapy might be effective in patients at high risk of recurrence [7, 8]. Also, a subgroup analysis of the EORTC 40983 trial demonstrated beneficial progression free survival in patients with elevated CEA levels (>5 ng/ml) [13]. Furthermore, multiple previous studies have shown that the survival of patients with non-dHGP tumours is worse. Also,

non-dHGP is associated with several aggressive biological characteristics [14]. Therefore, the observed higher effectiveness of adjuvant chemotherapy in patients with non-dHGP tumours is in line with previous research, although validation of these findings is needed.

The current study demonstrates that the predictive value of HGPs regarding the effect of systemic chemotherapy might be limited after preoperative chemotherapy. No clear explanation for this phenomenon is available yet. Patients with non-dHGP not responding to preoperative chemotherapy might remain non-dHGP, while the CRLM in those that do respond convert to dHGP. Patients that did not respond to preoperative chemotherapy remain non-dHGP after pretreatment, and consequently also might not benefit from adjuvant systemic chemotherapy. This could explain the limited value of HGPs in pretreated patients. Unfortunately, evaluation of HGPs is only possible after resection.

Biological explanations of why only patients with non-dHGP appear to benefit from adjuvant systemic chemotherapy are lacking. One hypothesis is that a difference in tumour blood supply plays a role. Tumour cells in the non-dHGP use pre-existing liver vasculature from the surrounding liver parenchyma, instead of inducing the growth of new vessels through angiogenesis. However, in dHGP CRLM angiogenesis is observed [11]. This difference in tumour blood supply may also exist in occult micrometastases after curative-intent resection of CRLM, and could account for the fact that adjuvant systemic chemotherapy seems only effective in non-dHGP tumours due to a more effective supply of chemotherapy into the tumours.

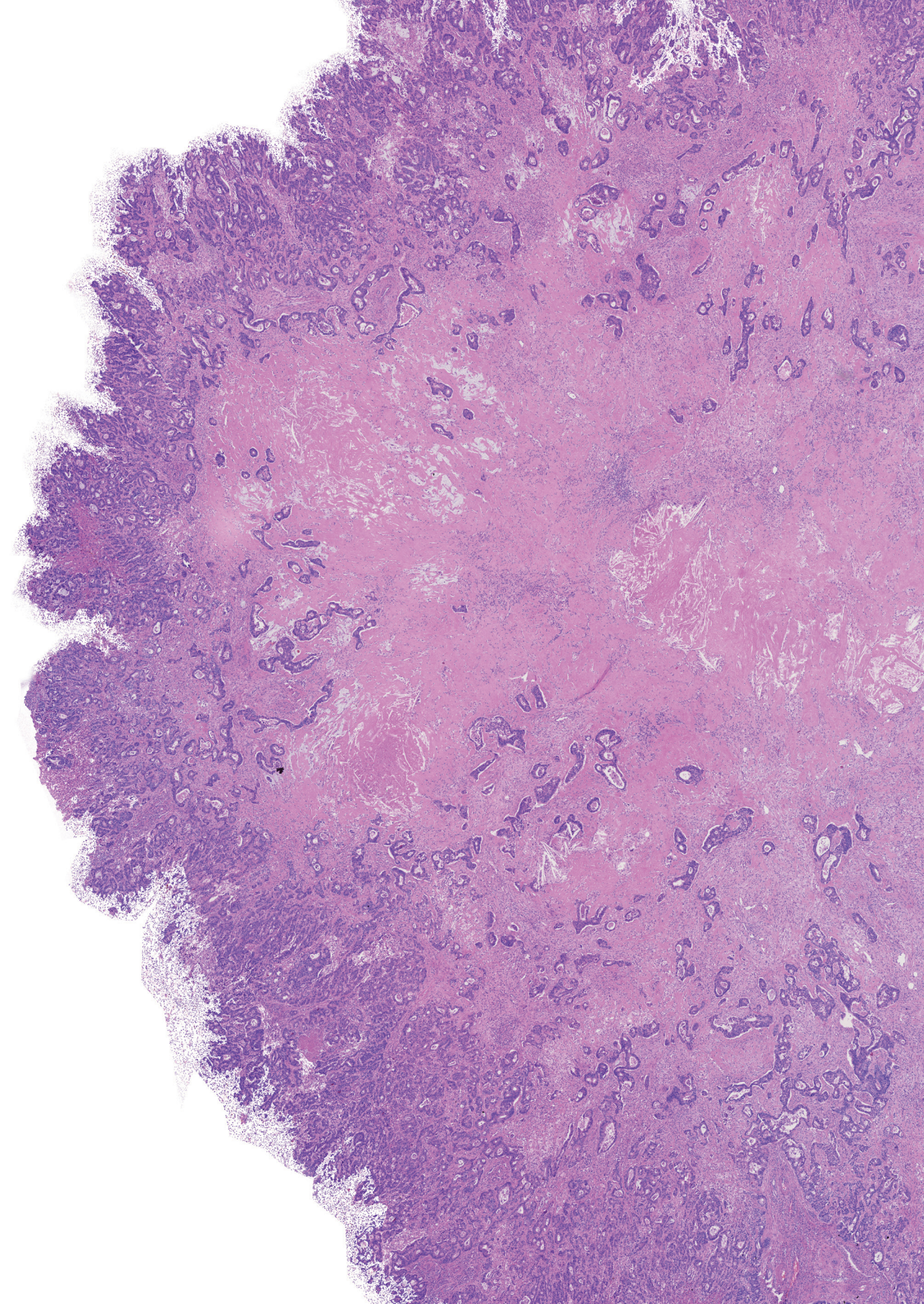
Since HGPs are determined after resection, this new biomarker can be used to guide the choice whether adjuvant systemic chemotherapy should be administered or not. However, future prospective studies should confirm the results of this study prior to clinical application. Future research should determine if pre-operative surrogate markers for HGPs can be identified (i.e., imaging [15], HGP gene expression in circulating tumour cells [16], or urine peptides [17]). Those surrogate markers may be helpful for predicting the effectiveness of preoperative systemic chemotherapy.

The results of this study should be interpreted in the light of several limitations. Most importantly, the retrospective nature of this study and the fact that two different centers were involved with different treatment policies. Moreover, the administration of adjuvant systemic chemotherapy was not determined at random. In Erasmus MC, no standard adjuvant systemic chemotherapy is given, according to the national guidelines. Also, a large number of patients were excluded due to missing H&E sections. This is the first study that demonstrates the predictive value of HGPs for adjuvant systemic chemotherapy after resection of CRLM. Other studies should are needed to confirm our findings.

In conclusion, the current study demonstrated that HGPs can predict the effectiveness of adjuvant systemic chemotherapy after resection of CRLM. Patients with non-dHGP seem to benefit from adjuvant systemic chemotherapy, while patients with dHGP do not benefit. After preoperative chemotherapy, adjuvant treatment seems not beneficial in either patient with dHGP or non-dHGP.

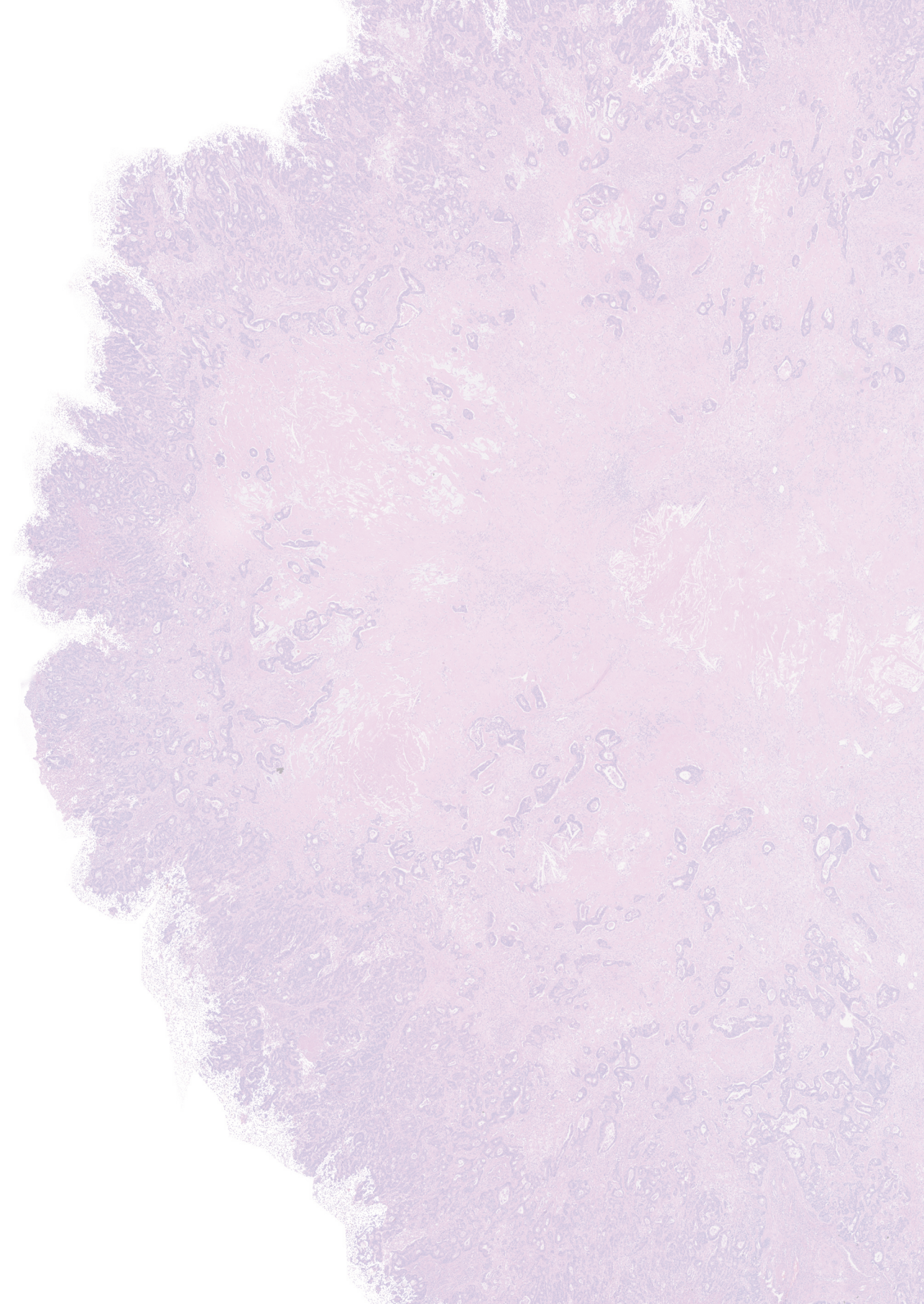
REFERENCES

1. Bismuth, H., et al., Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. Ann Surg, 1996. 224(4): p. 509-20; discussion 520-2.
2. van der Geest, L.G., et al., Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis, 2015. 32(5): p. 457-65.
3. Manfredi, S., et al., Epidemiology and management of liver metastases from colorectal cancer. Ann Surg, 2006. 244(2): p. 254-9.
4. Elferink, M.A., et al., Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis, 2015. 30(2): p. 205-12.
5. Tomlinson, J.S., et al., Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol, 2007. 25(29): p. 4575-80.
6. Nordlinger, 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(12): p. 1208-15.
7. Ayez, N., et al., The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator. Eur J Surg Oncol, 2015. 41(7): p. 859-67.
8. Rahbari, N.N., 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: p. 174.
9. Fong, Y., et al., Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. Ann Surg, 1999. 230(3): p. 309-18; discussion 318-21.
10. van Dam, P.J., et al., International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer, 2017.
11. Frentzas, S., et al., Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med, 2016. 22(11): p. 1294-1302.
12. Vermeulen, P.B., et al., Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol, 2001. 195(3): p. 336-42.
13. Sorbye, H., et al., Predictive factors for the benefit of perioperative FOLFOX for resectable liver metastasis in colorectal cancer patients (EORTC Intergroup Trial 40983). Ann Surg, 2012. 255(3): p. 534-9.
14. van Dam, P.J., et al., International consensus guidelines for scoring the histopathological growth patterns of liver metastasis. Br J Cancer, 2017. 117(10): p. 1427-1441.
15. Rao, S.X., et al., CT texture analysis in colorectal liver metastases: A better way than size and volume measurements to assess response to chemotherapy? United European Gastroenterol J, 2016. 4(2): p. 257-63.
16. Mostert, B., et al., KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue. Int J Cancer, 2013. 133(1): p. 130-41.
17. Broker, M.E., et al., Collagen peptides in urine: a new promising biomarker for the detection of colorectal liver metastases. PLoS One, 2013. 8(8): p. e70918.



PART III

Surgical Management of Colorectal Liver Metastases



CHAPTER 10

Management of liver metastases in colorectal cancer patients: A retrospective case-control study of systemic therapy versus liver resection

J.A.M. de Ridder
E.P .van der Stok
L.J.M. Mekenkamp
B. Wiering
M. Koopman
C.J.A. Punt
C. Verhoef
J.H.W. de Wilt

European Journal of Cancer. 2016 May;59:13-21

ABSTRACT

OBJECTIVE

To evaluate and compare the overall survival (OS) in case-matched patient-groups treated either with systemic therapy or surgery for colorectal liver metastases (CRLM).

METHODS

Patients with CRLM, without extra-hepatic disease, treated with chemotherapy with or without targeted therapy in two phase III studies (n=480) were selected and case-matched to patients who underwent liver resection (n=632). Matching criteria were sex, age, established prognostic factors for survival (clinical risk score (CRS)). Available CT scans of patients treated with systemic therapies were reviewed by three independent liver surgeons for resectability. Survival was compared between patients with resectable CRLM (based on CT scan review) who were treated with systemic therapy versus patients who underwent liver resection.

RESULTS

A total of 96 patients treated with systemic therapy were included. Main reasons for excluding patients were missing data on the CRS, extra-hepatic disease, >10 CRLM and surgery after initial systemic therapy. Pre-treatment CT scans of the liver were available for review in 56 of the systemically treated patients, and metastases were unanimously considered (complex) resectable in 36 (64.3%) patients. These 36 patients were case matched with 36 patients who underwent liver resection. Median OS in the patient group treated with systemic therapy was 26.5 months (range 0-81 months), which was significantly lower than the OS in case-matched patients who underwent liver resection (median OS 56 months; range 6-116) (p=0.027).

CONCLUSIONS

In this case-matched control study, surgery provided superior overall survival rates compared to systemic therapy for CRLM. These findings suggest surgery remains the preferred treatment strategy for CRLM. Resection of CRLM should always be considered, preferably in a dedicated centre, since not all patients that qualify for resection are identified as such.

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-death world-wide [1]. CRC patients develop metastases in 30-40%, depending on various factors such as T stage, N stage or histological subtype of colorectal cancer (i.e. mucinous, signet ring cell or adenocarcinoma) [2]. Approximately 20% of patients present with synchronous distant metastases (stage IV disease) [3] and another 20% will develop metachronous metastases, predominantly located in the liver [4].

In terms of treatment, liver resection is considered the standard of care in patients with resectable colorectal liver metastases (CRLM), with 5-year survival rates ranging from 35-60% [5-7]. In recent years an increasing number of patients are considered eligible for surgical resection of CRLM due to improved treatment strategies, both surgical and non-surgical. These improvements include two-staged liver resections [8], portal vein embolization [9] and preoperative systemic therapy downsizing initially unresectable CRLM [10].

In order to predict prognosis of patients with CRLM considered for surgery, various groups have assessed risk factors [11, 12] and multiple prognostic scoring systems have been developed [13-17]. The clinical risk score (CRS) by Fong et al. [18] is the most used scoring system, and its prognostic value has been validated by several independent investigators [19-21]. According to this CRS the following items are assigned one point: positive nodal status of the primary tumour, tumour size >50mm, >1 metastases, CEA-level >200ng/ml and an interval between primary tumour and development of liver metastases <12 months. Patients with extrahepatic disease are excluded. The total sum of the CRS divides patients into 'low risk' (0-2 points), and 'high risk' (3-5) of disease recurrence and overall survival after surgery [18].

Due to extra-hepatic disease and location, number or size of the liver metastases, only a minority of patients is, or will become, eligible for liver resection [13, 22]. Two issues play an important role in the treatment of patients with CRLM. First, there is no consensus on the criteria for resectability. Blinded retrospective reviews on this topic illustrated great variability in the assessment of resectability, even between dedicated liver surgeons [10, 23]. Second, chemotherapy regimens combining multiple drugs enriched with targeted agents, result in excellent median overall survival of >30 months in patients with initially unresectable colorectal metastases [24, 25]. Despite this, there is little doubt that surgical resection of CRLM offers the best chance for long-term survival [26] [27]. A randomized controlled trial on this topic is not considered to be ethical. Therefore, the challenge is to identify all patients who may be candidates for radical surgery of CRLM. Although the majority of cancer patients is currently being assessed in multidisciplinary teams, specific expertise in liver surgery is often lacking in these teams.

Therefore, we investigated the baseline resectability status in the subgroup of patients with CRLM in two well-defined and prospectively established patient cohorts who were considered to have unresectable CRLM and received systemic therapy within a clinical trial. The survival of patients who were considered resectable at baseline was compared to a matched control group of patients who underwent surgical resection of CRLM during the same period.

METHODS

PATIENT POPULATION AND DATA-COLLECTION

PATIENTS TREATED WITH SYSTEMIC THERAPY

We analysed patients with presumed unresectable CRLM at baseline who were included in two phase III randomized clinical trials from the Dutch Colorectal Cancer Group (DCCG). Starting in 2003, the CAIRO study randomized 820 metastatic colorectal cancer patients between first-line sequential or a combination treatment with capecitabine, irinotecan and oxaliplatin [28]. The CAIRO2 study included 755 metastatic colorectal cancer patients, who were randomly assigned to receive first-line treatment with capecitabine, oxaliplatin, and bevacizumab, or the same schedule with the addition of weekly cetuximab [29]. One of the inclusion criteria in both studies was that the metastases were unresectable. However a discussion of the individual patient in a multidisciplinary liver team was not mandatory for inclusion in both studies. Patients in the CAIRO study were required to have a WHO performance status of 0-2, and in the CAIRO2 study of 0-1. The details of both studies have been presented previously [28, 29].

Since patients with more than 10 CRLM are rarely candidates for curative surgery, CAIRO and CAIRO2 patients with less than 10 CRLM and without extra-hepatic disease were selected. Patients in both trials who underwent liver resection after initial systemic therapy were excluded as well as patients with the primary colorectal tumour still in situ. Another criterion for exclusion was incomplete data on the items of the CRS [18]. These criteria were pre-operative CEA level, number of CRLM, size of the CRLM, lymph node status of the primary tumour, and the time between surgery of the primary tumour and 'treatment' (systemic therapy) of the metastases. These data were not necessary to be known for inclusion in the CAIRO and CAIRO2 studies, and therefore were not available in the majority of patients.

PATIENTS TREATED WITH LIVER RESECTION

Erasmus MC Cancer Institute Rotterdam and Radboud University Medical Centre Nijmegen are tertiary referral hospitals for CRLM surgery. Post-operative follow-up consisted of clinical examination, measurement of CEA-levels, and imaging using computed tomography (CT) imaging. In order to compare patients from similar time periods, all patients who underwent primary liver resection for CRLM between January 2003 (start of the CAIRO study) and September 2011 were analysed in the present study. Patients who received induction, neo-adjuvant systemic therapy were excluded from the present analysis. Patients who underwent liver resection together with RFA of other lesions during the same operation were also excluded. Liver resection was considered to be complete (R0) when the pathologist assessed free resection margins.

DATA-COLLECTION AND MATCHING

Demographics and clinical-pathological factors of the primary tumour as well as the liver metastases were collected. Fong's CRS[18] was used for matching patients' oncological risk profiles. Thus, all 5 variables included in this CRS were collected: CEA level, tumour size and number of metastases recorded at baseline, the disease free interval between resection of the primary tumour and treatment of liver metastases (either surgery or randomization for systemic therapy) and nodal status of the primary tumour. Systemically treated patients were selected and case-matched to patients who underwent liver resection only, in terms of gender, age, CRS and the absence of extra-hepatic metastases.

REVIEW OF RESECTABILITY

In order to assess the potential surgical options and agreement on proposed treatment for CRLM, all baseline CT-scans of patients treated with systemic therapy were requested. Review of resectability, based on radiological images only, was performed by 3 dedicated liver surgeons. After reviewing the images of the CT scans, liver lesions were classified:

- resectable;
- complex resectable (e.g. two-staged procedures, including portal vein embolization, resection in combination with radio frequent ablation, or the need for induction chemo therapy);
- unresectable;
- CT images were of insufficient quality for the assessment of resectability. Quality of images was based on the system used by Jones et al. [23].

OUTCOME VARIABLES:

The primary endpoint of the current analysis was overall survival (OS). This was defined as the time from liver resection or from randomization to systemic therapy, until date of last follow up or death. As described in the protocols of CAIRO and CAIRO II, the maximum time from randomization to initiation of systemic treatment had to be within 7 days [28, 29].

STATISTICAL ANALYSIS:

The comparison between categorical variables was performed using the Chi-square tests. Means and medians of the items from the CRS were compared using the Mann-Whitney-U test. Survival analysis was performed by using the Kaplan Meier survival analysis, and compared by using log-rank tests. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences version 18.0 (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

PATIENTS AND TREATMENT CHARACTERISTICS

SYSTEMIC THERAPY

A total of 480 patients with CRLM without extra-hepatic metastases were treated with systemic therapy between January 2003-December 2004 (CAIRO, n=256) and between June 2005-December 2006 (CAIRO 2, n=224). The majority of patients (n=259; 54%) could not be included in the present study due to missing or incomplete data with respect to the CRS. Most frequently the pre-operative CEA-level was absent. Other reasons for exclusion are listed in figure 1a. Eventually 36 patients were eligible for inclusion from either the CAIRO (n=14), or CAIRO 2 (n=22). Of these 36 patients, 6 patients were treated with first-line sequential chemotherapy, 8 patients received first-line combination therapy, 16 patients were treated with first-line chemotherapy with bevacizumab, and 6 patients received first-line chemotherapy in combination with bevacizumab and cetuximab. **Figure 1a.** Selection process of patients with colorectal liver metastases treated with systemic therapy. tx: treatment. CRLM: colorectal liver metastases

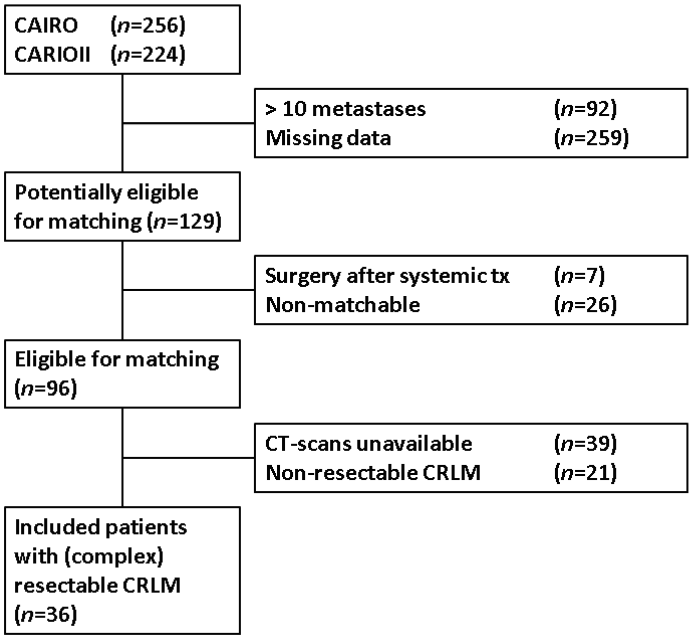


Figure 1a. Selection process of patients with colorectal liver metastases treated with systemic therapy. tx: treatment. CRLM: colorectal liver metastases

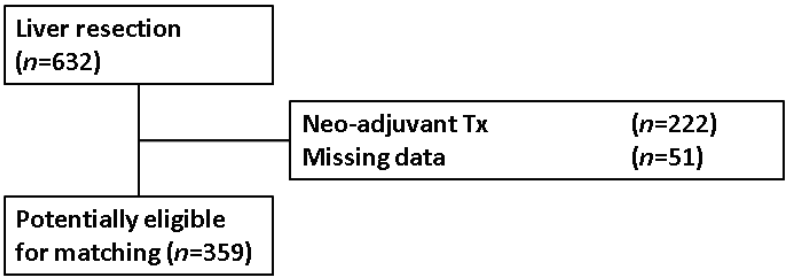


Figure 1b. Selection process of patients with colorectal liver metastases treated with liver resection. Tx: treatment; CRS: clinical risk score.

RETROSPECTIVE REVIEW OF RESECTABILITY

Baseline CT images of 57 patients out of 96 patients selected from the CAIRO studies could be retrieved from the different hospitals. These images were not available in 39 patients, because they were stored on microfilm only, or not stored digitally. In one patient, all three surgeons considered the CT images of "insufficient quality for review", which left 56 patients (58.3%) eligible for analyses. In five patients one or more surgeons were unable to make a decision on resectability as a result of insufficient quality of the CT images. The majority of patients were considered (complex) resectable (n=36; 64.2%), while only 2 patients were considered unresectable by all three reviewers (Figure 2).

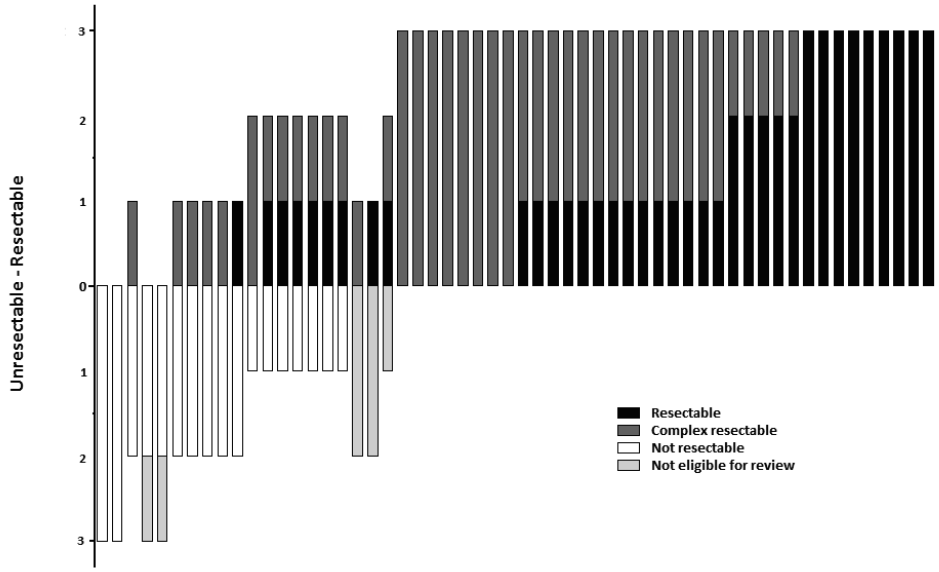


Figure 2. Forrest plot showing decisions of 3 surgeons on resectability of colorectal liver metastases in 56 patients who were treated with systemic therapy, based on computer tomography images. The number of reviewers who made a decision is shown on the Y-axis and each bar on the X-axis represents one patient.
Complex resectability was defined as the need for neo-adjuvant treatment or complex surgery (two-staged procedures including portal vein embolization or resection in combination with radiofrequent ablation).

LIVER RESECTION

Between January 2003 until September 2011 a total of 632 patients underwent liver resection. After excluding patients treated with neo-adjuvant systemic treatment (n=222), patients of whom data were missing on one of the items of the CRS (n=25), or patients with extra-hepatic disease (n=26), 358 patients were eligible and could be included in the current study.

CASE-MATCHING

A total of 36 patients who were considered (complex) resectable by the liver surgeons were matched with patients who underwent liver surgery. The clinical-pathological characteristics used to case-match both treatment groups are summarized in Table 1. The types of performed liver resection were: wedge resection (n=15); segmental resection (n=11); hemihepatectomy (n=10). A microscopic incomplete resection (R1) seemed to be present in 6 patients (16.7%). After resection, 7 patients (19.4%) were treated with adjuvant systemic therapy (fluoropyrimidine only (n=1) and fluoropyrimidine plus oxaliplatin (n=6)). Of which, 1 patient was also treated with bevacizumab as part of a multicentre randomized clinical trial [30].

Table 1: Demographic and tumour clinical-pathological factors of case-matched patients treated with systemic therapy or liver resection. CEA: carcinoembryonic antigen

		Systemic therapy N=36	Surgery N=36	p-value
Gender	Male	20 (55.6%)	20 (55.6%)	-
Male	Female	16 (44.4%)	16 (44.4%)	
Median age in years (range)		66.5 (36-79)	66 (32-79)	0.813
Primary tumour				
	Colon	29 (80.6%)	22 (61.1%)	**0.023****
	Rectum	5 (13.9%)	14 (38.9%)	
	Unknown	2 (5.5%)	0	
T-stage primary tumour				
	T1-3	26 (72.2%)	30 (83.3%)	0.257
	T4	10 (27.8%)	6 (16.7%)	
Lymph node	Negative	16 (44.4%)	11 (30.6%)	0.224
	Positive	20 (55.6%)	25(69.4%)	
Liver metastases				
Median CEA level (range)		18.5 (1-635)	26.6 (1-910)	0.907
Median interval (range)*		4 (1-109)	3 (0-91)	0.907
Median number metastases (range)		4 (1-7)	3 (1-10)	**0.009****
Median size largest metastasis in mm (range)		30 (12-160)	39 (12-120)	**0.044****
Fong-score[18]	1	5 (13.9%)	4 (11.1%)	0.907
	2	10 (27.8%)	12 (33.3%)	
	3	18 (50.0%)	16 (44.5%)	
	4	3 (8.3%)	4 (11.1%)	

* interval between treatment of the primary tumour and the liver metastases (either systemic therapy or liver resection)

** significant with $p<0.05$

SURVIVAL

Median follow-up of all patients treated with systemic therapy was 43 months (range 0-81 months) and 31 months (range 0-101) for patients who were surgically treated.

Median overall survival (OS) in the total group of patients treated with systemic therapy (n=480) was 21 months (range 0-84 months) and 52 months (range 0-101 months) in the total group of patients who underwent liver resection (n=632). The 5-year OS was 11.9% in patients treated with systemic therapy only versus 45.6% in surgically treated patients.

In the matched cohort of patients treated with systemic therapy (n=36) median overall survival was 27 months (range 0-81 months), which was significantly higher (p=0.002) than in the total group of patients treated with systemic therapy (n=480). However, the OS in the matched cohort of patients treated with systemic therapy was significantly lower (p=0.027) compared to the median OS of 56 months (range 1-116 months) in case-matched patients treated with liver resection (n=36) (Figure 3).

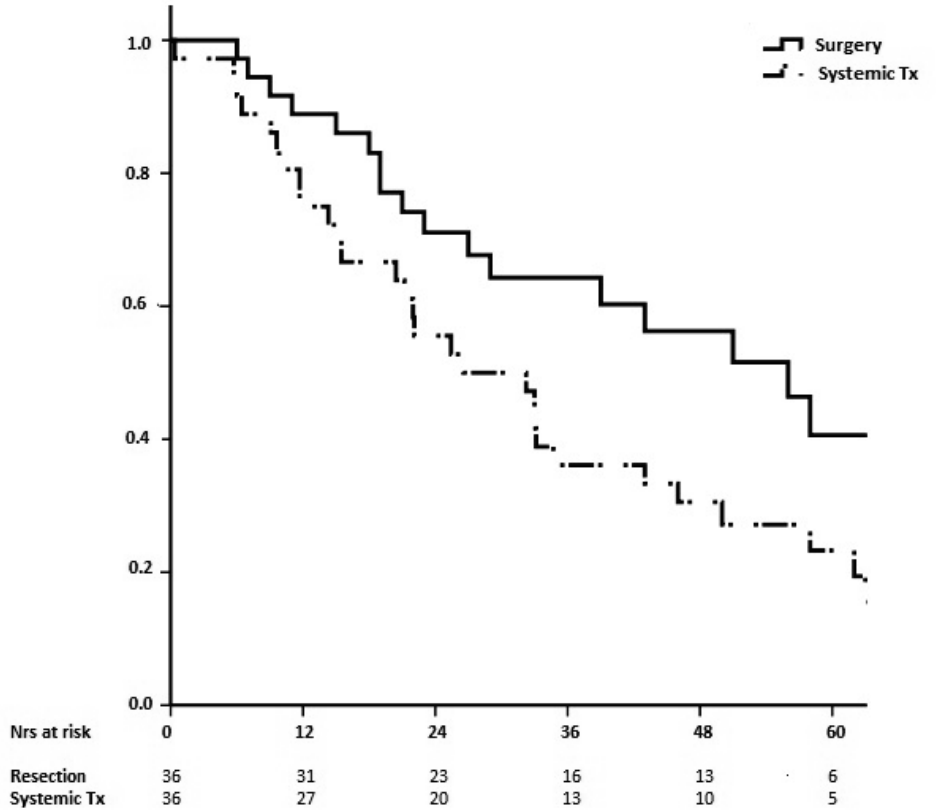


Figure 3. Overall survival in case-matched patients with colorectal liver metastases (considered resectable based on reviewing the computed tomography images) treated with either systemic therapy or liver resection (p=0.027). On the Y-axis the proportion of patients, on the X-axis survival in months. Tx: treatment. Nrs: numbers

DISCUSSION

This study showed that patients with CRLM are not always identified and offered surgery with curative intent. In the retrospective evaluation of CT-images performed by dedicated liver surgeons in a patient group treated with systemic, palliative regimens for CRLM, a significant number appeared to be resectable. The case-matched patient groups with resectable liver-only disease showed significant differences in overall survival after surgical or systemic treatment strategies. Patients undergoing surgery for CRLM had superior overall survival rates as compared to patients where systemic therapies were administered. Thus, the current study confirms surgery is the preferred treatment strategy in patients with CRLM. These findings emphasize the importance of adequate patient selection for surgery.

In the current study, patients were selected from two completed multicentre randomized clinical trials focusing on systemic therapy for CRLM, and from two large liver surgery databases. On ethical grounds, a true randomized clinical trial comparing both treatment strategies in patients with resectable CRLM has not been, and will not be performed. By case-matching the patients for known prognostic factors, this study is the second best alternative to compare both treatment strategies in patients with resectable CRLM.

After liver surgery patients showed a longer OS (median 56 months) compared to patients who were treated with systemic therapy for (retrospectively) resectable liver metastases (median 27 months).

Kopetz et al. (2009) previously reported a survival benefit in patients with CRLM who underwent liver resection compared to patients treated with systemic therapy [26]. Patients undergoing liver surgery received pre-operative systemic therapy, suggestive of a selection bias in this study. Also, data were derived from unmatched patient cohorts, which could make the results susceptible for additional bias and should be interpreted cautiously. Brouquet et al. (2011) performed an intention-to-treat analysis to evaluate OS of "high risk patients" with CRLM (patients undergoing at least the first stage of a 2-staged surgical approach) after treatment with systemic agents versus patients treated with systemic therapy only [27]. In the surgery group, only non-progressors on systemic therapy were selected for comparison. In the group of patients treated with systemic therapy only, responders were selected, suggesting that only patients with a favourable tumour biology were used for comparison. This could induce a potential bias for the survival rates demonstrated in the group of patients receiving systemic therapy only (favourable tumour biology). However, even though patients with excellent response to systemic therapy were selected for comparison, surgery proved to yield superior overall survival.

In the study of Brouquet et al. response to systemic therapy was accounted for in the analysis, in contrast to the study of Kopetz et al. [26, 27]. In the present study, only patients who underwent surgical treatment without neo-adjuvant/induction chemotherapy were selected in order to rule out a potential bias of selection of less aggressive cancers in resected patients. On the other hand, in the group treated with systemic therapy only, patients with favourable characteristics (< 10 liver only metastases) were selected from the CAIRO studies. This was demonstrated by a high median OS of 27 months, which was significantly better compared to the complete group of patients with treated with systemic therapy in both CAIRO studies (21 months; p=0.002) [28, 29]. Matching the surgical and systemic patient groups was performed using the CRS, age and gender. After case matching, the OS of systemically treated patients with resectable CRLM (based on reviewing the CT images, N=36), was compared to the OS of patients who underwent liver resection. Reviewing the CT scans with respect to resectability in the patient group treated with systemic therapy rules out the potential unfavourable effects of unresectability on survival. Median OS was 56 months (range 1-101) in patients who were treated with liver resection, which was superior compared to systemically treated patients (median OS 27 months; range 0-81) (p=0.027).

Moreover, 5-year survival in the surgically treated group was 46.4% which is comparable to survival rates in the literature after liver resection with a median OS of 43-64 months and 5-year OS rates of 51%-58% [7, 31]. The results of this study support the concept of a surgical treatment strategy as the gold standard for CRLM, although this has never been validated in a prospective randomized clinical trial.

Unfortunately, there was no information available whether patients treated with systemic therapy were discussed in MDTs and evaluated for potential resectability in the CAIRO trials. Jones et al. reported the importance of MDTs and especially the involvement of specialist liver surgeons in those teams [23]. In their study, 63% of the patients with liver only colorectal metastases who were treated with palliative systemic treatment were retrospectively considered to have potentially resectable CRLM by a majority of the reviewing liver surgeons. In the present study the CT images of the systemically treated patients were reviewed and a majority of liver surgeons agreed on CRLM being resectable in 79% of cases, compared to 63% in the study by Jones et al. [23].

In the CAIRO studies 4.8% (n=23) of all systemically treated patients with liver-only metastases underwent subsequent liver resection after a good response. For the comparison of survival in the present study, patients were only included if they continued systemic treatment and if they were not invited for surgery. Importantly, the decision on whether to perform a liver resection for CRLM is subject to bias as demonstrated by 13 of 56 patients (23.2%) in which at least one of the liver surgeons (being the expert panel of this study) considered lesions unresectable, while one of the other surgeons considered the same lesions (potentially) resectable. Folprecht et al. (2010) previously demonstrated critical disagreement between experienced liver surgeons in 7% of assessed patients, when they evaluated resectability on CT images of patients and deciding whether surgery or (induction) chemotherapy was the preferred treatment strategy [10].

The current study confirms that surgery yields superior survival rates in resectable, liver-only CRLM as compared to systemic treatment only. These results emphasize the importance of assessing each patient with CRLM by a dedicated MDT, including specialised liver surgeons. Furthermore, consensus on resectability between liver surgeons is essential. Standardised assessment of all patients with CRLM by specialist teams, might ensure that potentially all that qualify for surgery are identified accordingly, offering those patients the best prospects in terms of survival. A useful tool to assess resectability might be the Met-Assist program, which was developed to indicate the likelihood that experts in the field would judge surgery as feasible under given circumstances [32, 33]. Currently, the CAIRO5 trial is performed in which patients with potentially resectable CRLM are selected for different induction chemotherapy regimens [32]. This prospective trial uses a central panel consisting of one radiologist and three liver surgeons. Possibly, this trial will add to the definition of resectability of CRLM in the future.

Separate from the (retrospective) observation that resection for CRLM yields superior survival rates as compared to systemic therapy, another point of interest is the cost effectiveness of

treatment strategies. Recently, Roberts et al. performed a cost-utility analysis of operative versus non-operative treatment for CRLM [34]. The results of their study show surgery is more effective and less costly than non-operative treatment for CRLM. Again, this emphasises the importance of patient selection for resection.

A limitation of the present study may be that patients who underwent systemic therapy in the CAIRO and CAIRO2 trial underwent CT imaging demonstrating liver only disease, but did not all receive additional diagnostics for extra-hepatic metastases (e.g. FDG-PET scan). However, a recent randomized clinical trial evaluating the treatment changes in patients with CRLM scheduled for surgery after FDG-PET CT scan, reported cancellation of the suggested surgical procedure in only 2.7% of the patients [35]. Additionally, survival in patients who underwent liver surgery did not differ between patients who were selected with or without FDG-PET [36]. Because of the retrospective character of the current study and despite the thorough case matching, the performance status and co-morbidity may differ between surgery and systemically treated patients. However, the systemically treated patients all had a WHO performance status of 0-2 in the CAIRO study and WHO status of 0-1 in the CAIRO 2 study, which is probably not inferior to the surgically treated patients.

