Evaluation of prognosis and treatment response in colorectal cancer with focus on biomarkers

Kethe Hermunen

ACADEMIC DISSERTATION

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Unigrafia Oy
Helsinki 2020
To my family
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LIST OF ORIGINAL PUBLICATIONS

I Hermunen K., Soveri L-M., Karsbøl Boisen M., Mustonen H.K., Dehlendorff C., Haglund C., and Sidenius Johansen J. and Osterlund P. Postoperative serum CA19-9, YKL-40, CRP and IL-6 in combination with CEA as prognostic markers for recurrence and survival in patients resected for colorectal cancer 
SUBMITTED

II Soveri L-M., Hermunen K., de Gramont A., Poussa T., Quinaux E., Bono P., Andre T. and Osterlund P. Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity? 
European Journal of Cancer 2014;50(17):2966-2974

III Hermunen, K., Haglund, C. and Osterlund, P. 2013, CEA fluctuation during a single fluorouracil-based chemotherapy cycle for metastatic colorectal cancer 

IV Hermunen K., Lantto E., Poussa T., Haglund C. and Osterlund P. Can carcinoembryonic antigen replace computed tomography in response evaluation of metastatic colorectal cancer? 
Acta Oncologica 2018;57(6):750-758

*Publication II was also included in the thesis of Leena-Maija Soveri

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ABBREVIATIONS

AE    adverse event
AJCC  the American Joint Committee on Cancer
ALP   alkaline phosphatase
APE   abdominoperineal excision
AR    anterior resection
ASCO  American Society for Clinical Oncology
BRAF  proto-oncogene BRAF or v-Raf murine sarcoma viral oncogene homolog B
BSC   best supportive care
CA19-9 carbohydrate antigen 19-9
CAPIRI capecitabine and irinotecan
CAPOX capecitabine and oxaliplatin
CEA   carcinoembryonic antigen
CHI3L1 chitinase-3-like protein 1, also called YKL-40
CI95% 95% confidence interval
CIN   chromosomal instability
CME   complete mesocolic excision
CMS   consensus molecular subtypes
CpG   Cytocine-phosphate-Guanine
CR    complete response
CRC   colorectal cancer
CRP   c-reactive protein
CRT   chemoradiotherapy
CT    computed tomography
CEA   carcinoembryonic antigen
DCR   disease-control rate
DFS   disease-free survival
DLT   dose-limiting toxicity
ECOG  Eastern Cooperative Oncology Group
EGFR  epidermal growth factor receptor
EGTM  European Group on Tumour Markers
ELAPE extralevator abdominoperineal excision
ESMO  European Society for Medical Oncology
EUS   endoscopic ultrasound
FIT   faecal immunochemical test
FOLFOX folinic acid, 5-fluorouracil as bolus injection and continuous infusion and oxaliplatin
FOLFIRI folinic acid, 5-fluorouracil as bolus injection and continuous infusion and irinotecan
5-FU  5-fluorouracil
FOBT  faecal occult blood test
GCP   good clinical practice
GIST  gastrointestinal stromal tumour
GPS   Glasgow prognostic score
mGPS  modified Glasgow prognostic score
HAI   hepatic arterial infusion
HIPEC hyperthermic intraperitoneal chemotherapy
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>HFS</td>
<td>hand-foot syndrome</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>IFL</td>
<td>short infusion irinotecan combined with intravenous bolus 5-fluorouracil and leucovorin</td>
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<tr>
<td>IL-6</td>
<td>interleukin-6</td>
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<tr>
<td>IROX</td>
<td>irinotecan and oxaliplatin</td>
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<tr>
<td>KRAS</td>
<td>Kirsten rat sarcoma viral oncogene</td>
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<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
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<tr>
<td>LV</td>
<td>leucovorin, folic acid, calciumfolinate</td>
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<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>mCRC</td>
<td>metastatic colorectal cancer</td>
</tr>
<tr>
<td>MDT</td>
<td>multidisciplinary teamwork</td>
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<tr>
<td>MMR</td>
<td>mismatch repair</td>
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<tr>
<td>mCRC</td>
<td>metastatic colorectal cancer</td>
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<tr>
<td>MRF</td>
<td>mesorectal fascia</td>
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<tr>
<td>MSI</td>
<td>microsatellite instability</td>
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<tr>
<td>NPV</td>
<td>negative predictive value</td>
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<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NRS</td>
<td>nutritional risk screening</td>
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<tr>
<td>NRAS</td>
<td>neuroblastoma RAS viral (v-ras) oncogene homolog</td>
</tr>
<tr>
<td>OS</td>
<td>overall survival</td>
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<tr>
<td>PD</td>
<td>progressive disease</td>
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<td>PFS</td>
<td>progression-free survival</td>
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<tr>
<td>PLGF</td>
<td>placental growth factor</td>
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<tr>
<td>PPV</td>
<td>positive predictive value</td>
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<tr>
<td>PR</td>
<td>partial response</td>
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<tr>
<td>PS</td>
<td>performance status</td>
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<tr>
<td>QoL</td>
<td>quality of life</td>
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<tr>
<td>RECIST</td>
<td>Response Evaluation Criteria in Solid Tumours</td>
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<tr>
<td>RCT</td>
<td>randomised controlled study</td>
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<tr>
<td>RFA</td>
<td>radio frequency ablation</td>
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<td>RFS</td>
<td>relapse-free survival</td>
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<tr>
<td>RR</td>
<td>response rate</td>
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<td>SCRT</td>
<td>short-course radiotherapy</td>
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<td>SIRT</td>
<td>selective internal radiation therapy</td>
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<td>TACE</td>
<td>transarterial chemoembolisation</td>
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<td>TAMIS</td>
<td>transanal mini-invasive surgery</td>
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<tr>
<td>TME</td>
<td>total mesorectal excision</td>
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<tr>
<td>TNM</td>
<td>Tumour node metastasis staging system</td>
</tr>
<tr>
<td>TS</td>
<td>thymidylate synthase</td>
</tr>
<tr>
<td>TTP</td>
<td>time to progression</td>
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<tr>
<td>UFT</td>
<td>an oral fluoropyrimidine consisting of tegafur and uracil</td>
</tr>
<tr>
<td>UICC</td>
<td>Union Internationale Contre le Cancer</td>
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<tr>
<td>VATS</td>
<td>video-assisted thoracic surgery</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>VEGFR</td>
<td>vascular endothelial growth factor receptor</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell count</td>
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<td>WHO</td>
<td>World Health Organization</td>
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YKL-40 Tyrosine (Y), Lysine (K) and Leucine (L) and its molecular mass of 40 kDa, also called chitinase-3-like protein 1 (CHI3L1)
ABSTRACT

**Background and aims:** Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer death in Finland. Improvements in surgical techniques, diagnostics, pathological evaluation of the specimen and oncological treatments have all improved its 5-year survival. Early diagnosis, proper surgery and multidisciplinary teamwork are the keys to a cure. Improvements in the oncological treatment of metastatic CRC (mCRC) have prolonged survival, but in oligometastatic disease, only metastasectomies are curative. Biomarkers are useful in follow-up of CRC patients and in prediction of prognosis and treatment response, both in radically operated CRC patients and in mCRC.

**Patients and methods:** In this thesis, the patients included were from four different trials: the randomised LIPSYT trial investigating bolus versus infusional 5-fluorouracil-based adjuvant treatment for radically operated stage I to IV CRC patients at the Department of Oncology at Helsinki University Hospital and the multicentre French GERCOR C96.1 trial with the same design in radically operated stage II to III colon cancer patients. In addition were the MEPSYT TNF trial consisting of mCRC patients treated with three different chemotherapy regimens and the MEPSYT phase I to II trial with raltitrexed combined with peroral carmofur for mCRC, which both took place at the Department of Oncology at Helsinki University Hospital.

Study I included 147 patients from the LIPSYT trial with archived post-operative blood samples available for biomarker assessment, YKL-40 (also known as chitinase-3-like protein 1 (CHI3L1)), and interleukin-6 (IL-6), combined with routine measurement of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and c-reactive protein (CRP). The study aim was to look at relapses, disease-free survival (DFS), and overall survival (OS) in the group of elevated versus normal post-operative markers.

Study II included 153 stage II to III CRC patients from the LIPSYT trial and 880 stage II to III colon cancer patients from the GERCOR C96.1 trial eligible for analysis of adverse events and their impact on DFS and OS.

Study III included 60 patients from the MEPSYT- TNF trial who were treated with fluoropyrimidines (n=20), raltitrexed alone (n=20), or raltitrexed combined with the peroral fluoropyrimidine carmofur (n=20); weekly CEA values, liver function tests, and inflammatory markers during one chemotherapy cycle were available for evaluation regarding fluctuation and correlation with prognosis.

Study IV included 66 patients from the MEPSYT trial with CEA values and computed tomography (CT) examinations available for evaluation at baseline (before chemotherapy) and at 2-month intervals regarding the possibility of replacing CT with CEA in treatment response evaluation.
**Results:** Study I showed that post-operatively elevated CEA had a high positive predictive value (PPV) of 89% (CI$_{95\%}$ 65-99%) and specificity 97% (CI$_{95\%}$ 91-100%) and sensitivity 31% (CI$_{95\%}$ 21-48%). Post-operatively elevated CEA was a significant marker for relapses (HR 7.91; CI$_{95\%}$ 3.43-18.24), DFS (HR 8.63; CI$_{95\%}$ 3.82-19.50), and OS (HR 10.17; CI$_{95\%}$ 4.35-23.79) in multivariate analysis. Normal post-operative CEA combined with elevated YKL-40 was linked with impaired DFS (HR 2.30; CI$_{95\%}$ 1.27-4.16) and OS (HR 2.40; CI$_{95\%}$ 1.28-4.52) or CRP with impaired DFS (HR 3.54; CI$_{95\%}$ 1.57-8.02) and OS (HR 3.10; CI$_{95\%}$ 1.29-7.45). An elevated CEA combined with an elevated CA19-9, YKL-40, CRP, or IL-6 was linked to very high relapse rates: CA19-9 with PPV 100%, YKL-40 with PPV 90%, CRP with PPV 100%, and IL-6 with PPV 100%.

In Study II, 47% of the patients receiving adjuvant 5-FU chemotherapy developed neutropenia, 54% developed nausea/vomiting, and 43% developed mucositis. Those patients experiencing these adverse events, especially if mild to moderate, had the best outcome. On the other hand, patients experiencing no adverse events had the worst survival rates.

In Study III, CEA fluctuated during a fluorouracil-based chemotherapy cycle. A non-significant decrease occurred at day 7 and an increase at day 14. A significant CEA increase occurred during the evaluation cycle (55.4 $\mu$g/l vs. 148.2 $\mu$g/l; P=0.024) in progressive disease, but in patients with disease control, their CEA level was stable (10.6 $\mu$g/l vs. 17.8 $\mu$g/l; P=0.58).

Study IV showed that at a certain measuring point, a decreasing CEA or a CEA at the same level compared to baseline (before chemotherapy) or the lowest value noticed was equivalent to disease control. In 23% to 47% of the cases, CEA could replace CT in the response evaluation of mCRC.

**Conclusions:** In radically operated stage II to IV CRC patients, a post-operatively elevated CEA or a normal CEA combined with an elevated YKL-40 or an elevated CRP may aid us in finding high-risk patients who would benefit from more aggressive adjuvant therapy and more intensive follow-up. Moreover, adverse events during adjuvant chemotherapy may serve as clinical markers for the evaluation of treatment efficacy. Because CEA fluctuates during a chemotherapy cycle, measurement of CEA should not take place during the cycle, but just before the next cycle is due. If CEA replaces CT in response evaluation of mCRC during chemotherapy for mCRC, response evaluation becomes easier for the patient and cost-saving becomes possible for the health care system.
TIIVISTELMÄ


Neljäs osatyö koostui 66 levinneestä kolorektaalisyöpäpotilaasta, joilla oli CEA-arvot ja tietokonetomografiatutkimukset (TT) ennen solunsalpaajahoidon aloitusta ja 2 kuukauden välisen vastearvionta. Jos CEA oli samalla tasolla tai laskussa verrattuna lähtötasoon tai matalampaan arvoon kysessä oli stabiilin tautitilanteen eikä progressio. Tulosten perusteella 23–47 %:ssa CEA voisi korvata TT:n hoitovasteen arviointissa.

Yhteenvetona voi todeta, että radikaalisti hoidettuakin levinneisyysasteen II–IV kolorektaalisyöpäpotilailla postoperatiivisesti kohonnut CEA tai normaali...
SAMMANFATTNING

Kolorektalcancer är den tredje vanligaste cancern i Finland och den näst vanligaste orsaken till cancerdöd. Bättre kirurgisk teknik, diagnostik, patologisk bedömning av vävnadsproverna och onkologiska behandlingar har förbättrat femårsöverlevnaden. Tidig diagnos och god kirurgi i kombination med multidisciplinärt teamarbete är nyckeln till bot. Förbättringar i den onkologiska behandlingen av metastaserad kolorektalcancer har lett till förlängd överlevnad. Bot kan uppnås med metastasektomier vid oligometastatisk sjukdom. Biomarkörer används vid uppföljning och för bedömning av prognos och behandlingsrespons både vid kurativ och metastaserad kolorektalcancer.

Studie I inkluderade 147 kurativt behandlade stadium II-IV kolorektalcancerpatienter med postoperativa blodprover tillgängliga för analys av YKL-40 och IL-6, samt CEA, CA19-9 och CRP från rutinmätning. I studien jämfördes patienter med postoperativt förhöjda markörvärden med patienter med normala värden. I multivariantanalys var ett förhöjt CEA en signifikant markör för återfall, sjukdomsfri och total överlevnad. Normalt CEA kombinerat med förhöjt YKL-40 eller CRP korrelerade med en kortare sjukdomsfri och total överlevnad. Ett förhöjt CEA kombinerat med ett förhöjt CA19-9, YKL-40, CRP eller IL-6 korrelerade med hög risk för återfall.