In conclusion, this case-matched controlled comparison of patients undergoing either systemic therapy or surgery for resectable CRLM demonstrate a significant survival benefit in patients treated with liver resection. Surgery should remain the gold standard treatment for patients with CRLM. This finding emphasises the importance of adequate patient selection for surgery. Consensus on resectability and standardised assessment of all patients presenting with CRLM by dedicated liver surgeons in specialised MDTs optimises patient selection for surgery.

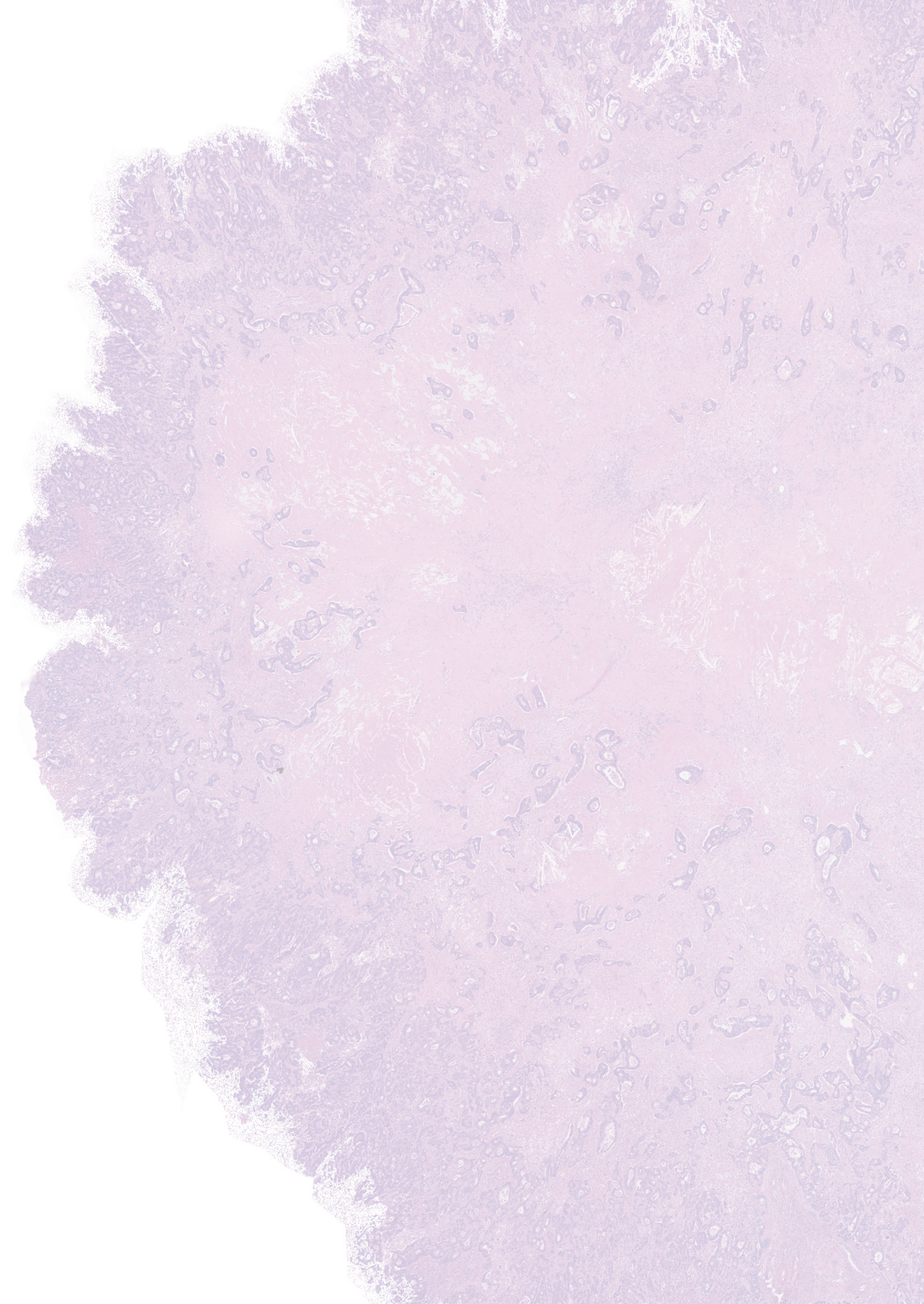
REFERENCES

1. Jemal, A., et al., *Global cancer statistics*. CA Cancer J Clin, 2011. **61**(2): p. 69-90.
2. Hugen, N., et al., *Metastatic pattern in colorectal cancer is strongly influenced by histological subtype*. Ann Oncol, 2014. **25**(3): p. 651-7.
3. van der Geest, L.G., et al., *Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases*. Clin Exp Metastasis, 2015. **32**(5): p. 457-65.
4. Elferink, M.A., et al., *Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands*. Int J Colorectal Dis, 2015. **30**(2): p. 205-12.
5. Choti, M.A., et al., *Trends in long-term survival following liver resection for hepatic colorectal metastases*. Ann Surg, 2002. **235**(6): p. 759-66.
6. Zakaria, S., et al., *Hepatic resection for colorectal metastases: value for risk scoring systems?* Ann Surg, 2007. **246**(2): p. 183-91.
7. House, M.G., et al., *Survival after hepatic resection for metastatic colorectal cancer: trends in outcomes for 1,600 patients during two decades at a single institution*. J Am Coll Surg, 2010. **210**(5): p. 744-52, 752-5.
8. van der Pool, A.E., et al., *'Staged' liver resection in synchronous and metachronous colorectal hepatic metastases: differences in clinicopathological features and outcome*. Colorectal Dis, 2010. **12**(10 Online): p. e229-35.
9. Wicherts, D.A., et al., *Impact of portal vein embolization on long-term survival of patients with primarily unresectable colorectal liver metastases*. Br J Surg, 2010. **97**(2): p. 240-50.
10. 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**(1): p. 38-47.
11. Kanas, G.P., et al., *Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors*. Clin Epidemiol, 2012. **4**: p. 283-301.
12. Knijn, N., et al., *Histopathological evaluation of resected colorectal cancer liver metastases: what should be done?* Histopathology, 2013. **63**(2): p. 149-56.
13. Nordlinger, B., et al., *Surgical resection of colorectal carcinoma metastases to the liver*. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. Cancer, 1996. **77**(7): p. 1254-62.
14. Nagashima, I., et al., *Proposal of criteria to select candidates with colorectal liver metastases for hepatic resection: comparison of our scoring system to the positive number of risk factors*. World J Gastroenterol, 2006. **12**(39): p. 6305-9.
15. Konopke, R., et al., *Prognostic factors and evaluation of a clinical score for predicting survival after resection of colorectal liver metastases*. Liver Int, 2009. **29**(1): p. 89-102.
16. Iwatsuki, S., et al., *Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system*. J Am Coll Surg, 1999. **189**(3): p. 291-9.
17. Hayashi, M., et al., *Clinicopathological analysis of recurrence patterns and prognostic factors for survival after hepatectomy for colorectal liver metastasis*. BMC Surg, 2010. **10**: p. 27.

18. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases*. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
19. Mala, T., et al., *Hepatic resection for colorectal metastases: can preoperative scoring predict patient outcome?* World J Surg, 2002. **26**(11): p. 1348-53.
20. Reissfelder, C., et al., *Validation of prognostic scoring systems for patients undergoing resection of colorectal cancer liver metastases*. Ann Surg Oncol, 2009. **16**(12): p. 3279-88.
21. Ayez, N., 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): p. 2757-63.
22. Scheele, J., et al., *Resection of colorectal liver metastases*. World J Surg, 1995. **19**(1): p. 59-71.
23. Jones, R.P., et al., *Effect of specialist decision-making on treatment strategies for colorectal liver metastases*. Br J Surg, 2012. **99**(9): p. 1263-9.
24. Heinemann, V., 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): p. 1065-75.
25. Loupakis, F., et al., *Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer*. N Engl J Med, 2014. **371**(17): p. 1609-18.
26. Kopetz, S., et al., *Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy*. J Clin Oncol, 2009. **27**(22): p. 3677-83.
27. Brouquet, A., 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): p. 1083-90.
28. Koopman, M., et al., *Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial*. Lancet, 2007. **370**(9582): p. 135-42.
29. Tol, J., et al., *Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer*. N Engl J Med, 2009. **360**(6): p. 563-72.
30. Snoeren, N., et al., *A randomized two arm phase III study in patients post radical resection of liver metastases of colorectal cancer to investigate bevacizumab in combination with capecitabine plus oxaliplatin (CAPOX) vs CAPOX alone as adjuvant treatment*. BMC Cancer, 2010. **10**: p. 545.
31. de Haas, R.J., et al., *Impact of expanding criteria for resectability of colorectal metastases on short- and long-term outcomes after hepatic resection*. Ann Surg, 2011. **253**(6): p. 1069-79.
32. Huiskens, J., 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**: p. 365.
33. Langenhoff, B.S., P.F. Krabbe, and T.J. Ruers, *Computer-based decision making in medicine: A model for surgery of colorectal liver metastases*. Eur J Surg Oncol, 2007. **33 Suppl 2**: p. S111-7.
34. Roberts, K.J., et al., *Cost-utility analysis of operative versus non-operative treatment for colorectal liver metastases*. Br J Surg, 2015. **102**(4): p. 388-98.
35. Moulton, C.A., et al., *Effect of PET before liver resection on surgical management for colorectal*

adenocarcinoma metastases: a randomized clinical trial. JAMA, 2014. **311**(18): p. 1863-9.

36. Ayez, N., et al., *Preoperative FDG-PET-scan in patients with resectable colorectal liver metastases does not improve overall survival: a retrospective analyses stratified by clinical risk score*. Dig Surg, 2013. **30**(4-6): p. 451-8.



CHAPTER 11

Regional and inter-hospital differences in the utilization of liver surgery for patients with synchronous colorectal liver metastases in the Netherlands

J. 't Lam-Boer
E.P. van der Stok
J. Huiskens
R.H.A. Verhoeven
C.J.A. Punt
M.A.G. Elferink
J.H. de Wilt
C. Verhoef

European Journal of Cancer. 2017 Jan;71:109-116

ABSTRACT

BACKGROUND

The objective of this study was to map referral patterns in patients with synchronous colorectal liver metastases (SCLM) and to investigate if type, volume and location of the hospital of diagnosis are associated with whether or not patients underwent liver resection.

METHODS

This population-based study includes all patients diagnosed with SCLM between 2008 and 2012, based on the Netherlands Cancer Registry. To study inter-hospital variation, the proportion of patients undergoing liver surgery was calculated per hospital of diagnosis. Multivariable multilevel logistic regression analysis was used to investigate the association be- tween hospital characteristics and liver resection.

RESULTS

Of 10,520 patients with SCLM, 12% (n = 1259) underwent liver surgery. Of these patients, 58% (n = 733) were referred to another hospital to undergo liver surgery. In 53% of the patients (n = 647), liver resection was performed in a university hospital, in 39% (n = 482) in a dedicated liver centre and in 8% (n = 102) in a general hospital. There was a large inter-hospital variation in the proportion of patients undergoing liver resection (2-26%). In a multi- level logistic regression model, the odds of undergoing liver surgery were higher when patients were diagnosed in hospitals where liver surgery was performed compared with the general hospitals (dedicated liver centre: odds ratio 1.36 [95% confidence intervals 1.08-1.70], university hospital: odds ratio 1.69 [95% confidence intervals 1.22-2.34]).

CONCLUSION

There is a large inter-hospital and inter-regional variation in the utilization of liver resection. Patients diagnosed with SCLM in expert centres had a higher chance of undergoing liver resection.

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of cancer worldwide. In 2014, more than 15,000 patients were diagnosed with CRC in the Netherlands. Metastases occur in a substantial number of patients, depending on the histological subtype of colorectal cancer (i.e. mucinous, signet ring cell or adenocarcinoma) [1]. Approximately, 20% of patients present with synchronous distant metastases (stage IV disease), and another 20% of patients develop metastases during follow-up (metachronous metastases) [2, 3]. Colorectal liver metastases (CLMs) are present in three out of four patients with stage IV disease and in the majority no extrahepatic metastases are found [3, 4].

Surgical resection of the primary tumour and all metastases offers a potential cure for patients with CLM; in particular for patients without extrahepatic metastases [5]. The five-year survival rates for patients undergoing liver resection are nowadays between 20% and 60%, depending on clinical risk factors [5-8]. As the criteria for resectability are evolving, the proportion of patients undergoing liver resection is increasing [9]. At present, metastasectomy is considered for patients with colorectal liver metastases if the patient is fit for surgery, if there is an expected remnant liver of at least 20-30% of the preoperative volume, if liver resection is anatomically possible with regard to vascular and biliary structures, and if no unresectable extrahepatic metastases are present [10, 11].

In the Netherlands, inter-hospital variation in the proportion of patients undergoing curative treatment has been demonstrated for various types of cancer [12-14]. A survey among surgeons showed a wide variety in the diagnostics and therapeutic workup for patients with metastatic CRC [15]. Recently, a regional study demonstrated variation in the utilization of liver resection in the south of the Netherlands [6]. This implies that substantial differences in the utilization of liver surgery might exist on a national level as well. Previous research has demonstrated that involvement of a hepatobiliary surgeon in the multidisciplinary colorectal cancer team improves overall survival [16-18]. Therefore, we hypothesize that patients with CLM diagnosed in a dedicated liver centre more often undergo liver surgery.

The objective of this study was to determine the variation between hospitals in the proportion of patients with CLM undergoing liver resection, using the Netherlands Cancer Registry (NCR). Because only synchronous metastases can be identified by the NCR, we focused on stage IV patients only. Moreover, we analysed referral patterns for liver surgery and investigated if type, volume and region of the hospital of diagnosis were associated with the probability that a patient will undergo liver resection.

METHODS

NETHERLANDS CANCER REGISTRY

Nationwide population-based data were extracted from the NCR. This database registers all newly diagnosed cancers since 1989. Identification of patients is primarily based on notification by the national automated pathology archive and hospital discharge notes [19]. Patient, tumour and treatment characteristics were retrieved from patient files by specially trained registration employees of the Netherlands Comprehensive Cancer Organization. Classification of tumour characteristics occurs according to the current versions of the TNM Classification of Malignant Tumours [20] and the International Classification of Diseases for Oncology [21]. Information on metachronous metastases is not registered in the NCR. Follow-up of vital status is obtained by annually linking the Registry to the Municipal Personal Records Database, which contains information on vital status of all Dutch inhabitants.

DATA SELECTION

All adult patients (18 years or older) who presented with SCLM between 2008 and 2012 were selected for this study. Patients diagnosed with SCLM during autopsy were excluded. Synchronous metastases were defined as metastases detected before the start of initial treatment and/or during surgical exploration. The extracted data included patient, tumour and treatment characteristics, as well as overall survival (OS). OS was defined as the interval between date of diagnosis of the cancer until date of death from any cause, or censored at date of end of follow-up (December 2015). Year of diagnosis was defined as the year of first histological confirmation. Hospital of diagnosis was defined as the hospital where the first histological confirmation of malignant disease was obtained: most often as the result of endoscopic biopsy of the primary tumour. Liver resection was defined as any removal of liver metastases (including surgical diagnostic biopsies, not including percutaneous biopsies or percutaneous treatments such as radiofrequency ablation).

ORGANIZATION OF DUTCH HOSPITAL HEALTH CARE

Hospital health care in the Netherlands during the studied time period comprised of 91 hospitals. These are commonly divided into general hospitals, teaching hospitals and university hospitals. University hospitals (n = 8) are allied with the Dutch universities and function as tertiary referral centres. There are 42 teaching hospitals that offer a surgical residency in collaboration with the university hospitals. General hospitals are often smaller and provide less complex care in high volume or diseases with a high incidence.

However, because not all teaching hospitals provide liver surgery, within this article, we scored type of hospital as university hospital, dedicated liver centre (i.e. teaching hospitals where liver surgery in performed) and general hospital (i.e. teaching or general hospitals not providing liver surgery). In the Netherlands, liver surgery may be performed if a hospital meets specific requirements set by the medical professional associations. These

requirements state that a centre should employ at least two hepatobiliary surgeons, have access to specific interventions (e.g. radiofrequency ablation, endoscopic retrograde cholangiopancreatography) and meet a volume requirement of at least twenty oncologic liver resections annually [22]. The number of surgical procedures presented in this study does not include liver resections performed for metachronous colorectal metastases, non-colorectal metastases or primary liver tumours. Volume of hospital of diagnosis was divided in low (<20 diagnoses), medium (20-35 diagnoses) and high (>35 diagnoses) according to the mean number of patients diagnosed with stage IV CRC annually in the studied time period. Regional differences were analysed using the nine regions of the Netherlands Comprehensive Cancer Organization. These nine regions, covering the whole of the Netherlands and all including rural and urban areas, are also the basis for regional collaboration between hospitals.

DATA ANALYSIS

Patient, tumour and treatment characteristics were presented for the total population. Any trend in the

utilization of liver resection over time was studied using chi-square tests. The number of hospitals performing liver resections was calculated per year. For each type of hospital, the proportion of patients diagnosed with SCLM undergoing liver resection was calculated. To identify factors associated with liver resection, a multi-variable multilevel logistic regression analysis was per- formed, taking into account the hierarchical structure of patients clustered within hospitals of diagnosis. The multivariable analysis included those factors that showed a p-value below 0.1 on univariable analysis. Regions of diagnosis were alphabetically numbered to ensure anonymity. The region with the lowest rate of liver resection was taken as the reference value in multivariable analysis. For all statistical analyses, STATA version 12.0 was used.

RESULTS

PATIENT CHARACTERISTICS

Patient, tumour and treatment characteristics are presented in Table 1. A total of 10,520 patients were diagnosed with SCLM in the Netherlands between 2008 and 2012. Over the years, there was a small increase in the number of diagnosed patients (from 1954 to 2173). The majority of patients were diagnosed in a general hospital (67%, n = 7036). Metastatic spread was limited to the liver in 60% (n = 6263) of the patients. Liver surgery was performed in 12% of patients (n = 1259). Most patients were treated with preoperative and/or postoperative systemic therapy (65%, n = 6869). Of 6263 patients with metastases confined to the liver, 19% underwent liver surgery (n = 1213). Over the years, the proportion of all patients undergoing liver resection increased from 9% to 15% ($p < .001$). Patients with colorectal liver metastases who underwent liver surgery had a 5-year OS of 46%, compared with 4% in patients who did

not undergo liver surgery. For patients with metastases confined to the liver specifically, this difference in 5-year OS was 47% versus 6%, respectively.

CONCENTRATION OF CARE AND REFERRAL PATTERNS

Between 2008 and 2012, there were a total of 91 hospitals in the Netherlands, divided into 9 university hospitals, 16 dedicated liver centres and 66 general hospitals. Table 2 shows that there was some variation in the number of hospitals performing at least one liver resection for synchronous colorectal metastases. This was mainly due to a variation in the number of hospitals performing only one (incidental) resection per year (varying from 3 to 14).
Table 3 shows the referral patterns for patients undergoing liver surgery. Of the 1259 patients who underwent liver surgery, 527 patients (42%) did so at the hospital of primary diagnosis. This number varied between the types of hospital of diagnosis: in university hospitals, only 4% of patients were referred to another centre before undergoing liver surgery; in dedicated liver centres, 16% of patients underwent liver surgery at another hospital than the hospital of diagnosis; and in the general hospitals this number was 88%.

Table 1: Characteristics of patients with synchronous colorectal liver metastases (n=10 520).

	Total	**Liver resection**
	N (%)	N (%)
Year of diagnosis		
2008	1 954 (19)	172 (14)
2009	2 020 (19)	191 (15)
2010	2 178 (21)	269 (21)
2011	2 195 (21)	315 (25)
2012	2 173 (21)	312 (25)
Gender		
Male	6 136 (58)	798 (63)
Female	4 384 (42)	461 (37)
Age		
Mean	68	63
<60	2 347 (22)	398 (32)
60-75	5 316 (51)	714 (57)
75+	2 857 (27)	147 (12)
Hospital of diagnosis		
General hospital	7 036 (67)	720 (60)
Dedicated liver centre	2 869 (27)	402 (32)
University hospital	615 (6)	97 (8)
Location primary		
Colon	7 402 (70)	787 (63)
Rectosigmoid	384 (4)	48 (4)
Rectum	2 734 (26)	424 (34)
Other metastatic locations		
None (liver-only)	6 263 (60)	1 213 (96)
1	3 097 (29)	42 (3)
2	918 (9)	3 (0)
>2	242 (2)	1 (0)
Liver resection *(+/- other treatment)*		
Yes	1 259 (12)	1 259 (100)
No	9 136 (87)	
Unknown	125 (1)	
Radiofrequency ablation *(+/- other treatment)*		
Yes	302 (3)	147 (12)
No	10 218 (97)	1 112 (88)
Perioperative systemic therapy *(+/- other treatment)*		
Chemotherapy	3 705 (35)	654 (52)
Targeted therapy	30 (0)	1 (0)
Combination therapy	3 134 (30)	357 (28)
None	3 651 (35)	247 (20)

Table 2: Number of hospitals performing treatment in patients with SCLM						
	Total	**2008**	**2009**	**2010**	**2011**	**2012**
Hospitals where liver resection was performed	53	30	42	34	37	30
Number of liver resections per hospital per year (%)						
1 resection		4 (13%)	14(33%)	5(15%)	9(24%)	3(10%)
2-10 resections		21(70%)	22(52%)	23(68%)	16(43%)	15(50%)
>10 resections		5(17%)	6(14%)	6(18%)	12(32%)	12(40%)

Table 3: Referral patterns of patients undergoing liver resection (n = 1259) with type of hospital						
Hospital of diagnosis	**Hospital of surgery (n, %)**					
	Not referred	**Referred**				**Total**
		General	**Dedicated**	**University**	**Foreign**	
General	95 (13)	4 (1)	136 (18)	499 (66)	26 (3)	760 (100)
Dedicated	339 (84)	3 (1)	4 (1)	54 (13)	2 (0)	402 (100)
University	93 (96)	0 (0)	3 (3)	0 (0)	1 (1)	97 (100)
Total	527 (42)	7 (1)	143 (11)	553 (44)	29 (2)	1259 (100)

UTILIZATION OF LIVER RESECTION

Fig. 1 shows the proportion of patients undergoing liver resection for each hospital of diagnosis. Overall, 12% of patients with SCLM underwent a liver resection. This proportion varied between hospitals from 2% to 26%. The variation was largest in general hospitals (2-26%), followed by the dedicated liver centres (4-24%) and the university hospitals (10-22%). The proportion was significantly higher in the university hospitals (16%) and the dedicated liver centres (14%), compared with the general hospitals (11%, $p < .001$).

In univariable logistic regression analysis, no association was found between volume of hospital of diagnosis and liver resection ($p = .12$). Type and region of hospital, as well as several other clinicopathological factors were associated with liver resection and were thus included in the multivariable analysis.

As shown in Fig. 2, patients diagnosed in a dedicated liver centre or in a university hospital were significantly more likely to undergo liver resection (odds ratio 1.36 [95% confidence intervals 1.14-1.62] and 1.64 [95% confidence intervals 1.25-2.14], respectively). There was an association between region of diagnosis and liver resection, with patients diagnosed in six out of nine regions (region B, C, F, G, H and I) being more likely to undergo liver resection compared with the reference region with the lowest probability of this treatment (region A).

With regard to chemotherapy, variation was smaller between type of hospital, as well as region. Only 6% of patients treated with chemotherapy were referred to another hospital for treatment. In the university hospital, 64% of patients were treated with chemotherapy, in the dedicated liver centres 65% and in the general hospitals 65%. Between regions, the percentage of patients treated with chemotherapy varied from 60% to 69%.

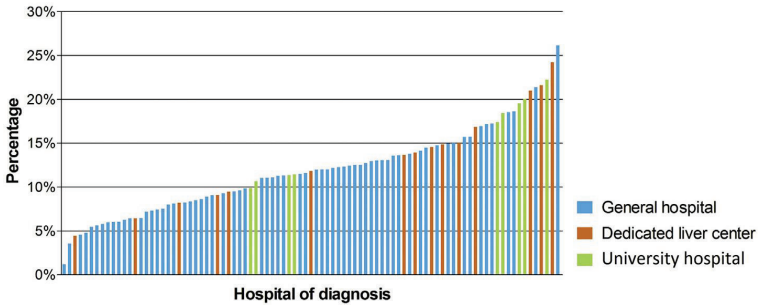


Figure 1. Proportion of patients undergoing liver resection (whether or not after referral to another hospital) per hospital of primary diagnosis.

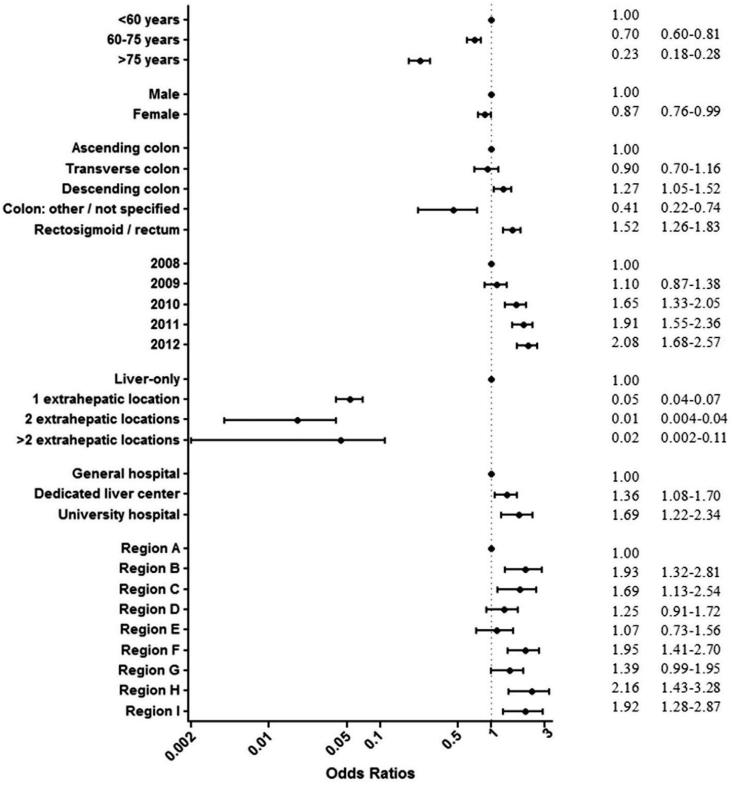


Figure 2. Forest plot of the probability to undergo liver resection, as found in a multilevel multivariable logistic regression analysis.

DISCUSSION

This nationwide population-based study shows that the rate of liver resection in patients with synchronous colorectal liver metastases (SCLMs) varies considerably between hospitals in the Netherlands. Patients diagnosed in general hospitals were less likely to undergo liver surgery, compared with patients diagnosed in a dedicated liver centre or in a university hospital. Besides this inter-hospital variation, an inter-regional variation in the utilization of liver resection was also found, which was sustained after correction for several confounders in a multivariable analysis.

Similar to these results, studies from the United Kingdom and Sweden showed considerable inter- hospital variation in the utilization of liver resection [8, 23]. Although patients included in the study by Morris et al. [8] 7] were diagnosed between 1998 and 2004, our study showed comparable results in more recent years, as did the study by Noren et al. [23]. In the Netherlands, inter-hospital variation was also found in the utilization of curative treatment for oesophageal cancer, lung cancer and gastric cancer [22, 24, 25]. Similar to the treatment of colorectal liver metastases, curative treatment for these cancer types is also concentrated in dedicated centres.

According to The Dutch National Guidelines on Colorectal Cancer, all patients who are diagnosed with colorectal cancer should be discussed by a multidisciplinary team (MDT) [26]. In the general hospitals, this colorectal MDT also identifies patients who should be presented to a tertiary liver MDT at one of the dedicated liver centres or university hospitals. An explanation for the lower resection rate among patients diagnosed in the general hospitals could be that the local MDTs are less proficient in identifying all patients potentially eligible for curative treatment. Previous studies showed that a large number of patients, who were initially diagnosed with unresectable liver metastases, were still considered potentially resectable by a hepatobiliary surgeon [27, 28]. Therefore, an obvious solution to reduce inter-hospital variation could be to involve a hepatobiliary surgeon in the local colorectal MDTs.

As there was also a substantial inter-hospital variation in the hospitals where liver resection was performed with a range of 4-24% in the dedicated liver centres and 10-22% in the university hospitals involving a hepatobiliary surgeon in the local MDTs would not disperse these inter-hospital and inter-regional variations completely. Folprecht et al. [29] showed that even between hepatobiliary specialists considerable inter-individual variation in the decision of resectability existed. This could also account for the inter-hospital and inter-regional variation.

The variation in treatment with chemotherapy was remarkably smaller, with an inter-regional variation of 60-69%. An important difference between chemotherapeutic treatment and liver surgery is that patients were seldom referred to another hospital, as there is no centralization of chemotherapeutic treatment in the Netherlands.

A possible solution to this problem was recently put forth in the design of a new trial investigating treatment strategies in patients with initially unresectable colorectal liver-only

metastases: the CAIRO5 study and the CHARISMA study [30, 31]. To prevent bias caused by inter-individual variation, a nationwide expert panel was appointed, that first reached consensus on the criteria of resectability. Second, a web-based system was developed where physicians can upload diagnostic imaging, which are then assessed by a radiologist on quality, and subsequently evaluated by at least three expert liver surgeons on resectability [31]. Although this expert panel is currently only operating as part of a clinical trial, it shows that establishing a nationwide expert panel is logistically and financially possible. It should therefore be advocated that this panel will be maintained for daily practice as well, as this will probably contribute to more consensus on resectability, as well as a reduction in inter-hospital and interregional variation.

Besides type and region of hospital of diagnosis, other predictors to undergo liver resection included location of the primary tumour, the absence of extra- hepatic disease and the year of diagnosis. The latter shows that the utilization of liver resection is still increasing, implying that the rates of liver resection might increase even further in the nearby future [9]. The association between a more distal location of the primary tumour and an increased probability to receive curative treatment is remarkable and has been previously demonstrated; however, no clear explanation for this phenomenon is available [6] [8]. It might be that patients with distal tumours are more often eligible for resection of the primary tumour, due to the beneficial effect of neoadjuvant therapies; or that new strategies such as the 'liver first' resection are more suitable for rectal cancer patients [32].

Although it is tempting to consider the presented inter-hospital and inter-regional differences as a direct reflection of differences in quality of care, this would not be correct. Several other factors might attribute to these differences as well [33]. First, liver resection rates might vary simply because of chance. A variation in case-mix between the hospitals can also lead to a variation in treatment strategies [33]. To minimize this potential bias, a multivariable analysis including various clinicopathological factors was performed. Several factors, such as comorbidity, number and size of liver metastases, were not available. Because information on these prognostic factors was missing, multivariable analysis to investigate the influence of the inter-hospital and inter-regional variation in treatment utilization on overall survival could not be performed. Another limitation of this study is that no information is available on the accuracy of clinical staging. Recent studies demonstrate that PET- CT imaging does not have an impact on resectability or prognosis of patients with colorectal liver metastases [7, 34]. Hence, despite these possible differences, the variation shown in this study should at least be partially contributed to differences in (quality of) care. This study does not include data on variation in overall survival, and future studies should investigate how inter-hospital and inter-regional differences influence survival.

In conclusion, there is a considerable variation in the utilization of liver resection for patients with SCLM in the Netherlands. As liver resection offers the only potential cure, it is very important that all eligible patients are identified by dedicated specialists. The formation of a national expert panel, including hepatobiliary surgeons, dedicated radiologists as well

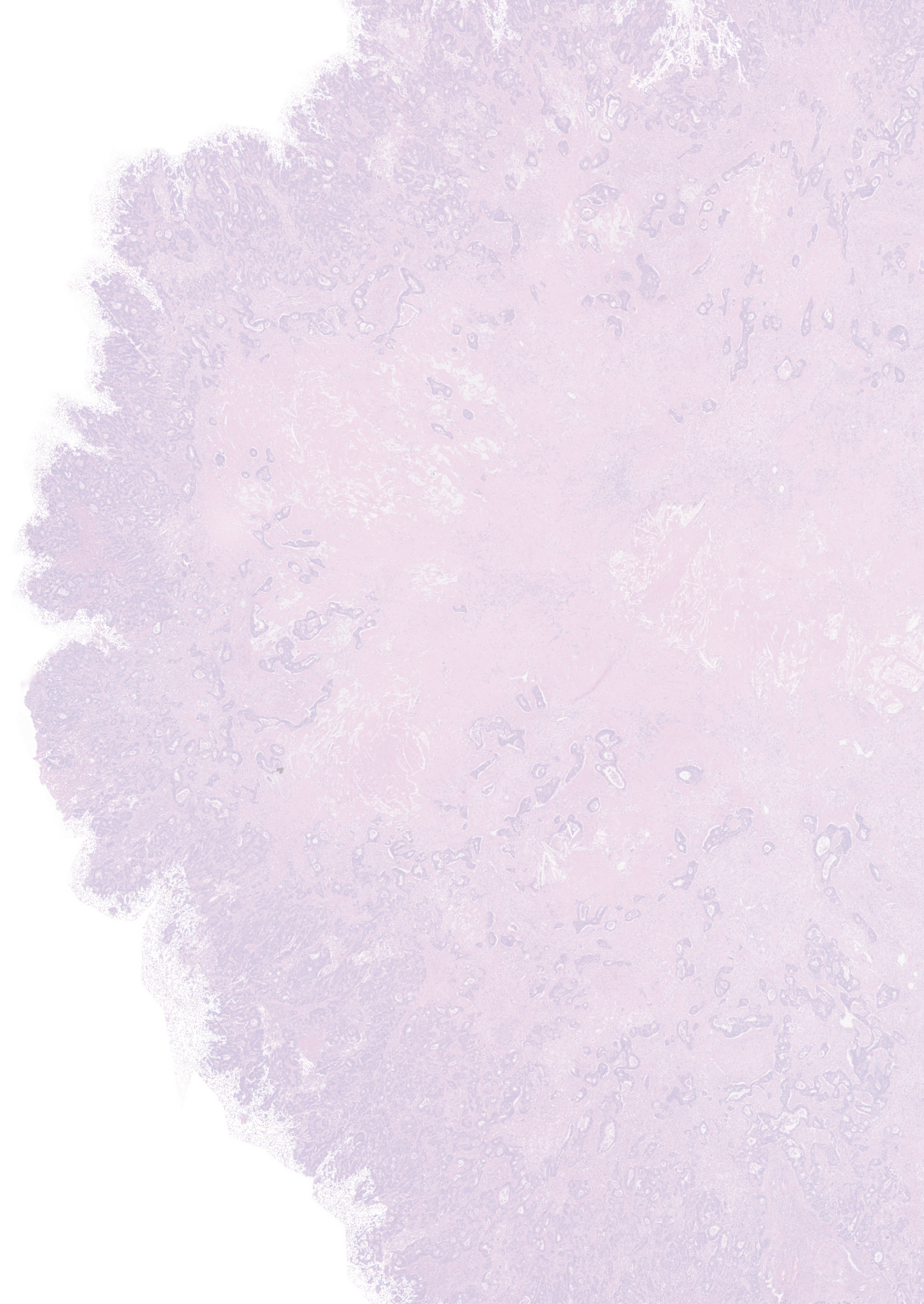
as dedicated medical oncologists, evaluating resectability in all patients with SCLM, will potentially lead to an important improvement in the identification of patients; and might even lead to an improvement in overall survival.

REFERENCES

1. Hugen, N., et al., *Metastatic pattern in colorectal cancer is strongly influenced by histological subtype*. Ann Oncol, 2014. **25**(3): p. 651-7.
2. Elferink, M.A., et al., *Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands*. Int J Colorectal Dis, 2015. **30**(2): p. 205-12.
3. van der Geest, L.G., et al., *Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases*. Clin Exp Metastasis, 2015.
4. van der Pool, A.E., et al., *Trends in treatment for synchronous colorectal liver metastases: differences in outcome before and after 2000*. J Surg Oncol, 2010. **102**(5): p. 413-8.
5. Kanas, G.P., et al., *Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors*. Clin Epidemiol, 2012. **4**: p. 283-301.
6. 't Lam-Boer, J., et al., *Large variation in the utilisation of liver resections in colorectal cancer patients with synchronous liver-only metastasis*. Eur J Surg Oncol, 2015. **In press**.
7. Ayez, N., et al., *Preoperative FDG-PET-scan in patients with resectable colorectal liver metastases does not improve overall survival: a retrospective analyses stratified by clinical risk score*. Dig Surg, 2013. **30**(4-6): p. 451-8.
8. Morris, E.J., et al., *Surgical management and outcomes of colorectal cancer liver metastases*. Br J Surg, 2010. **97**(7): p. 1110-8.
9. de Ridder, J.A., et al., *Liver resection for metastatic disease; a population-based analysis of trends*. Dig Surg, 2015. **In press**.
10. Alberts, S.R. and G.J. Poston, *Treatment advances in liver-limited metastatic colorectal cancer*. Clin Colorectal Cancer, 2011. **10**(4): p. 258-65.
11. Nordlinger, B., et al., *Does chemotherapy prior to liver resection increase the potential for cure in patients with metastatic colorectal cancer?* A report from the European Colorectal Metastases Treatment Group. Eur J Cancer, 2007. **43**(14): p. 2037-45.
12. Koeter, M., et al., *Hospital of diagnosis and probability to receive a curative treatment for oesophageal cancer*. Eur J Surg Oncol, 2014. **40**(10): p. 1338-45.
13. Siemerink, E.J., et al., *Effect of hospital characteristics on outcome of patients with gastric cancer: a population based study in North-East Netherlands*. Eur J Surg Oncol, 2010. **36**(5): p. 449-55.
14. Wouters, M.W., et al., *Variation in treatment and outcome in patients with non-small cell lung cancer by region, hospital type and volume in the Netherlands*. Eur J Surg Oncol, 2010. **36 Suppl 1**: p. S83-92.
15. Bipat, S., et al., *Imaging and treatment of patients with colorectal liver metastases in the Netherlands: a survey*. Neth J Med, 2006. **64**(5): p. 147-51.
16. Lordan, J.T., 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**(3): p. 302-6.
17. MacDermid, E., et al., *Improving patient survival with the colorectal cancer multi-disciplinary team*. Colorectal Dis, 2009. **11**(3): p. 291-5.
18. Tiernan, J., et al., *Evaluation of the introduction of a standardised protocol for the staging and*

follow-up of colorectal cancer on resection rates for liver metastases. Ann R Coll Surg Engl, 2010. **92**(3): p. 225-30.

19. Netherlands Comprehensive Cancer Organisation (IKNL). *Comprehensive Cancer Centre the Netherlands: Dutch Cancer Figures - About the Registration*. 2014, Mar 6.]; Available from: http://www.cijfersoverkanker.nl/about-the-registration-37.html.
20. Sobin LH, W.C. *The TNM Classification of Malignant Tumours - 7th edition*. 2009; Available from: http://www.uicc.org/resources/tnm/about.
21. Fritz, A.G. *International Classification of Diseases for Oncology (ICD-O-3), third edition*. 2000; Available from: http://www.who.int/classifications/icd/adaptations/oncology/en/.
22. Foundation for oncological cooperation (SONCOS). *Multidisciplinary standards for oncological are in the Netherlands*. 2014; Available from: http://www.soncos.org/soncos/download/11Normering_Rapport_SONCOS_Februari_2014.pdf.
23. Noren, A., H.G. Eriksson, and L.I. Olsson, *Selection for surgery and survival of synchronous colorectal liver metastases; a nationwide study*. Eur J Cancer, 2016. **53**: p. 105-14.
24. Bakens, M.J., et al., *Hospital of diagnosis and likelihood of surgical treatment for pancreatic cancer*. Br J Surg, 2015. **102**(13): p. 1670-5.
25. van Putten, M., et al., *Hospital of diagnosis and probability of having surgical treatment for resectable gastric cancer*. Br J Surg, 2016. **103**(3): p. 233-41.
26. National Working Group on Gastrointestinal Tumors. *Colorectal carcinoma - National Guideline*. 2014; 226]. Available from: http://www.oncoline.nl/richtlijn/doc/index.php?type=pda&richtlijn_id=933.
27. Jones, R.P., et al., *Effect of specialist decision-making on treatment strategies for colorectal liver metastases*. Br J Surg, 2012. **99**(9): p. 1263-9.
28. Young, A.L., et al., *Variation in referral practice for patients with colorectal cancer liver metastases*. Br J Surg, 2013. **100**(12): p. 1627-32.
29. 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**(1): p. 38-47.
30. Ayez, N., 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**: p. 180.
31. Huiskens, J., 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**(1): p. 365.
32. Ayez, N., et al., *Long-term results of the "liver first" approach in patients with locally advanced rectal cancer and synchronous liver metastases*. Dis Colon Rectum, 2013. **56**(3): p. 281-7.
33. Birkmeyer, J.D. and J.B. Dimick, *Understanding and reducing variation in surgical mortality*. Annu Rev Med, 2009. **60**: p. 405-15.
34. Moulton, C.A., et al., *Effect of PET before liver resection on surgical management for colorectal adenocarcinoma metastases: a randomized clinical trial*. JAMA, 2014. **311**(18): p. 1863-9.



CHAPTER 12

Posttreatment surveillance in patients with prolonged disease-free survival after resection of colorectal liver metastases

E.P. van der Stok*, B. Galjart*
J. Rothbarth
D.J. Grünhagen
C. Verhoef

* Both authors contributed equally

Annals of Surgical Oncology. 2016 Nov;23(12):3999-4007

ABSTRACT

INTRODUCTION

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

METHODS

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

RESULTS

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

CONCLUSION

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

INTRODUCTION

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

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

PATIENTS AND METHODS

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

FOLLOW-UP OF PATIENTS WITH CRLM

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

RECURRENT DISEASE

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

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

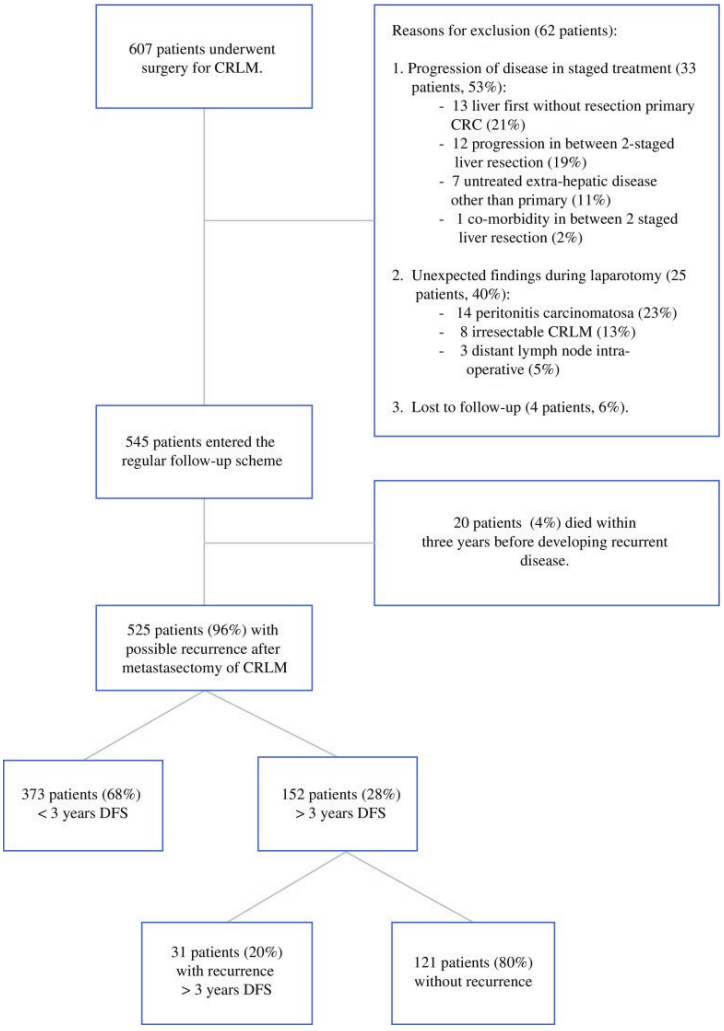


Figure 1. Flowchart of the study

DISEASE-FREE AND OVERALL SURVIVAL

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

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

STATISTICAL ANALYSIS

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

RESULTS

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

Eighty-one patients were disease-free for more than 5 years (15%). Median follow-up time in this group of patients (t0=60 months after first hepatectomy) was 31 months (IQR: 20-52 months). Seven recurrences (9%) and 6 deaths (7%) were observed and the estimated (Kaplan-Meier) probabilities of recurrence and mortality in the following 5 years were 11% and 12% respectively. Conditional OS and DFS curves are presented in figure 2, for both patients with 3 and 5 years of DFS.

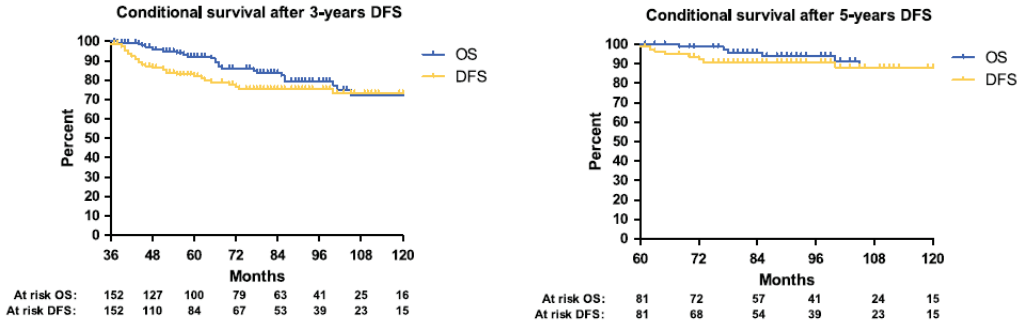


Figure 2. Conditional DFS and OS for patients with 3- and 5 years of DFS

In total 393 patients (72%) had a DFS of less than 3 years. When comparing the recurrence pattern of early (< 3 years DFS) and late recurrences (> 3 years DFS), no significant differences in tumour location were seen (table 1).

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

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

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

Risk categories for late recurrences were created, in which patients with node negative primary tumours and a DFI of less than 12 months (n=50, 33%) were considered at low-risk. All other patients (with either a N+ status, a DFI of more than 12 months or a combination of both characteristics) were considered at high-risk of late recurrence (n=101, 66%). In 1 patient no risk score could be determined. In the low-risk group 2 patients (4%) developed recurrence during the 2 following years of surveillance (after the initial 3 disease-free years),

compared to 22 patients (22%) in the high-risk group. The estimated 10 years recurrence rate in the low risk group was 5% and 25% in the high risk group (p=0,005). The sensitivity of this risk score for prediction of late recurrences during the last 2 years of follow-up is 92%. The estimated difference in recurrence rate between the "high-risk" group and the complete group of patients with 3 years of DFS is 2%. This means that 50 patients with a DFS of 3 years need to remain in follow-up for another 2 years, in order to detect 1 "low-risk" patient with late recurrent disease.

After 5 years of DFS one recurrence (3%) was observed in the low-risk group (n=32), compared to 6 recurrences (12%) in the high-risk group (n=49). The estimated 10 years recurrence rate in the following 5 years (after 5 years of DFS) is 3% in the low-risk group versus 15% in the high-risk group (p=0,207). Kaplan-Meier curves after 3 and 5 years of DFS are presented in figure 3. The created risk model had a moderate capacity to predict late disease recurrence (bootstrap corrected concordance index: 0,707) and acceptable calibration (See Supplementary Material).

Table 1: Recurrence pattern, surveillance and treatment results

	Recurrence < 3 years (N=373)	Recurrence > 3 years (N=31)	P-value
Location recurrence:			
Intrahepatic only	144 (39%)	9 (29%)	0.291
Extrahepatic location recurrences	229 (61%)	22 (71%)	0.904
Pulmonary recurrence	84 (23%)	11 (36%)	
Local recurrence	15 (4%)	1 (3%)	
Distant lymph nodes	21 (6%)	1 (3%)	
Hepatic and pulmonary	35 (9%)	1 (3%)	
Hepatic and other	28 (8%)	4 (13%)	
Pulmonary and other	15 (4%)	2 (7%)	
Multi-organ metastasis (≥3)	10 (3%)	1 (3%)	
Other locations	21 (6%)	1 (3%)	
Surveillance:			
Median CEA (IQR) µg/L	7.0 (2.9-20.0)	7.1 (3.9-12.7)	0.849
Elevated CEA (> 5,0 µg/L)	204 (55%)	22 (71%)	0.087
Non elevated CEA (≤ 5,0 µg/L)	152 (40%)	8 (26%)	
Missing CEA values	17 (5%)	1 (3%)	
Perc. increase (when normal CEA)	152 (40%)	8 (26%)	0.225
>25% compared to baseline	49 (29%)	4 (50%)	
1-25% compared to baseline	25 (15%)	2 (25%)	
Decreased compared to baseline	26 (16%)	2 (25%)	
Not calculated	52 (34%)	0 (0%)	
Treatment:			
Curative	168 (45%)	17 (55%)	0.293
Non-curative	205 (55%)	14 (45%)	

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

Variables:	Total (N=152)	Recurrence >3 years (N=31)	Chi-squared p-value	Univariable [HR (95%CI) p-value]	Multivariable [HR (95%CI) p-value]
Gender:					
Male	94	19 (20.2%)	0.943	0.942 (0.456-1.943)	
Female	58	12 (20.7%)		0.871	
Age:					
Median (range)	64 (30-86)	66 (30-86)	0.326	1.030 (0.994-1.067)	
Mean ± SD	63.3 ± 11.1	65.9 ± 13.2		0.106	
Primary CRC:					
Location:					
Colon	93	19 (20.4%)	0.989	0.978 (0.475-2.015)	
Rectum	59	12 (20.3%)		0.952	
T-stage:					
T3-4	37	3 (8.1%)	0.086	3.250 (0.989-10.682)	
T1-2	114	28 (24.6%)		0.052	
Node status:					
Positive	72	20 (27.8%)	0.035	2.316 (1.109-4.837)	2.279 (1.090 – 4.764)
Negative	79	11 (13.9%)		0.025	0.029
Adjuvant CTx:					
Yes	31	9 (29.0%)	0.181	1.890 (0.868-4.116)	
No	121	22 (18.2%)		0.109	
CRLM:					
DFI <12 months:					
Yes	93	12 (12.9%)	0.004	0.372 (0.180-0.766)	0.471 (0.215 – 1.029)
No	59	19 (32.2%)		0.007	0.059
Number of CRLM:					
1	77	17 (22.1%)	0.602	1.002 (4.94-2.033)	
>1	75	14 (18.7%)		0.996	
Size of tumours:					
≤4,99 cm	124	24 (19.4%)	0.386	1.382 (0.595-3.210)	
≥5,00 cm	26	7 (26.9%)		0.451	
CEA preoperative:					
≤199 µg/L	120	22 (18.3%)	0.305	0.045 (0.00- 46.585)	
≥200 µg/L	8	1 (12.5%)		0.381	
Bilobar metastases:					
Yes	43	9 (20.9%)	0.918	1.218 (0.560-2.647)	
No	109	22 (20.2%)		0.691	
Neoadjuvant CTx:					
Yes	70	8 (11.4%)	0.011	0.411 (0.184-0.920)	0.577 (0.241-1.380)
No	82	23 (28.0%)		0.03	0.216
Margin <1mm:					
Yes	22	4 (18.2%)	0.743	0.985 (0.344-2.815)	
No	127	27 (21.3%)		0.977	
EHD:					
Yes	3	0 (0.0%)	0.376	0.048 (0.00-8158.217)	
No	149	31 (20.8%)		0.621	
Clinical Risk Score:					
LR (1-2)	102	23 (22.5%)	0.550	0.809 (0.347-1.886)	
HR (3-5)	39	7 (17.9%)		0.624	

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

CTx = Chemotherapy; EHD = Extrahepatic disease; CEA = Carcinoembryonic antigen; LR = Low-risk; HR = High-risk

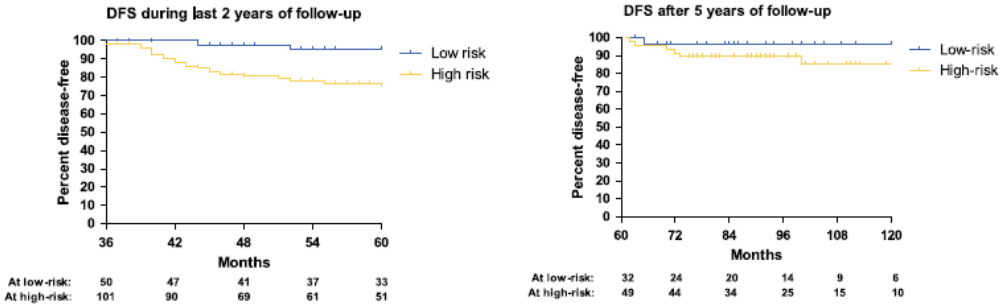


Figure 3. Risk stratification for late recurrences. The graph on the left illustrates the DFS during the last 2 years of follow-up (from 36 to 60 months after hepatectomy). The graph on the right illustrates the DFS after more than 60 months after hepatectomy.

DISCUSSION

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

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

Follow-up in the centre of the current study is carried out during 5 years for all patients after resection of CRLM, as is advised in the ASCRS and NCCN guidelines [23, 24]. Preferably cancer surveillance should only be performed in those patients benefiting from it. In order to decide in which patients follow-up is desirable, accurate prediction of outcome after metastasectomy is needed. Many efforts to determine prognosis of patients with CRLM have been made [4, 17, 25-27], of which the CRS is mostly practised [17]. Less evidence is available to predict the likelihood of late disease recurrence, which is demonstrated by the fact that patients with initially poor prognostic factors can still be cured from CRLM [28]. A study by Tan et al. shows that the currently used risk scores for CRLM have little predictive value in 3-years

survivors of CRLM with regards to the disease-specific survival and are therefore not suitable to decide whether long-term follow-up is appropriate [29]. In the current study the nodal status of the primary CRC showed to be the only significant prognostic factor with respect to developing late disease recurrence. The DFI was non-significant in multivariate analysis, but showed a trend towards significance. The interval between resection of the primary tumour and occurrence of CRLM has been used in most CRS, as a DFI of less than a year increases the chance of developing recurrent disease shortly after hepatectomy [4, 17, 25-27]. The results in this study indicate an opposite effect in patients with 3 years of DFS, as patients with a short interval (<12 months) between the primary CRC and the occurrence of CRLM had a favourable outcome in this particular group of patients. Although counterintuitive, this finding might not be illogical. The included study group is highly selected as patients with early recurrence (< 3 years) have been excluded from the study. A short DFI between resection of primary CRC and development of CRLM is generally considered a sign of aggressive tumour biology, inducing recurrences shortly after partial hepatectomy rather than late [17]. This effect is still found in the current study: a short DFI makes it unlikely that a patient will recur after 3 years, but more likely a patient will recur within 3 years. The protective effect of a short DFI found in patients with 3 years of DFS after partial hepatectomy may well be explained by the selection criteria of this study. Further research in an external cohort of patients is needed to validate the currently obtained results. Nonetheless this study shows that the DFI might still be an important factor in a selected group of patients, when considering long-term surveillance in patients with CRLM.

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

During the past decade several research groups have retrospectively evaluated the different aspects of follow-up after metastectomy, in order to define an optimal surveillance protocol [30-37]. Jones et al. [14] highlight the lack of evidence surrounding surveillance of patients with CRLM after reviewing all available literature on early intensive follow-up after metastasectomy and therefore remain inconclusive on how to perform optimal follow-up. In a review by Metcalfe et al. [38] 5 years of follow-up is proposed. As was shown in this and other studies, patients with a DFS of 5 years still have a probability of approximately 10% to develop recurrences after being discharged from surveillance. Recent literature states that

cure after resection of CRLM might only be achieved after 10 years of survival [28, 39]. This suggests that an extended follow-up protocol of more than 5 years could be worthwhile for some patients, again addressing the need for tailor-made follow-up schedules.

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

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

REFERENCES

1. Bengmark, S. and L. Hafström, *The natural history of primary and secondary malignant tumors of the liver I. The prognosis for patients with hepatic metastases from colonic and rectal carcinoma by laparotomy*. Cancer, 1969. **23**(1): p. 198-202.
2. van der Pool, A.E.M., et al., *Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series*. Colorectal Disease, 2012. **14**(1): p. 56-61.
3. Choti, M.A., et al., *Trends in long-term survival following liver resection for hepatic colorectal metastases*. Ann Surg, 2002. **235**(6): p. 759-66.
4. Rees, M., et al., *Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients*. Ann Surg, 2008. **247**(1): p. 125-35.
5. Butte, J.M., et al., *Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection*. Ann Surg Oncol, 2015. **22**(8): p. 2761-71.
6. de Jong, M.C., 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): p. 440-8.
7. Lam, V.W., et al., *A systematic review of repeat hepatectomy for recurrent colorectal liver metastases*. J Gastrointest Surg, 2013. **17**(7): p. 1312-21.
8. de Jong, M.C., et al., *Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis*. J Gastrointest Surg, 2009. **13**(12): p. 2141-51.
9. Adam, R., et al., *Repeat hepatectomy for colorectal liver metastases*. Ann Surg, 1997. **225**(1): p. 51-60; discussion 60-2.
10. Petrowsky, H., et al., *Second liver resections are safe and effective treatment for recurrent hepatic metastases from colorectal cancer: a bi-institutional analysis*. Ann Surg, 2002. **235**(6): p. 863-71.
11. Suzuki, H., et al., *Long-term outcomes after surgical resection of pulmonary metastases from colorectal cancer*. Ann Thorac Surg, 2015. **99**(2): p. 435-40.
12. Warwick, R. and R. Page, *Resection of pulmonary metastases from colorectal carcinoma*. Eur J Surg Oncol, 2007. **33 Suppl 2**: p. S59-63.
13. Pfannschmidt, J., H. Dienemann, and H. Hoffmann, *Surgical Resection of Pulmonary Metastases From Colorectal Cancer: A Systematic Review of Published Series*. The Annals of Thoracic Surgery. **84**(1): p. 324-338.
14. Jones, R.P., et al., *Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases*. Br J Surg, 2012. **99**(4): p. 477-86.
15. Tree, A.C., et al., *Stereotactic body radiotherapy for oligometastases*. Lancet Oncol, 2013. **14**(1): p. e28-37.
16. Abdalla, E.K., et al., *Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases*. Ann Surg, 2004. **239**(6): p. 818-25; discussion 825-7.
17. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic*

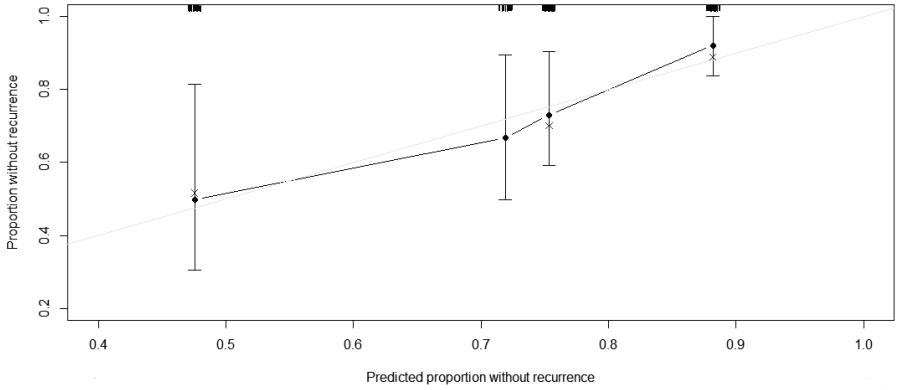
colorectal cancer: analysis of 1001 consecutive cases. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.

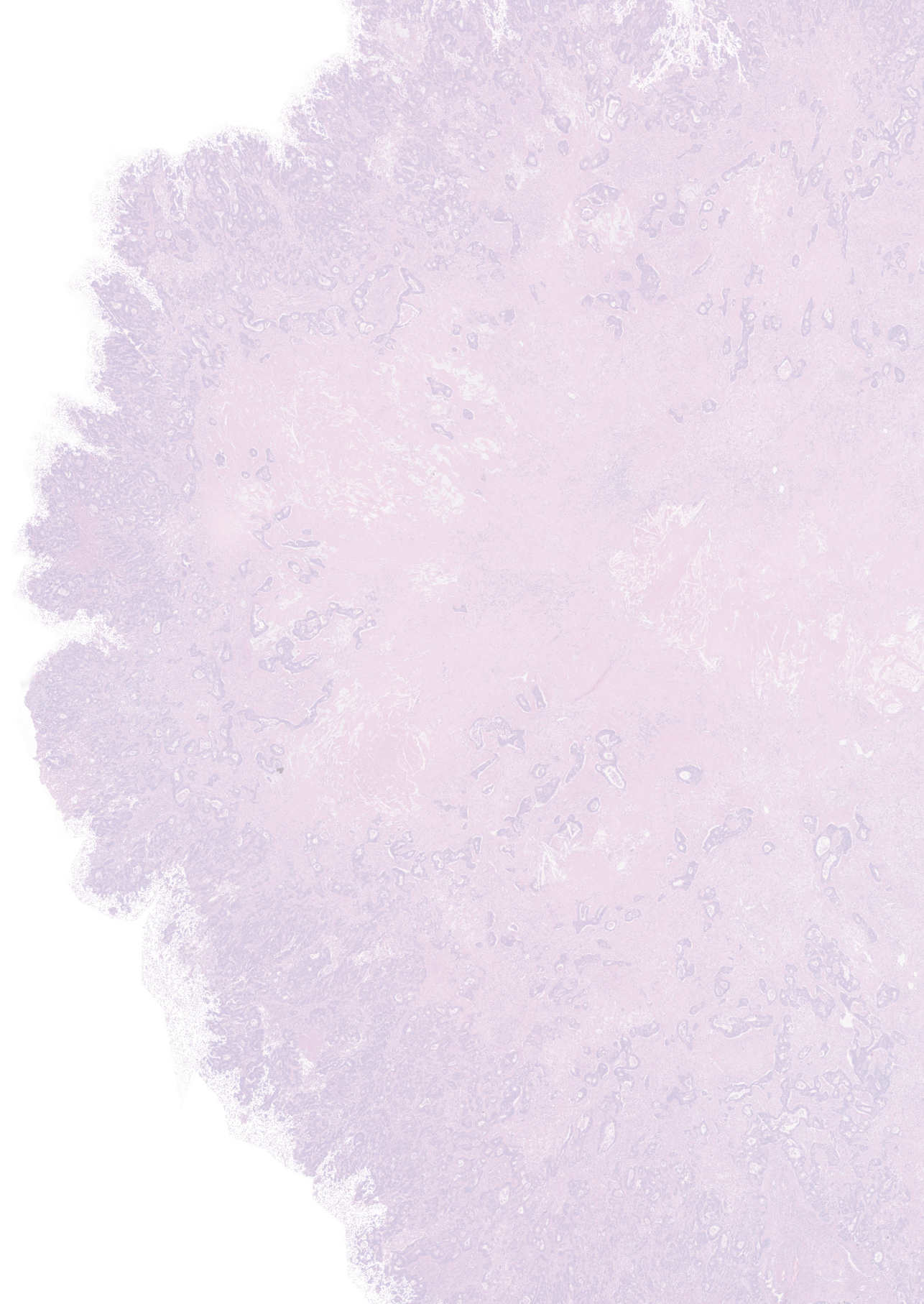
18. Oba, M., et al., *Survival benefit of repeat resection of successive recurrences after the initial hepatic resection for colorectal liver metastases*. Surgery, 2016. **159**(2): p. 632-40.
19. Kulik, U., et al., *Repeat liver resection for colorectal metastases*. Br J Surg, 2013. **100**(7): p. 926-32.
20. Wicherts, D.A., et al., *Repeat hepatectomy for recurrent colorectal metastases*. Br J Surg, 2013. **100**(6): p. 808-18.
21. Rusthoven, K.E., et al., *Multi-institutional phase I/II trial of stereotactic body radiation therapy for liver metastases*. J Clin Oncol, 2009. **27**(10): p. 1572-8.
22. Van Tilborg, A.A., et al., *Long-term results of radiofrequency ablation for unresectable colorectal liver metastases: a potentially curative intervention*. Br J Radiol, 2011. **84**(1002): p. 556-65.
23. Steele, S.R., et al., *Practice Guideline for the Surveillance of Patients After Curative Treatment of Colon and Rectal Cancer*. Dis Colon Rectum, 2015. **58**(8): p. 713-25.
24. Benson, A.B., 3rd, et al., *Colon cancer, version 3.2014*. J Natl Compr Canc Netw, 2014. **12**(7): p. 1028-59.
25. Kattan, M.W., et al., *A Nomogram for Predicting Disease-specific Survival After Hepatic Resection for Metastatic Colorectal Cancer*. Annals of Surgery, 2008. **247**(2): p. 282-287.
26. Iwatsuki, S., et al., *Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system*. J Am Coll Surg, 1999. **189**(3): p. 291-9.
27. Nordlinger, B., et al., *Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie.* Cancer, 1996. **77**(7): p. 1254-62.
28. Tomlinson, J.S., et al., *Actual 10-Year Survival After Resection of Colorectal Liver Metastases Defines Cure*. Journal of Clinical Oncology, 2007. **25**(29): p. 4575-4580.
29. Tan, M.C., et al., *Prognostic significance of early recurrence: a conditional survival analysis in patients with resected colorectal liver metastasis*. HPB (Oxford), 2013. **15**(10): p. 803-13.
30. Bakalakos, E.A., et al., *Is carcino-embryonic antigen useful in the follow-up management of patients with colorectal liver metastases?* Am J Surg, 1999. **177**(1): p. 2-6.
31. Gomez, D., et al., *Outcomes of intensive surveillance after resection of hepatic colorectal metastases.* Br J Surg, 2010. **97**(10): p. 1552-60.
32. Metcalfe, M., et al., *Detecting curable disease following hepatectomy for colorectal metastases*. ANZ J Surg, 2005. **75**(7): p. 524-7.
33. Bhattacharjya, S., R. Aggarwal, and B.R. Davidson, *Intensive follow-up after liver resection for colorectal liver metastases: results of combined serial tumour marker estimations and computed tomography of the chest and abdomen - a prospective study*. Br J Cancer, 2006. **95**(1): p. 21-6.
34. Connor, S., et al., *Follow-up and outcomes for resection of colorectal liver metastases in Edinburgh*. Eur J Surg Oncol, 2007. **33**(1): p. 55-60.
35. Hyder, O., et al., *Post-treatment surveillance of patients with colorectal cancer with surgically treated liver metastases*. Surgery, 2013. **154**(2): p. 256-65.
36. Verberne, C.J., et al., *Detection of recurrences during follow-up after liver surgery for colorectal metastases: both carcinoembryonic antigen (CEA) and imaging are important.* Ann Surg Oncol,

2013. **20**(2): p. 457-63.

37. Langenhoff, B.S., P.F. Krabbe, and T.J. Ruers, *Efficacy of follow-up after surgical treatment of colorectal liver metastases*. Eur J Surg Oncol, 2009. **35**(2): p. 180-6.
38. Metcalfe, M.S., E.J. Mullin, and G.J. Maddern, *Choice of surveillance after hepatectomy for colorectal metastases*. Arch Surg, 2004. **139**(7): p. 749-54.
39. Viganò, L., et al., *Liver Surgery for Colorectal Metastases: Results after 10 Years of Follow-Up. Long-Term Survivors, Late Recurrences, and Prognostic Role of Morbidity*. Annals of Surgical Oncology, 2008. **15**(9): p. 2458-2464.

SUPPLEMENTARY DATA





CHAPTER 13

Surveillance after curative treatment for colorectal cancer

E.P. van der Stok
M.C.W. Spaander
D.J. Grünhagen
C. Verhoef
E.J. Kuipers

Nature Reviews Clinical Oncology. 2017 May;14(5):297-315

ABSTRACT

Treatments for colorectal cancer (CRC) of all stages have evolved considerably over the past two decades, resulting in improved long-term outcomes. After curative treatment, 30% of patients with stage I–III and up to 65% of patients with stage IV CRC develop recurrent disease. Thus, patients are routinely offered surveillance in order to detect disease recurrence at an early, asymptomatic stage, with the intention of improving survival. Nevertheless, controversy continues to surround the optimal surveillance protocols. For patients with stage I–III CRC, more-intensive surveillance improves overall survival compared with less-intensive or no surveillance, probably owing to improved outcomes after cancer recurrence, as well as proactive treatment of other conditions detected opportunistically. The benefit of surveillance after curative treatment of stage IV CRC is more controversial, but might be justified because repeat resection can improve overall survival and 20% of these patients are eligible for such treatment with curative intent. No trials have assessed the optimal follow-up approach after curative resection of metastatic CRC, and similarly to surveillance of patients with stage I–III disease, most programmes are more intensive during the first 3 years than at later time points. Herein, we provide a comprehensive overview of surveillance strategies for patients with CRC, and discuss the future development of patient-centred programmes.

INTRODUCTION

Colorectal cancer (CRC) is a major cause of cancer-related deaths [1]: CRC is the third most commonly diagnosed cancer worldwide, with an estimated 614,000 women (9.2% of all new cancer cases) and 746,000 men (10% of all new cancer cases) affected, and an estimated 693,900 CRC-related deaths in 2012 [2]. Around 50% of patients with CRC present with localized disease (stage I–II), about 25% with locoregional advanced-stage disease (stage III), and the remainder with metastases in distant organs (stage IV) [3-5]. National screening programmes for CRC have been introduced in more than 50 countries, and this number is increasing [6]. Of note, screening increases the proportion of patients with CRCs that are diagnosed at an early disease stage [7], thereby increasing the potential for treatment with curative intent and improving patients outcomes. After treatment with curative intent, patients with CRC enter a surveillance programme that generally lasts for 5 years. Approximately 30% of patients with stage I–III disease develop recurrent disease after initial treatment [8, 9]; among patients with stage IV CRC, up to 65% have relapsed disease after treatment with curative intent [10-16].

The detection of local recurrent disease, metachronous lesions, and distant metastases at an early, asymptomatic, and treatable stage is the goal of surveillance after treatment of CRC with a curative intent. Indeed, early detection of recurrent CRC can increase patient eligibility for a range of effective treatments, reduce morbidity, and most importantly improve overall survival. In addition, treatment for early stage cancer tends to be less extensive than treatment for advanced-stage disease [17]. Modern curative treatments for locoregional and distant metastatic recurrences are increasingly successful, resulting in long-term survival [14, 18-22]. The early discovery of recurrent disease is only useful, however, if the patient's condition — in terms of performance status — allows for repeated therapeutic intervention, and if the patient's preference is to explore any opportunity for repeated treatment. Moreover, investigating whether treatment of asymptomatic recurrent disease ultimately provides an overall survival and/or quality-of-life benefit compared with treatment of patients only after they present with symptomatic disease is important. In addition, the total follow-up duration, the surveillance intervals, and the most-suitable diagnostic modalities must all be assessed in order to optimize the effectiveness of surveillance schemes.

Herein, we review the established evidence on the effectiveness of surveillance programmes used after treatment of patients with CRC with curative intent. We have focused our discussion on surveillance of three specific patient groups: patients with stage I–III CRC who have received conventional curative treatment; those with stage IV CRC who have undergone local treatment with curative intent; and patients who have received curative organ-sparing treatment for CRC.

KEY POINTS

- Pooled analyses of prospective trials have demonstrated an overall survival benefit of intensive postoperative surveillance in patients with stage I–III colorectal cancer (CRC); however, individual studies used highly heterogeneous surveillance schemes
- The overall survival benefit of intensive surveillance is only partly due to improved cancer-specific survival; other contributing factors include the treatment of comorbidities owing to frequent contact with medical professionals
- For patients with stage I–III CRC, no optimal diagnostic tool or frequency of patient visits has been established; regular follow-up assessment by a clinician seem to be the most-important factor
- Colonoscopies are generally performed at 6, 30 and 60 months after curative treatment of patients with stage I–III CRC; performing additional colonoscopies does not improve overall survival
- Limited evidence is available regarding surveillance after endoscopic resection of early neoplasia, and after organ-sparing treatment for rectal cancer; prospective randomized trials are needed
- Similarly, a lack of evidence exists on the effectiveness of surveillance after treatment of patients with stage IV CRC with curative intent, and thus randomized trials are also needed to address this issue

DIAGNOSTIC MODALITIES FOR SURVEILLANCE

The diagnostic modalities used during follow-up assessments of patients are fundamental to the effectiveness of surveillance schemes. Detection of locally recurrent disease requires different diagnostics compared with those used to detect distant metastases: imaging and serum biomarker analyses are important for detection of non-luminal local recurrences and distant metastases, whereas endoscopic investigations enable diagnosis of intraluminal recurrent disease and metachronous (pre)malignant lesions. With every technique, patient burden, test accuracy (TABLE 1), optimal intervals, and cost effectiveness need to be considered in the design of surveillance strategies. Moreover, follow-up programmes should be based on anatomical and transitory patterns of tumour recurrence. In an efficient surveillance scheme, each specific modality is implemented at optimal time points.

INTRALUMINAL RECURRENCES

Intraluminal locally recurrent disease and metachronous CRC occur after surgery in approximately 2–4% and 1.5–3% of patients, respectively [23-30]. In a long-term study of 10,283 Dutch patients with CRC [27], a mean 0.3% annual incidence of intraluminal metachronous cancer was observed, with a cumulative incidence of 1.1% at 3 years, rising to 2% at 6 years and 3.1% at 10 years after initial treatment. This incidence was significantly higher than the incidence of CRC in the age-matched and sex-matched general population (standardized incidence ratio 1.3, 95% CI 1.1–1.5) [27]. This finding is even more remarkable because most of the patients with metachronous lesions only had a hemicolon after previous surgical treatment to remove the primary CRC. The presence of synchronous intraluminal CRC at time of diagnosis was the most important risk factor for development of metachronous intraluminal CRC. Synchronous intraluminal lesions occur in ~4% of patients with CRC [27].

Table 1: Recurrence pattern, surveillance and treatment results

Anatomical location and modality	Test	Sensitivity	Specificity	References
All-site recurrences				
Laboratory tests	CEA	50–90%	80–90%	[58, 66, 77, 80]
	Circulating tumour DNA (methylation status) and/or circulating tumour cells (quantity, mRNA-expression profiles, KRAS and BRAF mutation status), and/or tumour-derived RNA in platelets (RNA-expression profiles)	57–87%	90–96%	[93-100]
Local recurrences				
Laboratory tests	Faecal tumour DNA (methylation analyses, mutation analyses, and immunoassays of various genes)	92%	90%	[7, 91, 93]
	FIT (for human haemoglobin)	74–88% (depending on cutoff used)	93–96%	[7, 92, 214]
	G-FOBT	51–100%	90–97%	[89, 90]
Imaging	CT	70–85%	50–92%	[57-60]
	MRI	66–95%	76–86%	[58, 62, 63]
	Endoscopy (colonoscopy)	95%	100%	[31]
	Endoscopic ultrasonography	67–95%	78–94%	[62, 215]
	18F-FDG-PET/CT	22–98%	93–98%	[66, 69, 70]
	CT-colonography	96–100%	NR	[48-55]
Liver metastasis				
Imaging	Ultrasonography	50–76%	50–60%	[58, 81, 82]
	CT	85%	90–95%	[81, 85]
	MRI	85%	90–95%	[81, 85]
	18F-FDG-PET–CT	97%	97%	[81, 85]
	Urine collagen peptides	85%	92%	[102]
Lung metastasis				
Imaging	CT	68–88%	87–97%	[86-88]
	18F-FDG-PET–CT	61–97%	95–96%	[86]

Test characteristics as reported in studies of test use in screening, staging, and surveillance. 18F-FDG-PET–CT, 2-[18F]fluoro-2-deoxy-D-glucose PET–CT; CEA, carcinoembryonic antigen; CRC, colorectal cancer; FIT, faecal immunochemical testing; G-FOBT, guaiac faecal occult blood test; NR, not reported.

Colonoscopy

Colonoscopy is considered the standard of care for detecting local or metachronous luminal recurrences of CRC, with a sensitivity of approximately 95% [31] (TABLE 1). Most guidelines recommend that colonoscopy is performed before initial surgery, or within 2–4 months of emergency surgery, to detect synchronous lesions, followed by repeat colonoscopy at 1 year to detect metachronous tumours [32-37] (TABLE 2). In a range of studies reporting the yield of surveillance colonoscopy after CRC resection, approximately 65% of metachronous tumours were of stage I or II, of which 56% were asymptomatic, and 87% of patients with metachronous CRC underwent repeat surgery with curative intent [24, 38-45]. These findings are considered sufficient to warrant colonoscopy 1 year after initial resection. The effectiveness of colonoscopy surveillance in increasing overall survival after curative treatment for CRC has been assessed in a trial [46], in which 326 patients with CRC were randomly assigned to receive either intensive or 'routine' colonoscopy surveillance (see Supplementary S1 (table) for details of the test frequencies). During 5 years of follow-up monitoring, recurrent intraluminal CRC was detected in 8% and 11% of patients in the intensive versus routine surveillance groups, respectively (P = 0.32). Among the patients with intraluminal recurrence, more than two-thirds of those in the intensive colonoscopy group could be treated with curative intent versus one-third in the routine surveillance group (P = 0.048; TABLE 3). Indeed, overall survival after CRC recurrence was significantly increased for patients in the intensive surveillance group (P = 0.03); however, this improvement had no statistically significant effect on 5-year overall survival for the intensive colonoscopy group as a whole (77% versus 72% in the routine surveillance group; P >0.05) [46]. These findings illustrate that intensive colonoscopy surveillance can improve outcomes for the subset of patients with locally recurrent and metachronous endoluminal CRC; altough, the effects of this approach on overall survival are limited because endoluminal recurrence only affects a minority of patients. Of note, the current emphasis on quality assurance in colonoscopy procedures and the improvements in endoscopic equipment since this study was conducted have substantially improved the diagnostic and therapeutic effectiveness of colonoscopy [47]. This progress, together with the biological behaviour of sporadic colorectal neoplasia and its anatomic location, probably make the use of colonoscopy surveillance intervals <1 year irrelevant, even during the first 2 years after CRC resection.

CT-colonography

CT-colonography is an alternative to colonoscopy as a surveillance tool for the detection of intraluminal recurrent disease. In a Korean study that included 548 patients with CRC [48], of whom six (1%) developed intraluminal recurrences, the sensitivity of CT-colonography in the detection of intraluminal recurrence approximated to 100% (TABLE 1). Across a range of studies focused on surveillance monitoring of patients after CRC treatment [48-55], the negative predictive value (NPV) of CT-colonography for recurrent adenocarcinoma, advanced-stage neoplasia, and any adenomatous lesions was 100%, 99%, and 97%, respectively. In

patients who are unwilling or unable to undergo colonoscopy, as well as in settings with limited colonoscopy resources, guidelines support the use of CT colonography as the best alternative tool for surveillance of intraluminal disease owing to the sensitivity and high NPV of this modality [48-55]. Notably, in a randomized trial that compared colonoscopy with CT-colonography as screening modalities for the detection of primary CRC [56], participants scored the latter approach as the most burdensome, although this opinion did not affect their patients' willingness to undergo repeat colonographic screening.