Studie II inkluderade 153 finska stadium II-III kolorektalcancerpatienter och 880 franska stadium II-III koloncancerpatienter, som erhöll 5-fluorouracil som adjuvant cytostatikabehandling. Av dem utvecklade 47 % neutropeni, 54% illamående eller kräkningar och 43% mukosit. De patienter som fick dessa biverkningar, särskilt då de var milda till måttliga, hade den bästa prognosen. Patienter som inte fick några biverkningar hade den sämsta prognosen.

Studie III inkluderade 60 patienter med metastaserad kolorektalcancer vilka erhöll fluoropyrimidinbaserad palliativ cytostatikabehandling. CEA, leverfunktionsvärden och inflammatoriska markörer hade tagits veckovis under en behandlingscykel. CEA fluktuerade under behandlingscykeln. En icke-signifikant minskning inträffade dag 7 och en ökning dag 14. Vid progressiv sjukdom noterades en signifikant CEA-stegring under pågående behandlingscykel medan patienter med stabil sjukdom hade en stabil CEA-nivå.

Studie IV inkluderade 66 patienter med metastaserad kolorektalcancer. CEA-bestämning och datortomografi (DT)-undersökning utfördes innan cytostatikabehandlingen påbörjades och uppföljning skedde med 2 månaders mellanrum. Resultaten visade att då CEA sjönk eller hölls på samma nivå jämfört med utgångsläget eller lägsta uppmätta värde hade patienten en stabil sjukdom. I 23 % - 47 % av patienterna kunde CEA ha ersatt DT i responsbedömning av metastaserad kolorektalcancer.
Sammanfattningsvis kan man konstatera att ett postoperativt förhöjt CEA eller ett normalt CEA kombinerat med förhöjt YKL-40 eller CRP hos kurativt behandlade stadium II-IV kolorektalcancerpatienter kan hjälpa oss att identifiera högriskpatienter vilka kunde dra nytta av mer aggressiv adjuvantbehandling och mer intensiv uppföljning. Dessutom kunde biverkningar under pågående adjuvantbehandling fungera som en klinisk markör för bedömning av effekten av behandlingen. Eftersom CEA fluktuerar under en behandlingscykel bör CEA inte mätas under pågående fluoropyrimidinbaserad behandlingscykel, utan precis innan nästa behandlingscykel påbörjas. Genom att i vissa fall ersätta DT med CEA vid responsevalueringen av metastaserad kolorektalcancer under pågående cytostatikabehandling kunde man underlätta uppföljningen för patienten och minska utgifterna för sjukvården.
1 INTRODUCTION

Colorectal cancer (CRC) is worldwide the fourth most common cancer after lung, breast and prostate cancer (1). Approximately 30% to 50% of all CRC patients die of CRC despite improvements in surgical and oncological treatments over recent decades (2). Early diagnosis and multidisciplinary teamwork are the keys to longer survival.

Carcinoembryonic antigen (CEA) is recommended pre-operatively for determining prognosis in patients with newly diagnosed CRC (3). Being the only biomarker recommended for monitoring of CRC patients both in curatively operated and metastatic disease (3,4), CEA is taken before oncological therapy and during ongoing oncological therapy every 2 to 3 months and after oncological therapy every 2 to 3 months up to 3 years, and then every 3 to 6 months for up to 5 years (4,5).

Several studies concern pre-operative CEA and its prognostic value (6–10). Lately, interest has been increasing in post-operative CEA and its prognostic value (11,12), and one group questions the present guideline recommendations for evaluating pre-operative CEA only as a prognostic marker (12).

Neutropenia in breast cancer patients receiving chemotherapy in the 1990s predicted improved survival (13,14). Neutropenia during chemotherapy has been a predictor of better survival in patients with different types of cancers (15). Among metastatic CRC (mCRC) patients, chemotherapy-induced neutropenia is also associated with improved survival (16).

In mCRC, CEA is the recommended tumour marker for monitoring patients receiving chemotherapy (4), and it should be taken at the start of treatment. Some studies show a CEA increase especially during the first 4 to 6 weeks of new treatment, an increase known as a surge or a tumour flare reaction (17,18).

ESMO (European Society for Medical Oncology) Guidelines recommend CT and CEA evaluation every 2 to 3 months for mCRC patients receiving palliative chemotherapy (19). Studies show a correlation between CEA and CT findings in mCRC patients with ongoing chemotherapy, which opens up the possibility of replacing computed tomography (CT) with CEA in their monitoring (20–22).

In this thesis, the focus was on the prognostic value of post-operative CEA in combination with tumour (CA19-9) and inflammatory (YKL-40, CRP, and IL-6) markers for long-term relapse-free survival (RFS) and overall survival (OS) of radically operated stage II to IV CRC patients. Other areas of study were grade of toxicity during six months of adjuvant therapy in stage II to III radically operated CRC and the impact of severity of common adverse events as clinical markers for disease-free survival (DFS) and for OS. In the metastatic setting, CEA fluctuation and whether CEA might replace CT in response evaluation in mCRC also proved of interest.
2 REVIEW OF THE LITERATURE

2.1 Epidemiology

CRC is the fourth most common cancer in the world after lung, breast and, prostate cancer, with an estimation of 1.8 million new cases worldwide in 2018 (1). When it comes to deaths from CRC, the estimate was of 881 000 in 2018, which ranks CRC second after lung cancer. Men showed a slight predominance, with 56% (1).

In Europe, CRC is the second most common cancer after female breast cancer, with an estimated number of 500 000 new cases in 2018, followed by lung cancer and prostate cancer (23). Together, these four cancers represented 49.7% of the estimated cancer burden. CRC was the second leading cause of cancer death after lung cancer, at an estimated 243 000 deaths in 2018, followed by breast and pancreatic cancer. There was a slight imbalance between the sexes, the male population amounting to 54% of the CRC cases.

In Finland, CRC is the third most common cancer after prostate and breast cancer with 3 356 new cases in 2017, and that year, CRC was the second leading cause of cancer death after lung cancer with 1 368 deaths. A slight imbalance emerged between the sexes, the male population contributing 51.5% of the CRC cases (24).

2.2 Aetiology

The exact cause of CRC is unknown, but several risk factors are known, such as old age, smoking, alcohol consumption, consumption of red and processed meat, body and abdominal fatness, and low fibre intake, as well as hereditary cancer syndromes and history of inflammatory bowel disease (25). Sporadic cancer is the most common CRC type (75-85%), and a specific genetic cause is known in approximately 5% of cases.

Lynch Syndrome, previously also known as hereditary non-polyposis colorectal cancer (HNPPC), is the most common hereditary CRC syndrome (26). It accounts for 3% to 5% of all CRC cases and is caused by mutations in DNA mismatch-repair (MMR) genes, which are inherited in an autosomal dominant pattern. The lifetime risk for CRC is 70% to 80%.

Familial adenomatous polyposis (FAP) results from mutations in the adenomatous polyposis coli (APC) gene and shows an autosomal dominant pattern of inheritance (27). It is characterised by the development of multiple adenomas in the rectum and colon during the second decade of life. Its incidence at birth of about 1/8 300, it manifests equally in both sexes, and accounts for less than 1% of all CRC cases (28). It poses a lifetime risk for CRC of 100%, and therefore prophylactic surgery is advocated by the late teens or early twenties.
Patients with ulcerative colitis (UC) and Crohn’s colitis have an increased risk of developing CRC (29). Risk factors in UC include duration and extent of colitis, early age of onset of colitis, family history of CRC, and severity of microscopic inflammation. The incidence rate increases with each successive decade of disease activity, with cumulative probabilities of 2% at 10 years, 8% at 20 years, and 18% at 30 years (29). A meta-analysis by Canavan et al. showed the overall relative risk for CRC in Crohn’s disease to be 2.5 and for patients with Crohn’s disease affecting the colon to be 4.5 (30). In another meta-analysis, Crohn’s disease was a risk factor for CRC, small bowel cancer, and fistula cancer (31). The risk for CRC was increased by a factor of 2 to 3 and the risk for small bowel cancer by a factor of 19 compared to an age-matched standard population.

Over recent years, an increasing number of studies have been evaluating the differences between adenocarcinoma located in the right and the left colon. Proximal and distal segments of the colon have different embryologic origins; the caecum, appendix, ascending colon, hepatic flexure, and proximal two-thirds of the transverse colon derive from the midgut, whereas the distal one-third of the transverse colon, splenic flexure, sigmoid colon, descending colon, and rectum derive from the hindgut (32).

Right- and left-sided tumours exhibit different histologies; right-sided tumours show sessile serrated adenomas or mucinous adenocarcinomas, and left-sided tumours show tubular, villous, and typical adenocarcinomas (33). Moreover, right-sided tumours tend to be larger, to occur at older ages, to more often be poorly differentiated, to be more microsatellite instability-high (MSI-high), and to metastasize to the peritoneal region; these predominantly occur in females.

The CALGB 80405 trial, with 1,139 patients, showed strong evidence of tumour sidedness (34). All RAS wild-type patients with left-sided tumours had a mOS of 39.3 months in the cetuximab arm and 32.6 months in the bevacizumab arm (HR 1.36 CI95% 0.93-1.99), regardless of chemotherapy backbone, whereas all RAS wild-type patients with right-sided tumours had a mOS of 13.3 months in the cetuximab arm and 29.2 months in the bevacizumab arm (HR CI95% =0.93-1.99).

### 2.3 Pathways

Oncogenes and tumour suppressor genes are the two main types of genes involved in cancer (35). When a mutation in a proto-oncogene is activated, cell growth continues beyond control, leading to cancer. This activated gene is then an oncogene. Tumour-suppressor genes slow down cell division, repair DNA, and regulate apoptosis. Inactivation of tumour-suppressor genes causes cancer.

Vogelstein et al. discovered an important pattern of colorectal carcinogenesis, the so-called adenoma-carcinoma sequence (36,37). Chromosomal instability
predominates, with accumulation of mutations in genes APC, KRAS, and p53; gradually normal mucosa transforms into malignant epithelium.

The serrated neoplasia pathway which has been established more recently describes the progression of serrated polyps, including sessile serrated adenomas and traditional serrated adenomas, to colorectal cancer (38). The primary mutations are in the BRAF gene, followed by epigenetic methylation and gene deletions.

Parallel to these two pathways, three other molecular pathways lead to CRC, namely chromosomal instability (CIN), microsatellite instability (MSI), and Cytocine-phosphate-Guanine (CpG) island methylator phenotype (CIMP) (35,39). Most CRC cases arise through the CIN pathway which consists of mutations in the tumour-suppressor gene APC. About 15% of CRCs have a high degree of MSI, and these tumours are usually less likely to send lymph-node- or distant metastases. The MSI is caused by mutations in the DNA mismatch repair (MMR) genes. Their hypermethylation leads to silencing of the tumour-suppressor gene (CIMP) phenotype (39).

To resolve inconsistencies among the reported gene expression-based CRC classifications and to facilitate clinical translation, an international consortium was formed (40). It was possible to identify four different consensus molecular subtypes (CMS) of CRC based on gene expression, molecular, mutational, histological, and clinical data, namely: CMS1 (14%, MSI Immune subtype, hypermutated, microsatellite unstable, strong immune activation), CMS2 (37%, Canonical subtype, epithelial, chromosomally unstable), CMS3 (13%, Metabolic subtype, epithelial, evident metabolic
Important associations between CMS groups and clinical variables emerged (40). CMS1 were more common in female patients with right-sided lesions; these presented with higher histopathological grade, and the patients had poor survival upon relapse. CMS2 were predominantly left-sided. CMS4 were at a more advanced stage at diagnosis and led to impaired survival also in the adjuvant setting (40,41).

2.4 CRC screening

Early diagnosis is the key to a cure. Since early CRC symptoms are vague and easily missed by both patients and doctors, what would be ideal is to have a screening programme that identifies all CRC patients at an early stage (i.e. stage I or stage II).

Large randomised trials have shown an effect on mortality of CRC screening using a faecal occult blood test (FOBT), resulting in an estimated mortality reduction of 12% to 21% (42,43).

In the first report of the UK study, with a median follow-up of 7.8 years, the difference in CRC mortality between the screening and control arms was 15%, and this effect began to emerge 3 to 4 years after study entry. The authors’ interpretation of their findings suggested, to reduce CRC mortality, a national programme of FOBT screening (44).

In Finland, a population-based randomised screening study began in 2004, and the last patients were included in 2014. In the Finnish study, with a median follow-up time of 4.5 years, no effect was evident of FOBT screening on CRC mortality (45). The follow-up study showed sex differences among the findings and also that in particular men with a left-sided tumour benefitted from screening.

The EU recommends population-based screening for CRC using evidence-based methods with quality assurance of the entire screening process (46).

A new Finnish CRC screening programme began in 2019. During 2019 to 2020, all 60-, 62-, 64-, and 66-year-old men and women in 7 towns in Finland will be invited to the screening programme. The screening test will be a faecal immunochemical test (FIT) (24).

The FIT is an immunoassay specific for human haemoglobin and shows improved analytical and clinical sensitivity for CRC and also provides better detection of advanced adenomas and greater screenee participation compared to that with traditional guaiac-based FOBT (47).

The American Cancer Society Guidelines for CRC screening recommends that people at average risk start regular CRC screening at the age of 45 either with an annual stool-based test (FOBT or FIT) or a colonoscopy every 10 years (48).
According to ESMO guidelines, those at average risk should have organized access to CRC screening, if resources are available at a national level, but they give no exact recommendation regarding screening method or frequency (49).

2.5 Diagnosis and evaluation

2.5.1 Symptoms and clinical findings

Typical symptoms or a combination of symptoms may or may not be apparent in CRC patients, those such as rectal bleeding or blood in the stool, change in bowel habits, abdominal pain or discomfort, anaemia, fatigue, or weight loss. Upon clinical examination, a large tumour can be found by abdominal palpation, with approximately 50% of rectal cancers found by digital rectal examination (50).

Delay in diagnosis can be categorized as patient delay and doctor delay, with doctor delay further divided into family-doctor delay and hospital delay (51). Patient delay is accounted for by the patient being embarrassed, unaware of the importance of the symptoms, or afraid of the diagnosis (52,53). Patient delay is more common among male patients, among younger patients, and among those from lower socio-economic groups or ethnic minorities (53). Many studies have come to the conclusion that improving health education and raising awareness of CRC-related symptoms could shorten patient delay (51,53,54).

2.5.2 Endoscopy

Colonoscopy allows examination of the colon and rectum to determine tumour location, size, and histology, and it also makes tattooing of the tumour possible when necessary. The prevalence of synchronous CRC ranges from 1.1% to 8.1%, making it important to examine the entire colon (55).

2.5.3 Laboratory tests

Pre-operative laboratory tests include, at a minimum, haemoglobin, electrolytes, creatinine, and albumin, as part of nutritional-risk screening (NRS), and CEA, which is the only tumour marker recommended for CRC diagnosis and monitoring (4).

2.5.4 Imaging

For localized-CRC patients, contrast-enhanced computed tomography (CT) of the thorax and abdomen is essential for evaluation of possible distant metastases (25,49).

CT also aids in evaluating location and size of the tumour as well as its invasion depth and possible invasion into adjacent organs. Liver metastases are detectable by CT at a sensitivity of 74% to 84% and a specificity of 95%, and by magnetic resonance imaging (MRI) with a sensitivity of 80% to 88%.
and a specificity of 93% to 97% (56). For rectal cancer patients, pre-operative imaging also includes MRI of the pelvis to evaluate tumour location and size, depth of extra-mural spreading and nodal status (57). However, MRI seems to be poor at separating adenomas from T1 tumours, whereas endoscopic ultrasound (EUS) examination is highly accurate in the assessment of superficial tumours (58,59).

Imaging serves as a tool for planning the surgical and oncologic treatments whether for localized or metastatic CRC. It is also a tool for evaluating possible neoadjuvant oncological treatment for rectal cancer patients. Imaging is also useful to evaluate treatment response in the neoadjuvant and metastatic treatment settings.

The first global criteria for response evaluation in oncological therapy were published in 1982 (60). The World Health Organization (WHO)/Eastern Cooperative Oncology Group (ECOG) criteria have been replaced by the Response Evaluation Criteria in Solid Tumours (RECIST) 1.0 (61) and RECIST 1.1 (62). The RECIST criteria are comparable to the WHO/ECOG criteria in response evaluation of mCRC, and the RECIST guidelines are simple and reproducible (63). RECIST 1.1 defines CT as the gold standard in response evaluation in mCRC patients receiving chemotherapy.

At baseline, before chemotherapy, tumour lesions or lymph nodes or both will be categorized as measurable or non-measurable. The longest diameter in the plane of measurement is to be recorded, with the minimum size on CT being 10 mm for tumours and 15 mm for lymph nodes for measurable lesions. All other lesions are considered non-measurable. The number of lesions required to assess tumour burden is limited to a maximum of five in total and to a maximum of two per organ. The baseline sum diameters (also known as the target sum) will serve as the reference for response evaluation.

Response evaluation utilizes the following terminology: Complete response (CR) means disappearance of all target lesions and reduction in size of the pathological lymph nodes to <10 mm. Partial response (PR) means at least a 30% decrease in the sum of diameters of the target lesions. Progressive disease (PD) means at least a 20% increase in the sum of diameters of target lesions and also the appearance of one or more new lesions. Stable disease (SD) means neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. Now an Update and Clarification article from the RECIST committee is available, with commonly asked questions regarding RECIST 1.1 and answers (64).

2.6 CRC treatment

Multidisciplinary teamwork (MDT) improves tumour control and patients’ prognosis (65), and evidence even indicates patients with mCRC may be the ones to benefit the most from MDT (66).

MDT meetings consist of representatives of different specialities such as surgery, radiology, oncology, and pathology. A pre-operative MDT meeting
evaluates the patients' comorbidities, the possible need for neoadjuvant treatment, operation technique (laparoscopic or open surgery), extent of colon/rectum resection, possible protective stoma, need for extended pathological examinations (for instance if there exists a suspicion of hereditary CRC) and possible participation in ongoing trials. The MDT meeting's consensus plan reveals whether the aim is cure or palliation and also whether the primary tumour should be resected in cases with metastatic disease.

2.6.1 Treatment of localized colon cancer

2.6.1.1 Neoadjuvant treatment of localized colon cancer

Neoadjuvant treatment is administered before surgery to cause tumour shrinkage, to eradicate micrometastases, and prevent tumour-cell shedding during surgery, with the ultimate goal of improving the colon cancer patients' prognosis (67). As of today, neoadjuvant chemotherapy is not yet recommended for colon cancer patients, but several trials are ongoing (clinicaltrials.gov).

2.6.1.2 Surgery for localized colon cancer

Standardized operations for colon cancer include right hemicolectomy for cancer in the caecum or ascending colon, extended right hemicolectomy for cancer in the hepatic flexure, extended left-sided hemicolectomy for cancer in the splenic flexure, and left hemicolectomy for cancer in the descending or sigmoid colon. One should aim for a 5-cm margin proximal and distal to the tumour, the primary vessels should be ligated at the root of the mesentery, and a wide mesenteric resection is required to ensure a harvest of at least 12 lymph nodes (68). For small and local tumours, endoscopic excision may sometimes be an option, especially for older and fragile patients (69).

These operations can be performed by open or mini-invasive (laparoscopic- or robotic-assisted) surgery. Laparoscopic hemicolecctomy has the advantages of faster recovery, less post-operative pain, and better cosmetic results, without jeopardizing the oncological radicality (70–72). Evidence shows that complete mesocolic excision (CME) leads to a larger number of regional lymph nodes in the specimen, but evidence is limited that CME improves the oncological outcomes (73–75). Oncological outcomes between laparoscopic and open colon surgery are similar, but some evidence leads one to recommend laparoscopy because of the shorter hospital stay (76).

2.6.1.3 Adjuvant chemotherapy of localized colon cancer

Adjuvant therapy plays an established role in high-risk stage II and stage III colon cancer patients, and since 1990, 5-fluorouracil (5-FU) has been the standard treatment (77,78). These two studies by Laurie and colleagues (77) and Moertel and colleagues (78) showed a 40% to 41% risk reduction of recurrence in the 5-FU+surgery group compared to that of the surgery-alone group. Several studies such as the NSABP C-04 trial (79), the Intergroup study
0089 (80), the GERCOR C96.1 (81), and the PETACC-2 (82) have evaluated the efficacy of adjuvant therapy with 5-FU modulated by leucovorin and levamisole; the results of these trials are explained in section 5.8.1.1, Intravenous 5-FU.

Single-agent 5-FU is standard therapy in high-risk stage II tumours, but oxaliplatin can be considered on an individual basis, for instance in cases involving a T4 tumour. The benefit of oxaliplatin in the adjuvant setting has been demonstrated in these three trials: the MOSAIC study (83), the NSABP C-07 trial (84), and the XELOXA international phase III study (85). The results of the trials are explained in more detail under 5.8.2.1.1, Oxaliplatin in the adjuvant setting.

According to an ESMO eUpdate on early colon cancer recommendations (86), follow-up is recommended for patients with low-risk stage II colon cancer, and 5-FU should be considered for high-risk stage II colon cancer patients. Stage II colon cancers are considered high-risk if they present with at least one of the following clinical characteristics: T4 tumour, number of examined lymph nodes <12, tumour grade 3, vascular or lymphatic or perineural invasion, tumour presentation with obstruction or perforation, and absence of MSI (86). Patients with very high-risk stage II colon cancer (MSS and T4 tumour or more than one validated risk factor) may be considered for the addition of oxaliplatin (86). Updated ESMO guidelines are awaited.

According to NCCN Guidelines (based on results from the IDEA Collaboration) evidence is sufficient to divide stage III colon cancer into a low-risk (T1–3N1) and a high-risk group (T4N1–2) (87,88). For the low-risk stage III group, the recommendation is 3-month adjuvant treatment with CAPOX (capesitabine and oxaliplatin) or 3- to 6-month adjuvant treatment with FOLFOX. For the high-risk stage III group, the recommendation is 3- to 6-month adjuvant treatment with CAPOX or 6-month adjuvant treatment with FOLFOX.

2.6.2 Treatment of localized rectal cancer

2.6.2.1 Neoadjuvant therapy of localized rectal cancer

The aim of neoadjuvant radiotherapy is to improve local control by reducing the risk of local recurrence, improving resectability to enable R0-resection (when the mesorectal fascia is involved or threatened or when the patient has a T4 tumour), and preserving sphincter function.

The two different types of neoadjuvant therapy options are short-course radiotherapy (SCRT) with 5 × 5 Gy followed by immediate (within 1 week) or delayed (after 6-8 weeks) surgery (especially for older and fragile patients) versus long-course chemoradiotherapy (CRT) with 50.4 Gy in 25 to 28 fractions, with surgery after a 7- to 10-week break (89,90). Chemotherapy sensitises the tumour to radiotherapy, and the recommended chemotherapy is continuous infusion of 5-FU or oral capecitabine during CRT (49). Oxaliplatin is not recommended as a radiosensitiser, because it enhances acute toxicity and lacks long-term oncological benefits (89).
It is difficult to define exactly which T and N sub-stages require SCRT and which require long-course CRT (89). The pre-operative approach in locally advanced rectal cancer is based on an MDT decision regarding the risk of a positive circumferential resection margin at total mesorectal excision (TME) surgery. Finnish national guidelines have been available since 2019 (76).

2.6.2.2 Surgery for localized rectal cancer

Among standardized operations for rectal cancer are anterior resection (AR) for rectal cancer in the upper and middle third of the rectum and abdominoperineal excision (APE) with a permanent colostomy for distal rectal cancer beyond a sphincter-preserving possibility. Transanal mini-invasive surgery (TAMIS) is the choice for elderly patients as well as for superficial tumours (T1N0), with the avoidance of permanent colostomies (91).

The most common operation is AR (60-70%) with a colorectal or coloanal anastomosis often combined with a protective stoma (loop-ileostomy or loop-colostomy) (92–94). A protective stoma prevents anastomotic leak and reduces the need for urgent reoperations, but seems to offer no advantages regarding 30-day or long-term mortality (95). After the anastomosis has healed and a verifying endoscopic examination has been performed, the protective stoma can be reversed. APE is indicated when the tumour invades the anal sphincters, when the patient is unfit for an anastomosis (high risk of anastomotic leak), or when the patient has a history of anal incontinence (96).

Both operations include TME, which means removal of the entire rectal mesentery - including that distal to the tumour - as an intact unit. This method raises the likelihood of negative lateral margins and also facilitates nerve preservation (97). The completeness of the mesorectal fascia (MRF) is the most important prognostic factor for the quality of rectal surgery (98). As said, poor surgical quality cannot be treated with chemotherapy. A distal margin of 5 cm has been recommended for decades, but for distal rectal cancers, a margin of 1 to 2 cm can be acceptable when combined with a temporary protective stoma to prevent anastomotic leak (99,100).

Local recurrences are more common after APE than after AR, and introduction of extralevator abdominoperineal excision (ELAPE) was meant to address this problem (101). Indications for ELAPE would therefore be either T3-T4 tumours or tumours threatening the circumferential resection margin (CRM) (96). The literature provides evidence favouring ELAPE over APE (102,103), but also provides conflicting results (104). One Swedish prospective registry-based population study showed significantly more short-term complications after ELAPE, and selective use of ELAPE was favoured (101).

These operations can be performed by conventional open surgery, by laparoscopic-assisted surgery, or by robotic-assisted surgery (105). Some evidence supports laparoscopic rectal cancer surgery in upper and middle rectal cancer (106) and in patients with rectal cancer without invasion of adjacent tissues (107). On the other hand, the findings in two RCTs comparing open surgery to laparoscopic surgery did not support laparoscopic resection for rectal cancer patients, and these recommend longer follow-up (108,109).
A meta-analysis of laparoscopic vs. open mesorectal excision questioned the oncologic safety of laparoscopy and argued for long-term results (110).

### 2.6.2.3 Adjuvant chemotherapy of localized rectal cancer

Evidence regarding the effectiveness of adjuvant therapy is controversial. In a randomised study by the Dutch Colorectal Cancer Group on stage II and III rectal cancer patients undergoing R0 resection after neoadjuvant treatment, adjuvant chemotherapy (5-FU/LV or capecitabine) failed to improve DFS, OS, or recurrence rate (111). Two more RCTs on adjuvant chemotherapy following SCRT or long-course CRT and surgery failed to find improvement in DFS, OS, or recurrence rate (112,113).

A Cochrane review comprising 20 RCTs and 8 530 stage III rectal cancer patients showed a 25% reduction in the risk of recurrence among patients receiving 5-FU-based chemotherapy and surgery compared to undergoing surgery alone, and this supports the use of adjuvant 5-FU-based chemotherapy (114). However, only a few studies included in that meta-analysis required TME surgery or neoadjuvant SCRT or long-course CRT or both.