LOCOREGIONAL EXTRALUMINAL RECURRENCES

Data on surveillance for local extraluminal disease after treatment of CRC with curative intent are scarce: most reports relate to disease staging prior to treatment. Detection of extraluminal locoregional disease, and differentiation between relapse and postoperative fibrosis can be achieved using CT, MRI, ultrasonography, and PET, with varying levels of accuracy reported in both surveillance and staging studies (TABLE 1). The approaches used to detect locoregional recurrence differ for colon and rectal cancer, as outlined in the following sections.

Table 2: Disease surveillance guidelines for patients with stage I–IV colorectal cancer

Modality	ASCO 2013[33]	ASCRS 2015 [36]	ESMO (I–III: 2013; IV: 2014) [32] [37]		NCCN 2015 [34]		UK 2011 [35]
	II–III	I–IV: colon/rectum*	I–III	IV	I–III	IV	I–IV
History and/or physical exam	Every 3–6 mo. for 5 yrs	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	Every 3–6 mo. for 3 yrs Every 6–12 mo. in yrs 4–5	Every 3–6 mo. for 3 yrs	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	NA
Serum CEA test	Every 3–6 mo. for 5 yrs	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	Every 3–6 mo. for 3 yrs Every 6–12 mo. in yrs 4–5	Every 3–6 mo. for 3 yrs	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	Every 3–6 mo. for 2 yrs Every 6 mo. in yrs 3–5	Every 3–6 mo. for 3 yrs
Chest CT	Annually, or every 6–12 mo. for high-risk pts, for 3 yrs	Annually for 5 yrs	Every 6–12 mo. for 3 yrs	Every 3–6 mo. for 3 yrs	Annually for 5 yrs	Every 3–6 mo. for 2 yrs Every 6–12 mo. in yrs 3–5	Two times in first 3 years
Abdominal CT	Annually, or every 6–12 mo. for high-risk pts, for 3 yrs	Annually for 5 yrs	Every 6–12 mo. for 3 yrs	Every 3–6 mo. for 3 yrs	Annually for 5 yrs	Every 3–6 mo. for 2 yrs Every 6–12 mo. in yrs 3–5	Two times in first 3 yrs
Pelvic CT	Annually or every 6–12 mo. for high-risk pts, for up to 3 yrs, or up to 5 yrs for pts with rectal cancer	Annually for 5 yrs	NA	NA	Annually for 5 yrs	Every 3–6 mo. for 2 yrs Every 6–12 mo. in yrs 3–5	Two times in first 3 yrs
Liver CEUS	NA	NA	Can substitute for abdominal CT	NA	NA	NA	NA
Colonoscopy	At 1 yr; every 5 yrs thereafter	At 1 yr	At 1 yr ; every 3–5 yrs there-after	NA	At 1 and 4 yrs, then every 5 yrs; annually if advanced adenoma is detected	At 1 and 4 yrs, then every 5 yrs; annually if advanced adenoma is detected	At 1 yr; then every 5 yrs
Proctoscopy (+/- ERUS)	NA	Colon cancer: NA Rectal cancer: Every 6–12 mo. for those with anastomosis, or every 6 mo. after local excision, for 3–5 yrs	NA	NA	NA	NA	NA

ASCO, American Society of Clinical Oncology; ASCRS, American Society of Colon and Rectal Cancer Surgeons; CEA, carcinoembryonic antigen; CEUS, contrast-enhanced ultrasonography; ERUS, endorectal ultrasonography; ESMO, European Society for Medical Oncology; mo., months; NA, not applicable; NCCN, National Comprehensive Cancer Network; pts, patients; UK, United Kingdom; yr(s), year(s). *Same recommendations for colon and rectal cancer, unless noted.

CT scans

CT is the standard modality used to detect locoregional extraluminal disease during colon cancer staging. The sensitivity and specificity of CT for the detection of lymph-node metastases is approximately 70% and 78%, respectively, based on a meta-analysis of data from 674 patients [57] (TABLE 1). CT is also the most widely used imaging technique in the

setting of follow-up surveillance for CRC recurrence. In a pooled analysis including 17 studies comprising a total of 1,226 patients, CT had a sensitivity of 85% and a specificity of 92% for detecting locally recurrent CRC when using pathological confirmation as the 'gold standard' diagnostic test [58]. Only limited data are available on the effectiveness of CT in the detection of recurrent rectal cancer specifically: two studies involving a total of 43 patients reported 76% and 82% sensitivity, and a specificity of 50% [59, 60]; however, these studies were performed before the introduction of standardized surgical techniques and neoadjuvant therapies for rectal cancer.

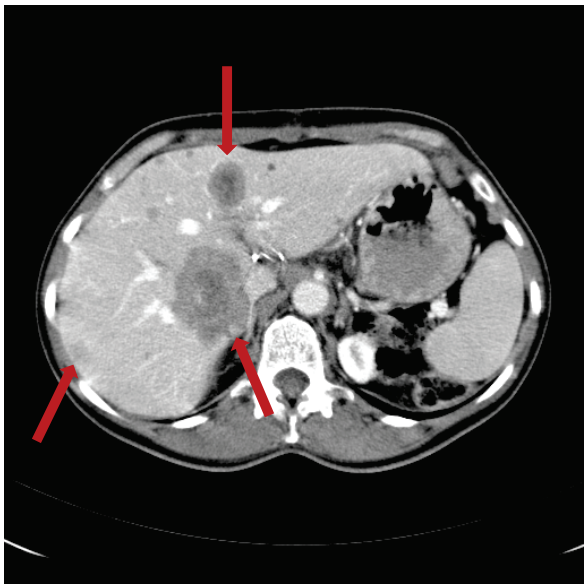


Figure 1. CT imaging surveillance for the diagnosis of colorectal liver metastases. An axial contrast-enhanced CT image of a patient who was previously treated for a primary colorectal cancer with curative intent reveals three liver metastases (arrows).

MRI assessments

MRI is the modality of choice for rectal cancer staging [61]. With respect to lymph-node assessment in the setting of initial staging, a large meta-analysis has been performed to assess the accuracy of MRI (in a total of 1,003 patients), compared with endorectal ultrasonography (in 3,879 patients), and CT (in 1,123 patients). All three modalities had a moderate sensitivity of 66%, 67%, and 55%, respectively, which no statistically significant difference observed between the techniques in this regard[62]. Specificities were also similar: 76% for MRI, 78% for endorectal ultrasonography, and 74% for CT[62]. MRI is not used routinely for surveillance after colorectal cancer resection; however, the authors of one study[58] reported that the sensitivity of MRI for the detection of local recurrence is approximately 95%, with a specificity

of 82%. Routine surveillance of rectal or left-sided colon cancer using MRI has also been assessed in a study involving 226 patients [63]. Local recurrence was identified in 30 patients (13%) using colonoscopy, with 26 recurrences localized to perirectal tissue and the other four occurring at the anastomosis [63]. MRI enabled the diagnosis of 26 of these recurrences (87%); of note, three out of four anastomotic recurrences were not detected using MRI[63]. Only two patients had local recurrences that were deemed resectable [63]; therefore, routine MRI offered a potential survival advantage (via curative treatment of relapsed disease) for <1% of the patients included in this study. These findings raise important questions regarding the utility of routine MRI for rectal cancer surveillance, and support the notion that MRI should be reserved for staging of recurrent CRC after diagnosis by other means. MRI does have a role in intraluminal evaluation of complete clinical response after organ-sparing treatment for rectal cancer [64, 65], and will be discussed in this context later in this Review.

PET analysis

The use of 2-[18F]fluoro-2-deoxy-D-glucose (18F-FDG)-PET–CT has been assessed for the detection of locoregional spread of CRC in several reports, with a sensitivity of 22–84% for nodal metastases [66-68](TABLE 1); the accuracy for detection of positive nodes in close vicinity to the primary tumour is lower owing to the limited spatial resolution of PET–CT [66]. 18F-FDG-PET-CT imaging is valuable, however, for the differentiation of post-treatment changes in nonmalignant tissue from a recurrent tumour. Indeed, in this context, PET with 18F FDG can have advantages over CT and MRI in terms of sensitivity and specificity for detection of tumour recurrence. A meta-analysis of data from 336 patients indicated a 94% sensitivity with a 98% specificity for diagnosis of local and/or pelvic recurrences using 18F FDG-PET, when compared with the gold standard of histopathological confirmation [69] (TABLE 1). The performance of combined PET and CT (PET–CT) was addressed in a retrospective study involving 62 patients [70], in which pelvic sites were rated separately using PET and PET–CT. PET alone had a sensitivity of 82% with a specificity of 65% for the detection of malignant lesions, whereas PET–CT enabled greater accuracy with a sensitivity of 98% and a specificity of 96% [70]. This study emphasised the importance of careful evaluation of the data obtained using PET–CT. The use of CT alone resulted in the identification of 30 pelvic abnormalities, of which seven were recurrent tumours and 23 were benign lesions; PET–CT enabled the differentiation of these lesions with a sensitivity of 100% and a specificity of 96% [70]. The use of 18F-FDG-PET specifically in the follow-up surveillance of patients after curative treatment for stage III–IV CRC has been assessed in one prospective randomized trial [71]. The addition of two 18F-FDG-PET scans to a conventional surveillance scheme (used alone in the 'control' group) at 9 and 15 months after surgery was associated with a substantial increase in the number of recurrences that could be treated with curative intent (10 versus two) [71]. The study was not powered to detect survival differences, and only examined the surveillance period between 9 and 24 months after treatment of CRC with a curative intent. The value of PET-CT surveillance has also been evaluated a retrospective study that included a highly selected group of 88 patients with CRC who had been under surveillance for at least 5 years after treatment with

curative intent [72]. These patients had an elevated CEA and subsequent nonconclusive findings upon subsequent conventional imaging (with CT, ultrasonography, and/or colonoscopy). In this group, PET–CT enabled the diagnosis of recurrent disease with a sensitivity of 88% and a specificity of 88% [72]. These data suggest that PET–CT as 'a last resort' surveillance modality has a low specificity for detecting recurrent disease, raising the question of whether it should be used routinely in this setting.

PET-based surveillance has some inherent limitations. First, diagnosis of relapse is dependent on tumour size and 18F-FDG uptake [73]. Second, the specificity of PET is reduced in the first 12 months after radiotherapy, owing to the presence of radiation-induced acute inflammatory and proliferative changes to tissues [74]. Finally, the sensitivity of 18F-FDG-PET is limited in patients receiving chemotherapy, which can reduce the metabolic activity of tumour cells [75]. In general, PET is not routinely used in surveillance after treatment for CRC with curative intent (TABLE 2).

Table 3: Randomized trials comparing different postoperative surveillance strategies in patients with stage I–III CRC

Study (year of publication)	Number of pts (recruitment period)	Stage of CRC	Surveillance strategy	Recurrences in intensive vs less-intensive surveillance arms			Mean ± SD or median time to recurrence (months)	OS rate* or HR (intensive vs less-intensive approach)
				Total (% of pts)	Treatable (% of pts)	% of total that were treatable		
Treasure *et al.* [122] (2014)	216 (1982–1993)	Dukes A,B,C	Second-look surgery vs no second-look surgery in the case of elevated CEA	83 (77%) vs 89 (82%)	62 (57%) vs 26 (24%)	75% vs 29%	NR	Death rate up to 1993: 84% vs 81%; NS
Ohlsson *et al.* [116] (1995)	107 (1983–1986)	Dukes A,B,C	Intensive vs minimal surveillance	17 (32%) vs 18 (33%)	5 (9%) vs 3 (6%)	29% vs 17%	20 vs 24	75% vs 67%; NS
Kjeldsen *et al.* [43] (1997)	597 (1983–1994)	Dukes A,B,C	Intensive vs less-intensive surveillance	81 (28%) vs 83 (27%)	17 (6%) vs 5 (2%)	21% vs 6%	18 vs 27	70% vs 68%; NS
Schoemaker *et al.* [44] (1998)	325 (1984–1990)	Dukes A,B,C	Intensive imaging vs less-intensive imaging in surveillance	56 (34%) vs 64 (41%)	6 (4%) vs 5 (3%)	11% vs 8%	NR	78% vs 72%‡; NS
Pietra *et al.* [117] (1998)	207 (1987–1990)	Dukes B,C	Intensive vs less-intensive surveillance	41 (39%) vs 42 (41%)	21 (20%) vs 6 (6%)	51% vs 14%	10.3 ± 2.7 vs 20.2 ± 6.1	73% vs 58%; P = 0.02
Mäkela *et al.* [115] (1995)	106 (1988–1990)	Dukes A,B,C	Intensive imaging vs less-intensive imaging in surveillance	22 (42%) vs 21 (39%)	5 (10%) vs 3 (6%)	22% vs 14%	10 ± 5 vs 15 ± 10	59% vs 54%; NS
Secco *et al.* [121] (2002)	358 (1988–1996)	Low or high risk§	Risk-adapted: intensive for high-risk group, or low-intensity for low-risk group vs minimal follow-up testing	Low risk: 27 (32%) vs 25 (40%) High risk: 74 (69%) vs 58 (69%)	Low risk: 6 (7%) vs 6 (10%) High risk: 25 (23%) vs 7 (8%)	Low risk: 22% vs 24% High risk: 34% vs 12%	Low risk: 16 vs 14 High risk: 13.5 vs 8	Low risk: 80% vs 50%; P <0.001 High risk: 60% vs 32%; P <0.01
Wang *et al.* [46] (2009)	326 (1995–2001)	Dukes A,B,C	Intensive vs 'routine' colonoscopy surveillance	13 (8%) vs 18 (11%)	9 (5%) vs 6 (4%)	69% vs 33%	22 ± 17.6 vs 35 ± 23.9	77% vs 73%; NS
Rodriguez-Moranta *et al.* [119] (2006)	259 (1997–2001)	AJCC II–III	Intensive imaging vs less-intensive imaging in surveillance	35 (27%) vs 34 (26%)	18 (14%) vs 10 (8%)	51% vs 29%	39 ± 21 vs 38 ± 19	HR 0.87; NS Pts with AJCC stage II CRC: HR 0.34; P = 0.045 Pts with rectal cancer: HR 0.09; P = 0.03
Wattchow *et al.* [123] (2006)	203 (1998–2001)	Dukes A,B,C; no rectal cancer	Intramural (surgeon) vs extramural (general practitioner) surveillance	NR	NR	NR	8 vs 9.5	Median: 31 months vs 20 months; NS
GILDA‖: Grossmann et al. [114] (2004)	985 (1998–2004)	Dukes B2,C	Intensive vs less-intensive surveillance	75 (15%) vs 64 (13%)	NR	NR	NR	Death rate up to 2004: 7% vs 5% ; P = NR
GILDA: Rosati *et al.* [120] (2016)	1,228 (1998–2006)	Dukes B2,C	Intensive vs less-intensive surveillance	135 (22%) vs 115 (19%)	57 (9%) vs 46 (8%)	42% vs 40%	NR	HR 1.14; NS
Sobhani *et al.* [71] (2008)	130 (2001–2004)	AJCC III–IV	Surveillance with addition of 18F FDG-PET vs surveillance without 18F FDG-PET	23 (35%) vs 21 (32%)	10 (15%) vs 2 (3%)	43% vs 10%	12 ± 4.9 vs 15.3 ± 4.9	NR
Primrose *et al.* [118] (2014)	1,202 (2003–2009)	AJCC I–III	1: CEA only 2: CT only 3: CEA and CT 4: Minimal follow-up testing	1: 57 (19%) 2: 57 (19%) 3: 48 (16%) 4: 37 (12%)	1: 20 (7%) 2: 24 (8%) 3: 20 (7%) 4: 7 (2%)	1. 35% 2. 42% 3. 42% 4. 19%	1: 45 ± 18 2: 44 ± 19 3: 45 ± 19 4: 44 ± 20	Death rate at mean of 4.4 years (SD 0.8) 18.2% (arms 1–3 combined) vs 15.9% (arm 4); NS
Augestad *et al.* [113] (2013)	110 (2007–2011)	Dukes A,B,C	Intramural (surgeon) vs extramural (general practitioner) surveillance	8 (15%) vs 6 (11%)	4 (7%) vs 3 (5%)	50% vs 50%	NR	Death rate 7% vs 2% at 2 years

See Supplementary data S1 (table) for an unabridged of this table that show details of the surveillance schemes used in each arm of each trial, as well as the primary end points of the trials (for which they were powered). 18F-FDG-PET, 2-[18F]fluoro-2-deoxy-D-glucose PET; AJCC: American Joint Committee on Cancer; CEA, carcinoembryonic antigen; CRC, colorectal cancer; HR, hazard ratio; NR, not reported; NS, not statistically significant; OS, overall survival; pts, patients; SD, standard deviation. *5-year OS unless otherwise stated. ‡Estimated visually from survival curve. §High-risk patients had CEA >7.5 ng/ml; Dukes B3 or T3 left colon cancer; Dukes C disease; low anterior resection for rectal cancer; or a poorly differentiated, mucinous, or signet-ring histology. Low-risk patients had any other form of disease. ‖Interim analysis.

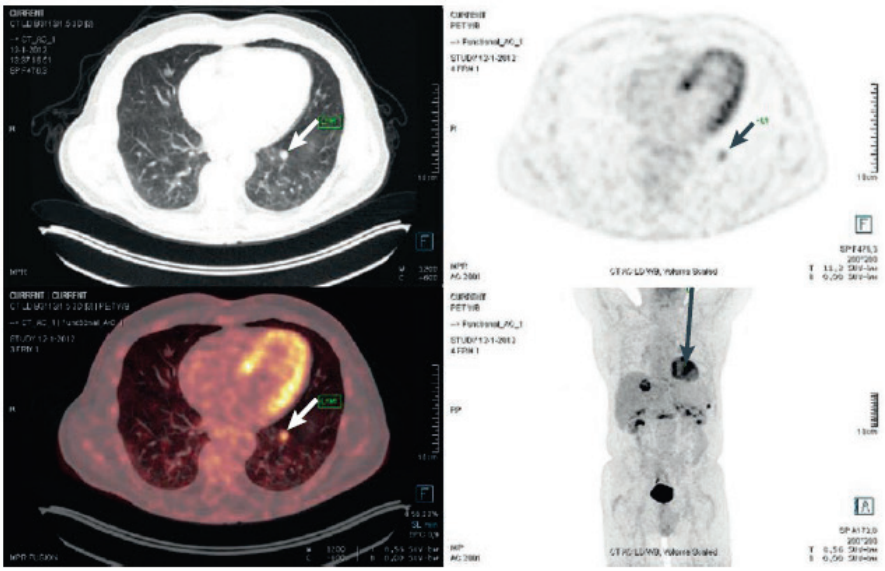


Figure 2. PET–CT imaging surveillance for the diagnosis of pulmonary metastases. The upper-left panel shows an axial CT image of a patient who was previously treated for a primary colorectal cancer with curative intent. A lesion that was later confirmed as a pulmonary metastasis of colorectal origin is evident in the inferior lobe of the left lung (arrow). The upper-right and lower-right panels are 2 [18F] fluoro 2 deoxy-d-glucose (18F FDG) PET images from axial and coronal planes, respectively, with the same hypermetabolic lesion indicated (arrows). The lower-left panel shows a corresponding 18F FDG PET–CT fusion image demonstrating tracer accumulation and thus elevated metabolic activity in the pulmonary metastasis.

DISTANT RECURRENCES

Approximately 25% of patients with CRC have metastatic disease at presentation and another 20% will subsequently develop metachronous metastases [3-5, 76]. Most distant metastases occur within the liver (FIG. 1), which is affected in 65% of patients with metachronous metastases [76]. The lungs are the second most-common site of metachronous metastasis (FIG. 2), and are affected in 43% of patients; in 12% of patients, liver and lung metastases are present simultaneously [76]. Biomarkers, such as CEA, can be indicative of distant, systemic disease; however, discovery of distant lesions requires a range of imaging techniques.

Serum CEA testing

Serum CEA is the most widely used biomarker for the diagnosis of disseminated CRC. The sensitivity of CEA for identifying patients with distant metastases of CRC depends on the

cutoff level used, and ranges from 50–70%, with a specificity of 80–90% [58] . One meta-analysis that included 4,285 patients with CRC established an optimal serum CEA cutoff of 2.2 ng/ml, which had a sensitivity of 64% and a specificity of 90% for detection of CRC metastases [77].

The diagnostic accuracy of CEA testing further depends on the algorithm used for interpretation of the findings [77-80]. After resection of colorectal liver metastases (CRLM), assessment of the extent of the increase in serum CEA levels (in particular, a slope ≥25%) rather than the absolute values can help to identify patients with recurrent disease, with a sensitivity of 69% [79]. Bimonthly surveillance with CEA slope analyses (and further investigation using imaging if serum CEA levels increase by ≥20%) after resection of CRLM resulted in increased detection of recurrences that could be treated with a curative intent, compared with standard surveillance with less frequent CEA testing according to Dutch guidelines (42% versus 30%; P = 0.0048) [80]; however, the increased opportunities for curative treatment of recurrent disease did not translate into an overall survival benefit. The latter study used a 'stepped wedge' randomization method, whereby most patients were at least partly covered by both the conventional and investigational surveillance schemes, thus introducing a potentially important confounding variable.

Of note, surveillance for disease recurrence involving referral for targeted imaging based on CEA levels is less expensive than the use of intensive imaging without triage. In one study [79], the costs per detected recurrence using either CEA-directed or imaging-based surveillance schemes were estimated at €2,196 and €6,721, respectively.

Imaging-based surveillance

Sensitivity for the detection of distant metastases from CRC differs between imaging techniques. Ultrasonography of the liver is inexpensive, non-burdensome and does not expose patients to radiation; however, this modality is not suitable for the detection of extrahepatic metastases, and has a lower sensitivity (approximately 60%) for the detection of CRLM than CT or MRI [58, 81, 82] (TABLE 1). The use of contrast-enhanced ultrasonography (CEUS) has improved the detection and characterization of liver lesions, compared with non-contrast-enhanced approaches [83]. Even with the use of contrast, limitations of ultrasonography remain, including: operator-dependency, low sensitivity for small lesions (<5 mm in diameter), difficulty in the evaluation of the subdiaphragmatic liver (especially segments VII and VIII), visual impairment by interposition of the intestine, and signal interference caused by steatosis and fibrosis. These various limitations of ultrasonography can increase the possibility of missing lesions [84]. Both CT and MRI overcome the mentioned drawbacks of ultrasonography, and both are associated with a sensitivity for CRLM of approximately 85% and a specificity of 90–95% [85] (TABLE 1). In addition, 18F-FDG-PET–CT enables detection of CRLM with a sensitivity of 97% and a specificity of 97% [81, 85].

The diagnostic value of CT scanning for the detection of extrahepatic abdominal metastases has not been systematically investigated. In a systematic review in which CT and 18F-FDG-

PET–CT surveillance were compared in 178 patients, CT had a sensitivity of 64–88% with a specificity of 87–97% for the detection of pulmonary metastases [86]. Thus, the routine use of thoracic CT scans in patients with negative chest radiographs seems be of limited diagnostic value [87, 88]. In a retrospective analysis of the outcomes of 100 patients referred for resection of CRLM [88], all of whom had no signs of pulmonary metastasis on chest radiographs, additional assessments using chest CT revealed pulmonary lesions in 11 patients (11%); three patients had histologically confirmed pulmonary metastases, one patient had primary lung cancer, and the seven other patients had lesions were not malignant. Thus, in addition to negative findings of chest radiographs, the yield of a chest CT scan in the detection of malignant lesions was 4%, with a positive predictive value of 36% [88]. 18F-FDG-PET–CT has a sensitivity of 61–97% and a specificity of 95–96% for pulmonary metastases from CRC[88] (TABLE 1; FIG. 2); thus the specificity of 18F-FDG-PET–CT is comparable to that of CT.

NOVEL DIAGNOSTIC TOOLS

The field of screening and surveillance for CRC is evolving rapidly owing to the introduction of new tests. The novel approaches differ in character and scope, and comprise biomarker or imaging tests focused on the detection of intraluminal or systemic disease. Biomarker tests can be based on assessment of stools, blood, urine, or, potentially, breath.

Faecal immunochemical testing (FIT)

Faecal immunochemical testing (FIT) is aimed at the detection of faecal occult blood using antibodies specific to human globin, and is more sensitive than the traditional guaiac faecal occult blood tests (G-FOBT) [7, 89, 90] (TABLE 1). FIT enables the selective detection of the human globin-protein in stool, making it rather specific to colonic blood loss, as globin from blood lost proximal to the colon will mostly be degraded before entering the colon. FIT and G-FOBT are used primarily as a population screening tool for CRC, and the evidence supporting the use these methodologies to detect disease recurrence is less strong or non-existant. In 1,244 patients with previous CRC undergoing surveillance colonoscopies, G-FOBT enabled the identification of only three of nine patients with local recurrence (33%) and two of 13 patients with metachronous intraluminal disease (15%) [91].

Faecal DNA testing

Quantification of tumour DNA in stool samples can enable the detection of intraluminal disease. In the setting of primary screening for CRC, stool DNA testing for changes in multiple genes, including quantitative molecular assays for KRAS mutations, NDRG4 and MBP3 methylation, and ACTB (encoding β-actin), in combination with FIT has been compared with the use of FIT alone in 9,989 patients [92]; the combined stool DNA-testing plus FIT approach had a sensitivity of 92.3% and a specificity of 89.8% versus 73.8% and 96.4% for FIT alone (P

= 0.002) [92] (TABLE 1). These data support the conclusion that the inclusion of stool DNA testing improves on the accuracy of FIT alone, although the difference between both arms would have been reduced substantially if the investigators had achieved a similar rate of referral to colonoscopy in both arms by simply applying a lower FIT cutoff.

Analysis of circulating tumour DNA and circulating tumour cells.
umour material can be shed from invasive CRCs into the circulation; therefore, interest in the use of blood-based tests — termed 'liquid biopsies' — to tailor treatment and evaluate therapy response is increasing. Such tests might also be helpful tools in surveillance for disease recurrence. Detection of mutations involving genes such as KRAS, as well as methylation markers, in circulating cell-free tumour DNA (ctDNA) can indicate the presence of metastatic disease with a sensitivity of 57–87% and a specificity of 90–96% in a primary screening setting [93-95] (TABLE 1). Other promising alternative liquid biopsy approaches, such as analysis of circulating tumour cells (and their gene-expression profiles) [96-98] and tumour RNA in thrombocytes [99, 100], and other serum biomarkers need to be further investigated in terms of their effectiveness as surveillance tools after treatment for CRC with curative intent. Novel biomarkers might be superior in terms of the prediction and detection of malignant spread at an earlier stage — that is, before symptoms occur, or long before disease recurrence can be detected using imaging modalities.

Urine peptides
The urinary proteome might also provide opportunities for the early detection of cancer [101]. Similar to stool and blood sampling, urine collection is noninvasive and can be performed in primary care. At present, data on this approach is limited, but urine collagen peptides have been used to diagnose CRC metastases [102].

PET–MRI
Lymph nodes with and without tumour metastases vary in size. In patients with lung cancer, up to 21% of lymph nodes <1 cm harbour metastases, while ~40% of those >1 cm do not [103, 104]. These findings are relevant to the assessment of lymph nodes with any imaging technique and for any cancer type. The combination of PET data with CT findings can result in superior accuracy of nodal staging compared with the use of MRI scans [105]. Similarly, the additional use of PET might improve the diagnostic accuracy of MRI. Indeed, no major differences in the accuracy of lymph-node staging would be expected when comparing PET–CT and PET–MRI [106], as differentiation of malignant and benign lesions is mainly based on functional information obtained using PET. With regard to distant metastases, studies comparing the effectiveness of 18F-FDG-PET–CT with that of whole-body MRI for the detection of pulmonary lesions demonstrated that the CT-based approach is more accurate [105, 107-109]; however, this advantage was attributable to the increased sensitivity of CT imaging for small pulmonary lesions (typically ≤3 mm) that are not detectable with MRI.

Thus, no additional benefit can be expected when adding PET to MRI in this setting [106]. Likewise, addition of functional PET imaging to classical morphological imaging is unlikely to increase the accuracy of detection of intraluminal lesions [106].

SURVEILLANCE INTERVALS

In patients with stage I–III CRC, approximately 60% of disease recurrences occur within the first 2 years, 80% within the first 3 years, and >90% within the first 5 years after resection of the primary tumour [110]. After 5 years, the recurrence rates are <1.5% per year, and after 10 years, are <0.5% per year [110]. For patients with stage IV CRC who are treated with a curative intent, 80% of those with relapse develop recurrent disease within the first 2 years after metastasectomy [10, 13, 111]. Thus, as the majority of disease recurrences are within the first 3 years after treatment with curative intent, most surveillance programmes involve frequent, 3–6 monthly follow-up assessments during this period, with a decreased frequency of testing between years 3–5, and no further specified surveillance thereafter. These intervals are not evidence-based, however, and no level 1 evidence exists regarding the optimal total duration of surveillance after treatment for CRC. In a study that included 207 patients who had undergone resection of CRC [112], 121 patients were alive and disease free at 5 years. 81 of these 121 patients (67%) had received a 5-year surveillance CT scan, and none of these scans had revealed recurrent CRC, although other malignancies were detected in six of the 81 patients (7%) [112]. Notably, although fewer asymptomatic recurrences will be detected per additional year of surveillance beyond the traditional 5 years, a high proportion of these late recurrences are likely to have a relatively mild tumour biology and might, therefore, be curable.

SURVEILLANCE FOR STAGE I–III CRC

After conventional curative treatment

As can be distilled from the various international guidelines for surveillance after treatment of stage I–III CRC with curative intent (TABLE 2), no clear consensus has been reached regarding the frequency and modalities for intraluminal, extraluminal, and distant-organ evaluation. Over the past three decades, 14 randomized clinical trials examining different surveillance programmes after curative surgery for stage I–III CRC have been conducted [43, 44, 46, 113-123] (TABLE 3 and Supplementary S1 (table), and one trial is ongoing [124]. The effectiveness of the various surveillance schemes was evaluated based primarily on 5-year overall survival rates. Intensive schemes applying frequent visits and/or the use of multiple diagnostic modalities were compared with less-intensive schemes or to a nihilistic approach of watchful waiting with diagnostic and therapeutic interventions based purely on the presence of symptoms.

The FACS trial [118], published in 2014, evaluated the potential benefit of scheduled serum CEA measurement and/or CT of the chest and abdomen in the detection of recurrent disease,

compared with minimal surveillance (TABLE 3 and Supplementary S1 (table)). The original primary end point, overall survival, was abandoned owing to accrual issues, and was replaced with the detection rate of recurrent disease that could be treated with curative intent (from here on termed 'treatable recurrence'). This rate was significantly higher in the CEA and/or CT groups compared with the minimal surveillance group, with odds ratios of 3.0 (95% confidence interval (CI) 1.2–7.3) for CEA only and 3.6 (95% CI 1.5–8.7) for CT only. The odds ratio for combined CEA and CT surveillance was similar to those reported for CEA or CT alone. No significant difference in overall survival between the minimal surveillance group and the combined more-intensive surveillance groups was reported (81.8% versus 84.1% after a mean follow-up duration of 4.4 years). Mature results of the FACS trial were recently presented at the ESMO Congress 2016 [125]. At a median follow up duration of 8.7 years, use of the intensive surveillance strategies resulted in identification of recurrences that were amenable to treatment in a higher proportion of patients (7.5% versus 2.7%, P = 0.003); however, this apparent benefit in terms of earlier diagnosis had no effect on overall survival (P = 0.45) [125]. Subgroup analysis according to primary tumour location showed that the detection rate of treatable recurrences did not differ significantly between the various surveillance protocols among the patients with rectal cancer (9.8% versus 6.9%, P = 0.41) [125]. By contrast, in those with colon cancer, more patients had treatable recurrences detected with more-intensive than with minimal surveillance (left-sided tumours: 7.3% versus 0.9%, P = 0.01; right-sided: 5% versus 0%, P = 0.02) [125]. Interestingly, an overall survival benefit was reported for a highly selected patient group: among the patients with recurrent disease from a left-sided primary colon cancer, those in the intensive surveillance group had a median overall survival of 4.4 years versus 3.1 years among those in the minimal surveillance protocol (P = 0.03) [125]. These findings suggest follow-up should be tailored based on the risk of recurrence, or more specifically, based on tumour biology.

In 2016, the GILDA working group published the largest surveillance trial in patients with stage II–III CRC (n = 1,242) [120]. An interim analysis of this trial had been published previously, in 2004 [114]. The final results showed that intensive surveillance imaging enabled the earlier diagnosis of disease recurrence than a protocol involving less-frequent imaging (but with CEA testing and physical examination at similar intervals), as demonstrated by differences in mean disease-free survival of 5.9 months (95% CI 2.71–9.11) between the groups [120]. This association with earlier diagnosis had no significantly effect on overall survival (hazard ratio (HR) 1.14, 95% CI 0.87–1.48; TABLE 3 and Supplementary S1 (table)) [120].

In one randomized trial [119], the costs associated with follow-up procedures and additional work-up for any suspected disease recurrence were estimated to be higher for use of 'intensive' surveillance including multiple imaging assessments versus 'simple' surveillance comprising only CEA testing and physical examinations (€300,315 versus €188,630). Among 132 patients undergoing simple surveillance, 34 recurrences were observed, of which 10 (29%) were treatable. During intensive surveillance of 127 patients, 36 recurrences were detected, of which 18 (51%) could be treated [119]. Thus, the costs per detected treatable recurrence were

€16,684 for the intensive scheme versus €18,863 for the simple scheme [119]. Nonetheless, total costs are increased with more-intensive surveillance. If the aim of surveillance is to detect treatable recurrence, more-intensive surveillance might be cost-effective compared with less-intensive surveillance; however, whether intensive surveillance truly is cost-effective, when measured in terms of overall survival benefit (which is the ultimate end point for measuring cost-effectiveness), remains unknown.

Of all randomized surveillance trials published to date, only two have demonstrated a statistically significant overall survival benefit from use of intensive surveillance (TABLE 3 and Supplementary S1 (table)) [117, 121]. In a meta-analysis based on 11 of the 13 published randomized trials [8], comprising 1,511 patients who underwent intensive surveillance versus 1,559 patients who received less-intensive or minimal surveillance, an overall survival difference of 3.3% was demonstrated in favour of intensive surveillance (25.8% versus 29.1%, with 5-year overall survival assessed in most of the trials included, but often without reporting of the median follow-up duration) [8]. This finding implies that 30 patients have to undergo an intensive surveillance programme to avoid one premature death (95% CI 16–458) [8]. The beneficial effect of intensive surveillance on overall survival expressed as hazard ratios have been confirmed in five other meta-analyses that included 5–8 of the same randomized prospective trials [126-130] (TABLE 4). Another meta-analysis [131], published in 2016, included a marginally different selection of seven randomized trials; this study did not demonstrate any improvement in overall survival with intensified surveillance. Interestingly, the investigators also performed a subset analysis based on data from only the three most-recently published randomized trials, which confirmed this finding (HR 1.05, 95%CI 0.87–1.27) [131]. Further, none of the randomized trials, nor meta-analyses, demonstrated a CRC-specific survival benefit from intensive surveillance [8, 43, 46, 114, 116, 118, 126, 131]. Thus, any influence of surveillance on overall survival might arise through various mechanisms other than improved detection and curative treatment of recurrent CRC. Indeed, in one meta-analysis [129], the authors estimated that only one-fifth of the overall survival benefit could be attributed directly to the curative treatment of disease relapse. The additional gain in overall survival is probably related to other factors, and thus trials only focusing on the number of asymptomatic recurrences detected and resected might underestimate the true potential of follow-up surveillance after curative-intent treatment of CRC [129]. In particular, effects associated with intensive surveillance that might contribute to overall survival improvements include increased psychological support and general wellbeing [132, 133], improvements in dietary and lifestyle factors [134, 135], and proactive treatment of other comorbidities [134].

The findings of six meta-analyses have confirmed that the use of intensive surveillance is associated with a shorter time to detection of relapse, although the total number of recurrences identified was not affected by the intensiveness of the surveillance programme [8, 126-130] (TABLE 4). Moreover, earlier detection can increase the frequency of treatment with curative intent, as demonstrated in seven trials and five meta-analyses [8, 43, 46, 71, 117-119, 121, 126, 127, 129, 130]. Ultimately, these effects might translate into an overall survival benefit.

The effect of different surveillance strategies on health-related quality of life (HRQoL) has

been assessed in five trials. The results of two of these trials demonstrated no difference in HRQoL between standard and intensive surveillance [16, 120]. Investigators of another two of these five trials assessed QoL, anxiety, depression, and patient satisfaction, and found no differences in outcomes between intramural (surgeon) or extramural (general practitioner) follow-up strategies [113, 123]. The findings of one trial indicated that intensive surveillance was associated with a marginal benefit in HRQoL [136]. Overall, intensive surveillance seems to have a limited, or even no effect on HRQoL.

Table 4: Meta-analyses of prospective randomized trials on surveillance strategies for patients with stage I–III CRC

Study (year of publication)	Studies included	Number of patients	OS HR (95% CI)	Main conclusions regarding intensive follow-up surveillance
Renehan *et al*. [128] (2002)	5	1,342	0.81 (0.70–0.94)	• Significantly improved OS (P = 0.007) • Resulted in earlier detection of recurrences (P = 0.011)
Figueredo *et al*. [126] (2003)	6	1,679	0.80 (0.70–0.91)	• Significantly improved OS (P = 0.0008) • Rate of recurrence was similar between surveillance schemes of different intensity • Increased the detection of asymptomatic recurrences and more often enabled attempted curative surgery, compared with less-intensive schemes
Renehan *et al*. [129] (2005)	6	1,679	0.76 (0.67–0.86)	• Significantly improved OS • Only ~2% of the total survival benefit was attributed to treatment of recurrences detected through follow-up surveillance specifically: other factors are involved
Jeffery *et al*. [127] (2007)	8	2,141	0.73 (0.59–0.91)	• Significantly improved OS • Absolute number of recurrences detected was similar between different surveillance schemes; however, more curative surgical procedures were attempted in the intensive follow-up groups • Survival benefit was associated with more tests versus fewer tests, and with liver imaging versus no liver imaging
Tjandra *et al*. [130] (2007)	8	2,923	0.74 (0.59–0.93)	• Significantly improved OS (P = 0.01) • No statistically significant difference in all-site recurrence nor in local or distant recurrence between follow-up schemes • Increased the detection of asymptomatic recurrences (P = 0.00001) and the rate of attempted curative surgery (P = 0.0002) • No statistically significant difference, however, in DSS between follow-up schemes
Pita-Fernández *et al*. [8] (2014)	11	4,055	0.75 (0.66–0.86)	• Significantly improved OS • No statistically significant DSS benefit • Absolute number of recurrences was similar between follow-up schemes • Increased the detection of asymptomatic recurrences and the rate of attempted curative surgery • Shorter time to detection of recurrence
Mokhles *et al*. [131] (2016)	7	3,325	0.98 (0.87–1.11)	• No improvement in OS • Pooled subset analysis of the three most-recently published randomized trials also showed no benefit in terms of OS for intensified surveillance (HR 1.05, 95% CI 0.87–1.27).

CI, confidence interval; CRC, colorectal cancer; DSS, disease-specific survival; HR, hazard ratio; OS, overall survival.

After organ-sparing treatment

In the past, surgery was the mainstay of treatment for all patients with stage I–III CRC; however, some patients are now treated with curative intent without surgery. This organ-sparing approach pertains to two major groups of patients.