According to ESMO guidelines, it is reasonable to consider adjuvant chemotherapy after neoadjuvant therapy in stage III and high-risk stage II rectal-cancer patients. The level of scientific evidence for sufficient benefit of adjuvant therapy is, however, much lower in rectal cancer than in colon cancer and is probably limited to DFS rather than OS (89). High-risk features in stage II rectal cancer are positive margins, lymphovascular invasion, poorly differentiated tumours, or submucosal invasion into the lower third of the submucosa (88). Moreover, the decision on adjuvant chemotherapy, either 5-FU-based alone or in combination with oxaliplatin, should be risk-balanced, evaluating the predicted toxicity for a particular patient and the risk of relapse, and should be made by the patient and the clinician together.

### 2.6.3 Treatment of mCRC

In 20% to 25% of all cases, CRC is diagnosed with synchronous liver-, lung-, peritoneal-, or other metastases, and in 30% to 50% of the cases, patients treated with curative intent develop metastatic disease (115,116). The most common metastatic sites are the liver and lungs, and the next most common metastatic sites are the peritoneum, the central nervous system, and the skeleton (116).

In mCRC, it may be possible to offer the patient treatment with curative intent, such as a liver- or lung resection, but it is also possible that best supportive care (BSC) is all that can be offered. Resection of the primary tumour is not always necessary or even possible in the metastatic setting, and sometimes a palliative operation such as a decompressing stoma or by-pass surgery can be more helpful for the patient than very extensive surgery for the primary.
Conversion therapy is a treatment option for patients with borderline resectable liver or lung metastases to make the metastases resectable. Palliative chemotherapy, first line, second line, third line, and so on may be a treatment option for other patients. Interventional radiological treatments, selective internal radiation therapy (SIRT), cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC), liver transplantation, and so on, are all different treatment options for mCRC. The list is long of possible treatment options to offer to patients with mCRC. The MDT plays an important role in decision-making as to the best treatment option for the patient, taking into consideration the patient's ECOG performance status, liver and kidney function, tumour burden, and location of metastases (116).

2.6.3.1 Colorectal liver metastases

The liver is the most common metastatic target organ for CRC (117), and around 20% of the patients with liver metastases can be offered a curative resection (118).

Patients with liver metastases have a median survival of 5 to 20 months if left untreated, 2-year survival is unusual, and 5-year survival is extremely rare (119). The 5-year OS rate is 30% to 50% for patients after liver resection compared to 10% to 15% for patients receiving palliative chemotherapy (115,120). However, more than 70% of patients with liver metastases who have had a liver resection do develop recurrence in the remnant liver (121).

Patients with liver-limited unresectable metastases or borderline resectable liver metastases can be offered conversion therapy to make the initially unresectable metastases resectable (122). The 5- and 10-year survival rates after conversion therapy have been 33% and 23%, compared to a respective 48% and 30% in patients with primarily resectable liver metastases (P=0.01) (122).

Chemotherapy may be an option for patients prior to liver resection, either as conversion therapy or as neoadjuvant chemotherapy in potentially curative liver resections. The conversion therapy, as well as the neoadjuvant therapy, consists of a fluoropyrimidine backbone (intravenous 5-FU or oral capecitabine) combined with oxaliplatin or irinotecan, with the addition of a biological agent (116).

2.6.3.2 Colorectal pulmonary metastases

Most patients with pulmonary metastases have non-resectable locally advanced disease or concurrent metastases to other organs and are therefore excluded from curative pulmonary metastasectomy (123). Patients with lung-limited, initially unresectable metastases can be offered conversion therapy in order to make the lung metastases resectable (116).

Video-assisted thoracic surgery (VATS) is now the method of choice for the treatment of stage I non-small cell lung cancer, but for colorectal pulmonary
metastases, some surgeons still prefer the open approach, since it allows for manual palpation of the entire lung parenchyma (6,124).

Clinical variables associated with prolonged survival after surgery include prolonged disease-free interval between primary tumour and pulmonary metastases, normal pre-thoracotomy CEA, absence of thoracic node involvement, and a single pulmonary lesion (125).

Resection of resectable pulmonary metastases in carefully selected patients offers a 5-year survival rate of 30% to 50% (126).

2.6.3.3 Neoadjuvant and adjuvant treatment for liver- or lung metastases

Typical adjuvant chemotherapy has a fluoropyrimidine backbone (i.e. intravenous 5-FU or oral capecitabine) and is combined with oxaliplatin, because FOLFOX or CAPOX provides a marginally better DFS and a non-significantly better OS than resection alone (127–130). Fluoropyrimidines have been tested in the adjuvant setting (128), and oxaliplatin-based in the perioperative setting (with neo- and adjuvant setting) (129). Hepatic arterial infusion (HAI) has shown some benefit in three randomized studies (131–133).

The monoclonal antibody bevacizumab (anti-VEGF) has been tested in the conversion/neo- and adjuvant setting with some benefit (134,135). Cetuximab (anti-EGFR) showed high conversion rates in the CELIM study (136), but showed impaired survival in the NEO-EPOC study (137), even in RAS-wild type patients (116).

2.6.3.4 Other treatment options for mCRC

Cytoreductive surgery and HIPEC
Peritoneal metastases are usually associated with poor prognosis. In highly selected patients with peritoneal metastases, ones who are sufficiently fit, complete cytoreductive surgery and HIPEC may provide survival benefit (116,138,139). However, the Prodige 7 trial showed no differences between the two groups in OS (41.2 months in the non-HIPEC group vs. 41.7 months in the HIPEC group) or RFS (11.1 months in the non-HIPEC group vs. 13.1 months in the HIPEC group) (140).

Interventional radiology and local ablative therapy
For patients with liver-limited and non-resectable liver metastases, several loco-ablative treatment options are available such as radio frequency ablation (RFA), transarterial chemoembolisation (TACE), and SIRT (116).

Liver transplantation
Some patients with liver-limited and non-resectable liver metastases may be candidates for liver transplantation. Organ shortage is, however, a limiting factor in most countries. Liver transplantation for 21 patients with liver metastases not suitable for R0 resection has shown initial results as follows:
OS at 1 year 95%, at 3 years 68%, and at 5 years 60%, and DFS was 35% at 1 year and 0% at 2 years (141).

2.7 Pathology and staging of CRC

Pathologic examination of biopsies, polypectomies, and resection specimens is crucial to adequate patient management, prognosis assessment, and family counselling (142). More than 90% of CRCs are adenocarcinomas with their origin from epithelial cells of the colorectal mucosa (143). Other rare histological types include neuroendocrine, squamous cell, adenosquamous, spindle cell, melanoma, and undifferentiated carcinomas.

Conventional adenocarcinomas are characterized by glandular formation, which is the basis for histologic tumour grading, and they are divided into three groups: well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, and poorly differentiated adenocarcinoma (143). Most adenocarcinomas (approximately 70%) are diagnosed as moderately differentiated, whereas well- and poorly differentiated adenocarcinomas account for 10% and 20%. Many have demonstrated that a two-scale grading system with low-grade (including well and moderately differentiated adenocarcinoma) and high-grade (including poorly differentiated) reduces interobserver variation and improves prognostic significance compared to a three-scale grading system (with well, moderately and poorly differentiated adenocarcinoma) (144,145).

According to the WHO classification, the histologic variants of adenocarcinomas are mucinous, signet ring cell, medullary, micropapillary, serrated, cribriform comedo-type, adenosquamous, spindle cell, and undifferentiated (143). Mucinous adenocarcinomas typically have large glandular structures with pools of extracellular mucin, and many mucinous adenocarcinomas occur in patients with Lynch syndrome (142). Signet ring cell adenocarcinomas are rare, representing less than 1% of all colorectal adenocarcinomas and are poorly differentiated (high grade) and carry a worse outcome than do conventional adenocarcinoma (146). Medullary, micropapillary, serrated, cribriform comedo-type, adenosquamous, spindle cell, and undifferentiated adenocarcinomas are extremely rare (143).

In 1950, the Union Internationale Contre le Cancer (UICC) published the tumour node metastasis (TNM) staging system (Nomenclature classification des cancers) (147). Approximately one decade later, in 1959, the American Joint Committee on Cancer (AJCC) included prognostic TNM subgroups in their staging system. Since 1980, the work of UICC and AJCC have been coordinated. The latest TNM staging manual for malignant diseases, the 8th edition, appeared in 2016 (148).

Staging of colorectal cancer serves as a prognostic tool and also as a tool for planning of surgical and oncological treatments.
<table>
<thead>
<tr>
<th><strong>Primary tumour (pT)</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>no evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>carcinoma in situ; intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)</td>
</tr>
<tr>
<td>T1</td>
<td>tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria)</td>
</tr>
<tr>
<td>T2</td>
<td>tumour invades muscularis propria</td>
</tr>
<tr>
<td>T3</td>
<td>tumour invades through the muscularis propria into the pericolorectal tissues</td>
</tr>
<tr>
<td>T4a</td>
<td>tumour invades through the visceral peritoneum (including gross perforation of the bowel through tumour and continuous invasion of tumour through areas of inflammation to the surface of the visceral peritoneum)</td>
</tr>
<tr>
<td>T4b</td>
<td>tumour directly invades or is adherent to other organs or structures</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Regional lymph node metastases (pN)</strong></th>
<th></th>
</tr>
</thead>
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<tr>
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<td>regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>No</td>
<td>no regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>metastasis in 1 - 3 regional lymph nodes</td>
</tr>
<tr>
<td>N1a</td>
<td>metastasis in 1 regional lymph node</td>
</tr>
<tr>
<td>N1b</td>
<td>metastasis in 2 - 3 regional lymph nodes</td>
</tr>
<tr>
<td>N1c</td>
<td>no regional lymph nodes are positive but there are tumour deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues</td>
</tr>
<tr>
<td>N2</td>
<td>metastasis in 4 or more regional lymph nodes</td>
</tr>
<tr>
<td>N2a</td>
<td>metastasis in 4 - 6 regional lymph nodes</td>
</tr>
<tr>
<td>N2b</td>
<td>metastasis in 7 or more regional lymph nodes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Distant metastases (M)</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>no distant metastasis by imaging</td>
</tr>
<tr>
<td>M1</td>
<td>distant metastasis</td>
</tr>
<tr>
<td>M1a</td>
<td>metastasis confined to 1 organ or site without peritoneal metastasis</td>
</tr>
<tr>
<td>M1b</td>
<td>metastasis to 2 or more sites or organs is identified without peritoneal metastasis</td>
</tr>
<tr>
<td>M1c</td>
<td>metastasis to the peritoneal surface is identified alone or with other site or organ metastases</td>
</tr>
</tbody>
</table>

Table 1  TNM staging of colorectal cancer by UICC / AJCC, 8th edition, 2017
<table>
<thead>
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<th>Stage</th>
<th>T</th>
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<th>M</th>
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<td>No</td>
<td>Mo</td>
</tr>
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<td>Mo</td>
</tr>
<tr>
<td>IIC</td>
<td>T4b</td>
<td>No</td>
<td>Mo</td>
</tr>
<tr>
<td>IIIA</td>
<td>T1-2</td>
<td>N1 / N1c</td>
<td>Mo</td>
</tr>
<tr>
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<td>T1</td>
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<td>Mo</td>
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<td>N1 / N1c</td>
<td>Mo</td>
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<td>Mo</td>
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<td>M1b</td>
</tr>
<tr>
<td>IVC</td>
<td>any T</td>
<td>any N</td>
<td>M1c</td>
</tr>
</tbody>
</table>

Table 2  Stage grouping by UICC / AJCC, 8th edition, 2017

CRC prognosis is predicted by TNM stage. Based on the SEER (Surveillance, Epidemiology, and End Results) Program, in colon cancer patients diagnosed between 2008 and 2014, 5-year survival rates were for localized cancer (stages I, IIA, and IIB) 90%, regional cancer (stages IIC and III) 71%, and distant cancer (stage IV) 14%. Corresponding survival rates for rectal cancer were localized cancer (stages I, IIA, and IIB) 89%, regional cancer (stages IIC and III) 70%, and distant cancer (stage IV) 15% (48).

Figure 2 shows the 5- and 10-year survival rates of 17 526 colon cancer patients extracted from the Taiwan Cancer Registry, 2002-2009. Five-year survival rates were 87.8% for stage I, 76.8% for stage II, 62.2% for stage III, and 14.2% for stage IV. The ten-year survival rates were 66.8% for stage I, 63.2% for stage II, 47.1% for stage III, and 10.0 for stage III %.
Chemotherapeutic agents in the treatment of CRC

Chemotherapy is possible in a neoadjuvant, in an adjuvant, or in a metastatic setting.

In the neoadjuvant setting, chemotherapy (usually continuous infusion of 5-FU or oral capecitabine) is the choice for treatment of certain rectal cancers to sensitize the tumour to radiotherapy (49).

In the adjuvant setting, the aim is to reduce risk of recurrence and thus improve survival. Generally, adjuvant treatment is recommended for stage III and high-risk stage II CRC patients. The 5-year survival after surgical resection alone according to the TNM classification is 99% for stage I, 68% to 83% for stage II, and 45% to 65% for stage III (148).

The 5-FU-based therapy combined with surgery improved the DFS to 67% compared to 55% for the surgery-only group (62). Adding oxaliplatin to the 5-FU treatment further improved the delta for 5-year DFS from 6.2% to 7.5% and the OS from 2.7% to 4.2% in stage III colon cancer patients (149).

In the metastatic setting, the aim is to achieve increased PFS, OS, and quality of life (QoL). Randomized studies comparing chemotherapy with BSC have showed prolonged survival and QoL in the chemotherapy group (150,151).
Today, the median OS for mCRC patients is reaching 30 months and is more than twice as long as it was 20 years ago (116).

2.8.1 Antimetabolites as single agents in CRC

2.8.1.1 Intravenous 5-fluorouracil (5-FU)

5-FU is an antimetabolite that is intracellularly converted into three active metabolites which disrupt RNA and DNA synthesis, thus leading to cell death. Its main anticancer effect is based on an irreversible inhibition of thymidylate synthase (TS), which leads to cell death in cancer cells. (152).

Ullman and colleagues were first to report that inhibition of TS by 5-FU in leukaemia cells could be potentiated by increased intracellular levels of the reduced folate leucovorin (153). Since then, modulation strategies have been developed to enhance the anticancer activity of 5-FU by co-treatment with leucovorin (LV) in mCRC (154,155). The antihelminthic drug levamisole has also reduced the toxicity of 5-FU (77).

5-FU is used both as a single agent or in combination with other chemotherapies and can be administered as an intravenous bolus or as a continuous intravenous infusion or orally as a prodrug (capecitabine, an oral prodrug of 5-FU).

The nature of 5-FU drug resistance is either primary or secondary. Multiple factors may contribute to 5-FU resistance, and one explanation for primary resistance may be that 5-FU is being pumped out of the tumour cells. Secondary drug resistance appears after 3 to 4 months (116). The overall response rate (RR) for mCRC with 5-FU alone is still 10% to 15%, and the combination of 5-FU with other anti-tumour drugs has improved its RR to 40% to 50% (156).

Several intravenous bolus regimens have been developed over time such as Roswell Park, Nordic FLv, and Mayo. (80,157,158). Because of toxicity issues, and in particular haematologic toxicity, Mayo and Roswell Park are no longer as widely used as in the beginning of the 5-FU era, but they are both still included in the present ESMO and NCCN guidelines.

Several continuous infusion regimens have been studied. Today, the widely accepted standard regimen is the modified or simplified de Gramont regimen developed by the French GERCOD (Groupe d’Etude et de recherches sur les Cancers de l’Ovarie et Digestifs) group; it consists of infusion of leucovorin followed by a bolus of 5-FU and a continuous infusion of 5-FU over 46 hours (159).
2.8.1.1 Intravenous 5-FU in the adjuvant setting

The cornerstone of adjuvant therapy is 5-FU (5). In 1989, the North Central Cancer Treatment Group (NCCTG) reported a 40% reduced risk of recurrence in stage III colon cancer patients receiving adjuvant 5-FU compared to results from surgery alone (77). In another study, stage II and III colon cancer patients had a 41% relative risk reduction for recurrence in the 5-FU/levamisole group compared to the surgery+ levamisole group or the surgery alone group (78). The median 5-year OS rate was 60% for the 5-FU/levamisole group vs. 47% for observation and 49% for levamisole alone (P=0.0007). In 1990, after these findings, adjuvant treatment with 5-FU became the new standard for stage II and stage III CRC.

In addition to these trials, among the many trials in the adjuvant setting of stage II and III colon cancer have been the NSABP C-04 trial (79), the Intergroup study 0089 (80), the GERCOR C96.1 (81), and the PETACC-2 (82).

The NSABP C-04 trial randomised stage II and III colon cancer patients to receive 5-FU/LV, 5-FU/levamisole, or 5-FU/LV/levamisole (79). In stage II patients, 5-FU/LV reduced the risk of recurrence and enhanced survival better than did 5-FU/LV/levamisole (5-year DFS rate 75% vs. 71%, and 5-year OS rate 84% vs. 81%). In stage III patients, as well, 5-FU/LV reduced the risk of recurrence and enhanced survival better than did 5-FU/LV/levamisole (5-year DFS rate 57% vs. 53%, and 5-year OS rate 67% vs. 63%). The combination of LV and levamisole added no further benefit.

The Intergroup study 0089 included high-risk stage II and stage III patients to investigate the effect of biochemical modulation of 5-FU by comparing 5-FU + low-dose LV, 5-FU + high-dose LV, 5-FU + levamisole, and 5-FU + levamisole + low-dose LV (80). No statistically significant differences between regimens appeared in either DFS or OS.

The GERCOR C96.1 study compared the Mayo regimen with LV5FU2 in stage II and III patients. The study also compared the duration of treatment, 24 weeks vs. 36 weeks (81). Both chemotherapy regimens were equal in terms of DFS (HR 1.01) and OS (HR 1.02). No statistically significant differences appeared in DFS or OS between the two treatment-duration groups. The PETACC-2 (the Pan European Trial in Adjuvant Colon Cancer 2 study) trial compared infusional high-dose 5-FU regimens to a bolus 5-FU/LV regimen (82). The infusional high-dose 5-FU regimens were either the Spanish TTD regimen or the German AIO regimen, and the bolus 5-FU/LV regimen was the Mayo regimen. The patients were randomised to receive one of these regimens. No significant differences in RFS or OS were detectable between infusional high dose 5-FU regimens compared to those of the bolus 5-FU/LV regimen, but the latter regimen was the more toxic.
<table>
<thead>
<tr>
<th>Study/publishing year</th>
<th>Stage</th>
<th>Patients (n)</th>
<th>Duration (months)</th>
<th>Effect on relapse</th>
<th>Effect on mortality</th>
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</thead>
<tbody>
<tr>
<td>5-FU+levamisole</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCCTG and Mayo Clinic</td>
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<td>262</td>
<td>12</td>
<td>Reduced*</td>
<td>Reduced*</td>
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<td>(77) 1987</td>
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<tr>
<td>Intergroup study</td>
<td>III</td>
<td>929</td>
<td>12</td>
<td>Reduced 41%*</td>
<td>Reduced 33%*</td>
</tr>
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<td>(160) 1995 (78) 1990</td>
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<tr>
<td>Dutch Colon and rectal cancers</td>
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<td>12</td>
<td>Not reported</td>
<td>Reduced 25%*</td>
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<td>(161) 2001</td>
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<td>NSABP C-03</td>
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<td>(165) 1997</td>
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</table>

Table 3  Studies with Surgery + 5-FU +levamisole / 5-FU+leucovorin vs. surgery alone in the adjuvant treatment of colon cancer

*P<0.05

2.8.1.1.2 Intravenous 5-FU in the metastatic setting

The mOS in mCRC patients who received BSC and underwent no oncological treatments was 2 to 5 months (150,167). Single-agent intravenous 5-FU improved the mOS by raising it to 15.8 months (167).

An RCT with 348 patients compared the Mayo regimen (bolus 5-FU) to the LV5FU2 regimen (continuous infusion 5-FU) and noted a favourable RR in the FV5FU2 group of 32.6% vs. 14.4% (P=0.0004) (168). The mPFS was 27.6 weeks in the LV5FU2 group and 22 weeks in the Mayo regimen group (P=0.0012). The mOS was 62 weeks the LV5FU2 regimen and 56.8 weeks in the Mayo regimen (P=0.067).

A meta-analysis of 6 RCTs with 1 219 patients comparing bolus 5-FU with continuous infusion of 5-FU showed a significantly higher RR and OS in the continuous-infusion 5-FU group than in the bolus 5-FU group (RR 22% vs. 14%, P=0.0002 and OS overall HR 0.88; 95% CI, 0.78 to 0.99; P=0.04) (169). Regarding toxicity, grade 3 or 4 haematologic toxicity was more frequent in patients receiving bolus 5-FU (31% v 4%; P<10(-16)), whereas hand-foot syndrome (HFS) was more frequent in the continuous infusion 5-FU group (34% vs. 13%; P<10(-7)) (169).

Today, single-agent intravenous 5-FU is still a treatment option for frail and elderly patients (88,116,170).
2.8.1.1.3 Treatment-related adverse events during 5-FU-based chemotherapy

Generally speaking, intravenous bolus regimens are more toxic than continuous infusion regimens. Grade 3-4 leukopenia and stomatitis are associated with the 5-FU bolus regimens, and HFS is associated with the continuous infusion regimens (82).

<table>
<thead>
<tr>
<th>Chemotherapy regimen</th>
<th>Mayo LV5FU2 %</th>
<th>Mayo PVI 5-FU %</th>
<th>Mayo Roswell Park %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
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<td>55.6</td>
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<tr>
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<td>3.5</td>
<td>&lt;1</td>
</tr>
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</table>

Table 4 Toxicity related to 5-FU (166)

2.8.1.2 Capecitabine

Oral 5-FU has not gained widespread clinical acceptance because of its erratic absorption related to the varying levels of dihydrophymidine dehydrogenase in the gastrointestinal tract (172). The oral 5-FU prodrugs are absorbed as intact molecules and are then converted to 5-FU to exert antitumour activity. Several oral prodrugs are available for CRC treatment such as capecitabine, UFT (tegafur-uracil), carmofur, doxifluridine, tegafur, and S-1 (173).

Capecitabine, an oral prodrug of 5-FU, is activated to cytotoxic 5-FU by thymidine phosphorylase and has a pharmacokinetic profile similar to that of continuous infusion of 5-FU (174). In CRC, it is administered orally twice a day for 2 weeks, followed by a week of rest.

2.8.1.2.1 Capecitabine in the adjuvant setting

A randomised trial with 1 987 patients, the X-ACT (Xeloda in Adjuvant Colon Cancer Therapy) trial, showed that treatment with capecitabine led to significant improvement in RFS (HR 0.86, CI95% 0.74–0.99) over bolus 5-FU/LV in stage III colon cancer patients, but produced no significant difference in DFS (HR 0.87, CI95% 0.75–1.00) or OS (HR 0.84, CI95% 0.69–1.01) (175). Capecitabine was also associated with significantly fewer adverse events than FU/LV. After these results appeared, capecitabine was approved for the adjuvant setting.

2.8.1.2.2 Capecitabine in the metastatic setting

When results from two randomised trials (1 207 patients) comparing capecitabine to bolus 5-FU/LV were integrated, capecitabine offered a
superior response rate (26% vs. 17%, P<0.0002) and equivalent time to progression (TTP) (HR 0.997, 95% CI 0.885 – 1.123, P=0.95; median 4.6 months with capecitabine vs. 4.7 months with 5-FU/LV) and survival compared with 5-FU/LV (176–178). The median survival was 12.9 months in the capecitabine group and 12.8 months in the 5-FU/LV group (HR 0.95, 95% CI 0.84–1.06, P=0.48).

2.8.1.2.3 Treatment-related adverse events during capecitabine treatment

An integrated analysis showed that capecitabine had a more improved safety profile than did 5-FU/LV, with a significantly lower incidence of diarrhoea (grade 1-4 47% vs. 58%), stomatitis (24% vs. 61%), nausea (37% vs. 47%), or alopecia (6% vs. 20%) (179). The only more frequently occurring adverse event in the capecitabine group was hand-foot syndrome (53.5 vs. 6.2% with 5-FU/LV).

Because of its superior response rate, equivalent overall survival, its improved safety profile, and improved convenience when compared with 5-FU/LV, capecitabine is a suitable replacement for 5-FU (178).

<table>
<thead>
<tr>
<th>Chemotherapy regimen</th>
<th>Grade 3-4 toxicity</th>
<th>Capecitabine</th>
<th>FU/LV</th>
<th>Capecitabine</th>
<th>FU/LV</th>
<th>Capecitabine</th>
<th>FU/LV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td></td>
<td>2.0</td>
<td>19.8</td>
<td>2.6</td>
<td>25.9</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td>10.7</td>
<td>10.4</td>
<td>15.4</td>
<td>13.9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Stomatitis</td>
<td></td>
<td>1.3</td>
<td>13.3</td>
<td>3.0</td>
<td>16.0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Hand-foot syndrome</td>
<td></td>
<td>16.2</td>
<td>0.3</td>
<td>18.1</td>
<td>0.7</td>
<td>17</td>
<td>0.6</td>
</tr>
<tr>
<td>Study</td>
<td></td>
<td>(178)</td>
<td>(177)</td>
<td>(175)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Capecitabine vs. FU/LV toxicity (166)

2.8.1.3 Carmofur

Carmofur (1-hexylcarbamoyl-5-fluorouracil abbreviated HCFU) is an oral 5-FU derivate metabolized to 5-fluorodeoxyuridine monophosphate (FdUMP); it acts similarly to continuous infusion of 5-FU (180).

Carmofur has been used and studied mostly in Japan because intravenous 5-FU combined with leucovorin was unavailable on the Japanese market. It was sold in Finland because a Finnish pharmaceutical company had the marketing authorization of carmofur. At the time it was the only peroral fluoropyrimidine available on the Finnish market, both in the adjuvant (181) and the metastatic setting (182) as monotherapy as well as in combination therapy (183). Carmofur was never pursued for Food and Drug Administration (FDA) approval in the USA and has never gained wider acceptance in Europe, because of the development of better-tolerated peroral fluoropyrimidines.
2.8.1.3.1 Carmofur in the adjuvant setting

In an individual patient-based meta-analysis with 2,152 CRC patients, carmofur as the adjuvant chemotherapy was compared with surgery alone (181). The 5-year OS rates were 80.4% vs. 76.4%, and the 5-year DFS rates were 76.9% vs. 71.0%, in the carmofur and surgery group compared to the surgery-alone group. Oral carmofur had a significant advantage over surgery alone in terms of both OS (HR 0.82; CI95%=0.68–0.99; P=0.043) and DFS (HR 0.77; CI95% 0.65–0.91; P=0.003). This individual patient-based meta-analysis demonstrated that oral carmofur significantly improves both OS and DFS in patients with curatively resected colon cancers.

2.8.1.3.2 Carmofur in the metastatic setting

Carmofur is active as monotherapy in mCRC with a RR ranging from 12% to 27% (182). The RR in combination therapy (carmofur and raltitrexed) was 44%, the median TTP was 5.2 months, and median survival was 9.1 months (183).