The first group consists of patients diagnosed with a malignant colorectal polyp. The number of patients diagnosed with these lesions is increasing owing to implementation of CRC screening programmes [6]. Concurrently, the introduction of new endoscopic and surgical techniques has enabled these patients to be treated using organ-sparing techniques, such as endoscopic polypectomy, mucosal resection (EMR), and submucosal dissection (ESD), or transanal endoscopic microsurgery (TEM) for rectal tumours [137, 138]. Neoplastic polyps are classified as either malignant polyps, or T1 cancer when the lesion has invaded the submucosa [139]. Achieving cure via endoscopic resection of malignant polyps or T1 CRC is dependent on complete resection of the lesion, and is only feasible in the absence of involved lymph nodes [140-143]. Even in the case of radical endoscopic resection of superficial T1 cancer, however, local recurrence — including lymph-node involvement — is an issue and should, therefore, be evaluated through surveillance schemes [143, 144]. The depth of tumour invasion within the submucosa is correlated with the rate of recurrence, including lymph-node metastasis: tumours that invade the upper, middle, and lower third of the submucosa are associated with a 2%, 9%, and 35% rate of lymph-node metastasis, respectively [143, 144]. Other prognostic factors predict the development of recurrences, including lymph-node metastases, after endoscopic resection of malignant polyps include tumour morphology, differentiation grade, and lymphovascular invasion [140-144] (TABLE 5). For example, preliminary evidence suggests that having T1-stage sessile lesions is associated with a higher risk of recurrence than T1-stage pedunculated polyps [145, 146]. Pedunculated polyps confined to the submucosa and without unfavourable histological characteristics, such as vascular and lymphatic invasion or poor differentiation, are associated with a 0.3% risk of cancer recurrence or lymph-node metastasis after complete endoscopic removal, whereas similar sessile polyps are associated with a 4.8% risk [147]. Of note, some studies have reported recurrence rates of up to 55% after piecemeal resection of malignant CRC polyps [144, 148-152]. When colon or rectal cancer is resected endoscopically and additional surgery is not planned (owing to complete resection of a lesion with a favourable histology, or to increased surgical risk as a result of comorbidities), endoscopic surveillance with inspection and biopsy sampling of the resection site is mandatory [140, 153, 154], and is typically performed 3–6 months after initial endoscopic resection [155]. Residual and/or recurrent neoplasia can be successfully treated via further endoscopic resection or ablation [149]. After endoscopic confirmation of the absence of residual disease, most guidelines recommend use of colonoscopy surveillance, according to adenoma surveillance guidelines [150, 156].

Table 5: Estimated risk of pelvic rectal cancer recurrence at 3 years after organ-sparing treatment [144]

Depth of invasion	Lymphovascular invasion	Maximum tumour diameter (cm)					
		≤1	1.1–2	2.1–3	3.1–4	4.1–5	>5
pT1; sm1	No	3%	3.6%	4.4%	5.4%	6.6%	8.1%
	Yes	5.2%	6.4%	7.7%	9.4%	11.4%	13.7%
pT1; sm2-3	No	10.5%	12.7%	15.3%	18.5%	22.1%	26.4%
	Yes	17.8%	21.4%	25.5%	30.3%	35.7%	41.8%
pT2	No	9.8%	11.9%	14.3%	17.3%	20.7%	24.7%
	Yes	16.7%	20%	23.9%	28.5%	33.7%	39.5%
pT3	No	19.7%	23.6%	28%	33.2%	39%	45.4%
	Yes	32.2%	37.9%	44.1%	51%	58.3%	65.7%

pT, pathological T stage; sm, Kikuchi submucosal stage. Adapted with permission from John Wiley and Sons © Bach *et al. Br. J. Surg*. **96**, 280–290 (2009).

The second group that is amenable to nonsurgical management of stage I–III disease comprises patients with rectal cancer and a clinical complete response to chemoradiotherapy (CRT). CRT for rectal cancer results in a pathological complete response in 9–24% of patients [157]. This finding has increased interest in rectum-conserving treatment strategies based on watchful waiting, as an alternative to immediate radical surgery that is inherently associated with morbidity [158, 159]. The presence of microscopic residual disease is a caveat of this 'wait-and-see' approach in patients with a clinical (macroscopic) complete response. In two studies involving 109 and 488 patients undergoing CRT for rectal cancer [160, 161], concordance between achievement of a clinical and pathological complete response was only 22–25%. Moreover, in a detailed review of the outcomes of 509 patients with ypT0 rectal cancer (that is, a pathological complete response of the primary tumour to CRT), 26 patients (5%) had pathological lymph-node involvement confirmed by resection specimens [159]. In patients with rectal tumours invading the submucosa (ypT1) and muscularis propria (ypT2) after CRT, the rate of lymph-node involvement is increased to 15% and 17%, respectively [162].

In addition, definite confirmation of a clinical complete response to CRT for rectal cancer remains challenging, and similar complicating factors also pertain to the diagnosis of locally recurrent disease. For example, the identification of residual or recurrent viable tumour after CRT is hampered by the presence of post-treatment fibrosis. In general, the use of endoscopy leads to overestimation of the response of rectal cancer to CRT. The combination of diffusion-weighted MRI, digital rectal examination (DRE), and endoscopy can enable confirmation of a clinical complete response with a sensitivity of up to 98% [163].

Seven published studies reporting outcomes of a wait-and-see strategy have described a systematic surveillance protocol (TABLE 6) [64, 65, 164-170]. These schemes consist of endoscopic examinations with targeted biopsy sampling of suspected neoplastic tissues, as well as DRE, MRI, CT or PET–CT, and CEA monitoring [64, 65, 164-170]. Surveillance is more intensive in the first 6–12 months after CRT, mainly to confirm a clinical complete response. In some protocols, patient visits are planned every 4 weeks during the first year, every 3 months

during the second year, and then biannually in the third, fourth, and fifth years after CRT [64, 65, 164-170] (a detailed overview of these surveillance protocols is provided in TABLE 6). No prospective evidence is available to support these proposed surveillance schemes, and no national or international guidelines exist for surveillance of patients with a complete response to CRT for rectal cancer.

The Dutch/UK/Danish phase II/III STAR-TREC trial (a successor to the CARTS study [138] discussed previously) has been approved by the Dutch Cancer Society (reference: KUN2014 7448). This trial will incorporate both wait-and-see and local excision as organ-sparing treatment options in patients with cT1sm3-T3N0M0 rectal cancer. This study will involve three-way randomization to treatment with either standard TME surgery; organ-sparing resection or ablation after long-course CRT; or organ-sparing treatment after short-course preoperative radiation. For the latter two arms, secondary stratification to either wait-and-see organ-sparing local excision, or TME is being performed depending on the extent of treatment response: patients with a clinical complete response to radiation or CRT will undergo a wait-and-see follow up schedule (similar to that described at the end of this paragraph); those with limited residual disease will undergo local excision; and nonresponders will be treated with standard TME sur¬gery. In addition, a multicentre, prospective, observational and implementation study has also been approved by the Dutch Cancer Society (reference: UM2015-7738, a successor to the NCT00939666 trial [171, 172]). The aim of this study is to set up a national network of dedicated centres for organ-preservation studies, in order to offer such treatment to all patients who are considered adequate candidates and to set up a national registry that will generate more evidence on the oncological and functional outcome of this approach. Patients with a complete response to CRT (as determined using endoscopy, diffusion-weighted MRI, digital rectal exam and lymph-node imaging) will be offered organ-sparing treatment (using a wait-and-see policy). In the follow-up schedule of this study, patients will undergo clinical and physical evaluations, serum CEA testing, and pelvic MRI every 3 months for the first year, then at 18, 24, and 36 months, with sigmoidoscopy ± endorectal ultrasonography except at 36 months when colonoscopy with be performed instead; chest and abdominal CT scans will be conducted at 24 months.

STAGE IV CRC

Treatment with curative intent

For patients presenting with resectable stage IV CRC, surgery with or without systemic therapy offers the only potential for cure and is associated with better long-term outcomes than those of patients with unresectable metastatic CRC treated with palliative systemic therapy [21, 22, 76, 173-176]; patients with unresectable stage IV CRC who are treated with modern chemotherapy with or without biologic agents have a median overall survival of approximately 30 months [174, 175]. Unfortunately, only 10–20% of patients with metastatic CRC are eligible for curative treatment strategies [21, 76]. Most of the literature on treatment for stage IV CRC with curative intent is focused on patients with CRLM and no extrahepatic disease. Partial hepatectomy offers

the potential for cure in these patients (FIG. 3), with various case series indicating 5-year survival rates of 30–60% [21, 22, 177-179], and 10-year survival of approximately 20% [21, 111, 180, 181].

Table 6: Surveillance in studies of organ-sparing 'wait-and-see' strategies after clinical CR of rectal cancer to CRT

Test	Habr-Gama et al. [166] (2006)	Maas et al. [167] (2011)	Dalton et al. [165] (2011)	Yu et al. [170] (2011)	Yeo et al. [169] (2013)	Smith et al. [168] (2012)	Araujo et al. [164] (2015)
Physical examination	Every 1–2 mo. in yr 1	NA	NA	NA	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5; then annually	Every 3 mo. in yr 1 Every 4–6 mo. thereafter	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5
Complete blood counts	NA	NA	NA	NA	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5; then annually	NA	NA
Serum CEA testing	NA	Every 3 mo. in years 1–3 Every 6 mo. in yrs 4–6	NA	At 1, 2, 3, 4, and 6 mo., then every 3 mo. until yr 3 Every 6 mo. in yrs 3–5; then annually until yr 10	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5 Annually thereafter	NA	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5
Direct rectal examination	Every 1–2 mo. in yr 1	Every 3 mo. in yr 1 Every 6 mo. in yrs 2–5	NA	At 1, 2, 3, 4, and 6 mo., then every 3 mo. until yr 3 Every 6 mo. in yrs 3–5; then annually until yr 10	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5; then annually	NA	NA
Endoscopy (± biopsies)	Every 1–2 mo. in yr 1	Every 3 mo. in yr 1 Every 6 mo. in yrs 2–5	At 3 and 12 mo.	At 3, 6, 9, 18, and 24 mo.; then annually	Annual sigmoidoscopy or video colonoscopy	Every 3 mo. in yr 1 Every 4–6 mo. thereafter	Every 3 mo. in yrs 1–2 Every 6 mo. in yrs 3–5
Colonoscopy (± biopsies)	NA	NA	NA	At 12, 24, 60 and 120 mo.	NA	NA	NA
Pelvic MRI	NA	Every 3 mo. in yr 1 Every 6 mo. in yrs 2–5	Every 6 mo. in yr 1 Annually in yrs 2–5	At 1, 2, 3, 4, 6, 9, and 12 mo. Every 6 mo. in yr 2 Annually in yrs 3–7	NA	NA	NA
^{18}F-FDG-PET–CT	NA	NA	Every 6 mo. in yr 1 Annually in yrs 2–5	At 4, 16, and 52 weeks	NA	NA	NA
Pelvic CT	Every 6 mo. in yr 1 Annually thereafte	NA	NA	NA	NA	Not standardized	NA
Abdominal CT	Every 6 mo. in yr 1; then annually	Every 6 mo. in yr 1 Annually in yrs 2–5	NA	NA	Every 6 mo. in yrs 1–5; then annually	Not standardized	NA
Chest CT	NA	Every 6 mo. in yr 1 Annually in yrs 2–5	NA	NA	NA	NA	NA
Chest radiography	Every 6 mo. in yr 1; then annually	NA	NA	NA	NA	NA	NA

CEA, carcinoembryonic antigen; CR, complete response; CRT, chemoradiotherapy; 18F-FDG-PET–CT; 2-[18F] fluoro-2-deoxy-D-glucose-PET–CT; mo., months; NA, not applicable; yr(s), year(s).

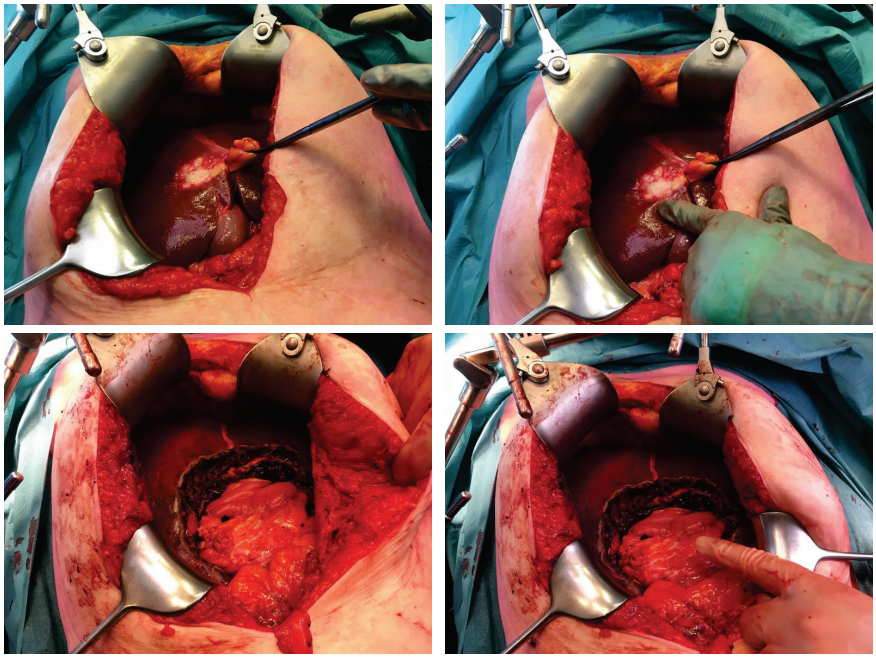


Figure 3. Resection of colorectal liver metastases. The photographs in the upper left and right frames show the appearance of the liver immediately before resection of a colorectal liver metastasis, with the metastasis clearly visible in segment IV (indicated by the surgeon's finger in the upper-right image). This lesion was detected during follow up by CT imaging after treatment with curative intent for colorectal cancer. Lower-left and lower-right frames demonstrate the appearance of the liver immediately after resection of the metastasis.

Surgery with curative intent for isolated pulmonary metastases of colorectal origin is less well studied than resection of liver-limited disease, but such surgery is increasingly performed; the 5-year overall survival rates are around 40–50% [14, 16, 182, 183]. A randomized trial [184, 185] has been initiated to investigate the benefits of adding metastasectomy to active monitoring (with regular CT scans and CEA testing) in patients with pulmonary CRC metastases.

At present, many patients with peritoneal carcinomatosis from CRC are also treated with curative intent. For such patients, cytoreductive surgery combined with use of perioperative hyperthermic intraperitoneal chemotherapy (HIPEC) can yield promising outcomes, with 5-year survival rates of around 40% [11, 12, 15, 186-188]. Moreover, increasing evidence supports the use of surgery with curative intent for liver metastases and concurrent extrahepatic disease in selected patients, with reported 5-year overall survival rates of approximately 30% [19, 189]. The randomized ORCHESTRA trial that has opened in the

Netherlands is being conducted to assesses the value of maximal tumour debulking in patients with multiorgan CRC metastases (NCT 01792934) [190]. Yet more patients with stage IV CRC could become eligible for surgical treatment as the indications for use of curative therapies continue to expand.

Surveillance schemes

Follow-up schemes for patients with stage IV CRC treated with curative intent have been poorly studied, and few surveillance guidelines include protocols specifically for those with stage IV CRC (TABLE 2). For patients with CRLM specifically, clinical risk scores are based on surrogates of tumour load (including number of tumours, serum CEA, tumor size, and nodal status), which is an important prognostic factor for disease recurrence after resection [191]. Patients with high-risk disease (high tumour load) have a 5-year overall survival of around 30% versus 60% for those with low-risk disease (low tumour load) [178]. In patients with pulmonary metastases, the number of metastases removed during metastasectomy or diagnosed using imaging is of prognostic value. One study demonstrated a higher mortality in patients who underwent resection of multiple pulmonary lesions versus patients with a solitary resected pulmonary lesion (HR 2.04, 95% CI 1.72–2.41) [182] — again tumour load dictates oncological outcome, representing aggressiveness of tumour biology. Likewise, the outcomes after maximal tumour debulking of peritoneal metastases combined with use of HIPEC or systemic therapy are related to peritoneal tumour load [11, 187]. One study in 523 patients revealed 5-year overall survival rates between 10% and 50% depending on the peritoneal tumour load [11]. In general, tumour load influences the survival of patients with stage IV CRC after treatment with a curative intent; therefore, intensive follow-up surveillance is likely to be relevant to these patients because early detection of disease recurrence might increase the possibility of further treatment with curative intent. Indeed, accumulating evidence indicates that repeat resection of CRLM improves survival [10, 192-196]. Similarly, reports have described survival benefits of patients undergoing repeated resection of pulmonary metastases of colorectal origin [14, 16, 197]. Thus, the high recurrence rates in patients with stage IV CRC, and the fact that a considerable proportion of patients can nowadays be successfully treated for recurrent disease, might justify the use of intensive surveillance for patients with stage IV CRC treated with curative intent.

Most centres offer a more-intensive surveillance protocol for the first 3 years after partial hepatectomy in patients with CRLM. Surveillance during this initial period usually consists of CT of the thorax and abdomen, liver MRI, chest radiography, liver ultrasonography, and serum CEA testing every 3–6 months (TABLE 2). In the subsequent 2 years, the frequency of follow-up assessments generally decreases to annual imaging combined with serum CEA testing. Studies evaluating follow-up schemes after surgery for those with stage IV CRC are limited to patients who underwent resection for CRLM [13, 198-203] (TABLE 7). In a meta-analysis based on data from a total of 7,330 patients, the effectiveness of surveillance schemes with uniform test intervals over the entire follow-up period of 5 years was compared to that of schemes

with more-intensive follow-up assessments in the early years after partial hepatectomy and then less-frequent testing thereafter. The meta-analysis did not demonstrate an overall survival benefit of initial more-intensive follow-up surveillance [13]. Of note, however, the studies included were heterogeneous in terms of patient characteristics, treatments used, surveillance protocols, and follow-up durations. The surveillance costs per operated recurrence after treatment with curative intent for stage IV CRC have been estimated to range from £23,338 to £31,000, depending on the scheme used (and excluding treatment costs) [198, 200]. No prospective data are available on cost-effectiveness. The limited available data on surveillance of patients with stage IV CRC after treatment with curative intent highlights the need for prospective randomized trials, in particular, to compare the effectiveness and cost-effectiveness of surveillance schemes of different intensities.

Table 7: Overview of studies on patient follow-up surveillance after curative-intent treatment for stage IV CRC

Authors (year of publication)	Study type	Number of patients included	Main conclusions
Jones *et al*. [13] (2012)	Meta-analysis	7,330	Intensive early follow-up surveillance after resection of CRLM fails to improve 5-year OS (42% vs 40%; NS)
Metcalfe *et al*. [203] (2004)	Systematic review	5,745	• Most surveillance protocols involved serum CEA measurements combined with CT or US of the abdomen • Data relating to follow-up protocols used to detect recurrence or record patient outcome were almost completely absent
Metcalfe *et al*. (2005) [202]	Retrospective cohort study	41	• Most recurrences were detected using CT • Serum CEA testing did not result in earlier detection of curable relapses than the use of CT or presentation of symptoms
Langenhoff *et al*. [201] (2009)	Retrospective cohort study	103	• Resectable recurrent disease rate of 24% • Repeat resection resulted in superior OS compared with patients treated with palliative chemotherapy for recurrent disease (median 51 months versus 34 months, respectively; P value not reported) • Serum CEA levels are not a sensitive maker for treatable recurrence • 3 monthly CT scanning is too frequent: more pulmonary recurrences were detected at 6 months interval than liver recurrences at 3 month intervals
Bhattacharja *et al*. [198] (2006)	Prospective cohort study	76	• Relapses were predominantly hepatic, rather than extra hepatic • Serum CEA levels before resection of the primary liver metastases did not correlate with CEA levels at occurrence of recurrent disease • Use of CT enabled detection of recurrences earlier than CEA testing
Connor *et al*. [199] (2007)	Retrospective cohort study	191	• Low rate of repeat resections for recurrent disease (9%) • High rate of interval recurrences (58%)
Gomez *et al*. [200](2010)	Retrospective cohort study	705	Repeat resection of recurrent disease (liver or lung ± liver metastases) was superior to palliative systemic therapy in terms of 5-year OS (31% and 30% for patients who underwent resection, compared with 3.9% in the palliative systemic therapy group; P <0.001)

CEA, carcinoembryonic antigen; CRC, colorectal cancer; CRLM, colorectal liver metastases; NS, not significant; OS, overall survival; US, ultrasonography.

FUTURE DIRECTIONS

The wide range of diagnostic modalities that can be used to detect disease recurrence after treatment of patients with CRC with a curative intent differ in terms of their invasiveness, accuracy, burden, costs as well as cost-effectiveness. Moreover, these modalities can be used in different combinations and at various intervals for surveillance after curative therapy. Thus, determining the optimal surveillance protocols for use among the diverse populations of patient who are eligible for curative treatment is extremely challenging. The numerous studies conducted to address this issue have varied in quality, cohort size, and the specific follow-up schemes investigated (in terms of diagnostic modalities, intervals, total duration). In addition, diagnostic and treatment modalities have evolved considerably during the timeframe in which these studies were conducted, raising further questions regarding the relevance and comparability of their results. The absence of strong conclusive evidence is reflected in the lack of consensus between surveillance guidelines (TABLE 2). Three decennia of meta-analyses, in some cases combining data from more than 5,000 patients, have therefore brought us only a little closer to defining the optimal surveillance protocol. Ongoing further meta-analytical evaluations of the available data will not add useful information: new prospective randomized trials are needed, in a risk-adapted setting aiming for tailored protocols [120].

Currently, patients with malignant colorectal polyps or early T1-stage cancers, and those with rectal cancer with a complete response to CRT are increasingly treated with organ-sparing techniques, or even a watch-and-wait strategy for the latter group [64, 65, 137, 138]. In addition, local treatment of stage IV disease with curative intent is being performed at an increasing frequency for an expanding range of indications, owing to reports of improved survival in selected patients [21, 22, 76, 173-176]. Nevertheless, after organ-sparing treatment and metastasectomy, recurrence rates can be high, and evidence indicates that repeat resections might provide overall survival benefits [10, 14, 149, 194-197]. These findings emphasize the importance of surveillance for different patient populations. Clinicians should realize, however, that no prospective evidence supports the use of any of the surveillance schemes proposed to date. Thus, a clear need exists for prospective evaluation of the optimal surveillance strategy for these categories of patients, in particular, and for the development of evidence-based guidelines.

Apart from an increased oncological risk of recurrence (according to clinical stage or molecular subgroup, for example), other factors are known to influence the risk of CRC recurrence [204]. These factors include tumour perforation [205, 206], obstructive tumours [207], anastomotic leakage and subsequent bacterial infection after resection of CRC [208], and perioperative blood transfusion [209]. The existing surveillance guidelines do not recommend use of specific surveillance protocols for these different risk subgroups. Disease recurrence has been investigated in patients at a high risk of developing colorectal peritoneal carcinomatosis, owing to tumour perforation, resected minimal synchronous

macroscopic peritoneal carcinomatosis, or synchronous ovarian metastases, via systematic 'second-look' surgery performed 1 year after the initial surgery [210, 211]. In these patients, who had no signs of recurrence on imaging studies, the recurrence rate at second-look surgery was ~55% [210, 211]. These findings led to two prospective trials of mandatory second-look surgery for such patients, one in the USA [212] (NCT01095523) and the other in France [213] (NCT00005944). These studies provide examples of risk-adapted follow-up protocols for patients with CRC. Future research on risk-adapted surveillance should implement considerations of the complete spectrum of risk factors for recurrence after curative treatment for CRC, including biological variance (clinical and molecular), radicality of surgery, anastomotic leakage, postoperative infection, and tumour perforation. Ideally, a nomogram integrating all of these risk factors should be developed, to direct the use of personalized surveillance.

Importantly, in future surveillance trials, attention should be paid to the design of the study arms in order to avoid ambiguity regarding the definition of 'intensive' surveillance. The heterogeneity between published randomized trials of surveillance for patients with stage I–III CRC impairs evidence-based selection of any of the protocols assessed, despite the fact that most meta-analyses have validated an overall survival benefit from 'more-intensive surveillance'. Preferably, individual studies should be focused purely on comparing the efficacy of single diagnostic modalities, or the frequency of follow-up assessments, but not both. Computer-simulation modelling could then contribute to the development of more-effective surveillance schemes via analysis of comprehensive information on patterns of disease recurrence, oncological risks (clinical and molecular), costs, sensitivity and specificity of diagnostic tests, follow-up intervals, follow-up duration, and survival outcomes gathered in the prospective trials. This approach would also facilitate further research on risk-adapted follow-up surveillance approaches for individual patients, preferably based on the intrinsic (molecular and clinical) characteristics of the disease.

CONCLUSIONS

Patients with stage I–IV CRC can benefit from surveillance after treatment with curative intent. The aim of surveillance is to detect recurrent disease at an early, asymptomatic, and treatable stage, with the ultimately goal of achieving an overall survival benefit. This approach is important because treatment for disease recurrence can nowadays be applied repeatedly in individual patients. The surveillance frequency and methods used partly depend on the location and stage of the primary tumour. Meta-analyses of data from a range of studies over the past two decades confirm that frequent patient visits improve overall survival after curative resection of stage I–III CRC, although most individual trials demonstrate minimal to no overall survival benefit. The effect of surveillance for stage I–III CRC on overall survival can only partly be explained by improved cancer-specific survival, with repeated clinical

assessment contributing to the overall survival benefit. Further (model-based) research on surveillance after CRC treatment should focus on risk-stratification and should incorporate current knowledge on risk of recurrence in relation to the biology of the tumour. Consensus guidelines and prospective research investigating the optimal follow-up protocols are urgently needed for surveillance of patients with stage IV CRC who are treated with curative intent, as well as patients treated according to a 'wait and see' strategy after a complete clinical response to CRT.

REFERENCES

1. Kuipers, E.J., Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, van de Velde CJH, Watanabe T., *Colorectal cancer*. Nature Reviews Disease Primers, 2015. **1**: p. 1-25.
2. Torre, L.A., et al., *Global cancer statistics, 2012*. CA Cancer J Clin, 2015. **65**(2): p. 87-108.
3. Elferink, M.A., et al., *Marked improvements in survival of patients with rectal cancer in the Netherlands following changes in therapy, 1989-2006*. Eur J Cancer, 2010. **46**(8): p. 1421-9.
4. van der Pool, A.E., et al., *Trends in incidence, treatment and survival of patients with stage IV colorectal cancer: a population-based series*. Colorectal Dis, 2012. **14**(1): p. 56-61.
5. van Steenbergen, L.N., et al., *Improved survival of colon cancer due to improved treatment and detection: a nationwide population-based study in The Netherlands 1989-2006*. Ann Oncol, 2010. **21**(11): p. 2206-12.
6. Schreuders, E.H., et al., *Colorectal cancer screening: a global overview of existing programmes*. Gut, 2015. **64**(10): p. 1637-49.
7. Kuipers, E.J., T. Rosch, and M. Bretthauer, *Colorectal cancer screening--optimizing current strategies and new directions.* Nat Rev Clin Oncol, 2013. **10**(3): p. 130-42.
8. Pita-Fernandez, S., et al., *Intensive follow-up strategies improve outcomes in nonmetastatic colorectal cancer patients after curative surgery: a systematic review and meta-analysis*. Ann Oncol, 2015. **26**(4): p. 644-56.
9. Rose, J., K.M. Augestad, and G.S. Cooper, *Colorectal cancer surveillance: what's new and what's next*. World J Gastroenterol, 2014. **20**(8): p. 1887-97.
10. de Jong, M.C., 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): p. 440-8.
11. Elias, D., et al., *Peritoneal colorectal carcinomatosis treated with surgery and perioperative intraperitoneal chemotherapy: retrospective analysis of 523 patients from a multicentric French study*. J Clin Oncol, 2010. **28**(1): p. 63-8.
12. Glehen, O., et al., *Cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for the management of peritoneal carcinomatosis from colorectal cancer: a multi-institutional study*. J Clin Oncol, 2004. **22**(16): p. 3284-92.
13. Jones, R.P., et al., *Systematic review and meta-analysis of follow-up after hepatectomy for colorectal liver metastases*. Br J Surg, 2012. **99**(4): p. 477-86.
14. Pfannschmidt, J., H. Dienemann, and H. Hoffmann, *Surgical resection of pulmonary metastases from colorectal cancer: a systematic review of published series.* Ann Thorac Surg, 2007. **84**(1): p. 324-38.
15. Verwaal, V.J., et al., *Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer*. J Clin Oncol, 2003. **21**(20): p. 3737-43.
16. Warwick, R. and R. Page, *Resection of pulmonary metastases from colorectal carcinoma*. Eur J Surg Oncol, 2007. **33 Suppl 2**: p. S59-63.
17. Winawer, S.J., et al., *Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup*. N Engl J Med, 1993. **329**(27): p. 1977-81.

18. Bhangu, A., et al., *Meta-analysis of survival based on resection margin status following surgery for recurrent rectal cancer*. Colorectal Dis, 2012. **14**(12): p. 1457-66.
19. Chua, T.C., et al., *Hepatectomy and resection of concomitant extrahepatic disease for colorectal liver metastases--a systematic review.* Eur J Cancer, 2012. **48**(12): p. 1757-65.
20. Dresen, R.C., et al., *Radical resection after IORT-containing multimodality treatment is the most important determinant for outcome in patients treated for locally recurrent rectal cancer*. Ann Surg Oncol, 2008. **15**(7): p. 1937-47.
21. Kanas, G.P., et al., *Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors*. Clin Epidemiol, 2012. **4**: p. 283-301.
22. Rees, M., et al., *Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients*. Ann Surg, 2008. **247**(1): p. 125-35.
23. Cali, R.L., et al., Cumulative incidence of metachronous colorectal cancer. Dis Colon Rectum, 1993. **36**(4): p. 388-93.
24. Green, R.J., et al., *Surveillance for second primary colorectal cancer after adjuvant chemotherapy: an analysis of Intergroup 0089*. Ann Intern Med, 2002. **136**(4): p. 261-9.
25. Hohenberger, W., et al., *Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome*. Colorectal Dis, 2009. **11**(4): p. 354-64; discussion 364-5.
26. Kapiteijn, E., et al., *Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer.* N Engl J Med, 2001. **345**(9): p. 638-46.
27. Mulder, S.A., et al., *The incidence and risk factors of metachronous colorectal cancer: an indication for follow-up*. Dis Colon Rectum, 2012. **55**(5): p. 522-31.
28. Obrand, D.I. and P.H. Gordon, *Incidence and patterns of recurrence following curative resection for colorectal carcinoma*. Dis Colon Rectum, 1997. **40**(1): p. 15-24.
29. Peeters, K.C., et al., *The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma*. Ann Surg, 2007. **246**(5): p. 693-701.
30. Ringland, C.L., et al., *Second primary colorectal cancers (SPCRCs): experiences from a large Australian Cancer Registry*. Ann Oncol, 2010. **21**(1): p. 92-7.
31. Pickhardt, P.J., et al., *Colorectal cancer: CT colonography and colonoscopy for detection--systematic review and meta-analysis*. Radiology, 2011. **259**(2): p. 393-405.
32. Labianca, R., et al., *Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up*. Ann Oncol, 2013. **24 Suppl 6**: p. vi64-72.
33. Meyerhardt, J.A., et al., *Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement*. J Clin Oncol, 2013. **31**(35): p. 4465-70.
34. NCCN, *Guidelines for Treatment of Colorectal Cancer*. http://www.nccn.org, 2015.
35. NICE, *Colorectal cancer: diagnosis and management [CG131]*. http://www.nice.org.uk/guidance/CG131, 2011.
36. Steele, S.R., et al., *Practice Guideline for the Surveillance of Patients After Curative Treatment of*

Colon and Rectal Cancer. Dis Colon Rectum, 2015. **58**(8): p. 713-25.

37. Van Cutsem, E., et al., *Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up*. Ann Oncol, 2014. **25 Suppl 3**: p. iii1-9.
38. Barillari, P., et al., *Surveillance of colorectal cancer: effectiveness of early detection of intraluminal recurrences on prognosis and survival of patients treated for cure*. Dis Colon Rectum, 1996. **39**(4): p. 388-93.
39. Chen, F. and M. Stuart, *Colonoscopic follow-up of colorectal carcinoma*. Dis Colon Rectum, 1994. **37**(6): p. 568-72.
40. Granqvist, S. and T. Karlsson, *Postoperative follow-up of patients with colorectal carcinoma by colonoscopy*. Eur J Surg, 1992. **158**(5): p. 307-12.
41. Grobbee, E.J., et al., *Second-Look Colonoscopies and the Impact on Capacity in FIT-Based Colorectal Cancer Screening*. Am J Gastroenterol, 2015. **110**(7): p. 1072-7.
42. Juhl, G., et al., *Six-year results of annual colonoscopy after resection of colorectal cancer.* World J Surg, 1990. **14**(2): p. 255-60; discussion 260-1.
43. Kjeldsen, B.J., et al., *A prospective randomized study of follow-up after radical surgery for colorectal cancer*. Br J Surg, 1997. **84**(5): p. 666-9.
44. Schoemaker, D., et al., *Yearly colonoscopy, liver CT, and chest radiography do not influence 5-year survival of colorectal cancer patients*. Gastroenterology, 1998. **114**(1): p. 7-14.
45. Togashi, K., et al., *Predictive factors for detecting colorectal carcinomas in surveillance colonoscopy after colorectal cancer surgery*. Dis Colon Rectum, 2000. **43**(10 Suppl): p. S47-53.
46. Wang, T., et al., *The role of postoperative colonoscopic surveillance after radical surgery for colorectal cancer: a prospective, randomized clinical study*. Gastrointest Endosc, 2009. **69**(3 Pt 2): p. 609-15.
47. Robertson, D.J., M.F. Kaminski, and M. Bretthauer, *Effectiveness, training and quality assurance of colonoscopy screening for colorectal cancer*. Gut, 2015. **64**(6): p. 982-90.
48. Kim, H.J., et al., *CT colonography for combined colonic and extracolonic surveillance after curative resection of colorectal cancer*. Radiology, 2010. **257**(3): p. 697-704.
49. Amitai, M.M., et al., *Contrast-enhanced CT colonography with 64-slice MDCT compared to endoscopic colonoscopy in the follow-up of patients after colorectal cancer resection*. Clin Imaging, 2009. **33**(6): p. 433-8.
50. Fletcher, J.G., et al., *Contrast-enhanced CT colonography in recurrent colorectal carcinoma: feasibility of simultaneous evaluation for metastatic disease, local recurrence, and metachronous neoplasia in colorectal carcinoma*. AJR Am J Roentgenol, 2002. **178**(2): p. 283-90.
51. Laghi, A., et al., *Contrast-enhanced computed tomographic colonography in the follow-up of colorectal cancer patients: a feasibility study*. Eur Radiol, 2003. **13**(4): p. 883-9.
52. Lee, J.H., et al., *CT colonography in patients who have undergone sigmoid colostomy: a feasibility study*. AJR Am J Roentgenol, 2011. **197**(4): p. W653-7.
53. Leonardou, P., et al., *Screening of patients after colectomy: virtual colonography*. Abdom Imaging, 2006. **31**(5): p. 521-8.
54. Neri, E., et al., *Post-surgical follow-up of colorectal cancer: role of contrast-enhanced CT colonography*. Abdom Imaging, 2010. **35**(6): p. 669-75.

55. You, Y.T., et al., *Evaluation of contrast-enhanced computed tomographic colonography in detection of local recurrent colorectal cancer*. World J Gastroenterol, 2006. **12**(1): p. 123-6.
56. de Wijkerslooth, T.R., et al., *Burden of colonoscopy compared to non-cathartic CT-colonography in a colorectal cancer screening programme: randomised controlled trial*. Gut, 2012. **61**(11): p. 1552-9.
57. Dighe, S., et al., *Diagnostic precision of CT in local staging of colon cancers: a meta-analysis*. Clin Radiol, 2010. **65**(9): p. 708-19.
58. Kievit, J., *Follow-up of patients with colorectal cancer: numbers needed to test and treat*. Eur J Cancer, 2002. **38**(7): p. 986-99.
59. Blomqvist, L., et al., *MR imaging, CT and CEA scintigraphy in the diagnosis of local recurrence of rectal carcinoma*. Acta Radiol, 1996. **37**(5): p. 779-84.
60. Pema, P.J., et al., *CT vs MRI in diagnosis of recurrent rectosigmoid carcinoma*. J Comput Assist Tomogr, 1994. **18**(2): p. 256-61.
61. Group, M.S., *Diagnostic accuracy of preoperative magnetic resonance imaging in predicting curative resection of rectal cancer: prospective observational study*. BMJ, 2006. **333**(7572): p. 779.
62. Bipat, S., et al., *Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging--a meta-analysis*. Radiology, 2004. **232**(3): p. 773-83.
63. Titu, L.V., et al., *Routine follow-up by magnetic resonance imaging does not improve detection of resectable local recurrences from colorectal cancer*. Ann Surg, 2006. **243**(3): p. 348-52.
64. Glynne-Jones, R. and R. Hughes, *Critical appraisal of the 'wait and see' approach in rectal cancer for clinical complete responders after chemoradiation*. Br J Surg, 2012. **99**(7): p. 897-909.
65. Glynne-Jones, R. and R. Hughes, *Complete Response after Chemoradiotherapy in Rectal Cancer (Watch-and-Wait): Have we Cracked the Code?* Clin Oncol (R Coll Radiol), 2016. **28**(2): p. 152-60.
66. Furukawa, H., et al., *Positron emission tomography scanning is not superior to whole body multidetector helical computed tomography in the preoperative staging of colorectal cancer*. Gut, 2006. **55**(7): p. 1007-11.
67. Shin, S.S., et al., *Preoperative staging of colorectal cancer: CT vs. integrated FDG PET/CT*. Abdom Imaging, 2008. **33**(3): p. 270-7.
68. Tsunoda, Y., et al., *Preoperative diagnosis of lymph node metastases of colorectal cancer by FDG-PET/CT*. Jpn J Clin Oncol, 2008. **38**(5): p. 347-53.
69. Huebner, R.H., et al., *A meta-analysis of the literature for whole-body FDG PET detection of recurrent colorectal cancer*. J Nucl Med, 2000. **41**(7): p. 1177-89.
70. Even-Sapir, E., et al., *Detection of recurrence in patients with rectal cancer: PET/CT after abdominoperineal or anterior resection*. Radiology, 2004. **232**(3): p. 815-22.
71. Sobhani, I., et al., *Early detection of recurrence by 18FDG-PET in the follow-up of patients with colorectal cancer*. Br J Cancer, 2008. **98**(5): p. 875-80.
72. Khan, K., et al., *Survival Outcomes in Asymptomatic Patients With Normal Conventional Imaging but Raised Carcinoembryonic Antigen Levels in Colorectal Cancer Following Positron Emission Tomography-Computed Tomography Imaging*. Oncologist, 2016.
73. Fukunaga, H., et al., *Fusion image of positron emission tomography and computed tomography*

for the diagnosis of local recurrence of rectal cancer. Ann Surg Oncol, 2005. **12**(7): p. 561-9.