2.8.1.3.3 Treatment-related adverse events during carmofur treatment

Carmofur has a manageable toxicity profile, with increased urinary frequency and hot flushes being the most common adverse events (182). Diarrhoea, nausea/vomiting, anorexia, fatigue, and haematological toxicity also occur.

2.8.1.4 Raltitrexed

Raltitrexed is a folate-based inhibitor of TS with a specific mode of action (184); it predominantly enters the cell via the reduced folate carrier and then undergoes polyglutamation, whereafter, it remains in the cell and prevents TS from binding to its folate cofactor (185).

2.8.1.4.1 Raltitrexed in the adjuvant setting

Raltitrexed is not used in the adjuvant setting. The PETACC-1 trial, a randomised trial with 1,921 colon cancer patients, compared raltitrexed and 5-FU/LV in the adjuvant setting (186). The trial closed prematurely because 17 (1.9%) deaths occurred related to raltitrexed treatment.

2.8.1.4.2 Raltitrexed in the metastatic setting

Raltitrexed has showed comparative efficacy in treatment of mCRC versus the standard Mayo clinic 5-FU+leucovorin combination, in which 5-FU 425 mg/m² is given on 5 successive days together with 20 mg/m2 leucovorin in 4-week cycles (187). In one study, the Mayo regimen appeared to be somewhat more efficient than raltitrexed (172), but what needs to be borne in mind is that this was a single drug versus a two-drug comparison.

The COMET study was a retrospective study focusing on mCRC patients receiving raltitrexed as a single agent or in combination therapy, patients who had experienced cardiotoxicity or disease progression with prior 5-FU/LV
treatment. This study also recorded cardiovascular risk factors and cardiovascular history (188). The results showed raltitrexed as effective in heavily pretreated mCRC patients with tumour progression after 5-FU therapy and thus could prove itself a safe therapeutic alternative to 5-FU for patients with mCRC and prior cardiac toxicity (188).

Raltitrexed has been combined with fluoropyrimidines in several studies (189,190). In one phase I study, mCRC patients received raltitrexed and carmofur, and their RR was 50%; at the time, this chemotherapy combination had a manageable toxicity and a promising efficacy in mCRC (183). The phase II results have been published as part of Study IV (191).

2.8.1.4.3 Treatment-related adverse events with raltitrexed

The most frequently reported DLT are malaise, diarrhoea, and neutropenia. Elevated liver transaminases also occur, but these are usually reversible. Sometimes also a rash is evident (Table 6). The overall safety profile for raltitrexed is acceptable, and some advantages such as less severe neutropenia and mucositis have been apparent (183,192).

The MEPSYT-TNF study (60 patients) findings showed that mCRC patients treated with single-agent raltitrexed or the combination of raltitrexed and carmofur often developed fever, fatigue, and CRP elevation, plus elevated proinflammatory cytokine levels, which peak a few days after raltitrexed infusion and may recur after subsequent cycles (193). These features are suggestive of a drug-related systemic inflammation which may be detrimental and cause excess treatment-related deaths (186).

2.8.1.4.4 Raltitrexed in clinical use

Raltitrexed never gained widespread acceptance in clinical use because of its unacceptably high treatment-related death rate (186), the cytokine-release syndrome (193), and in part because of its lack of superiority to 5-FU (186). Raltitrexed is mostly the choice in fluoropyrimidine-related cardiotoxicity nowadays (194,195). Cardiotoxicity occurs in approximately 5% of all patients receiving fluoropyrimidines (196).

2.8.2 Combination chemotherapy in CRC

2.8.2.1 Oxaliplatin

Oxaliplatin is a platinum-based compound that exerts its cytotoxic effect through DNA damage, thus leading to apoptosis (197). It also possesses synergistic effects with other cytotoxic drugs, but the underlying mechanism is unclear. Oxaliplatin has shown poor effects as a single agent.
2.8.2.1.1 Oxaliplatin in the adjuvant setting

The benefit of oxaliplatin in the adjuvant setting has been demonstrated in these three trials: The MOSAIC study (83), The NSABP C-07 trial (84), and the XELOXA international phase III study (85).

In the MOSAIC study, 2,246 stage II and stage III colon cancer patients were randomised to receive either 5-FU/LV in continuous infusion+bolus (LV5FU2) alone or in combination with oxaliplatin (FOLFOX). The 3-year DFS rate was 78.2% in the FOLFOX group and 72.9% in the LV5FU2 group (P=0.002). Ten-year follow up results appeared in 2015; for the whole group, OS was 71.7% in the FOLFOX group and 67.1% in the LV5FU2 group (P=0.043) (198), with, however, no benefit observable for either DFS or OS in patients with stage II disease. In high-risk stage II patients (defined as having a T4 tumour, perforation, or fewer than 10 lymph nodes examined), the estimated 10-year probability of OS was 75.4% for FOLFOX and 71.7% for LV5FU2 (P=0.058). For low-risk stage II patients, the addition of oxaliplatin to FU did not provide a survival benefit.

The NSABP C-07 trial evaluated the impact on DFS of adding oxaliplatin to bolus weekly fluorouracil (FU) combined with leucovorin as surgical adjuvant therapy for 2,409 stage II and III colon cancer patients (84). The 3-year DFS rates were 71.8% for FU combined with leucovorin (FULV) and 76.1% for FULV+oxaliplatin (FLOX). The updated results of this trial, with 8 years of median follow-up, showed no increase in the OS in the FLOX group, but the DFS effect remained strong (199).

The Xeloxa trial consisted of 1,886 patients with stage III colon cancer comparing bolus FU/folinic acid (FA) with capecitabine and oxaliplatin (XELOX) (85). The 3-year DFS rate was 70.9% with XELOX and 66.5% with FU/FA. The 5-year OS for XELOX was 77.6% and for FU/FA 74.2%.

The IDEA collaboration (International Duration Evaluation of Adjuvant therapy) was established in 2007 to do a prospective pre-planned analysis of pooled data from six concurrent phase III clinical studies. A total of 12,800 stage III colon cancer patients were followed for a median of 39 months, and the aim was to determine whether adjuvant therapy for 3 months was as effective as it was for 6 months. The results showed little difference: for all patients: the rate of DFS at 3 years was 74.6% in the 3-month group and 75.5% in the 6-month group.

The rate of clinically meaningful nerve damage was higher in the 6-month group than in the 3-month group (45% vs. 15% with FOLFOX and 48% vs. 17% with CAPOX). The rate of 3-year DFS differed by chemotherapy regimen and duration of treatment, with 3- and 6-month treatments with CAPOX showing a DFS rate of 75.9% and 74.8%, and 3- and 6-month treatments with FOLFOX showing a DFS rate of 73.6% and 76.0%.

According to NCCN Guidelines (based on results from the IDEA Collaboration), evidence is sufficient to divide stage III colon cancer into a
low-risk (T1–3N1) and a high-risk group (T4N1–2) (88). For the low-risk group, adjuvant therapy would be recommended for a duration of 3 months if CAPOX was chosen, and 3 to 6 months if FOLFOX was chosen. For the high-risk group, 3 to 6 months of adjuvant therapy would be recommended with CAPOX and 6 months with FOLFOX.

According to an ESMO eUpdate on Early colon cancer recommendations (86), follow-up is recommended for patients with low-risk stage II colon cancer, and 5-FU should be considered for high-risk stage II colon cancer. Stage II colon cancers are considered high risk if they present with at least one of the following clinical characteristics: T4 tumour, number of examined lymph nodes <12, tumour grade 3, vascular or lymphatic or perineural invasion, tumour presentation with obstruction or perforation, and absence of MSI (86). Patients with very high-risk stage II colon cancer (MSS and T4 tumour or more than one validated risk factor) may be considered for the addition of oxaliplatin (86). Updated ESMO guidelines are awaited.

2.8.2.1.2 Oxaliplatin in the metastatic setting

Oxaliplatin has been standard in mCRC since 2000 when de Gramont and colleagues showed that adding oxaliplatin to LV5FU2 significantly improved the mPFS (9.0 vs. 6.2 months, P=0.003) and the RR (50.7% vs. 22.3%, P=0.001) compared to LV5FU2 alone (200). The improved OS in the oxaliplatin and LV5FU2 group at 16.2 months compared to the LV5FU2 group alone of 14.7 months did not reach statistical significance. Oxaliplatin has also showed efficacy combined with chronomodulated 5-FU (201).

In one randomised controlled study, patients with mCRC were divided into three groups, one receiving IFL (irinotecan, bolus 5-FU, and leucovorin), another receiving FOLFOX, and a third receiving IROX (irinotecan and oxaliplatin) (202). The FOLFOX group showed a median TTP of 8.7 months, a RR of 45%, and median survival time of 19.5 months. These results were significantly superior to those with IFL for all end points and with IROX for TTP and RR.

In a study by Ducreux, CAPOX was not inferior to FOLFOX as first-line chemotherapy for mCRC (203).

Oxaliplatin has also shown efficacy in combination with raltitrexed (204).

2.8.2.1.3 Treatment-related adverse events during oxaliplatin-containing chemotherapy

Oxaliplatin causes adverse reactions mainly in the haematopoietic system, the peripheral nerves, and the gastrointestinal system (197). It is moderately myelotoxic and a common cause of peripheral neuropathy. It can also cause nausea, vomiting, and diarrhoea. Peripheral neuropathy is the most common dose-limiting adverse event and can be characterized as acute or chronic (200,201).
2.8.2.2 Irinotecan

Irinotecan hydrochloride is a camptothecin derivative that exerts antitumor activity against a variety of tumors (206). The active metabolite of irinotecan is SN-38. In early clinical development, the DLT of irinotecan hydrochloride was manifested as severe neutropenia and delayed diarrhea (206).

### 2.8.2.2.1 Irinotecan in the adjuvant setting

Irinotecan is not a choice in the adjuvant setting. Several randomised trials involved irinotecan in the adjuvant setting and none of them showed significant improvement in DFS or OS (207–210).

### 2.8.2.2.2 Irinotecan in the metastatic setting

Irinotecan was introduced as single-agent treatment in second- or later line mCRC therapy and showed clinically meaningful survival benefit after fluoropyrimidine failure (211).

In 2000, the results from two trials comparing 5-FU to 5-FU and irinotecan in mCRC revealed that irinotecan combined with 5-FU was well-tolerated and raised the RR, the TTP, and the survival (211,212). After these findings, irinotecan (together with 5-FU) became an established first-line treatment option in mCRC.

Today, FOLFIRI, irinotecan with infusional 5-FU/LV, is one of the standard treatment options for first-line treatment of mCRC (116).

Irinotecan has also been combined with raltitrexed (213).

### Table 6 Toxicity related to oxaliplatin-based regimens (166)

<table>
<thead>
<tr>
<th>Chemotherapy regimen Grade 3-4</th>
<th>LV5FU2 FOLFOX %</th>
<th>LV5FU2 FOLFOX %</th>
<th>XELOX FOLFOX %</th>
<th>FU/LV FLOX %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia/Neutropenia Infection</td>
<td>5.3 41.7</td>
<td>4.7 41.1</td>
<td>5 47</td>
<td>19.4 28.3</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>0.2 1.8</td>
<td>3.2 10.9</td>
<td>0 6</td>
<td>0.2 1.8</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2.4 2.7</td>
<td>6.6 10.8</td>
<td>14 7</td>
<td>14 7</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5.3 11.9</td>
<td>0 18.2</td>
<td>11 26</td>
<td>32.2 38</td>
</tr>
<tr>
<td>Hand-foot syndrome Neurotoxicity</td>
<td>0 18.2</td>
<td>0.2 12.4</td>
<td>0.7 9</td>
<td>(84)</td>
</tr>
<tr>
<td>Study</td>
<td>(200)</td>
<td>(205)</td>
<td>(203)</td>
<td>(84)</td>
</tr>
</tbody>
</table>
2.8.2.2.3 Treatment-related adverse events during chemotherapy including irinotecan

Irinotecan typically causes toxicity such as diarrhoea, nausea, neutropenia, alopecia, and cholinergic symptoms; of these, the most common grade 3-4 toxicities are diarrhoea and neutropenia (151).

The randomised BICC-C trial, a phase III trial, compared three different irinotecan-containing regimens comparing IFL, FOLFIRI, and CapeIRI (capecitabine and irinotecan) (214). It demonstrated the superior safety and efficacy of FOLFIRI compared to that of the two other regimens. The lowest rate of diarrhoea and febrile neutropenia occurred in the FOLFIRI group. The PFS was significantly better in the FOLFIRI group (median, 8.0 months) when compared with the IFL group (median, 5.9 months; P=0.006) or for the CapeIRI group (median, 6.2 months; P=0.01).

![Chemotherapy regimen Grade toxicity 3-4](image)

![Table 7 Toxicities reported with irinotecan-based regimens](table)

2.9 Biological agents in CRC treatment

2.9.1 VEGF inhibitors bevacizumab, aflibercept, and ramucirumab

Angiogenesis, the formation of new blood vessels, is a physiological process during embryogenesis and in normal tissue growth and repair, but it is also typical in cancer, in which new blood vessels are required for tumour growth (217,218). Several proteins identified as angiogenic activators include vascular endothelial growth factor (VEGF). VEGFs comprise a large family of growth factors such as placental growth factor (PLGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E (219). VEGF-A, VEGF-B, VEGF-C, and VEGF-E are involved in angiogenesis, whereas VEGF-C and VEGF-D are involved in lymphangiogenesis (218). The VEGF binds to its receptor, the vascular endothelial growth factor (VEGFR), including subtypes such as VEGFR-1, VEGFR-2, and VEGFR-3, and activates a transmitting signal into the nucleus of the endothelial cell, which prompts a group of genes to make products needed for endothelial cell growth.

Bevacizumab is a recombinant humanised monoclonal antibody which binds to soluble VEGF-A and hence prevents receptor binding and inhibits endothelial cell proliferation and vessel formation (217,220).
Aflibercept is a fusion protein consisting of the extracellular domains of VEGFR-1 and VEGFR-2 fused with the Fc domain of human IgG (221). It acts as a VEGF trap which blocks the activity of VEGF-A, VEGF-B, and placental growth factor (PLGF).

Ramucirumab, a human IgG1 monoclonal antibody, binds specifically to the extracellular domain of VEGFR-2 with high affinity and prevents VEGF-A, VEGF-C, and VEGF-D from binding to VEGFR-2 (221).

2.9.1.1 Bevacizumab in the adjuvant setting

Bevacizumab failed to prolong DFS in the AVANT and in the NSABP-08 studies and should therefore not be utilised in adjuvant settings (222,223). The updated results from the AVANT study with a follow-up of 10 years failed as well to show an improved DFS (224). The other VEGF inhibitors have not been studied in the adjuvant setting.

2.9.1.2 Bevacizumab in the metastatic setting

For first-line treatment in a phase III study, 813 patients with mCRC were randomly assigned to receive either IFL plus bevacizumab or IFL plus placebo (225). A significantly better RR (44.8% vs. 34.8%), median duration of survival (20.3 months vs. 15.6 months), and PFS (10.6 months vs. 6.2 months) appeared in the group of patients receiving IFL plus bevacizumab. Bevacizumab has been the focus of several randomised first-line mCRC trials in combination with 5-FU, capecitabine, irinotecan, and oxaliplatin; those trials have shown RR, PFS, and OS benefits in the bevacizumab arm (225–227).

A randomised trial with 820 patients from 220 centres in Europe assessed second-line treatment of bevacizumab (228). Median OS was 11.2 months for bevacizumab plus chemotherapy and 9.8 months for chemotherapy alone (HR 0.81, CI95% 0.69-0.94).

A geriatric analysis from the PRODIGE 20 trial showed that patients with mCRC over the age of 75 did not suffer from impaired autonomy or impaired QoL when treated with bevacizumab+chemotherapy (229).

2.9.1.3 Aflibercept in the metastatic setting

In the VELOUR trial (1 401 patients), in the second-line treatment of patients pretreated with oxaliplatin-based chemotherapy either with or without bevacizumab, aflibercept improved the OS from 12.1 months in the placebo arm to 13.5 months in the aflibercept arm (HR=0.82). (230). Aflibercept also significantly improved mPFS from 4.7 months with placebo to 6.9 months with aflibercept (HR=0.76), as well as improving the RR (11.1% vs. 19.8%).
2.9.1.4 Ramucirumab in the metastatic setting

After the RAISE trial (1,072 patients) demonstrated a statistically significant OS benefit for patients treated with ramucirumab, it received approval for second-line treatment with an irinotecan-based regimen (231). The mOS was 13.3 months for the ramucirumab group versus 11.7 months for the placebo group (HR 0.84).

2.9.1.5 Treatment-related adverse events during therapy including bevacizumab

The most frequent adverse events (AE) of bevacizumab have been transient minor epistaxis, gastrointestinal haemorrhage, thrombosis, and hypertension (220).

Frequencies of grade 3 to 4 or serious AEs observed were as follows: hypertension (5.0-22.0%), bleeding (2.2-3.0%), arterial thromboembolic event (1.0-2.0%), gastrointestinal perforation (1.9-2.0%), wound-healing complications (1.0-4.4%), and proteinuria (all grades, 1.0%) (209,232,233). The mechanism behind bevacizumab-induced hypertension, the most common toxicity, is not yet fully understood (234).

2.9.2 EGFR inhibitors cetuximab and panitumumab

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane glycoprotein, a member of the ErbB family of receptors, and is sometimes referred to as ErbB1 or HER1. It consists of a ligand-binding extracellular domain, a lipophilic transmembrane region, and an intracellular tyrosine kinase domain (235). The EGFR is overexpressed in many malignancies, including CRC. EGFR inhibitors like cetuximab and panitumumab are monoclonal antibodies directed against the EGFR that target the ligand-binding domain of the EGFR (235).

2.9.2.1 Cetuximab in the adjuvant setting

Two studies are available in the adjuvant setting comparing FOLFOX vs. FOLFOX with cetuximab, and neither of these trials demonstrated benefits from adding cetuximab (236,237). One study available in the neoadjuvant setting compares neoadjuvant FOLFOX to neoadjuvant FOLFOX with cetuximab and shows no survival benefit (238).

2.9.2.2 Cetuximab and panitumumab in the metastatic setting

Jonker and colleagues showed that cetuximab treatment improved OS (6.1 months vs. 4.6 months) and PFS compared to BSC in mCRC patients who had had prior treatment with 5-FU, irinotecan, and oxaliplatin (or had contraindications for treatment with these drugs) (239). In one randomised study, patients with mCRC received FOLFIRI or FOLFIRI plus cetuximab (240). The addition of cetuximab to FOLFIRI in patients with KRAS wild-type disease resulted in significant improvements in OS (23.5 vs. 20.0 months), in
PFS (9.9 vs. 8.4 months), and in RR (57.3% vs. 39.7%) compared with FOLFIRI alone. KRAS and NRAS mutation status was confirmed as a powerful predictive biomarker for the efficacy of cetuximab plus FOLFIRI (241). The OPUS study showed that addition of cetuximab to FOLFOX significantly improved PFS in the first-line treatment of those mCRC patients belonging to the wild-type RAS group (242). The mutant RAS group exhibited detrimental effects upon addition of cetuximab to FOLFOX. BRAF tumour mutation was a strong indicator of poor prognosis and had a negative predictive effect on cetuximab efficacy (243).

A study by the Amado group showed that 43% of the patients with mCRC had KRAS mutations (244). The treatment effect of panitumumab, another EGFR inhibitor, on PFS (12.3 weeks vs. 7.3 weeks) and on OS was significantly greater in the wild-type KRAS group than in the mutant group, suggesting KRAS-status evaluation prior to initiation of panitumumab treatment (244). Panitumumab has been combined with oxaliplatin in the first-line setting (245,246) and with irinotecan in the second-line setting (247,248) and showed significant clinical benefit.

ESMO and NCCN guidelines clearly state that all patients with mCRC should have their tumour tissue genotyped prior to cetuximab and panitumumab treatment, which should only be considered for patients with RAS wild-type mCRC (88,116). Cetuximab and panitumumab have only been effective in left-sided colon and rectal cancer with possible detrimental effect in right-sided colon cancer (249).

2.9.2.3 Treatment-related adverse events during therapy containing EGFR-inhibitors

Cetuximab and panitumumab are usually reasonably well tolerated, and the most common toxicity is an acne-like rash (235,250). Rash intensity is associated with clinical efficacy and may in the future serve as a marker for optimal drug exposure (235).

2.10 Adverse events and their impact on prognosis

The National Cancer Institute (NCI) of the National Institutes of Health (NIH) has published standardized definitions for adverse events, ones known as the Common Terminology Criteria for Adverse Events (CTCAE), to describe the severity of organ toxicity for patients receiving cancer therapy. The most recent CTCAE (version 5.0) appeared in November 2017 (www.ctep.cancer.gov). The adverse events (AEs) are categorized from grade 1 to 4, with grade 1 being the mildest possible AE, and grade 4 the worst possible AE; grade 5 is death (Table 8).
<table>
<thead>
<tr>
<th>Grade</th>
<th>Explanation</th>
<th>Intervention plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Mild</td>
<td>asymptomatic or mild symptoms</td>
<td>clinical or diagnostic observation only, intervention not indicated</td>
</tr>
<tr>
<td>2 Moderate</td>
<td>moderate symptoms</td>
<td>minimal, local or non-invasive intervention indicated</td>
</tr>
<tr>
<td>3 Severe</td>
<td>severe or medically significant symptoms but not immediately life-threatening</td>
<td>hospitalization or prolongation of hospitalization indicated</td>
</tr>
<tr>
<td>4 Life-threatening</td>
<td>life-threatening consequences</td>
<td>urgent intervention indicated</td>
</tr>
<tr>
<td>5 Death</td>
<td>death related to AE</td>
<td></td>
</tr>
</tbody>
</table>

Table 8  The Common Terminology Criteria for Adverse Events (CTCAE) according to The National Cancer Institute (NCI) of the National Institutes of Health (NIH).

Neutropenia during chemotherapy has been reported to be a predictor of better survival in patients with different types of cancers (15). The first studies to demonstrate improved survival among patients with neutropenia were in breast cancer patients receiving chemotherapy in the 1990s (13,14).

In a retrospective analysis, both grade 1 to 2 and grade 3 to 4 neutropenia during FOLFOX therapy were associated with improved survival in patients with mCRC (15). In a meta-analysis comprising eight studies and 2 745 stage II to IV CRC patients, improved survival was evident among patients with chemotherapy-induced neutropenia (16).

The AIO KRK-0104 randomised phase II trial investigated the efficacy and safety in first-line treatment of mCRC of two capecitabine-based regimens: CAPIRI plus cetuximab (CAPIRI-C) and CAPOX plus cetuximab (CAPOX-C) (251). Capecitabine-attributed skin toxicity (ST) grade 1–3 was associated with a significantly higher disease control rate (DCR) than was grade 0 toxicity. Capecitabine-attributed grade 1–3 skin toxicity was associated with a longer PFS (9.9 vs. 5.6 months, P<0.001) and OS (32.8 vs. 22.4 months, P=0.008).

Two meta-analyses have shown that bevacizumab-induced hypertension is associated with significant improvement in PFS and OS (252,253).

The Everest study investigated the effect of cetuximab dose escalation in patients with irinotecan-refractory metastatic colorectal cancer who had developed no skin reactions or mild ones after 21 days of treatment at the standard dose (254). Dose escalation showed no significant benefit regarding OS.

2.11 Follow-up of CRC

2.11.1 Follow-up of localized CRC

The primary aim of the follow-up programmes is to detect recurrence of the disease at an early stage in order to improve the chance of cure, but also to find a possible second CRC and also to collect data on the natural history of the disease (255). Intensive follow-up after CRC surgery is common practice, in many hospitals for up to 5 years, but this is based on limited evidence (256).
The FACS randomised clinical trial evaluated four follow-up regimens for stage I-III CRC: CEA only, CT only, CEA+CT, and minimum follow-up (follow-up only if symptoms occurred). The results showed that follow-up with CT or CEA provided an increased rate of surgical treatments of recurrence with curative intent compared with the rate with minimal follow-up, but there emerged no advantage in combining CEA and CT. If there exists some survival advantage to any strategy, it is likely to be small, according to these authors (256)

In the COLOFOL trial, an unblinded randomised trial, patients with stage II or III CRC had follow-ups with CT and CEA (257). A group with more frequent follow-ups was compared with another group with less frequent follow-ups, and results showed no significant rate reduction between groups in 5-year overall mortality or in colorectal cancer-specific mortality.

According to ESMO guidelines, follow-up is meaningful only if it improves survival (5). Four systematic reviews show that intensive follow-up results in improved survival compared to survival during minimal or no follow-up; ESMO recommendations are based on these reviews (258–261). The suggested recommendations include physical examination and CEA every 3 to 6 months for 3 years and then every 6 to 12 months for up to 5 years; colonoscopy at year 1 and then every 3 to 5 years, CT of chest and abdomen every 6 to 12 months up to 3 years and even longer for patients who are at higher risk of recurrence.

According to ASCO guidelines for early-stage CRC, follow-up differs depending on setting (basic, limited, enhanced, or maximal) (262). In summary, follow-up with CEA is recommended every 6 months for 3 to 5 years, follow-up with imaging every 6 to 12 months for the first 3 years and follow-up with colonoscopy from once in the first 1 to 2 years to 1 year after surgery and then every 5 years up to the age of 75.

According to the Finnish recommendations, a minimum follow-up of 2 years is recommended for rectal cancer patients who have undergone curative surgery, because 75% of all the recurrences appear within 2 years of the primary operation (76).
2.11.2 Follow-up of mCRC

Patients with mCRC may typically achieve an OS of approximately 30 months as a result of MDT management with first-, second-, third-, and possibly fourth-line therapy followed by BSC (116). ASCO recommends follow-up with CEA every 1 to 3 months during ongoing treatment (4).

Follow-up is indicated after a curative resection of metastases (88,170). Only the NCCN recommends a surveillance schedule after curative resection of metastases that includes CEA testing, imaging, and clinical evaluation every 3 to 6 months for 2 years, and then every 6 to 12 months in years 3 to 5. Periodic endoscopy is also recommended. It is unclear if there is cost-effective surveillance strategy for mCRC after curative resection.

2.12 Prognosis of CRC

Prognosis of CRC both localized and metastatic has improved over the decades. In 1953, when statistics by the Cancer Registry began, the mortality rate was 85%, but it is only around 35% today (24). The 5-year survival after surgical resection alone according to the TNM classification is 99% for stage I, 68% to 83% for stage II, and 45% to 65% for stage III (148). (Figures 3 to 5 show 60 years of Nordic-region rates.). Figures below show the incidence and mortality of CRC from 1953 -2016 in the Helsinki region and Finland as well as from 1960-2016 in Nordic countries.

Figure 3 NORDCAN Colorectal cancer incidence and mortality in Helsinki region 1953-2016
http://www-dep.iarc.fr/NORDCAN/English/StatsFact.asp?cancer=590&country=2460
Predictive and prognostic biomarkers

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (263). A WHO coordinated meeting has defined a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease” (264).