74. Engenhart, R., et al., *Therapy monitoring of presacral recurrences after high-dose irradiation: value of PET, CT, CEA and pain score*. Strahlenther Onkol, 1992. **168**(4): p. 203-12.
75. Moore, H.G., et al., *A case-controlled study of 18-fluorodeoxyglucose positron emission tomography in the detection of pelvic recurrence in previously irradiated rectal cancer patients*. J Am Coll Surg, 2003. **197**(1): p. 22-8.
76. Elferink, M.A., et al., *Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands*. Int J Colorectal Dis, 2015. **30**(2): p. 205-12.
77. Tan, E., et al., *Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer*. Surg Oncol, 2009. **18**(1): p. 15-24.
78. Staab, H.J., et al., *Slope analysis of the postoperative CEA time course and its possible application as an aid in diagnosis of disease progression in gastrointestinal cancer*. Am J Surg, 1978. **136**(3): p. 322-7.
79. Verberne, C.J., et al., *Detection of recurrences during follow-up after liver surgery for colorectal metastases: both carcinoembryonic antigen (CEA) and imaging are important*. Ann Surg Oncol, 2013. **20**(2): p. 457-63.
80. Verberne, C.J., et al., *Intensified follow-up in colorectal cancer patients using frequent Carcino-Embryonic Antigen (CEA) measurements and CEA-triggered imaging: Results of the randomized "CEAwatch" trial*. Eur J Surg Oncol, 2015. **41**(9): p. 1188-96.
81. Bipat, S., et al., *Colorectal liver metastases: CT, MR imaging, and PET for diagnosis--meta-analysis*. Radiology, 2005. **237**(1): p. 123-31.
82. Kinkel, K., et al., *Detection of hepatic metastases from cancers of the gastrointestinal tract by using noninvasive imaging methods (US, CT, MR imaging, PET): a meta-analysis*. Radiology, 2002. **224**(3): p. 748-56.
83. Westwood, M., et al., *Contrast-enhanced ultrasound using SonoVue(R) (sulphur hexafluoride microbubbles) compared with contrast-enhanced computed tomography and contrast-enhanced magnetic resonance imaging for the characterisation of focal liver lesions and detection of liver metastases: a systematic review and cost-effectiveness analysis*. Health Technol Assess, 2013. **17**(16): p. 1-243.
84. Mainenti, P.P., et al., *Non-invasive diagnostic imaging of colorectal liver metastases*. World J Radiol, 2015. **7**(7): p. 157-69.
85. Niekel, M.C., S. Bipat, and J. Stoker, *Diagnostic imaging of colorectal liver metastases with CT, MR imaging, FDG PET, and/or FDG PET/CT: a meta-analysis of prospective studies including patients who have not previously undergone treatment*. Radiology, 2010. **257**(3): p. 674-84.
86. Patel, S., et al., *Positron emission tomography/computed tomographic scans compared to computed tomographic scans for detecting colorectal liver metastases: a systematic review*. Ann Surg, 2011. **253**(4): p. 666-71.
87. Kronawitter, U., et al., *Evaluation of chest computed tomography in the staging of patients with potentially resectable liver metastases from colorectal carcinoma*. Cancer, 1999. **86**(2): p. 229-35.
88. Povoski, S.P., et al., *Role of chest CT in patients with negative chest x-rays referred for hepatic colorectal metastases*. Ann Surg Oncol, 1998. **5**(1): p. 9-15.

89. Duffy, M.J., et al., *Use of faecal markers in screening for colorectal neoplasia: a European group on tumor markers position paper*. Int J Cancer, 2011. **128**(1): p. 3-11.
90. Whitlock, E.P., et al., *Screening for colorectal cancer: a targeted, updated systematic review for the U.S. Preventive Services Task Force*. Ann Intern Med, 2008. **149**(9): p. 638-58.
91. Jahn, H., et al., *Can Hemoccult-II replace colonoscopy in surveillance after radical surgery for colorectal cancer and after polypectomy?* Dis Colon Rectum, 1992. **35**(3): p. 253-6.
92. Imperiale, T.F., et al., *Multitarget stool DNA testing for colorectal-cancer screening*. N Engl J Med, 2014. **370**(14): p. 1287-97.
93. Bosch, L.J., et al., *Molecular tests for colorectal cancer screening*. Clin Colorectal Cancer, 2011. **10**(1): p. 8-23.
94. Lee, B.B., et al., *Aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes in plasma as a biomarker for early detection of colorectal cancer*. Clin Cancer Res, 2009. **15**(19): p. 6185-91.
95. Leung, W.K., et al., *Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer*. Am J Gastroenterol, 2005. **100**(10): p. 2274-9.
96. Lalmahomed, Z.S., et al., *Circulating tumor cells and sample size: the more, the better*. J Clin Oncol, 2010. **28**(17): p. e288-9; author reply e290.
97. Mostert, B., et al., *KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue*. Int J Cancer, 2013. **133**(1): p. 130-41.
98. Mostert, B., et al., *mRNA expression profiles in circulating tumor cells of metastatic colorectal cancer patients*. Mol Oncol, 2015. **9**(4): p. 920-32.
99. Best, M.G., et al., *RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics*. Cancer Cell, 2015. **28**(5): p. 666-76.
100. Nilsson, R.J., et al., *Blood platelets contain tumor-derived RNA biomarkers*. Blood, 2011. **118**(13): p. 3680-3.
101. Metzger, J., et al., *Urine proteomic analysis differentiates cholangiocarcinoma from primary sclerosing cholangitis and other benign biliary disorders*. Gut, 2013. **62**(1): p. 122-30.
102. Broker, M.E., et al., *Collagen peptides in urine: a new promising biomarker for the detection of colorectal liver metastases*. PLoS One, 2013. **8**(8): p. e70918.
103. Deslauriers, J. and J. Gregoire, *Clinical and surgical staging of non-small cell lung cancer*. Chest, 2000. **117**(4 Suppl 1): p. 96S-103S.
104. Staples, C.A., et al., *Mediastinal nodes in bronchogenic carcinoma: comparison between CT and mediastinoscopy*. Radiology, 1988. **167**(2): p. 367-72.
105. Antoch, G., et al., *Whole-body dual-modality PET/CT and whole-body MRI for tumor staging in oncology*. JAMA, 2003. **290**(24): p. 3199-206.
106. Antoch, G. and A. Bockisch, *Combined PET/MRI: a new dimension in whole-body oncology imaging?* Eur J Nucl Med Mol Imaging, 2009. **36 Suppl 1**: p. S113-20.
107. Pfannenberg, C., et al., *Prospective comparison of 18F-fluorodeoxyglucose positron emission tomography/computed tomography and whole-body magnetic resonance imaging in staging of advanced malignant melanoma*. Eur J Cancer, 2007. **43**(3): p. 557-64.
108. Schmidt, G.P., et al., *Comprehensive imaging of tumor recurrence in breast cancer patients using whole-body MRI at 1.5 and 3 T compared to FDG-PET-CT*. Eur J Radiol, 2008. **65**(1): p. 47-58.

109. Squillaci, E., et al., *Staging of colon cancer: whole-body MRI vs. whole-body PET-CT--initial clinical experience*. Abdom Imaging, 2008. **33**(6): p. 676-88.
110. Sargent, D.J., et al., *End points for colon cancer adjuvant trials: observations and recommendations based on individual patient data from 20,898 patients enrolled onto 18 randomized trials from the ACCENT Group*. J Clin Oncol, 2007. **25**(29): p. 4569-74.
111. Tomlinson, J.S., et al., *Actual 10-year survival after resection of colorectal liver metastases defines cure*. J Clin Oncol, 2007. **25**(29): p. 4575-80.
112. Walter, C.J., et al., *Fifth-year surveillance computed tomography scanning after potentially curative resections for colorectal cancer*. Surgeon, 2013. **11**(1): p. 25-9.
113. Augestad, K.M., et al., *Cost-effectiveness and quality of life in surgeon versus general practitioner-organised colon cancer surveillance: a randomised controlled trial*. BMJ Open, 2013. **3**(4).
114. Grossmann, E.M., et al., *Follow-up of colorectal cancer patients after resection with curative intent-the GILDA trial*. Surg Oncol, 2004. **13**(2-3): p. 119-24.
115. Makela, J.T., S.O. Laitinen, and M.I. Kairaluoma, *Five-year follow-up after radical surgery for colorectal cancer. Results of a prospective randomized trial*. Arch Surg, 1995. **130**(10): p. 1062-7.
116. Ohlsson, B., et al., *Follow-up after curative surgery for colorectal carcinoma. Randomized comparison with no follow-up*. Dis Colon Rectum, 1995. **38**(6): p. 619-26.
117. Pietra, N., et al., *Role of follow-up in management of local recurrences of colorectal cancer: a prospective, randomized study*. Dis Colon Rectum, 1998. **41**(9): p. 1127-33.
118. Primrose, J.N., et al., *Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial*. JAMA, 2014. **311**(3): p. 263-70.
119. Rodriguez-Moranta, F., et al., *Postoperative surveillance in patients with colorectal cancer who have undergone curative resection: a prospective, multicenter, randomized, controlled trial*. J Clin Oncol, 2006. **24**(3): p. 386-93.
120. Rosati, G., et al., *A randomized trial of intensive versus minimal surveillance of patients with resected Dukes B2-C colorectal carcinoma*. Ann Oncol, 2016. **27**(2): p. 274-80.
121. Secco, G.B., et al., *Efficacy and cost of risk-adapted follow-up in patients after colorectal cancer surgery: a prospective, randomized and controlled trial*. Eur J Surg Oncol, 2002. **28**(4): p. 418-23.
122. Treasure, T., et al., *The CEA Second-Look Trial: a randomised controlled trial of carcinoembryonic antigen prompted reoperation for recurrent colorectal cancer*. BMJ Open, 2014. **4**(5): p. e004385.
123. Wattchow, D.A., et al., *General practice vs surgical-based follow-up for patients with colon cancer: randomised controlled trial*. Br J Cancer, 2006. **94**(8): p. 1116-21.
124. Wille-Jorgensen, P., et al., *An interim analysis of recruitment to the COLOFOL trial*. Colorectal Dis, 2009. **11**(7): p. 756-8.
125. Pugh, S.A.M., D.; Shinkins B.; Mellor, J.; Perera, R.; Primrose, J., *Scheduled use of CEA and CT follow-up to detect recurrence of colorectal cancer: 6-12 year results from the FACS randomised controlled trial*. Annals of Oncology, Abstract Book of the 41st ESMO Congress (ESMO 2016), 2016. **27, Supplement 6**: p. Abstract 453O.
126. Figueredo, A., et al., *Follow-up of patients with curatively resected colorectal cancer: a practice guideline*. BMC Cancer, 2003. **3**: p. 26.

127. Jeffery, M., B.E. Hickey, and P.N. Hider, *Follow-up strategies for patients treated for non-metastatic colorectal cancer*. Cochrane Database Syst Rev, 2007(1): p. CD002200.
128. Renehan, A.G., et al., *Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trials*. BMJ, 2002. **324**(7341): p. 813.
129. Renehan, A.G., et al., *Mechanisms of improved survival from intensive followup in colorectal cancer: a hypothesis*. Br J Cancer, 2005. **92**(3): p. 430-3.
130. Tjandra, J.J. and M.K. Chan, *Follow-up after curative resection of colorectal cancer: a meta-analysis.* Dis Colon Rectum, 2007. **50**(11): p. 1783-99.
131. Mokhles, S., et al., *Meta-analysis of colorectal cancer follow-up after potentially curative resection*. Br J Surg, 2016. **103**(10): p. 1259-68.
132. Kuchler, T., et al., *Impact of psychotherapeutic support on gastrointestinal cancer patients undergoing surgery: survival results of a trial.* Hepatogastroenterology, 1999. **46**(25): p. 322-35.
133. Newell, S.A., R.W. Sanson-Fisher, and N.J. Savolainen, *Systematic review of psychological therapies for cancer patients: overview and recommendations for future research*. J Natl Cancer Inst, 2002. **94**(8): p. 558-84.
134. Braunholtz, D.A., S.J. Edwards, and R.J. Lilford, *Are randomized clinical trials good for us (in the short term)? Evidence for a "trial effect"*. J Clin Epidemiol, 2001. **54**(3): p. 217-24.
135. Calle, E.E., et al., *Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults*. N Engl J Med, 2003. **348**(17): p. 1625-38.
136. Kjeldsen, B.J., et al., *Influence of follow-up on health-related quality of life after radical surgery for colorectal cancer*. Scand J Gastroenterol, 1999. **34**(5): p. 509-15.
137. Bhangu, A., et al., *Survival outcome of local excision versus radical resection of colon or rectal carcinoma: a Surveillance, Epidemiology, and End Results (SEER) population-based study*. Ann Surg, 2013. **258**(4): p. 563-9; discussion 569-71.
138. Verseveld, M., et al., *Chemoradiation therapy for rectal cancer in the distal rectum followed by organ-sparing transanal endoscopic microsurgery (CARTS study)*. Br J Surg, 2015. **102**(7): p. 853-60.
139. Schlemper, R.J., et al., *The Vienna classification of gastrointestinal epithelial neoplasia*. Gut, 2000. **47**(2): p. 251-5.
140. Meining, A., et al., *Risk factors for unfavorable outcomes after endoscopic removal of submucosal invasive colorectal tumors*. Clin Gastroenterol Hepatol, 2011. **9**(7): p. 590-4.
141. Morson, B.C., et al., *Histopathology and prognosis of malignant colorectal polyps treated by endoscopic polypectomy*. Gut, 1984. **25**(5): p. 437-44.
142. Muller, S., et al., *Significance of venous and lymphatic invasion in malignant polyps of the colon and rectum*. Gut, 1989. **30**(10): p. 1385-91.
143. Nascimbeni, R., et al., *Risk of lymph node metastasis in T1 carcinoma of the colon and rectum*. Dis Colon Rectum, 2002. **45**(2): p. 200-6.
144. Bach, S.P., et al., *A predictive model for local recurrence after transanal endoscopic microsurgery for rectal cancer*. Br J Surg, 2009. **96**(3): p. 280-90.
145. ASGE, *Gastrointestinal Endoscopy, Guideline*. 2005. **61**(1).
146. Hassan, C., et al., *Histologic risk factors and clinical outcome in colorectal malignant polyp: a*

pooled-data analysis. Dis Colon Rectum, 2005. **48**(8): p. 1588-96.

147. Davila, R.E., et al., *ASGE guideline: the role of endoscopy in the diagnosis, staging, and management of colorectal cancer*. Gastrointest Endosc, 2005. **61**(1): p. 1-7.
148. Buchner, A.M., C. Guarner-Argente, and G.G. Ginsberg, *Outcomes of EMR of defiant colorectal lesions directed to an endoscopy referral center*. Gastrointest Endosc, 2012. **76**(2): p. 255-63.
149. Khashab, M., et al., *Incidence and predictors of "late" recurrences after endoscopic piecemeal resection of large sessile adenomas*. Gastrointest Endosc, 2009. **70**(2): p. 344-9.
150. Lieberman, D.A., et al., *Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer*. Gastroenterology, 2012. **143**(3): p. 844-57.
151. Winawer, S.J., et al., *Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society*. Gastroenterology, 2006. **130**(6): p. 1872-85.
152. Zlatanic, J., et al., *Large sessile colonic adenomas: use of argon plasma coagulator to supplement piecemeal snare polypectomy*. Gastrointest Endosc, 1999. **49**(6): p. 731-5.
153. Di Gregorio, C., et al., *Clinical outcome of low- and high-risk malignant colorectal polyps: results of a population-based study and meta-analysis of the available literature*. Intern Emerg Med, 2014. **9**(2): p. 151-60.
154. Kim, M.N., et al., *Clinical features and prognosis of early colorectal cancer treated by endoscopic mucosal resection*. J Gastroenterol Hepatol, 2011. **26**(11): p. 1619-25.
155. Ackland, S.P., et al., *A meta-analysis of two randomised trials of early chemotherapy in asymptomatic metastatic colorectal cancer*. Br J Cancer, 2005. **93**(11): p. 1236-43.
156. Cairns, S.R., et al., *Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002)*. Gut, 2010. **59**(5): p. 666-89.
157. Hartley, A., et al., *Pathological complete response following pre-operative chemoradiotherapy in rectal cancer: analysis of phase II/III trials*. Br J Radiol, 2005. **78**(934): p. 934-8.
158. Habr-Gama, A., et al., *Watch and wait approach following extended neoadjuvant chemoradiation for distal rectal cancer: are we getting closer to anal cancer management?* Dis Colon Rectum, 2013. **56**(10): p. 1109-17.
159. Maas, M., et al., *Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data*. Lancet Oncol, 2010. **11**(9): p. 835-44.
160. Hiotis, S.P., et al., *Assessing the predictive value of clinical complete response to neoadjuvant therapy for rectal cancer: an analysis of 488 patients*. J Am Coll Surg, 2002. **194**(2): p. 131-5; discussion 135-6.
161. Zmora, O., et al., *Does rectal wall tumor eradication with preoperative chemoradiation permit a change in the operative strategy?* Dis Colon Rectum, 2004. **47**(10): p. 1607-12.
162. Pucciarelli, S., et al., *Relationship between pathologic T-stage and nodal metastasis after preoperative chemoradiotherapy for locally advanced rectal cancer*. Ann Surg Oncol, 2005. **12**(2): p. 111-6.
163. Maas, M., et al., *Assessment of Clinical Complete Response After Chemoradiation for Rectal*

Cancer with Digital Rectal Examination, Endoscopy, and MRI: Selection for Organ-Saving Treatment. Ann Surg Oncol, 2015. **22**(12): p. 3873-80.

164. Araujo, R.O., et al., *Nonoperative management of rectal cancer after chemoradiation opposed to resection after complete clinical response. A comparative study*. Eur J Surg Oncol, 2015. **41**(11): p. 1456-63.
165. Dalton, R.S., et al., *A single-centre experience of chemoradiotherapy for rectal cancer: is there potential for nonoperative management?* Colorectal Dis, 2012. **14**(5): p. 567-71.
166. Habr-Gama, A., *Assessment and management of the complete clinical response of rectal cancer to chemoradiotherapy*. Colorectal Dis, 2006. **8 Suppl 3**: p. 21-4.
167. Maas, M., et al., *Wait-and-see policy for clinical complete responders after chemoradiation for rectal cancer*. J Clin Oncol, 2011. **29**(35): p. 4633-40.
168. Smith, J.D., et al., *Nonoperative management of rectal cancer with complete clinical response after neoadjuvant therapy*. Ann Surg, 2012. **256**(6): p. 965-72.
169. Yeo, S.G., D.Y. Kim, and J.H. Oh, *Long-term survival without surgery following a complete response to pre-operative chemoradiotherapy for rectal cancer: A case series*. Oncol Lett, 2013. **6**(6): p. 1573-1576.
170. Yu SK, B.G., Heald RJ., *Deferral of rectal surgery following a continued response to preoperative chemoradiotherapy (Watch and Wait) study: a phase II multicenter study in the United Kingdom*. J Clin Oncol, 2011. **29**(abstract 489).
171. *Minimal Invasive Strategies for Good and Complete Response to Chemoradiation in Rectal Cancer*. http://www.clinicaltrials.gov, 2009.
172. Martens, M.H., et al., *Long-term Outcome of an Organ Preservation Program After Neoadjuvant Treatment for Rectal Cancer*. J Natl Cancer Inst, 2016. **108**(12).
173. de Ridder, J.A., 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**: p. 13-21.
174. Heinemann, V., 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): p. 1065-75.
175. Loupakis, F., et al., *Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer*. N Engl J Med, 2014. **371**(17): p. 1609-18.
176. van der Geest, L.G., et al., *Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases*. Clin Exp Metastasis, 2015. **32**(5): p. 457-65.
177. Ayez, N., et al., *Long-term results of the "liver first" approach in patients with locally advanced rectal cancer and synchronous liver metastases*. Dis Colon Rectum, 2013. **56**(3): p. 281-7.
178. House, M.G., et al., *Survival after hepatic resection for metastatic colorectal cancer: trends in outcomes for 1,600 patients during two decades at a single institution*. J Am Coll Surg, 2010. **210**(5): p. 744-52, 752-5.
179. van der Pool, A.E., et al., *Optimizing the outcome of surgery in patients with rectal cancer and synchronous liver metastases*. Br J Surg, 2010. **97**(3): p. 383-90.
180. Chua, T.C., et al., *Predictors of cure after hepatic resection of colorectal liver metastases: an analysis of actual 5- and 10-year survivors*. J Surg Oncol, 2011. **103**(8): p. 796-800.
181. Vigano, L., et al., *Liver surgery for colorectal metastases: results after 10 years of follow-up. Long-*

term survivors, late recurrences, and prognostic role of morbidity. Ann Surg Oncol, 2008. **15**(9): p. 2458-64.

182. Gonzalez, M., et al., *Risk factors for survival after lung metastasectomy in colorectal cancer patients: a systematic review and meta-analysis*. Ann Surg Oncol, 2013. **20**(2): p. 572-9.
183. Zampino, M.G., et al., *Lung metastases from colorectal cancer: analysis of prognostic factors in a single institution study*. Ann Thorac Surg, 2014. **98**(4): p. 1238-45.
184. Migliore, M., et al., *Finding the evidence for pulmonary metastasectomy in colorectal cancer: the PulMicc trial*. Future Oncol, 2015. **11**(2 Suppl): p. 15-8.
185. Treasure, T. and F. Macbeth, *The GILDA trial finds no survival benefit from intensified screening after primary resection of colorectal cancer: the PulMiCC trial tests the survival benefit of pulmonary metastasectomy for detected asymptomatic lung metastases*. Ann Oncol, 2016. **27**(4): p. 745.
186. de Cuba, E.M., et al., *Cytoreductive surgery and HIPEC for peritoneal metastases combined with curative treatment of colorectal liver metastases: Systematic review of all literature and meta-analysis of observational studies*. Cancer Treat Rev, 2013. **39**(4): p. 321-7.
187. Elias, D., F. Quenet, and D. Goere, *Current status and future directions in the treatment of peritoneal dissemination from colorectal carcinoma*. Surg Oncol Clin N Am, 2012. **21**(4): p. 611-23.
188. Esquivel, J., et al., *The American Society of Peritoneal Surface Malignancies (ASPSM) Multiinstitution Evaluation of the Peritoneal Surface Disease Severity Score (PSDSS) in 1,013 Patients with Colorectal Cancer with Peritoneal Carcinomatosis*. Ann Surg Oncol, 2014. **21**(13): p. 4195-201.
189. Leung, U., et al., *Colorectal Cancer Liver Metastases and Concurrent Extrahepatic Disease Treated With Resection*. Ann Surg, 2016.
190. ORCHESTRA, *A Randomized Multicenter Clinical Trial for Patient With Multi-organ, Colorectal Cancer Metastases Comparing the Combination of Chemotherapy and Maximal Tumor Debulking Versus Chemotherapy Alone*. http://www.clinicaltrials.gov, 2012.
191. Sotiropoulos, G.C. and H. Lang, *Clinical scoring systems for predicting outcome after surgery for colorectal liver metastases: towards a better multidisciplinary approach*. Liver Int, 2009. **29**(1): p. 6-9.
192. Adam, R., et al., *Repeat hepatectomy for colorectal liver metastases*. Ann Surg, 1997. **225**(1): p. 51-60; discussion 60-2.
193. Butte, J.M., et al., *Recurrence After Partial Hepatectomy for Metastatic Colorectal Cancer: Potentially Curative Role of Salvage Repeat Resection*. Ann Surg Oncol, 2015. **22**(8): p. 2761-71.
194. de Jong, M.C., et al., *Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis*. J Gastrointest Surg, 2009. **13**(12): p. 2141-51.
195. Lam, V.W., et al., *A systematic review of repeat hepatectomy for recurrent colorectal liver metastases*. J Gastrointest Surg, 2013. **17**(7): p. 1312-21.
196. van der Pool, A.E., et al., *Local treatment for recurrent colorectal hepatic metastases after partial hepatectomy*. J Gastrointest Surg, 2009. **13**(5): p. 890-5.
197. Chen, F., et al., *Repeat resection of pulmonary metastasis is beneficial for patients with colorectal*

carcinoma. World J Surg, 2010. **34**(10): p. 2373-8.

198. Bhattacharjya, S., R. Aggarwal, and B.R. Davidson, *Intensive follow-up after liver resection for colorectal liver metastases: results of combined serial tumour marker estimations and computed tomography of the chest and abdomen - a prospective study*. Br J Cancer, 2006. **95**(1): p. 21-6.
199. Connor, S., et al., *Follow-up and outcomes for resection of colorectal liver metastases in Edinburgh*. Eur J Surg Oncol, 2007. **33**(1): p. 55-60.
200. Gomez, D., et al., *Outcomes of intensive surveillance after resection of hepatic colorectal metastases*. Br J Surg, 2010. **97**(10): p. 1552-60.
201. Langenhoff, B.S., P.F. Krabbe, and T.J. Ruers, *Efficacy of follow-up after surgical treatment of colorectal liver metastases*. Eur J Surg Oncol, 2009. **35**(2): p. 180-6.
202. Metcalfe, M., et al., *Detecting curable disease following hepatectomy for colorectal metastases*. ANZ J Surg, 2005. **75**(7): p. 524-7.
203. Metcalfe, M.S., E.J. Mullin, and G.J. Maddern, *Choice of surveillance after hepatectomy for colorectal metastases*. Arch Surg, 2004. **139**(7): p. 749-54.
204. Wilhelmsen, M., et al., *Determinants of recurrence after intended curative resection for colorectal cancer*. Scand J Gastroenterol, 2014. **49**(12): p. 1399-408.
205. Belt, E.J., et al., *Peri-operative bowel perforation in early stage colon cancer is associated with an adverse oncological outcome*. J Gastrointest Surg, 2012. **16**(12): p. 2260-6.
206. Honore, C., et al., *Definition of patients presenting a high risk of developing peritoneal carcinomatosis after curative surgery for colorectal cancer: a systematic review*. Ann Surg Oncol, 2013. **20**(1): p. 183-92.
207. Cortet, M., et al., *Patterns of recurrence of obstructing colon cancers after surgery for cure: a population-based study*. Colorectal Dis, 2013. **15**(9): p. 1100-6.
208. Krarup, P.M., et al., *Anastomotic leak increases distant recurrence and long-term mortality after curative resection for colonic cancer: a nationwide cohort study*. Ann Surg, 2014. **259**(5): p. 930-8.
209. Busch, O.R., et al., *Blood transfusions and prognosis in colorectal cancer*. N Engl J Med, 1993. **328**(19): p. 1372-6.
210. Elias, D., et al., *Results of systematic second-look surgery in patients at high risk of developing colorectal peritoneal carcinomatosis*. Ann Surg, 2008. **247**(3): p. 445-50.
211. Elias, D., et al., *Results of systematic second-look surgery plus HIPEC in asymptomatic patients presenting a high risk of developing colorectal peritoneal carcinomatosis*. Ann Surg, 2011. **254**(2): p. 289-93.
212. Ripley, R.T., et al., *Prospective randomized trial evaluating mandatory second look surgery with HIPEC and CRS vs. standard of care in patients at high risk of developing colorectal peritoneal metastases*. Trials, 2010. **11**: p. 62.
213. *Second-look Surgery With or Without Intraperitoneal Chemotherapy in Treating Patients With Recurrent Colorectal Cancer*. http://www.clinicaltrials.gov, 2000.
214. de Wijkerslooth, T.R., et al., *Immunochemical fecal occult blood testing is equally sensitive for proximal and distal advanced neoplasia*. Am J Gastroenterol, 2012. **107**(10): p. 1570-8.

215. Edelman, B.R. and M.R. Weiser, *Endorectal ultrasound: its role in the diagnosis and treatment of rectal cancer*. Clin Colon Rectal Surg, 2008. **21**(3): p. 167-77.

AUTHOR CONTRIBUTIONS

E.P.v.d.S., C.V. and E.J.K. researched the data for article. All author contributed substantially to discussions of content. E.P.v.d.S., C.V. and E.J.K. wrote the manuscript. M.C.W.S., D.J.G., C.V., and E.J.K. reviewed/edited the manuscript before submission.

COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REVIEW CRITERIA

We searched PubMed, Google Scholar, and the Cochrane Library databases using the following search terms: "colorectal neoplasms", "surveillance", "surgery", "metastases", "liver", "hepatectomy", "follow-up", "colonoscopy", "CT-colonography", "CT-scan", "MRI", "PET", "PET–CT", "PET–MRI", "faecal immunochemical testing", "faecal DNA", "blood DNA", "liquid biopsies", "breath test", and "urine peptides". The last search was completed on 15th April 2016. No search restrictions with respect to publication language or dates were applied. Other references for this Review were identified in the reference lists of the identified articles and by searching the authors' own bibliographic files. Reference were included based on relevance to the subject of the manuscript, with priority given to reports of prospective randomized trials and meta-analyses; if this level of evidence was absent, cohort studies or reviews of such studies were selected for inclusion.

S1 (table): Randomized trials comparing different disease postoperative surveillance strategies in patients with stage I–III CRC

Study (year of publication)	Number of pts (recruitment period)	Stage of CRC	Surveillance strategy	Recurrences in intensive vs less-intensive surveillance arms			Mean ± SD or median time to recurrence (months)	Primary end point	OS rate* or HR (intensive vs less-intensive approach)
				Total (% of pts)	Treatable (% of pts)	% of total that were treatable			
Treasure *et al.*S1 (2014)	216 (1982–1993)	Dukes A,B,C	**Second-look surgery arm:** in the case of elevated CEA, the clinician was informed and 'second look surgery' with intention to remove any recurrence discovered was performed. CEA was determined monthly for the first 3 years and every 3 months for the next 2 years **No second-look surgery arm:** in the case of elevated CEA, the clinician was not informed. CEA was determined monthly for the first 3 years and and 3 monthly for the next 2 years	83 (77%) vs 89 (82%)	62 (57%) vs 26 (24%)	75% vs 29%	NR	2-year OS	Death rate up to 1993: 84% vs 81%; NS
Ohlsson *et al.*S2 (1995)	107 (1983–1986)	Dukes A,B,C	**Intensive:** Visit every 3 months for 2 years, then 6 months for 3 years with physical examination, CEA testing, alkaline phosphatases, gamma-glutamyltransferase, faecal haemoglobin, rigid proctosigmoidoscopy, chest radiography. Endoscopic control of anastomosis at 9, 21 and 42 months. Complete colonoscopy at 3, 15, 30, 60 months. CT of the pelvis for patients undergoing abdominoperineal resection at 3, 6, 12, 18, 24 months **Minimal:** No visits planned. Written instruction recommending leaving faecal samples with the district nurse for haemoglobin examination every 3 months for 2 years, then every 12 months	17 (32%) vs 18 (33%)	5 (9%) vs 3 (6%)	29% vs 17%	20 vs 24	Disease-free survival	75% vs 67%; NS
Kjeldsen *et al.*S3 (1997)	597 (1983–1994)	Dukes A,B,C	**Intensive surveillance:** medical history, clinical examination, digital rectal examination, gynaecological examination, haemoccult test, colonoscopy, chest radiography, haemoglobin level, erythrocyte sedimentation rate, and liver enzymes at 6, 12, 18, 2, 30, 36, 48, 60, 120, 150, 180 months **Less-intensive surveillance:** medical history, clinical examination, digital rectal examination, gynaecological examination, haemoccult test, colonoscopy, chest radiography, haemoglobin level, erythrocyte sedimentation rate, and liver enzymes at 60, 120, 180 months	81 (28%) vs 83 (27%)	17 (6%) vs 5 (2%)	21% vs 6%	18 vs 27	5-year OS	70% vs 68%; NS
Schoemaker *et al.*S4 (1998)	325 (1984–1990)	Dukes A,B,C	**Intensive imaging surveillance:** visit every 3 months for 2 years, then every 6 months for 5 years, with medical history, clinical examination, complete blood profile, liver function tests, CEA testing, faecal occult blood test. Chest radiography, CT of the liver, and colonoscopy every 12 months **Less-intensive imaging surveillance:** visit every 3 months for 2 years, then every 6 months for 5 years, with medical history, clinical examination, complete blood profile, liver function tests, CEA testing, faecal occult blood test. Chest radiography, CT of the liver and colonoscopy only after completing 5 years of follow-up assessments	56 (34%) vs 64 (41%)	6 (4%) vs 5 (3%)	11% vs 8%	NR	5-year OS	78% vs 72%‡; NS

S1 (table): Randomized trials comparing different disease postoperative surveillance strategies in patients with stage I–III CRC (continue)

Study (year of publication)	Number of pts (recruitment period)	Stage of CRC	Surveillance strategy	Recurrences in intensive vs less-intensive surveillance arms			Mean ± SD or median time to recurrence (months)	Primary end point	OS rate* or HR (intensive vs less-intensive approach)
				Total (% of pts)	Treatable (% of pts)	% of total that were treatable			
Pietra et al.S5 (1998)	207 (1987–1990)	Dukes B,C	**Intensive surveillance:** visit every 3 months for 2 years, then every 6 months for 3 years, then every 12 months, with clinical examination, ultrasonography, and CEA testing. Colonoscopy at 3 months after surgery. Chest radiography, colonoscopy, and CT every 12 months **Less-intensive surveillance:** visit every 6 months for 1 year, then every 12 months, with clinical examination, ultrasonography, CEA testing. Colonoscopy at 3 months after surgery. Chest radiography and colonoscopy every 12 months	41 (39%) vs 42 (41%)	21 (20%) vs 6 (6%)	51% vs 14%	10.3 ± 2.7 vs 20.2 ± 6.1	5-year OS	73% vs 58%; P = 0.02
Mäkela et al.S6 (1995)	106 (1988–1990)	Dukes A,B,C	**Intensive imaging surveillance:** Visit every 3 months for 2 years, then every 6 months for 3 years, with medical history, clinical examination, complete blood-cell counts, tests for occult faecal bleeding, CEA testing, chest radiography. Colonoscopy with video imaging 3 months after surgery if not performed preoperatively, and every 12 months thereafter. Flexible fibre sigmoidoscopy with video imaging every 3 months for rectal or sigmoid cancers. Liver ultrasonography every 6 months, and CT of the liver and site of primary tumour every 12 months **Less-intensive imaging surveillance:** Visit every 3 months for 2 years, then every 6 months for 3 years, with medical history, clinical examination, complete blood-cell counts, tests for occult faecal bleeding, CEA testing, chest radiography. Rigid sigmoidoscopy for patients with rectal or sigmoid cancers; barium enema at 12 months and every 12 months thereafter	22 (42%) vs 21 (39%)	5 (10%) vs 3 (6%)	22% vs 14%	10 ± 5 vs 15 ± 10	5-year OS	59% vs 54%; NS
Secco et al.S7 (2002)	358 (1988–1996)	Low or high risk§	**High-risk group (intensive):** visit every 3 months for 2 years, every 4 months in year 3, every 6 months in years 4 and 5 with CEA testing. Abdominal and pelvic ultrasonography every 3 months for the first 3 years and every 12 months in years 4 and 5. Rigid rectosimoidoscopy for rectal cancer and chest radiography every 12 months **Low-risk group (low intensity):** visit every 6 months for 2 years, the every 12 months for 3 years. Abdominal and pelvic ultrasound every 6 months in the first 2 years, then every 12 months for 3 years. Rigid rectosigmoidoscopy for rectal cancer every 12 months for 2 years, then every 24 months. Chest X-ray every 12 months **Risk-stratified control groups (minimal follow-up testing):** not specified	Low risk: 27 (32%) vs 25 (40%) High risk: 74 (69%) vs 58 (69%)	Low risk: 6 (7%) vs 6 (10%) High risk: 25 (23%) vs 7 (8%)	Low risk: 22% vs 24% High risk: 34% vs 12%	Low risk: 16 vs 14 High risk: 13.5 vs 8	5-year OS	Low risk: 80% vs 50%; P <0.001 High risk: 60% vs 32%; P <0.01

S1 (table): Randomized trials comparing different disease postoperative surveillance strategies in patients with stage I–III CRC (continue)

Study (year of publication)	Number of pts (recruitment period)	Stage of CRC	Surveillance strategy	Recurrences in intensive vs less-intensive surveillance arms			Mean ± SD or median time to recurrence (months)	Primary end point	OS rate* or HR (intensive vs less-intensive approach)
				Total (% of pts)	Treatable (% of pts)	% of total that were treatable			
Wang et al.S8 (2009)	326 (1995–2001)	Dukes A,B,C	**Intensive colonoscopy surveillance:** Visit every 3 months for 1 year, then every 6 months for 2 years, then every 12 months for 2 years with medical history, clinical examination, CEA testing, chest radiography, liver imaging (CT or ultrasonography). Colonoscopy at each visit **Routine colonoscopy surveillance:** Visit every 3 months for 1 year, then every 6 months for 2 years, then every 12 months for 2 years with medical history, clinical examination, CEA testing, chest radiography, liver imaging (CT or ultrasonography). Colonoscopy at 6, 30, 60 months Intensive vs 'routine' colonoscopy surveillance	13 (8%) vs 18 (11%)	9 (5%) vs 6 (4%)	69% vs 33%	22 ± 17.6 vs 35 ± 23.9	5-year OS	77% vs 73%; NS
Rodriguez-Moranta et al.S9 (2006)	259 (1997–2001)	AJCC II–III	**Intensive imaging surveillance:** visit 3 months for 2 years, then every 6 months for 3 years with clinical examination, complete blood-cell count, liver function tests, CEA testing. Colonoscopy and chest radiography every 12 months. Abdominal CT for rectal cancer and ultrasonography for colon cancer every 6 months for 2 years, then every 12 months for 3 years **Less-intensive imaging surveillance:** visit every 3 months for 2 years, then every 6 months for 3 years with clinical examination, complete blood-cell count, liver function tests, and CEA testing. Colonoscopy within the first 12 months and in year 3	35 (27%) vs 34 (26%)	18 (14%) vs 10 (8%)	51% vs 29%	39 ± 21 vs 38 ± 19	5-year OS	HR 0.87; NS Pts with AJCC stage II CRC: HR 0.34; P = 0.045 Pts with rectal cancer: HR 0.09; P = 0.03
Wattchow et al.10 (2006)	203 (1998–2001)	Dukes A,B,C; no rectal cancer	**Intramural (surgeon-led):** visit every 3 months for 2 years, then every 6 months for 3 years. Faecal occult blood test every 12 months, colonoscopy every 36 months **Extramural (general-practitioner-led):** Extramural (general-practitioner -led): same as in intramural arm, but coordinated by general-practitioner	NR	NR	NR	8 vs 9.5	Quality of Life, depression, anxiety, and patient satisfaction	Median: 31 months vs 20 months; NS
GILDA‖: Grossmann et al.S11 (2004)	985 (1998–2004)	Dukes B2,C	**Intensive surveillance:** visit every 4 months for 2 years, then every 6 months for 3 years with CEA testing. Colonoscopy at 12 and 48 months. Liver ultrasonography at 4 and 16 months **Less-intensive surveillance:** Visit every 4 months for 2 years, then every 6 months for 3 years with CEA testing, complete blood count, liver function tests, and CA 19-9 testing. Colonoscopy at 12, 24, 36, 48, 60 months. Liver ultrasonography at 4, 8, 12, 16, 24, 36, 48, 60 months. Chest radiography at 12, 24, 36, 48 and 60 months	75 (15%) vs 64 (13%)	NR	NR	NR	5-year OS	Death rate up to 2004: 7% vs 5% ; P = NR

S1 (table): Randomized trials comparing different disease postoperative surveillance strategies in patients with stage I–III CRC (continue)

Study (year of publication)	Number of pts (recruitment period)	Stage of CRC	Surveillance strategy	Recurrences in intensive vs less-intensive surveillance arms			Mean ± SD or median time to recurrence (months)	Primary end point	OS rate* or HR (intensive vs less-intensive approach)
				Total (% of pts)	Treatable (% of pts)	% of total that were treatable			
GILDA: Rosati et al.S12 (2016)	1,228 (1998–2006)	Dukes B2,C	As reported above	135 (22%) vs 115 (19%)	57 (9%) vs 46 (8%)	42% vs 40%	NR	5-year OS	HR 1.14; NS
Sobhani et al.S13 (2008)	130 (2001–2004)	AJCC III–IV	**Surveillance including 18F FDG-PET:** Visit every 3 months with physical examination, serum CEA or CA19-9 testing (or both), and ultrasonography (except at 9 and 15 months). Chest radiography every 6 months, abdominal CT at 9 and 15 months, 18F-FDG-PET at 9 and 15 months **Surveillance excluding 18F FDG-PET:** same as in other arm, but omitting 18F FDG-PET The study follow-up protocol started from 9 months after surgery, and continued to 24 months or patient death. The total study comprised 6 visits per arm	23 (35%) vs 21 (32%)	10 (15%) vs 2 (3%)	43% vs10%	12 ± 4.9 vs 15.3 ± 4.9	Treatment of recurrence with curative intent	NR
Primrose et al.S14 (2014)	1,202 (2003–2009)	AJCC I–III	**1. CEA arm:** CEA testing every 3 months for 2 years, then every 6 months for 3 years, with single CT of the thorax/abdomen at 12–18 months, if requested at study entry by hospital clinician **2. CT arm:** CT of the thorax/abdomen every 6 months for 2 years, then every 12 months for 3 years **3. CEA + CT arm:** CEA testing every 3 months for 2 years, then every 6 months for 3 years, with CT of the thorax/abdomen every 6 months for 2 years, then every 12 months for 3 years **4. Minimal arm:** no scheduled follow-up assessments, except a single CT of the thorax/abdomen at 12–18 months, if requested at study entry by hospital clinician	1: 57 (19%) 2: 57 (19%) 3: 48 (16%) 4: 37 (12%)	1: 20 (7%) 2: 24 (8%) 3: 20 (7%) 4: 7 (2%)	1. 35% 2. 42% 3. 42% 4. 19%	1: 45 ± 18 2: 44 ± 19 3: 45 ± 19 4: 44 ± 20	Treatment of recurrence with curative intent	Death rate at mean of 4.4 years (SD 0.8) 18.2% (arms 1–3 combined) vs 15.9% (arm 4); NS
Augestad et al.S15 (2013)	110 (2007–2011)	Dukes A,B,C	**Intramural (surgeon-led):** clinical examination and CEA every 3 months, chest X-ray and liver ultrasound at 6, 12, 18 and 24 months, colonoscopy at 12 months **Extramural (general-practitioner -led):** same as in intramural arm, but coordinated by general-practitioner The study follow-up protocol started 1 month after surgery, and continued to 24 months or patient death. The total study comprised 9 visits per arm	8 (15%) vs 6 (11%)	4 (7%) vs 3 (5%)	50% vs 50%	NR	Quality of Life	Death rate 7% vs 2% at 2 years

^{18}F-FDG-PET, 2-[^{18}F]fluoro-2-deoxy-D-glucose PET; AJCC: American Joint Committee on Cancer; CA 19-9, Cancer antigen 19-9; CEA, carcinoembryonic antigen; CRC, colorectal cancer; HR, hazard ratio; NR, not reported; NS, not statistically significant; OS, overall survival; pts, patients; SD, standard deviation. *5-year OS unless otherwise stated. ‡Estimated visually from survival curve. §High-risk patients had CEA >7.5 ng/ml; Dukes B3 or T3 left colon cancer; Dukes C disease; low anterior resection for rectal cancer; or a poorly differentiated, mucinous, or signet-ring histology. Low-risk patients had any other form of disease. ||Interim analysis.

S1. Treasure, T. *et al*. The CEA Second-Look Trial: a randomised controlled trial of carcinoembryonic antigen prompted reoperation for recurrent colorectal cancer. *BMJ Open* **4**, e004385 (2014).

S2. Ohlsson, B. *et al*. Follow-up after curative surgery for colorectal carcinoma. Randomized comparison with no follow-up. *Dis. Colon Rectum* **38**, 619–626 (1995).

S3. Kjeldsen, B. J. *et al*. A prospective randomized study of follow-up after radical surgery for colorectal cancer. *Br. J. Surg.* **84**, 666–669 (1997).

S4. Schoemaker, D. *et al*. Yearly colonoscopy, liver CT, and chest radiography do not influence 5-year survival of colorectal cancer patients. *Gastroenterology* **114**, 7–14 (1998).

S5. Pietra, N. *et al*. Role of follow-up in management of local recurrences of colorectal cancer: a prospective, randomized study. *Dis. Colon Rectum* **41**, 1127–1133 (1998).

S6. Makela, J. T., Laitinen, S. O. & Kairaluoma, M. I. Five-year follow-up after radical surgery for colorectal cancer. Results of a prospective randomized trial. *Arch. Surg*. **130**, 1062–1067 (1995).

S7. Secco, G. B. *et al*. Efficacy and cost of risk-adapted follow-up in patients after colorectal cancer surgery: a prospective, randomized and controlled trial. *Eur. J. Surg. Oncol*. **28**, 418–423 (2002).

S8. Wang, T. *et al*. The role of postoperative colonoscopic surveillance after radical surgery for colorectal cancer: a prospective, randomized clinical study. *Gastrointest. Endosc*. **69**, 609–615 (2009).

S9. Rodriguez-Moranta, F. *et al*. Postoperative surveillance in patients with colorectal cancer who have undergone curative resection: a prospective, multicenter, randomized, controlled trial. *J. Clin. Oncol*. **24**, 386–393 (2006).

S10. Wattchow, D. A. *et al.* General practice vs surgical-based follow-up for patients with colon cancer: randomised controlled trial. *Br. J. Cancer* **94**, 1116–1121 (2006).

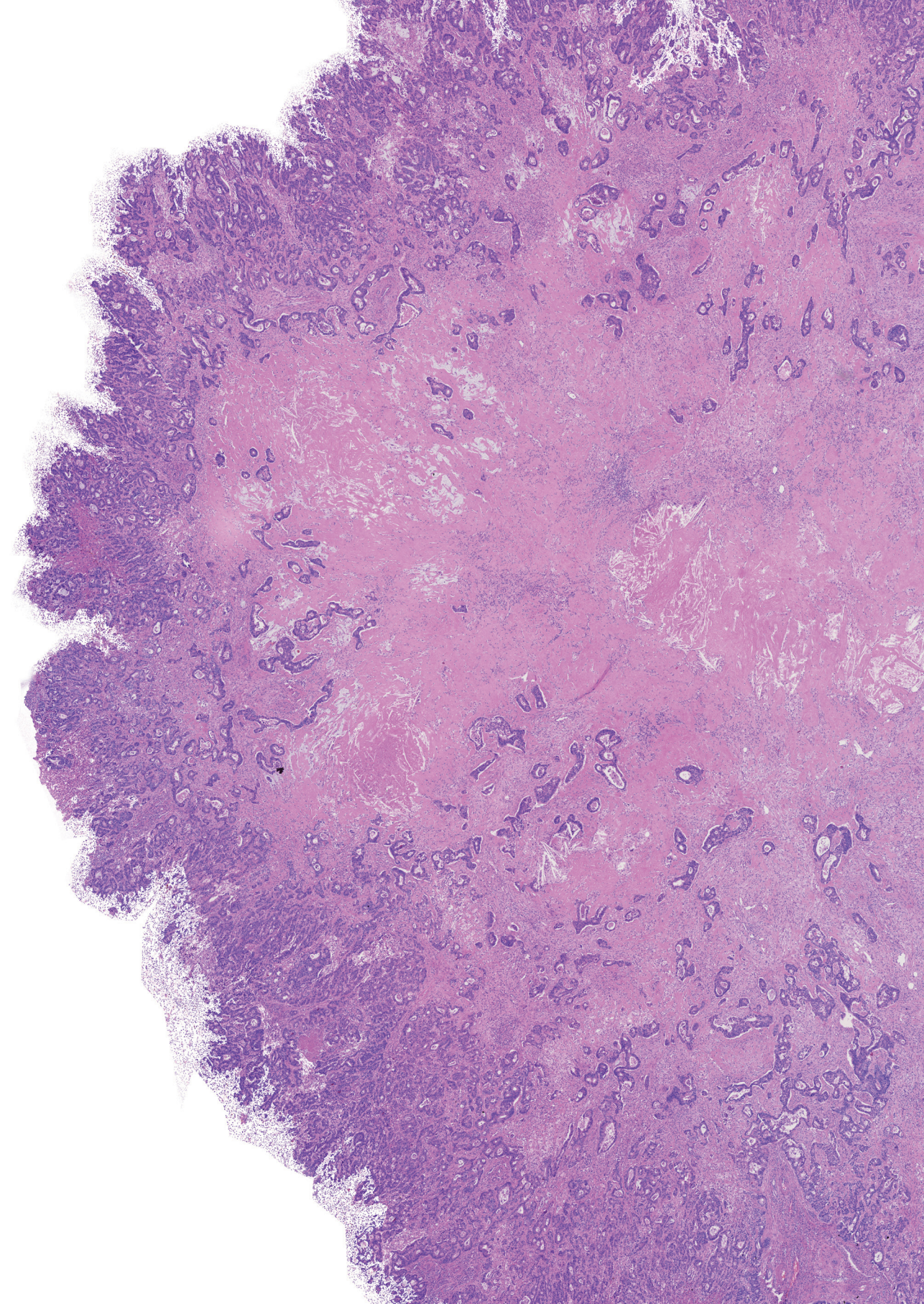
S11. Grossmann, E. M. *et al*. Follow-up of colorectal cancer patients after resection with curative intent-the GILDA trial. *Surg. Oncol*. **13**, 119–124 (2004).

S12. Rosati, G. *et al*. A randomized trial of intensive versus minimal surveillance of patients with resected Dukes B2–C colorectal carcinoma. *Ann. Oncol.* **27**, 274–280 (2016).

S13. Sobhani, I. *et al*. Early detection of recurrence by ^{18}FDG-PET in the follow-up of patients with colorectal cancer. *Br. J. Cancer* **98**, 875–880 (2008).

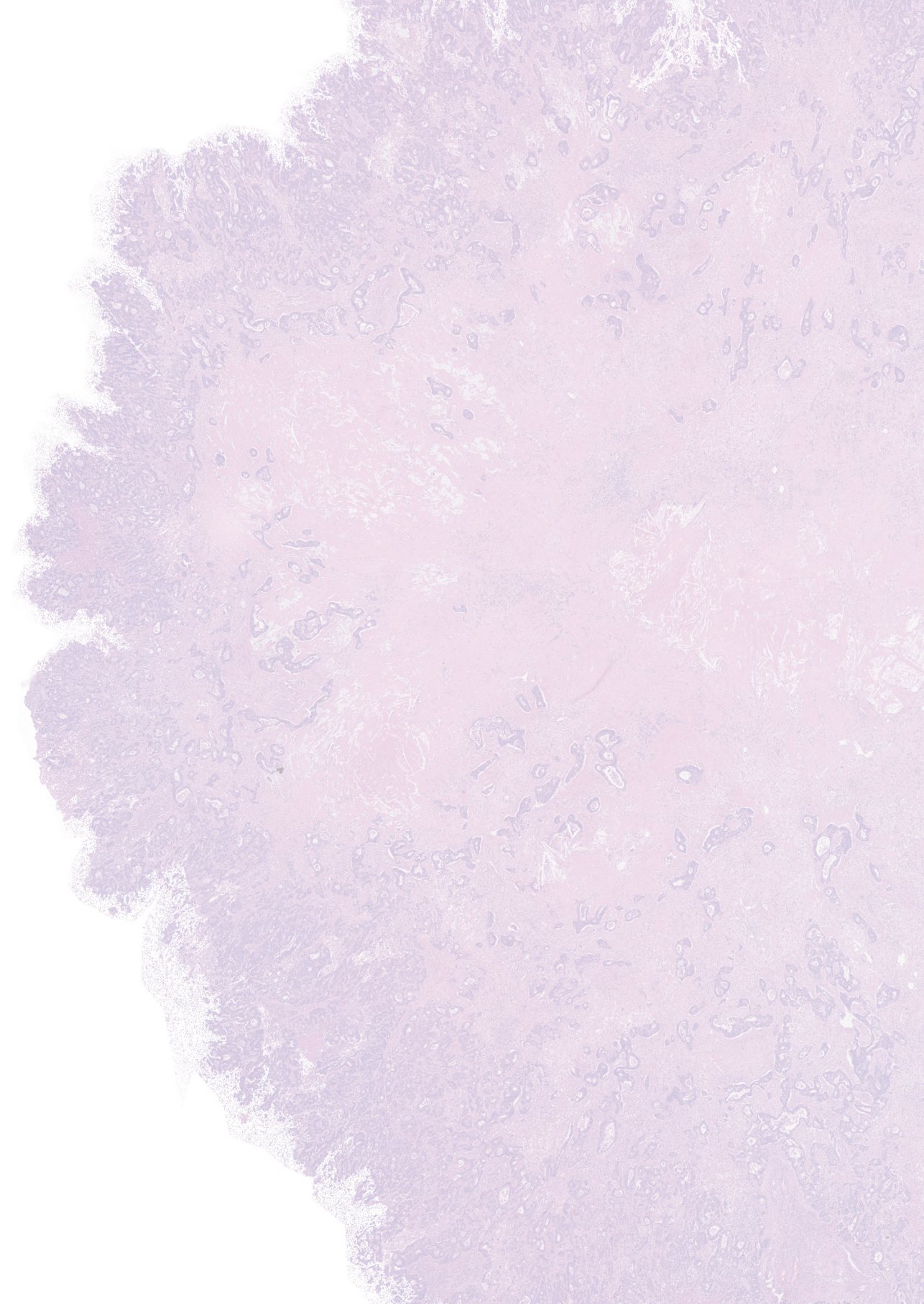
S14. Primrose, J. N. *et al.* Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. *JAMA* **311**, 263–270 (2014).

S15. Augestad, K. M. *et al*. Cost-effectiveness and quality of life in surgeon versus general practitioner-organised colon cancer surveillance: a randomised controlled trial. *BMJ Open* **3**, e002391 (2013).



PART IV

Discussion and Future Perspectives, Summary and Appendices



CHAPTER 14

General Discussion and Future Perspectives

CHEMOTHERAPY FOR RESECTABLE COLORECTAL LIVER METASTASES AND SURGERY FOR MULTI-ORGAN METASTASES

Systemic treatment for colorectal liver metastases (CRLM) is increasingly effective, with regains including oxaliplatin, irinotecan, and biologicals such as bevacizumab (monoclonal antibody against vascular endothelial growth factor) significantly improving overall survival (OS). Combining these regimens showed median overall survival of >30 months without local treatment in patients with metastasized colorectal cancer (mCRC) [2]. The high relapse rates after resection of CRLM, and the efficacy of modern systemic chemotherapy in the metastasized setting, have prompted investigators to perform studies to evaluate the role of systemic chemotherapy combined with liver resection. In four randomized trials, liver resection was followed by adjuvant systemic chemotherapy or observation [3-6]. Two of these trials included few patients and were inadequately powered [4, 5]. A Canadian/ European Intergroup study (ENG trial) closed early due to poor accrual [3]. In the other trial (a multicenter randomized trial) of 173 patients who received bolus FU/LV after liver resection vs. observation with median follow-up of 87.4 months, there was no survival benefit [6]. The small numbers accrued over 10 years and the outdated chemotherapy used, highlight the difficulties in conducting an adjuvant study after liver resection. The only successful trial is the EORTC 40983 trial that compared peri-operative FOLFOX-4 chemotherapy (6 cycles pre-operative and 6 cycles post-operative) in patients with resectable CRLM. Mature data showed no OS benefit from peri-operative chemotherapy in resectable patients [7].

In 2013 a questionnaire was sent to 27 Dutch hospitals with expertise in the area of hepatobiliary surgery [8]. The questionnaire focused on indications for (neo-) adjuvant chemotherapy for resectable colorectal liver metastases (CRLM) [8]. The results were striking: there was great variation in use of chemotherapy in resectable patients with great variation in indications for administration of chemotherapy. There seems to be no consensus, even though Dutch guidelines do not recommend standard administration of chemotherapy in resectable disease. However, various retrospective reports (including **chapter 4**), showed potential benefit in high-risk groups [9-11]. There was an obvious need for the CHARISMA trial, published in **chapter 1**.

The CHARISMA multicenter randomized trial opened in 13 Dutch hospitals. The trial included 14 patients in approximately 2 years. Main reasons for poor inclusion were patient preferences for surgical treatment without any delay; randomization for 6 cycles of chemotherapy would delay local treatment by 4 months. Another hurdle was the clinical risk score as an inclusion criterion. Very few patients present with a CEA>200ng/ml or a metastasis >5 cm. Patients with synchronous disease at presentation have no pathological lymph node status regarding their primary tumor (yet). Only a subset of patients would therefore become eligible (≥3 points according to the CRS [12]) for randomization. To overcome these challenges, the CHARISMA protocol was amended and patients may be randomized in the future for adjuvant chemotherapy instead (resolving the issue with patient preference and nodal status of primary CRC). There is an urgent need for clarification regarding the dogma of

"chemotherapy for resectable CRLM": even though (neo-) adjuvant randomized trials prove to be challenging we still need one.
For patients with a low-risk profile (Fong 0-2), a recent collaborative investigation (Erasmus MC & Memorial Sloan Kettering Cancer Center) objectified that this subgroup might benefit from hepatic arterial infusion (regional chemotherapy in addition to resection) [15]. The investigators initiated a randomized controlled trial in the Netherlands for this patient subgroup as well, randomizing between surgery alone versus adjuvant arterial infusion (PUMP trial). Results of both the CHARISMA-trial and the PUMP-trial will potentially enable us to tailor adjuvant systemic and regional treatment strategies for patients with CRLM.
In cases where patients present with multi-organ metastases of colorectal cancer, palliative systemic therapy is standard of care. The ORCHESTRA trial was initiated, randomizing patients for either systemic treatment or systemic treatment with additional tumor debulking. This is an ongoing trial, with significant logistic and safety implications. A safety and feasibility report was published in **chapter 2**. The protocol showed to be feasible and safe, now in place in over 30 hospitals in the Netherlands and including over 200 patients. In a few years we will see if additional tumor debulking of multi-organ metastases will lead to OS benefit.

MULTICENTER RESEARCH IN THE NETHERLANDS

The Netherlands ranks internationally among the countries with the highest scientific output, both in volume and quality [16, 17]. This reputation is at risk, as several reports produced by industry and academics on the initiation of trials show increasing delay and costs due to increasing complexity of procedures [18-23].
A recent evaluation of the procedure of obtaining local approval in individual hospitals of 2 large multicenter oncological trials is outlined in **chapter 3**. This procedure is inherent to initiating multicenter clinical trials in the Netherlands and subject to significant complexity. This issue is recognized in other countries as well [16, 24-29]. Adding the time of initiation to actual accrual of patients and follow-up means that implementation of potential novel therapies is delayed. Trial logistics should be on a national agenda, to benefit all future trials. Procedures should be standardized, centralized and digitized. The latter will be implemented by 2019 on a European level by the central committee on Research Involving Human Subjects (CCMO) and the Dutch Clinical Research Foundation (DCRF). For other challenges, senior investigators should be involved on a national level, together with the central committee on Research Involving Human Subjects. Hospitals should be dedicated to solving the issue as well; a potential part of the solution may be to merge their research infrastructures (research personnel, funds, expertise, material) on a regional level. An individual principal investigator initiating a trial would only have to tap in on a regional level (instead of hospital level).

BIOMARKERS FOR TAILORING TREATMENT STRATEGIES FOR COLORECTAL LIVER METASTASES

Currently, no biomarkers in patients with resectable colorectal liver metastases impact

clinical management [30]. The most widely used and validated marker would be the clinical risk score by Fong et al. established in 1999, but it is only used for scientific purposes [12]. Such scoring models permit comparisons of patient populations between studies, and provide prognostic information to the patient. **Chapter 5** showed that some prognostic elements of these clinical scores might alter due to systemic therapies, making them even less valid in daily practice. Although previous reports established serum CRP as a prognostic marker, the current thesis describes the largest cohort assessing CRP in patients with CRLM (**chapter 6**). No prognostic power could be validated. The future development of clinically relevant biomarkers for patients with CRLM requires larger patient populations and should focus on other factors than (classic) clinical parameters. Genetic and pathological markers are needed to provide a more detailed picture of colorectal cancer and inherently more accurate stratification of patients. Hopefully novel biomarkers will be able to provide a blueprint for clinical management.

The current thesis describes two promising biomarkers in **chapters 7, 8 and 9**. The mRNA signature described in **chapter 7** is currently being validated in ±100 chemo-naïve patients with resected CRLM, the "Winkelman II" study. Other prognostic signatures have been published, but these investigations remain scarce. This type of research remains expensive and technically challenging, with low yield in terms of clinically relevant markers.

The histopathological growth patterns (HGP) of CRLM published **chapter 8** show to have prognostic impact. This biomarker can be determined irrespective of hospital resources; it can be easily assessed by trained pathologists and has been objectified/validated in various patient cohorts around the world. **Chapter 9** involves an assessment of the predictive value of the histopathological growth patterns in relation to survival after hepatic arterial infusion therapy (PUMP); the patterns have been assessed in a cohort of patients in Memorial Sloan Kettering Cancer Center (MSKCC). HGP can be used to tailor treatment with hepatic arterial chemotherapy infusion, in this retrospective study. Future prospective studies, such as the PUMP-trial, should assess the relevance of HGP in clinical management of CRLM. Currently, the genetic background of the HGP's is assessed to clarify how the different patterns originate, and HGP's are related to recurrence patterns after resection of CRLM and their impact on radicality of surgery. All scored slides in MSKCC are digitized, and analyzed by machine learning (pathomics), in search of prognostic/predictive relevance.

Over recent years so called "liquid biopsies" have become increasingly relevant in the field of cancer [31]. By obtaining serum from patients, circulating biomarkers may represent more readily available methods to monitor, characterize and predict cancer biology. In addition to the abovementioned future studies, the MIRACLE study was initiated aiming to investigate whether circulating serum tumor DNA and circulating tumor cells (including their gene-expression pattern) have prognostic or even predictive value in resectable CRLM.

Possibly, for establishing relevant biomarkers in the complex setting of metastasized CRC, huge amounts of data from larger patient cohorts (preferably chemo naïve) should be analyzed with the help of artificial intelligence (AI) analysis [32]. Emergence of "Radiomics"

and "Pathomics", where image features are extracted from routine diagnostic radiology and pathology studies, are also evolving as valuable biomarkers [33]. This information explosion provides new and complex opportunities for an integrated, multi-scale investigation of cancer. Information synthesis is required across a broad spectrum, from the host itself to the molecular level (clinical, pathological, radiological, genetic). Possibly, the only way to reliably establish a set of biomarkers with clinical impact is with the help of machine learning (AI), integrating all these types of complex information and relating those to patient and treatment outcome. New biomarkers are urgently needed [34].

SURGICAL MANAGEMENT OF COLORECTAL LIVER METASTASES

As delineated in **chapters 10 and 11**, all patients with stage IV disease should be presented in a multidisciplinary expert panel. Since inter-hospital variations in the use of curative local treatment exist, and with expanding indications for local curative treatment expert panels are needed. Stage IV colorectal cancer patients should also be evaluated for participation in studies within this field. Optimal assessment of patients with stage IV CRC can therefore only be achieved by specialized (regional/national) collaborative efforts.

Chapter 13 outlines that for patients treated surgically for stage I-III colorectal cancer, some sort of surveillance should be organized. Literature does not support one specific follow-up scheme: there is no evidence for a specific frequency of follow-up visits nor for any kind of diagnostic modality. Meta-analyses have objectified a general health benefit from doctor's visits during surveillance for cancer. Future research should focus on cancer specific survival effects of follow-up, instead of overall survival. In almost all studies assessing surveillance, investigators concluded a tailored approach was needed. Indeed, in **chapter 12**, a potential subgroup was identified with stage IV CRC that may not need intensive long-term surveillance after surgery. Follow-up should be tailored to patient and tumor characteristics. Studies assessing the impact of surveillance for organ sparing treatments for CRC and in stage IV CRC are urgently needed. The FUTURE trial was initiated, where patients after resection of CRLM will be randomized for intensive in-hospital surveillance versus less intensive tele-surveillance.

REFERENCES

1. *Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project*. J Clin Oncol, 1992. **10**(6): p. 896-903.
2. Loupakis, F., et al., *Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer*. N Engl J Med, 2014. **371**(17): p. 1609-18.
3. Langer, B., et al., *Fluorouracil (FU) plus l-leucovorin (l-LV) versus observation after potentially curative resection of liver or lung metastases from colorectal cancer (CRC): results of the ENG (EORTC/NCIC CTG/GIVIO) randomized trial*. Proc Am Soc Clin Oncol, 2002. **21**: p. (abstr 592).
4. O'Connell, M.J., et al., *Clinical trial of adjuvant chemotherapy after surgical resection of colorectal cancer metastatic to the liver*. Mayo Clin Proc, 1985. **60**(8): p. 517-20.
5. Lopez-Ladron, A., et al., *Observation versus postoperative chemotherapy after resection of liver metastases in patients with advanced colorectal cancer*. Proc Am Soc Clin Oncol, 2003. **22**: p. (abstr 1497).
6. Portier, G., et al., *Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial*. J Clin Oncol, 2006. **24**(31): p. 4976-82.
7. Nordlinger, 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**(12): p. 1208-15.
8. Volders J.H., S.C., Verhoef C., de Wilt J.H.W., *Variation in chemotherapy policy for colorectal liver metastases in the Netherlands*. Nederlands Tijdschrift voor Oncologie, 2013. **10**(5).
9. Ayez, N., et al., *The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator*. Eur J Surg Oncol, 2015. **41**(7): p. 859-67.
10. Rahbari, N.N., 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**: p. 174.
11. Sorbye, H., et al., *Predictive factors for the benefit of perioperative FOLFOX for resectable liver metastasis in colorectal cancer patients (EORTC Intergroup Trial 40983)*. Ann Surg, 2012. **255**(3): p. 534-9.
12. Fong, Y., et al., *Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases*. Ann Surg, 1999. **230**(3): p. 309-18; discussion 318-21.
13. Araujo, R., et al., *Comparison Between Perioperative and Postoperative Chemotherapy After Potentially Curative Hepatic Resection for Metastatic Colorectal Cancer*. Ann Surg Oncol, 2013.
14. Mitry, E., et al., *Adjuvant chemotherapy after potentially curative resection of metastases from colorectal cancer: a pooled analysis of two randomized trials*. J Clin Oncol, 2008. **26**(30): p. 4906-11.
15. Groot Koerkamp, B., et al., *Perioperative Hepatic Arterial Infusion Pump Chemotherapy Is Associated With Longer Survival After Resection of Colorectal Liver Metastases: A Propensity Score Analysis*. J Clin Oncol, 2017. **35**(17): p. 1938-1944.
16. Kenter, M.J. and A.F. Cohen, *Re-engineering the European Union Clinical Trials Directive*. Lancet,

2012. **379**(9828): p. 1765-7.

17. Ahmed Ali, U., et al., *Trends in worldwide volume and methodological quality of surgical randomized controlled trials*. Ann Surg, 2013. **258**(2): p. 199-207.
18. Oude Rengerink, K., M. Kruijt, and B.W. Mol, *[Medical-ethical review of multicenter studies can be obtained faster: new directive leaves room for improvement]*

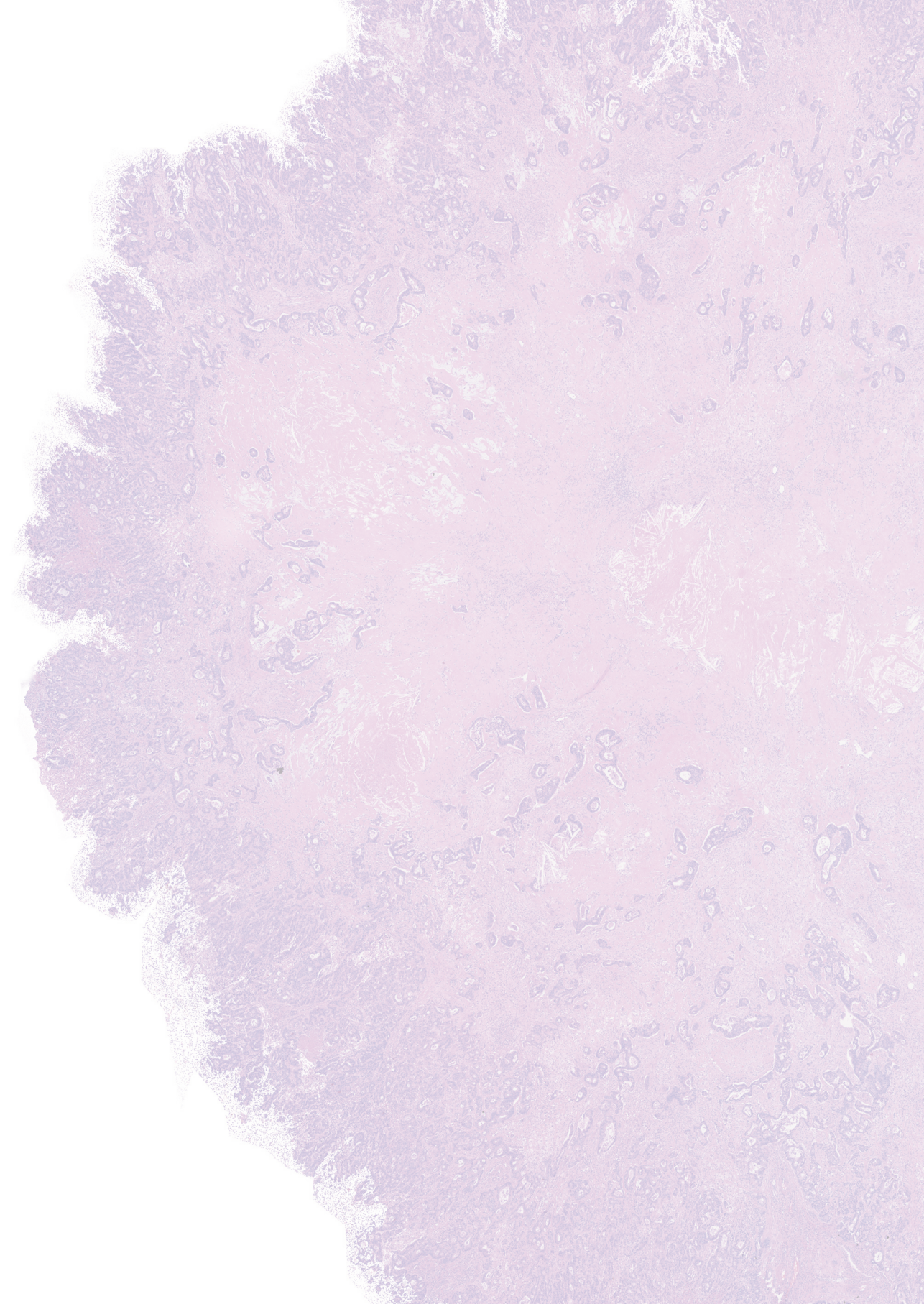
Medisch-ethische toetsing van multicentrisch onderzoek kan sneller: nieuwe richtlijn biedt ruimte voor verbetering. Ned Tijdschr Geneeskd, 2013. **157**(42): p. A6302.

19. van Santvoort, H.C., M.G. Besselink, and H.G. Gooszen, *[Obtaining medical ethical approval for a multicentre, randomised study: prospective evaluation of a ponderous process]*

Het verkrijgen van medisch-ethische goedkeuring voor een multicentrische, gerandomiseerde trial: prospectieve evaluatie van een moeizaam proces. Ned Tijdschr Geneeskd, 2008. **152**(38): p. 2077-83.

20. Evers, J.L.H., *Onnodig traag verlopende medisch-ethische toestemming voor multicentrische klinische trials*. Nederlands Tijdschrijft voor Geneeskunde 2008. **152**(38): p. 2062-2064.
21. Vissenberg R., H.W., Oudijk M., van Golde R., *Evaluatieonderzoek verzuipt in regelgeving.* Medisch Contact, 2013(45): p. 2354-2356.
22. E.A. Ooms, A.C.A., C.W. Burger, *Mensgebonden onderzoek traag op gang*. Medisch Contact, 2006(19): p. 803-805.
23. Nederland, n.-v.i.g., *Klinisch geneesmiddelen onderzoek in Nederland*. http://www.nefarma.nl/stream/com-nctd-monitor-2012, 2012.
24. Maskell, N.A., et al., *Variations in experience in obtaining local ethical approval for participation in a multi-centre study*. QJM, 2003. **96**(4): p. 305-7.
25. Ahmed, A.H. and K.G. Nicholson, *Delays and diversity in the practice of local research ethics committees*. J Med Ethics, 1996. **22**(5): p. 263-6.
26. Rawlins, M., *A new era for UK clinical research?* Lancet, 2011. **377**(9761): p. 190-2.
27. Christian, M.C., et al., *A central institutional review board for multi-institutional trials*. N Engl J Med, 2002. **346**(18): p. 1405-8.
28. Duley, L., et al., *Specific barriers to the conduct of randomized trials*. Clin Trials, 2008. **5**(1): p. 40-8.
29. Yusuf, S., et al., *Sensible guidelines for the conduct of large randomized trials*. Clin Trials, 2008. **5**(1): p. 38-9.
30. Zakaria, S., et al., *Hepatic resection for colorectal metastases: value for risk scoring systems?* Ann Surg, 2007. **246**(2): p. 183-91.
31. Diaz, L.A., Jr. and A. Bardelli, *Liquid biopsies: genotyping circulating tumor DNA*. J Clin Oncol, 2014. **32**(6): p. 579-86.
32. Libbrecht, M.W. and W.S. Noble, *Machine learning applications in genetics and genomics*. Nat Rev Genet, 2015. **16**(6): p. 321-32.
33. Saltz, J., et al., *Towards Generation, Management, and Exploration of Combined Radiomics and Pathomics Datasets for Cancer Research*. AMIA Jt Summits Transl Sci Proc, 2017. **2017**: p. 85-94.
34. Poston, G.J., et al., *Urgent need for a new staging system in advanced colorectal cancer*. J Clin Oncol, 2008. **26**(29): p. 4828-33.

35. Elske C. Gootjes, E.P.v.d.S., Lotte Bakkerus, Tineke E Buffart, Barbara M Zonderhuis, Martijn R. Meijerink, Cornelis J Haasbeek, Peter M van de Ven, Albert J. Ten Tije, Jan Willem De Groot, Sandra A Radema, Esther Van Meerten, Joost Nuyttens, Cornelis Verhoef, Henk M.W. Verheul, *Safety and feasibility of adding tumor debulking to palliative chemotherapy in multi-organ metastatic colorectal cancer: The ORCHESTRA trial*. Abstract ASCO 2017, 2017.



CHAPTER 15

Summary

In **chapter 1** a study protocol for a multicentre randomized trial is published, where patients with resectable colorectal liver metastases (CRLM) without extra hepatic disease and a clinical risk score of 3-5 will be included. The primary aim of this study, the CHARISMA trial, is to compare overall survival (OS) rates between patients randomized for treatment with chemotherapy followed by surgery, versus surgery alone. It is hypothesized that adding neo-adjuvant chemotherapy to surgical resection of CRLM will provide an improvement in OS in patients with a high-risk profile.

In **chapter 2** a report on safety and feasibility of the multicentre randomized ORCHESTRA trial is included, based on the first 100 included patients. This trial focuses on patients with multi-organ colorectal cancer metastases, and randomizes for palliative systemic treatment versus systemic treatment with maximal tumour debulking. Chapter 2 objectifies that the protocol is feasible as it operates in over 30 general and academic hospitals, and currently included over 200 patients. Complication rates from local treatment are comparable to literature. Patients in the intervention arm had an acceptable chemotherapy-free interval, and did not receive less cycles of chemotherapy compared to standard of care.

In **chapter 3** the logistics of initiating multicentre clinical trials in oncology were evaluated. Great variation exists in the procedures for obtaining approval for local feasibility of multicentre research in terms of time, content and costs. These variations are unpredictable and pose a serious obstacle in conducting scientific clinical research in The Netherlands. Delay in the process of initiation of studies decrease the chance of successful accrual of patients and thereby endanger their successful completion. Consensus on simplification of the procedure is urgently warranted. Collaboration with all stakeholders on further standardization, centralization and digitalization of the procedure would be of great value.

In **chapter 4** we demonstrate that stratifying patients with resectable colorectal liver metastases (CRLM) according to their clinical risk profile, as described by Fong et al, could provide a useful tool for selecting patients who are most likely to obtain survival benefit from neo-adjuvant chemotherapy. Although the indication for neo-adjuvant chemotherapy may not solely be based on overall survival benefit, we believe it should be included in the decision making process. Based on these findings and other retrospective evidence, the CHARISMA multicentre trial (chapter 1) was initiated.

Chapter 5 evaluated the prognostic value of lymph node status for overall survival after resection of colorectal liver. Patients who underwent resection of CRLM, lymph node status of the primary tumor was not of prognostic value in cases where the tumor was located in the colon. Interestingly, in primary rectal cancer lymph node status was a prognostic factor. This difference may be caused by the administration of effective adjuvant chemotherapy in node positive colon cancer.

In **chapter 6**, total post-operative inflammatory response as evidenced by CRP serum levels were evaluated as a prognostic marker in relation to overall survival after resection for colorectal liver metastases. To our knowledge, this is the largest patient cohort assessing post-operative CRP levels with respect to survival after surgery for CRLM and the first study stratifying for known risk factors and administration of neo-adjuvant chemotherapy. In the published study, post-operative CRP levels did not prove to be of prognostic value for survival after surgery for CRLM.

In **chapter 7**, a prognostic signature was constructed with the mRNA expression profiles of tumour tissue from resected CRLM. The signature consists of 11 genes of which the expression-patterns were able to discriminate between patients with early recurrences (≤12 months) versus no recurrences (≥36 months) after partial hepatectomy. This biomarker requires validation in a larger cohort representative of the complete clinical spectrum in terms of relapse and treated without (neo-) adjuvant therapy, including any other established prognostic molecular markers.

In **chapter 8** the international consensus guidelines for assessing the prognostic histopathological growth patterns (HGP) in CRLM are published. These guidelines allow for reproducible determination of liver metastasis HGPs. As HGPs impact overall survival after surgery for CRLM, they may serve as a novel biomarker for tailor made therapies.

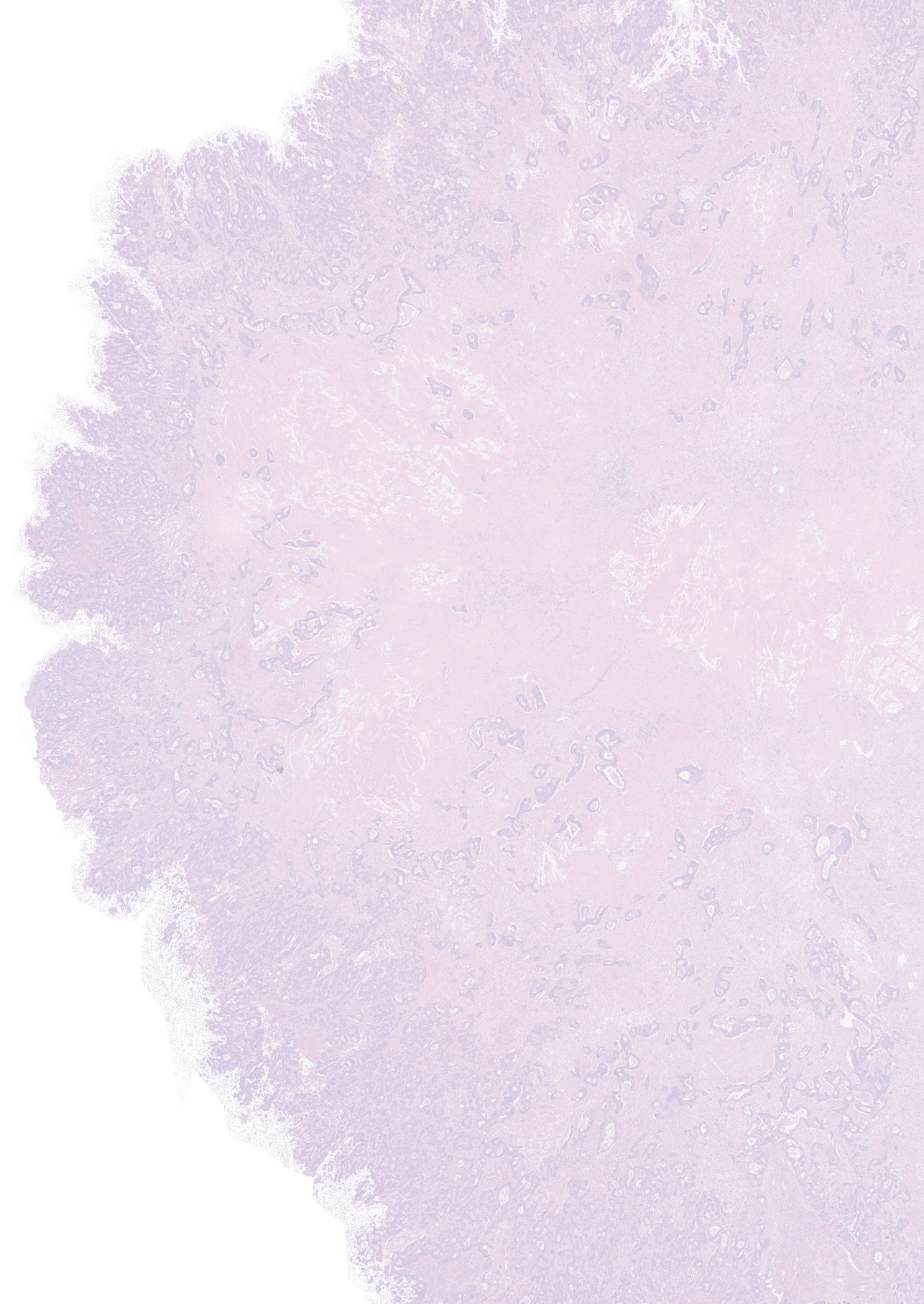
Chapter 9 demonstrated that HGPs could predict the effectiveness of adjuvant systemic chemotherapy after resection of CRLM. Patients with a non-desmoplastic type HGP seem to benefit from adjuvant systemic chemotherapy, while patients with a desmoplastic type HGP do not benefit.

Chapter 10 outlines a case-matched controlled comparison of patients undergoing either systemic therapy or surgery for resectable CRLM. It demonstrates a significant survival benefit in patients treated with liver resection. Surgery should remain the gold standard treatment for patients with CRLM. This finding emphasises the importance of adequate patient selection for surgery. Consensus on resectability and standardised assessment of all patients presenting with CRLM by dedicated liver surgeons in specialised MDTs optimises patient selection for surgery.

Chapter 11 objectified a considerable variation in the utilization of liver resection for patients with synchronous CRLM in the Netherlands. As liver resection offers the only potential cure, it is important that dedicated specialists identify all potentially eligible patients. The formation of a national expert panel, including hepatobiliary surgeons, dedicated radiologists as well as dedicated medical oncologists, evaluating resectability in all patients with CRLM, will potentially lead to an important improvement outcome.

Chapter 12 provides valuable insights regarding the follow-up of patients with 3 years of disease free survival after surgery for CRLM. The data suggests that follow-up in patients surviving 3 years without evidence of disease is useful and necessary in most patients. Some patients may not benefit from any additional surveillance after that period, as characterised by a risk profile. This chapter emphasizes the importance of a tailor-made long-term follow-up protocol after treatment of CRLM with curative intent.

Chapter 13 provides a review of literature on surveillance after curative treatment for stage I-IV colorectal cancer. Patients with stage I–IV CRC can benefit from surveillance after treatment with curative intent. Meta-analyses of data from a range of studies over the past two decades confirm that frequent patient visits improve overall survival after curative resection of stage I–III CRC, although most individual trials demonstrate minimal to no overall survival benefit. The effect of surveillance for stage I–III CRC on overall survival can only partly be explained by improved cancer-specific survival, with repeated clinical assessment contributing to the overall survival benefit. Further (model-based) research on surveillance after CRC treatment should focus on risk-stratification and should incorporate current knowledge on risk of recurrence in relation to the biology of the tumour. Consensus guidelines and prospective research investigating the optimal follow-up protocols are urgently needed for surveillance of patients with stage IV CRC who are treated with curative intent, as well as patients treated according to a 'wait and see' strategy after a complete clinical response to CRT.



CHAPTER 16

Nederlandse Samenvatting

In **hoofdstuk 1** wordt een studieprotocol voor een multicenter gerandomiseerde studie gepubliceerd, waarin patiënten met resectabele colorectale levermetastasen (CRLM) zonder extra hepatische uitzaaiingen en een klinische risicoscore van 3-5 worden geïncludeerd. Het primaire doel van deze studie, de CHARISMA-studie, is om de algehele overleving te vergelijken tussen patiënten die gerandomiseerd zijn voor behandeling met chemotherapie gevolgd door een operatie, of alleen een operatie. Er wordt verondersteld dat toevoeging van neo-adjuvante chemotherapie aan chirurgische resectie een verbetering in algehele overleving zal geven.

In **hoofdstuk 2** is een rapport opgenomen over veiligheid en haalbaarheid van de multicenter gerandomiseerde ORCHESTRA studie, gebaseerd op de eerste 100 geïncludeerde patiënten. Deze studie richt zich op patiënten met uitzaaiingen van darmkanker in meerdere organen en randomiseert voor palliatieve systemische behandeling versus systemische behandeling met maximale tumor debulking. Hoofdstuk 2 objectiveert dat het protocol logistiek uitvoerbaar is met meer dan 30 participerende algemene en academische ziekenhuizen en 200 geïncludeerde patiënten. Complicatie ratio's van lokale behandeling zijn vergelijkbaar met de literatuur. Patiënten in de interventie arm hadden een aanvaardbaar chemotherapie-vrij interval en kregen niet minder cycli van chemotherapie in vergelijking met de standaardbehandeling.

In **hoofdstuk 3** werd de logistiek van het initiëren van oncologische, multicentrische klinische studies geëvalueerd. Er bestaat een grote variatie in de procedures voor het verkrijgen van goedkeuring voor lokale uitvoerbaarheid van multicenter onderzoek in termen van tijd, inhoud en kosten. Deze variaties zijn onvoorspelbaar en vormen een ernstig obstakel bij het uitvoeren van wetenschappelijk klinisch onderzoek in Nederland. Vertraging in het proces van initiatie van studies vermindert de kans op succesvolle inclusie van patiënten en brengt voltooiing in gevaar. Een nationale agenda ter vereenvoudiging van de procedure is dringend nodig. Samenwerking met alle belanghebbenden omtrent verdere standaardisatie, centralisatie en digitalisering van de procedure zou van grote waarde zijn.

In **hoofdstuk 4** laten we zien dat patiënten met reseceerbare colorectale levemetastasen (CRLM) op basis van hun klinische risicoprofiel, zoals beschreven door Fong et al., overlevingswinst hebben na behandeling met neo-adjuvante chemotherapie. Op basis van deze bevindingen en die uit ander retrospectief bewijsmateriaal werd de CHARISMA multicenter trial (**hoofdstuk 1**) geïnitieerd.

Hoofdstuk 5 evalueerde de prognostische waarde van de lymfeklierstatus van de primaire colorectale tumor ten aanzien van de algehele overleving na resectie van colorectale lever metastasen. Bij patiënten die resectie van CRLM ondergingen, was de lymfklierstatus van de primaire tumor niet prognostisch in gevallen waarbij de tumor zich in het colon bevond.

Interessant is dat lymfeklierstatus bij primaire rectumkanker wel prognostisch was. Dit verschil kan worden veroorzaakt door de toediening van adjuvante chemotherapie bij lymfeklier positief colon carcinoom.

In **hoofdstuk 6** werd de totale postoperatieve inflammatoire respons, vertegenwoordigd door CRP-serumwaarden, geëvalueerd als prognostische marker in relatie tot de totale overleving na resectie voor colorectale levermetastasen. Voor zover ons bekend is dit het grootste patiënten cohort dat postoperatieve CRP-waarden beoordeelt met betrekking tot overleving na chirurgie voor CRLM en het eerste onderzoek dat stratificeert naar bekende risicofactoren en toediening van neo-adjuvante chemotherapie. In het gepubliceerde onderzoek bleken postoperatieve CRP-waarden geen prognostische waarde te hebben voor overleving na operatie voor CRLM.

In **hoofdstuk 7** werd een prognostische signatuur geconstrueerd met de mRNA-expressieprofielen van tumorweefsel van gereseceerde CRLM. De signatuur bestaat uit 11 genen waarvan de expressiepatronen onderscheid konden maken tussen patiënten met vroege recidieven (≤12 maanden) versus geen recidieven (≥36 maanden) na chirurgie voor CRLM. Deze biomarker vereist validatie in een groter cohort dat representatief is voor het volledige klinische spectrum in termen van recidieven, dat chemo-naïef is, en tezamen met andere prognostische moleculaire markers.

In **hoofdstuk 8** worden de internationale consensusrichtlijnen voor het beoordelen van de prognostische histopathologische groeipatronen (HGP) in CRLM gepubliceerd. Deze richtlijnen maken een universele, reproduceerbare bepaling van deze HGP's mogelijk. Aangezien HGP's van invloed zijn op de algehele overleving na een operatie voor CRLM, kunnen ze dienen als een nieuwe (veelbelovende) biomarker voor geïndividualiseerde therapieën.

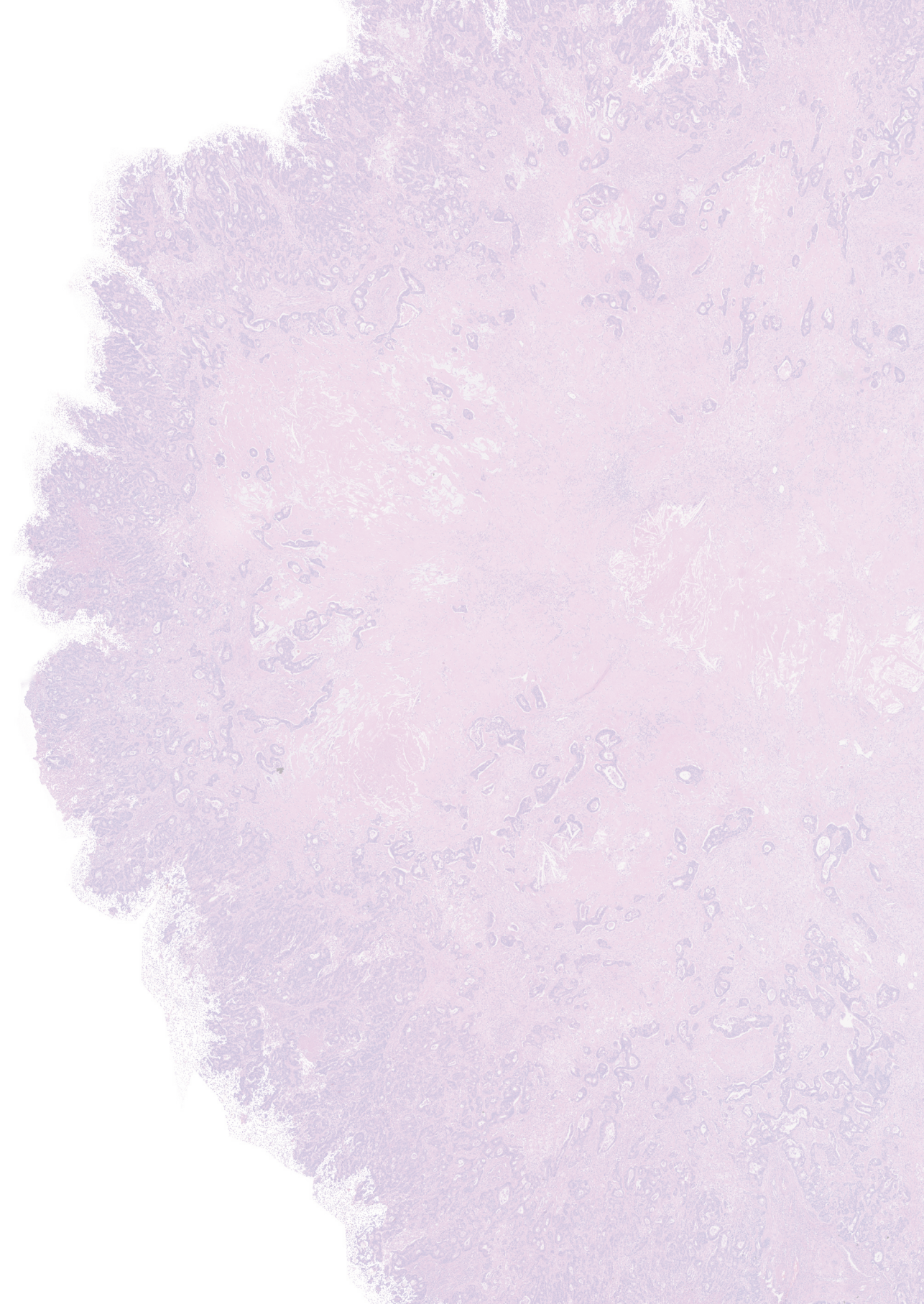
Hoofdstuk 9 toonde aan dat HGP's de effectiviteit van adjuvante systemische chemotherapie na resectie van CRLM kunnen voorspellen. Patiënten met een niet-desmoplastisch HGP lijken baat te hebben bij adjuvante systemische chemotherapie, terwijl patiënten met een desmoplastic type HGP niet profiteren in termen van algehele overleving.

Hoofdstuk 10 schetst een case-matched vergelijking van patiënten die alleen systemische therapie danwel een operatie ondergaan voor resectabele CRLM. Het vertoont een significant overlevingsvoordeel bij patiënten die worden behandeld met leverresectie. Chirurgie moet de gouden standaard behandeling blijven voor patiënten met CRLM. Deze bevinding benadrukt het belang van adequate selectie van patiënten voor chirurgie. Consensus over resectabiliteit en gestandaardiseerde beoordeling van alle patiënten die zich presenteren met CRLM door gespecialiseerde werkgroepen optimaliseert die patiënten selectie.

Hoofdstuk 11 liet een aanzienlijke variatie in het gebruik van leverresectie voor patiënten met synchrone CRLM in Nederland zien. Aangezien leverresectie de enige mogelijke genezing biedt, is het belangrijk dat alle patiënten die mogelijk voor genezing in aanmerking komen geïdentificeerd worden. De vorming van een regionaal of nationaal panel van deskundigen, waaronder hepatobiliaire chirurgen, toegewijde radiologen en toegewijde medische oncologen, die de resectabiliteit evalueren bij alle patiënten met CRLM, kan mogelijk leiden tot een belangrijke verbetering van selectie.

Hoofdstuk 12 biedt waardevolle inzichten met betrekking tot de follow-up van patiënten met een ziektevrije overleving van minstens 3 jaar na chirurgie voor CRLM. De gepubliceerde data suggereren dat follow-up bij patiënten die 3 jaar overleven zonder bewijs van terugkeer van ziekte bij de meeste patiënten nuttig en noodzakelijk is. Sommige patiënten profiteren na die periode mogelijk niet van extra controles, en deze groep is te identificeren middels een klinisch risicoprofiel. Dit hoofdstuk benadrukt het belang van een op maat gemaakt follow-up protocol na behandeling van CRLM.

Hoofdstuk 13 geeft een overzicht van de literatuur omtrent follow-up na curatieve behandeling voor stadium I-IV colorectale kanker. Meta-analyses van gegevens uit een reeks onderzoeken in de afgelopen twee decennia bevestigen dat frequente patiënten bezoeken de algehele overleving verbeteren na curatieve resectie van stadium I-III-CRC, hoewel de meeste individuele onderzoeken minimale tot geen algehele overlevingswinst vertonen. Het effect van follow-up bij stadium I-III CRC op de algehele overleving kan slechts gedeeltelijk worden verklaard door een verbeterde kanker specifieke overleving, omdat blijkt dat herhaalde klinische beoordeling bijdraagt aan een algeheel overlevingsvoordeel. Verder (modelmatig, digitaal) onderzoek naar follow-up na CRC-behandeling moet zich richten op risicostratificatie en waarbij tumor biologie wordt betrokken. Consensusrichtlijnen en prospectief onderzoek naar de optimale follow-up protocollen zijn dringend nodig voor patiënten met stadium IV CRC die curatief worden behandeld, evenals patiënten die worden behandeld volgens orgaan sparende therapieën bij primair CRC.



CHAPTER 17

Appendices

I Scientific Output
II Ph.D. Portfolio
III Acknowledgements
IV About the Author

LIST OF PUBLICATIONS

Safety and feasibility of additional tumor debulking to first line palliative chemotherapy for patients with multi-organ metastatic colorectal cancer in the multicenter randomized fase III ORCHESTRA trial
E.P. van der Stok*, E.C. Gootjes*, D.J. Grünhagen, J.W.A. Burger, T.E. Buffart, M.P. Tol, M.R. Meijerink, A.J. ten Tije, E. van Meerten, P.M. van de Ven, J. Nuyttens, C.J. Haasbeek, H.M.W. Verheul, C. Verhoef
Submitted

Histopathological growth patterns as a guide for adjuvant systemic chemotherapy in patients with resected colorectal liver metastases
E.P. van der Stok*, F.E. Buisman*, B. Galjart, J. Creasy, P.B. Vermeulen, E. Sadot, P.J. Allen, V.P. Balanchandran, W.R. Jarnagin, T.P. Kingham, D.J. Grünhagen, B. Groot Koerkamp, M.I. D'Angelica, C. Verhoef
Submitted

The desmoplastic growth pattern predicts improved survival after resection of colorectal liver metastases
B. Galjart *, P.M.H. Nierop*, **E.P. van der Stok**, R.R.J. Coebergh van den Braak, S. Daelemans, L.Y. Dirix, C. Verhoef, P.B. Vermeulen, D.J. Grünhagen
Submitted

Interrogation of transcriptomic changes associated with drug-induced hepatic sinusoidal dilatation in colorectal cancer.
Jarzabek MA, Proctor WR, Vogt J, Desai R, Dicker P, Cain G, Raja R, Brodbeck J, Stevens D, **van der Stok EP**, Martens JWM, Verhoef C, Hegde PS, Byrne AT, Tarrant JM.
PLoS One. 2018 Jun 7;13(6):e0198099. doi: 10.1371/journal.pone.0198099. eCollection 2018.

Quantitative imaging features and postoperative hepatic insufficiency: a multi-institutional expanded cohort
Pak LM, Chakraborty J, Gonen M, Chapman WC, Do RK, Koerkamp BG, Verhoef C, Lee SY, Massani M, **van der Stok EP**, Simpson AL
J Am Coll Surg. 2018 Feb 14. pii: S1072-7515(18)30122-4. doi: 10.1016/j.jamcollsurg.2018.02.001

Does portal vein embolization prior to liver resection influence the oncological outcomes – A propensity score matched comparison
Huiskens J, Olthof PB, **van der Stok EP**, Bais T, van Lienden KP, Moelker A, Krumeich J, Roumen RM, Grünhagen DJ, Punt CJA, van Amerongen M, de Wilt JHW, Verhoef C, Van Gulik TM.
Eur J Surg Oncol. 2018 Jan;44(1):108-114. doi: 10.1016/j.ejso.2017.09.017. Epub 2017 Sep 20.

Vitamin B_{12} deficiency after esophagectomy with gastric tube reconstruction for esophageal cancer
P. van Hagen, R. de Jonge, M.I. van Berge Henegouwen, G.J. Hotte, **E.P. van der Stok**, J. Lindemans, J.J.B. van Lanschot, B.P.L. Wijnhoven
Diseases of the Esophagus, Volume 30, Issue 12, 1 December 2017, Pages 1–8, https://doi.org/10.1093/dote/dox102

International consensus guidelines for scoring the histopathological growth patterns of liver metastasis
van Dam PJ, **van der Stok EP**, Teuwen LA, Van den Eynden GG, Illemann M, Frentzas S, Majeed AW, Eefsen RL, Coebergh van den Braak RRJ, Lazaris A, Fernandez MC, Galjart B, Laerum OD, Rayes R, Grünhagen DJ, Van de Paer M, Sucaet Y, Mudhar HS, Schvimer M, Nyström H, Kockx M, Bird NC, Vidal-Vanaclocha F, Metrakos P, Simoneau E, Verhoef C, Dirix LY, Van Laere S, Gao ZH, Brodt P, Reynolds AR, Vermeulen PB.
Br J Cancer. 2017 Nov 7;117(10):1427-1441. doi: 10.1038/bjc.2017.334. Epub 2017 Oct 5.

Systematic review of the influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases
Zhao J, van Mierlo KMC, Gómez-Ramírez J, Kim H, Pilgrim CHC, Pessaux P, Rensen SS, **van der Stok EP**, Schaap FG, Soubrane O, Takamoto T, Viganò L, Winkens B, Dejong CHC, Olde Damink SWM; Chemotherapy-Associated Liver Injury (CALI) consortium.
Br J Surg. 2017 Jul;104(8):990-1002. doi: 10.1002/bjs.10572. Epub 2017 May 25. Review.

Surveillance after curative treatment for colorectal cancer
van der Stok EP, Spaander MCW, Grünhagen DJ, Verhoef C, Kuipers EJ.
Nat Rev Clin Oncol. 2017 May;14(5):297-315. doi: 10.1038/nrclinonc.2016.199. Epub 2016 Dec 20. Review.

Regional and inter-hospital differences in the utilization of liver surgery for patients with synchronous colorectal liver metastases in the Netherlands
't Lam-Boer J, **van der Stok EP**, Huiskens J, Verhoeven RH, Punt CJ, Elferink MA, de Wilt JH, Verhoef C; CAIRO5/CHARISMA Group.
Eur J Cancer. 2017 Jan;71:109-116. doi: 10.1016/j.ejca.2016.10.026. Epub 2016 Dec 16.

mRNA expression profiles of colorectal liver metastases as a novel biomarker for early recurrence after partial hepatectomy
van der Stok EP, Smid M, Sieuwerts AM, Vermeulen PB, Sleijfer S, Ayez N, Grünhagen DJ, Martens JW, Verhoef C.
Mol Oncol. 2016 Dec;10(10):1542-1550. doi: 10.1016/j.molonc.2016.09.002. Epub 2016 Sep 20.

Local approval procedures act as a brake on RCTs
van der Stok EP, Huiskens J, Hemmes B, Grünhagen DJ, van Gulik TM, Verhoef C, Punt CJ.
Ned Tijdschr Geneeskd. 2016;160(0):D148. Dutch.

Posttreatment Surveillance in Patients with Prolonged Disease-Free Survival After Resection of Colorectal Liver Metastasis
van der Stok EP*, Galjart B*, Rothbarth J, Grünhagen DJ, Verhoef C.
Ann Surg Oncol. 2016 Nov;23(12):3999-4007. Epub 2016 Jul 8.

Chirurgische mogelijkheden bij hepatogene metastasen nemen toe
van der Stok EP, Grünhagen DJ, Verhoef C.
MAGMA. 2016 Juni; 22(2):65-68.

Management of liver metastases in colorectal cancer patients: A retrospective case-control study of systemic therapy versus liver resection
de Ridder JAM, **van der Stok EP**, Mekenkamp LJ, Wiering B, Koopman M, Punt CJA, Verhoef C, de Wilt JH.
Eur J Cancer. 2016 May;59:13-21. doi: 10.1016/j.ejca.2016.02.003. Epub 2016 Mar 16.

Short term and long term results of patients with colorectal liver metastases undergoing surgery with or without radiofrequency ablation
van Amerongen MJ, **van der Stok EP**, Fütterer JJ, Jenniskens SF, Moelker A, Grünhagen DJ, Verhoef C, de Wilt JH.
Eur J Surg Oncol. 2016 Apr;42(4):523-30. doi: 10.1016/j.ejso.2016.01.013. Epub 2016 Jan 23.

Neoadjuvante chemotherapie gevolgd door chirurgie versus chirurgie alleen bij hoogrisicopatiënten met resectabele colorectale levermetastasen: de CHARISMA-studie.
van der Stok EP, Verhoef C, Grünhagen DJ.
Nederlands Tijdschrift voor Oncologie. 2015 Dec; 12(8):321-326.

The Prognostic Value of Post-operative Serum C-reactive Protein Level for Survival after Surgery for Colorectal Liver Metastases
van der Stok EP, Grünhagen DJ, Rothbarth J, Verhoef C.
Acta Chir Belg. 2015 Sep-Oct;115(5):348-55.

The use of neo-adjuvant chemotherapy in patients with resectable colorectal liver metastases: Clinical risk score as possible discriminator
Ayez N, **van der Stok EP**, Grünhagen DJ, Rothbarth J, van Meerten E, Eggermont AM, Verhoef C.
Eur J Surg Oncol. 2015 Jul;41(7):859-67. doi: 10.1016/j.ejso.2015.04.012. Epub 2015 May 2.

The prognostic value of the primary tumor's nodal status after surgery for colorectal liver metastases in the era of effective systemic therapy
van der Stok EP, Grünhagen DJ, Alberda WJ, Reitsma M, Rothbarth J, Verhoef C.
Dig Surg. 2015;32(3):208-16.

Neo-adjuvant chemotherapy followed by surgery versus surgery alone in high-risk patients with resectable colorectal liver metastases: the CHARISMA randomized multicenter clinical trial
van der Stok EP*, Ayez N*, de Wilt H, Radema SA, van Hillegersberg R, Roumen RM, Vreugdenhil G, Tanis PJ, Punt CJ, Dejong CH, Jansen RL, Verheul HM, de Jong KP, Hospers GA, Klaase JM, Legdeur MC, van Meerten E, Eskens FA, van der Meer N, van der Holt B, Verhoef C, Grünhagen DJ.
BMC Cancer. 2015 Mar 26;15:180. doi: 10.1186/s12885-015-1199-8.

Pro-inflammatory and tumour proliferative properties of excess visceral adipose tissue
van der Stok EP*, Lysaght J*, Allott EH, Casey R, Donohoe CL, Howard JM, McGarrigle SA, Ravi N, Reynolds JV, Pidgeon GP.
Cancer Lett. 2011 Dec 15;312(1):62-72. doi: 10.1016/j.canlet.2011.07.034. Epub 2011 Aug 11.

Prognostic value of body mass index on short-term and long-term outcome after resection of esophageal cancer
Grotenhuis BA, Wijnhoven BP, Hötte GJ, **van der Stok EP**, Tilanus HW, van Lanschot JJ.
World J Surg. 2010 Nov;34(11):2621-7. doi: 10.1007/s00268-010-0697-8.

PHD PORTFOLIO

Name Ph.D. student:	Eric Pieter van der Stok
Erasmus MC department:	Surgical Oncology
Promotor:	Prof. Dr. C. Verhoef
Copromotor:	Dr. D.J. Grünhagen
Date thesis defense:	21-09-2018

	ORAL PRESENTATIONS	**ECTS**
2017	*Voordracht: Nationale leververpleegkundigendag, Utrecht*. Chirurgie voor colorectale levermetastasen.	**1.0**
2017	*Voordracht: symposium Thoraxchirurgie Erasmus MC. Management of liver metastases in colorectal cancer patients.*	**1.0**
2016	*Voordracht: annual meeting of the Liver Metastases Research Network, Umeå, Sweden*. mRNA expression profiles of colorectal liver metastases as a novel biomarker for early recurrence after partial hepatectomy.	**1.0**
2016	*Voordracht: clinical trial bureau Erasmus MC. Het colorectaal carcinoom.*	**1.0**
2016	*Voordracht: voorjaarsvergadering NVvH, Veldhoven*. RNA expressie van 11 genen in colorectale levermetastasen voorspelt vroege recidivering na leverchirurgie.	**1.0**
2015	*Voordracht: wetenschapsdag afdeling heelkunde Erasmus MC*. De prognostische waarde van RNA expressie in colorectale lever metastasen voor recidiveren na leverchirurgie.	**1.0**
2014	*Voordracht: annual meeting of ESSO, Liverpool, UK*. Surgical resection of colorectal liver metastases: Does nodal status of the primary tumour have prognostic value after surgery for CRLM?	**1.0**
2014	*Voordracht: voorjaarsvergadering NVvH, Veldhoven*. De invloed van de lymfklierstatus van primaire colorectale tumoren op overleving na resectie van colorectale levermetastasen.	**1.0**
2014	*Voordracht: trialbijeenkomsten DCCG, Amsterdam/Eindhoven/Zwolle.* Neo-adjuvant chemotherapy followed by surgery versus surgery alone in high-risk patients with resectable colorectal liver metastases. The CHARISMA randomized multicenter clinical trial.	**1.0**

	POSTER PRESENTATIONS	**ECTS**
2015	*Poster: annual meeting of ECCO, Vienna, Austria*. The relevance of follow-up in patients with prolonged disease free survival after surgery for colorectal liver metastases.	**1.0**
2015	*Poster: annual meeting of ECCO, Vienna, Austria*. The prognostic value of mRNA expression of colorectal liver metastases for recurrence after surgical resection.	**1.0**
2014	*Poster: annual meeting of ESSO, Liverpool, UK*. Oxaliplatin-induced hepatic sinusoidal injury in patients undergoing resection for colorectal liver metastases: An assessment of its reversibility.	**1.0**

	COURSES	**ECTS**
2014	Research Integrity.	**0.3**
2014	*BROK* (Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers).	**1.5**
2014	Basic introduction course on SPSS.	**1.0**

	TEACHING	**ECTS**
2017	Organisating the Liver Metastases Research Network annual meeting.	**2.0**
2014-2016	Organisating the "Wondcongres" + Pre-course (cursus snijzaal) 3x.	**10.0**
2016	Supervision master thesis 2x.	**4.0**
2014/2015	First-aid course 2x.	**0.5**

DANKWOORD

Promoveren kun je niet alleen. Dit proefschrift is tot stand gekomen dankzij de inzet, toewijding, kennis en het geduld van vele collega's, vrienden en familie, een aantal in het bijzonder. Als je zo veel aan anderen te danken hebt, kan het niet korter…

Geachte promotor, Professor Dr. C. Verhoef, beste Kees. Wat ben ik ongelofelijk blij dat ik (via Stijn) in "de Daniel" terecht ben gekomen. En wat ben ik dankbaar voor jouw mentorschap. In je begeleiding stond humor altijd op de voorgrond, ik heb er dagelijks van genoten en het leverde binnen het gehele team veel werkplezier op. De deur was altijd open (te krijgen), je was flexibel, en er was tijd. Toen er haast was, kwam je zelfs s 'weekends in huis om de stukken tijdig af te maken. Achter die ogenschijnlijk simpele grappenmaker schuilt een ware professor chirurgische oncologie, in de breedste zin. Je bent, letterlijk en figuurlijk, een groot voorbeeld. Dank…!

Geachte co-promotor, Dr. D.J. Grünhagen, beste Dirk. Ik ben trots op het werk dat ik met je heb mogen doen. Ik zal onze "CHARISMA roadshow" niet vergeten! Ik heb genoten van je impliciete(re), droge humor. Er was altijd een oplossing. Ik heb je nooit en te nimmer kunnen betrappen op een moment dat er geen tijd was voor overleg. En daarbij liep je meestal fluitend over A1. Hoe je dat voor elkaar krijgt weet ik nog steeds niet, maar ik streef ernaar het ook te kunnen! Ik waardeer ontzettend alle tijd en moeite die in mij en dit proefschrift geïnvesteerd is! Dank!

Geachte leden van de promotiecommissie. Ik wil u allen hartelijk danken voor uw tijd en expertise bij het beoordelen van dit proefschrift. Dank voor uw bereidheid van gedachten te wisselen over de inhoud ervan.

Beste Paranimfen, Stijn en Gijsbert. Wat is het mooi dat jullie vandaag aan mijn zijden staan! Bert, samen begonnen we (onder andere) ooit met onderzoek. We reisden zelfs af naar bepaalde laboratoria in Dublin waar menig "dry ice bomb" door ons werd afgestoken. Dit keer geen aanval, maar samen in de verdediging! Stijn, in 2013 dronken wij ter voorbereiding van Kaapstad een biertje in Melief, en drong je er sterk op aan over vervolgstappen te gaan praten met ene Kees. Vandaag ben jij erbij om het werk dat uit die gesprekken voortkwam met mij te verdedigen! Bert, Stijn, onze vriendschap is mij zeer dierbaar!

Dr. Koppert, Dr. Tilanus-Linthorst, Dr. Burger, Dr. Rothbart, Linetta, Madeleine, Pim, Joost. Mijn eerste stappen in de kliniek zette ik onder jullie vleugels. De vervolgstappen in het chirurgisch oncologisch onderzoek ook. Van daaruit kon ik verder! Ik ben zeer dankbaar voor jullie geduld, vertrouwen en goede voorbeeld.

Secretaressen van de chirurgische oncologie in de Daniel, inclusief en in het bijzonder Corine! Dank voor alle hulp, zonder kan het niet!

Daniel maatjes, in het bijzonder Boris, Maarten, Florian, Mirelle, Victorien, Wijnand, Charlotte, Elvira. Ondertussen bestaat "de Daniel" zoals wij haar kennen niet meer, maar vergeten zullen we haar niet. De kleine overdrachtsruimte op B3, de pingpong tafel op A1, de jaarlijkse zomer BBQ op het dak, het legendarische curling evenement in de winter… Het was mooi om er met jullie gewerkt te hebben! Boris, veel dank voor al je hulp in het winkeltje, én voor alle gewonnen potjes pingpong.

Dr. Vermeulen, beste Peter, ik wil u danken voor de prettige samenwerking. Al die uren achter de microscoop was doorzetten, maar zeer leerzaam en zowaar gezellig!

Dr. Wijnhoven, beste Bas, dank voor je introductie in Dublin en advies door de jaren heen. Het heeft zeker ook bijgedragen aan dit proefschrift!

Robert. In het weekend op zoek naar coupes in de kelder van het EMC. Urenlang achter de microscoop. Samen op congres, of ergens een -80 vriezer ophalen. Dank voor de gezelligheid bij met name die eentonige werkzaamheden!

Joost Huiskens. Het werk in Amsterdam had overeenkomsten met dat in Rotterdam, en dus liepen we tegen vergelijkbare uitdagingen aan. Dank voor al het overleg en de geboden hulp. Het was leuk om samen te werken!

Elske. Dank voor de samenwerking, het was leuk en leerzaam om met jou "onze" studies uit te rollen. En niet geheel onbelangrijk: dank voor je hulp bij het aller, allerlaatste stuk voor dit boek…

Dr. Groot-Koerkamp, beste Bas. Via jou werd het mogelijk om naar New York te gaan. Het was een hele bijzondere en mooie ervaring waar ik volop van genoten heb. Ik ben zeer dankbaar dat je die deur voor me geopend hebt.

Dear Dr. D'Angelica, Dr. Jarnagin, Dr. Simpson. I want to thank you for the opportunity of doing research in your department. Although my stay was relatively short, I learned much from you. Thank you!

Chirurgen en Forgerons van het Ikazia Ziekenhuis. De interesse voor de chirurgie werd gewekt op die hele kleine SEH... De werkzaamheden aldaar (in het bijzonder: koffiemelk opkloppen) en alle sociale events daarbuiten speelden een grote rol in mijn studententijd. Ik denk er met plezier aan terug, met mij vele anderen, dat is bijzonder. Ik wil jullie hiervoor danken! Dr. den Hoed, Ted, dank voor alle hulp en advies door de jaren heen…!

Wondcongres vrienden, in het bijzonder Gijs Welten, Jan Deroose, Simon, Dirk-Jan, Caroline, Cecile, Karel, Siebe, Victorien. Als een rode draad liep het wondcongres door de onderzoeksjaren heen. Het was prachtig om dit jaarlijkse evenement met jullie te organiseren en te doen groeien, met als kers op de taart het evenement in de van Nelle fabriek. Dank voor al het plezier hierbij!

Chirurgen en assistenten van het IJsselland Ziekenhuis, IJsvogels! Ik ben erg trots op mijn plek in jullie nest. Dank voor jullie geduld, vertrouwen en energie, wat nodig is om mij dit prachtige vak te leren. Ik geniet dagelijks van het werk dat we samen verzetten, en van de humor die zo'n grote rol speelt in onze groep. Er zijn weinig plaatsen waar het zo mooi is om opgeleid te worden als in Capelle City. Ik ben jullie zeer, …, zeer dankbaar!

Fietsvrienden (Fietsclub Elite ©), in het bijzonder **Sander Drost, Gijs Iordens, Simon, Karel, Stijn.** Ik kijk uit naar nog vele taaie kilometers te gaan. De fiets (of de schaatsen) en jullie gezelschap biedt een mooie gelegenheid te ontsnappen aan werk. Laten we proberen dat te blijven doen, het is genieten!

Lieve jongens van "JC Meisjes B1, Mokka Racers!". De boog kan niet altijd gespannen staan, en gelukkig is deze ook nooit gespannen geweest wanneer ik me in jullie gezelschap begaf. Van de Skihut tot op de top van de Cayambe. Wat heb ik een geluk met deze vriendschappen. Ik kijk uit naar nog veel meer!

Lieve Ferry, Morteza, Iman. Dank voor jullie eindeloze, onuitputtelijke gastvrijheid en gezelligheid. Dank voor al die (gewonnen) potjes Backgammon. Of wij elkaar nou treffen in Oud-Beijerland, in Rotterdam of helemaal in Teheran: het voelt altijd als thuis, en dat is bijzonder. Lieve Morteza, ik weet hoe ongelofelijk graag je hierbij was geweest, ik kom het boekje persoonlijk brengen.

Lieve Mustaba, Farah, Mali & Baba Joon.

مجتبی، ملیح، باباجون و فرح عزیز،
من از مهمان نوازی و محبت بی نهایت شما از صمیم قلب تشکر میکنم. با وجود اینکه ما به دلیل دوری از همدیگر لحظات کمی را با هم گذراندیم من خودم را در حضور شما بسیار راحت احساس میکنم. متشکرم.

Lieve Louise, Ruud, Sander. We hebben iets te vieren! Ik ben erg blij dat ik dat met jullie kan doen. Ik ben heel erg trots op jullie, op hoe ver we gekomen zijn. Soms hadden we de wind tegen, maar nu in de rug. We blijven doorgaan!

Lieve Moeder. Als er iets is waar ik graag voorbeeld aan neem is het je buitengewone doorzettingsvermogen. Je leerde me bovendien dat "arbeid adelt". Ik wil je bedanken voor het wijzen van de juiste richting, voor alle goede zorgen: ze kwamen ongevraagd en onvoorwaardelijk. En vaak allemaal in je eentje! Dank voor je steun, en je blinde vertrouwen. Dank..!

Lieve Mo! Dit boek kent geen verassingen voor jou. Je kent de inhoud van de hoofdstukken. Je weet (helaas) hoeveel tijd erin zit. Jij had allicht de verdediging kunnen doen, zo nodig. Misschien is het in die zin wel "ons" boek, maar ben je nergens opgenomen in de auteurslijst… Dank voor al jouw geduld, afleiding, en voor het relativeren op gezette momenten…

ABOUT THE AUTHOR

Eric Pieter van der Stok was born on March 13th, 1986 in Utrecht, as son of Esther Maria Frederica Kolb and Eric Johannes van der Stok. He grew up in the Netherlands and France; he was 12 when his family moved back to the Netherlands permanently. In 2004 he graduated from "de Breul" college in Zeist. He started studying International Business Administration at Erasmus University Rotterdam, and was admitted for medicine in Erasmus Medical Center, two years later (2006). From 2006 until 2011 he lived in "Huize Sionkaalie", at the Sionstraat in Rotterdam. During medical school he worked in the emergency room of the Ikazia hospital as a "Forgeron". This is where his interest in surgery originated. Thus, in 2011 Eric moved to Dublin, Ireland, for seven months to perform translational research in the laboratories of the Institute of Molecular Medicine, St. James Hospital, Trinity College. During his last three months of medical training, Eric worked in the trauma unit of Groote Schuur Hospitaal in Cape town, South Africa. After obtaining his M.D. in June 2013, Eric started as a resident in Erasmus MC Cancer Institute. Subsequently, he seized the opportunity to start a fulltime PhD program (this thesis) in the same institute. In July 2016, he went on to fulfill a research fellowship of four months in Memorial Sloan Kettering Cancer Center, New York, U.S.A. In January 2017, Eric started his General Surgery training in the IJsselland hospital in Capelle aan den IJssel (Dr. P.G. Doornebosch, Dr. I. Dawson).