Biomarkers can be divided into prognostic and predictive markers (265). Prognostic markers aim to evaluate the patient’s overall outcome objectively. Examples of prognostic biomarkers for colorectal cancer are CA19-9 (higher pre-operative CA19-9 levels associated with lower resectability, more advanced stage, and inferior survival), CEA (elevated pre-operative CEA levels in resectable CRC associated with poor prognosis), and MSI status (high frequency MSI CRCs associated with better prognosis in localized CRC and inferior prognosis in mCRC) (265). BRAF V600E is associated with
significantly inferior prognosis, whereas non-V600E might be associated with better prognosis (266).

As to negative predictive biomarkers in CRC: KRAS, NRAS, and BRAF are examples. KRAS mutation positivity in stage IV CRC patients predicts considerably less benefit from EGFR inhibitors like cetuximab and panitumumab (244). Biomarkers may be detected or monitored or both in tissue, blood, or relevant secretions such as urine, stool, sputum, or breast-nipple aspirates (267).

The European group on tumour markers (EGTM) updated the guidelines for colorectal cancer and other gastrointestinal cancers in 2014 (3). Recommended biomarkers for CRC include FIT for CRC screening and also pre-operative CEA in combination with clinical and histopathological criteria for determining prognosis in patients with newly diagnosed CRC. Moreover, post-operative CEA serves in surveillance of stage II and stage III CRC patients who may be eligible for further inventions such as liver resection or oncological treatments. CEA should also be evaluated every 2 to 3 months for mCRC patients receiving oncological treatment (4).

The ESMO consensus guideline recommends surveillance of MSI-H, KRAS, NRAS, and BRAF in mCRC (116). Microsatellite instability (MSI) or detection of mismatch repair (MMR) proteins or both may help to screen for Lynch syndrome and serve as a predictive marker for immuno-oncologic therapy (268). KRAS, NRAS, and BRAF mutations are negative predictive markers for EGFR therapy (3,116,269). BRAF is also a strong negative prognostic marker, possibly actionable with triplet (FOLFOXIRI) and bevacizumab biologic therapy (270,271).

Development of personalized treatment of CRC has been challenging compared with treatment for gastrointestinal stromal tumour (GIST) (c-KIT mutation), melanoma (BRAF), or lung cancer (EGFR, ALK, ROS), because therapeutically targeted mutations have been rare (265). Emerging targets are ALK, ROS1, NTRK fusion genes, and KRAS G12C, which may be targets in the near future (272,273).

This thesis research is a study of the serum biomarkers CEA, CA19-9, YKL-40, CRP, and IL-6, and hence the review of the literature is limited to these biomarkers. Biomarkers that have been standard in cancer are called tumour markers, and those biomarkers which are inflammatory mediators as well as measuring inflammation are called inflammatory markers.

2.13.1 Tumour markers

2.13.1.1 CEA

CEA was first described in 1965 by Gold and Freedman when they identified an antigen present in both foetal colon and colon adenocarcinoma but apparently absent from healthy adult colon (274,275). CEA is a glycoprotein considered a tumour-associated marker rather than a tumour-specific marker;
it is the only widely recommended serum tumour marker for CRC (3,262). The elimination of CEA occurs mainly in the liver (276).

Several factors affect the serum concentration of CEA, both benign and malignant. Benign reasons for an elevated CEA are smoking, inflammatory bowel disease, and liver disease/cirrhosis. Malignant reasons in CRC are higher stage, left-sidedness of the tumour, and bowel obstruction (275). Other adenocarcinomas also cause elevated CEA, ones such as pancreatic cancer (277) and breast cancer (278). Chemotherapy can cause a temporary increase in CEA known as a surge or a tumour-flare reaction (17).

**Pre-operative CEA**

Elevated pre-operative CEA is an important prognostic factor in addition to TNM stage and other prognostic factors such as perforation, high grade, and vascular invasion (8,279). Several studies report that in CRC, CEA is a prognostic marker (6–8,280,281). Most studies evaluate the pre-operative CEA value.

In a study of 9 083 stage II-IV CRC patients, elevated pre-operative CEA was independently associated with increased risk of overall mortality (HR=1.60) (8). In a Korean study of 2 230 consecutive CRC patients, pre-operative CEA was an independent prognostic marker (6).

The 5-year OS was significantly higher for groups with normal pre-operative CEA (81-82%) than for groups with elevated pre-operative CEA (47-76%) (7,9).

**Post-operative CEA**

Konishi and colleagues studied whether colon cancer patients with elevated pre-operative CEA that normalized after colon cancer resection were at increased risk of recurrence compared to patients with normal pre-operative CEA (12). In multivariate analyses, elevated post-operative CEA (HR 2.0) was independently associated with shorter RFS, but normalized post-operative CEA was not (HR 0.77). These authors judged routine measurement of post-operative, rather than pre-operative CEA to be warranted.

A post-hoc analysis of the MOSAIC trial showed that post-operative CEA is a strong prognostic factor for DFS and OS in stage II colon cancer (11). Patients were divided into a high-risk group and a low-risk group according to post-operative CEA level. The cut-off for CEA was set at 2.35 ng/ml. The 3-year DFS rate was 88.5% in the low-risk (CEA ≤2.35 ng/ml) and 78.7% in the high-risk group (CEA>2.35 ng/ml).

Nicholson and colleagues performed a meta-analysis of 52 studies with cut-off values for CEA in detecting recurrent CRC (282), showing sensitivity ranging from 41% to 97% and specificity from 52% to 100%. In the seven studies with a threshold of 2.5 μg/l, pooled sensitivity was 82% and pooled specificity 80%. In the 23 studies with a threshold of 5 μg/l, pooled sensitivity was 71% and pooled specificity 88%. In the seven studies with a threshold of 10 μg/l, pooled sensitivity was 68% and specificity 97%. The conclusion was that CEA was
insufficiently sensitive to serve as the sole biomarker, and lowering the
threshold did not work, since this only led to an increased number of false
positives. The recommendation was that two modalities are preferable for CRC
follow-up.

Screening and follow-up
CEA is of limited value in primary diagnosis and is unsuitable for screening
because of its low sensitivity (2,3,262).

CEA is recommended for post-operative surveillance of stage II and stage III
CRC patients who may be eligible for further inventions in case of recurrence,
such as liver resection or oncological treatments. The recommendation is that
CEA should be measured at baseline and after that every 2 to 3 months for at
least 3 years after diagnosis. After 3 years, CEA should be followed up every 6
months until 5 years after diagnosis. Similar recommendations have come
from expert panels at ASCO (4) and ESMO (2).

The expert panels at EGTM and ASCO recommend CEA monitoring in mCRC,
with a CEA baseline value to be determined before initiation of oncological
treatment and then CEA every 2 to 3 months during ongoing treatment (3,4).

2.13.1.2 CA19-9

Carbohydrate antigen (CA19-9), or sialylated Lewis a antigen, is a glycoprotein
which is synthesised by gastrointestinal epithelium (283). CA19-9 is a tumour
marker best validated for diagnosis of pancreatic cancer in diagnostics and for
monitoring patients receiving oncological treatment for pancreatic cancer (4).
Elevated CA19-9 has been a negative prognostic marker in mCRC (284–286).

Pre-operative CA19-9
In a large cohort of 4 794 curatively resected CRC patients, pre-operatively
elevated CA19-9 was a strong predictor for impaired survival (their 5-year OS
rate was 79.9% in the high pre-operative CA19-9 group and 91.9% in the
normal pre-operative CA19-9 group) (287). This finding is in line with these
published results (288,289).

Pre-operative CA19-9 and CEA
Pre-operatively elevated CA19-9 and CEA were prognostic for recurrence in
1 109 stage I to III CRC patients (290).

In 238 stage II CRC patients with pre-operatively elevated CEA and CA19-9,
there occurred impaired OS compared to the situation in patients with either
normal CEA and CA19-9 pre-operatively or with only one of their markers
elevated pre-operatively (291). A similar finding, with impaired OS (P=0.021),
emerged among 303 patients with locally advanced rectal cancer with elevated
pretreatment levels of both CA19-9 and CEA, when compared to OS among
those with normal pretreatment CA19-9 and CEA (292).
Post-operative CA19-9 and CEA
Post-operatively elevated CA19-9 was a significant predictor of early recurrence in 1,216 stage I-III CRC patients, whereas CEA was not (293). In radically resected stage IV CRC patients, post-operatively elevated CEA and CA19-9 were potent prognostic indicators for recurrence (280). Elevated post-operative CA19-9 was the only independent prognostic factor for OS.

Screening and follow-up
A majority of researchers have found that the sensitivity of CA19-9 is inferior to that of CEA in CRC and that elevated level of CA19-9 is a poor prognostic factor (5,294–297). Neither ESMO nor ASCO recommend CA19-9 in CRC for screening, follow up, or prognostics (2,4).

2.13.1.3 Other tumour markers
Other carbohydrate antigens under study such as CA 72-4, CA 242, and CA 195, with their relatively low sensitivities and specificities in screening and follow-up settings, gained no greater use in CRC (294,296). Neither ESMO nor ASCO have published any recommendations regarding these tumour markers in CRC (2,4,294).

2.13.2 Inflammatory markers
YKL-40, CRP, and IL-6 are inflammatory biomarkers or inflammatory mediators (298–300). Evidence is increasing that systemic inflammatory response plays an important role in carcinogenesis (301). Inflammatory markers have been important prognostic factors in randomised studies in mCRC (302).

The Glasgow prognostic score (GPS), a cumulative inflammation-based cancer-prognostic marker composed of serum elevation of CRP (>10 mg/l) and decrease in albumin (<35 g/l) concentration, is likely to reflect host systemic inflammatory response and has been significant as a prognostic indicator in cancer patients (303). Hypoalbuminemia alone is unlikely to be associated with reduced survival in CRC patients, so therefore the GPS has been modified (mGPS) (304), providing a score of 1 only if CRP is elevated, and a score of 0 for a case with only hypoalbuminemia or where neither is elevated.

The Köhne prognostic index or Köhne index is based these four parameters: performance status (PS), number of metastatic sites, alkaline phosphatase (ALP) level, and white blood cell (WBC) count, a measure of inflammatory activity (305). Based on these, patients with mCRC can be divided into a low-, an intermediate or a high-risk group, with mOS being 14.7, 10.5, and 6.4 months (305).
2.13.2.1 YKL-40

YKL-40 was identified in 1989 as being secreted in vitro in large amounts by the human osteosarcoma cell line MG63 (306). The protein was named YKL-40 because of its three N-terminal amino acids, namely, Tyrosine (Y), Lysine (K), and Leucine (L), and by its molecular mass of 40 kDa. In the literature, the protein has several names, human cartilage glycoprotein-39 (HC gp39) (307), breast regressing protein 39 Kd (brp-39) (308), 38-kDa heparin-binding glycoprotein (gp38k) (309), chitinase-3-like-1 (CHI3L1) (310), chondrex (311), and 40 kDa mammary-gland protein (MGP-40) (312).

The biological function of YKL-40 is unknown, but what is known is that it is a secreted protein produced by non-malignant cells such as macrophages, neutrophils, fibroblast-like synovial cells, chondrocytes, vascular smooth-muscle cells, and hepatic stellate cells and by different types of cancer cells such as breast, lung, osteosarcoma, glioblastoma, and colon (306). YKL-40 promotes cancer proliferation and inflammatory cytokine production, and several receptors are involved (298). YKL-40 and IL-6 are inflammatory biomarkers (301) and mediators for tumorigenesis, and are promoters of metastasis and angiogenesis (298,313–316).

The prognostic value of YKL-40 has been under study in patients with S. pneumoniae bacteraemia, rheumatoid arthritis, alcoholic liver disease, and six types of solid carcinomas, and all studies found that an elevated YKL-40 level was a prognostic biomarker of poor prognosis (306). The YKL-40 serum concentration is similar among men and women but increases with age, making it an age-dependent protein.

**Pre-operative YKL-40**

Elevated pre-operative YKL-40 in stage I-IV CRC patients was associated with significantly shorter survival than for patients with normal YKL-40 (HR 1.7) (317).

A prospective study including 4,496 Danish men and women referred for endoscopy because of symptoms or other risk factors of CRC showed that high YKL-40 levels were associated with increased risk for CRC in patients without comorbidities (318). Moreover, elevated YKL-40 was a biomarker for increased risk of CRC independent of CEA.

Fuksiewicz and colleagues had similar findings in stage I to III rectal cancer patients with significantly more recurrences in patients with elevated YKL-40 than in patients with normal YKL-40 (P=0.041) and found that elevated YKL-40 was a predictor of impaired OS (319).

**Post-operative YKL-40**

Stage I to IV CRC patients with elevated post-operative YKL-40 who had undergone curative surgery were at higher risk for tumour recurrence than were patients with normal post-operative YKL-40 (320).
**mCRC and prognosis**

Patients with mCRC and elevated post-operative YKL-40 had shorter PFS than did patients with normal YKL-40 (7.5 vs. 8.2 months, HR= 1.27, P=0.013) and shorter OS (16.8 vs. 23.9 months, HR = 1.33, P=0.024) (321). Elevated post-operative YKL-40 was an independent biomarker of short OS (HR = 1.12, P=0.033).

As of now, no guideline recommendations are available regarding YKL-40 and its use in CRC (3,4).

**2.13.2.2 CRP**

C-reactive protein (CRP) is a non-specific acute phase reactant that reflects tissue damage; it is also a sensitive and stable marker of inflammation (299). CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes (322). Elevated serum CRP is associated with inflammation and cachexia in cancer patients, including CRC, and with poor prognosis both in metastatic cancer and after radical surgery (323,324).

**Pre-operative CRP**

Pre-operatively elevated CRP (>10 mg/l) in CRC patients with liver metastases was a strong predictor of comprised survival (HR 1.72) (325). Patients with CRP ≤10 mg/l had a median survival of 4.27 years compared to 2.59 years in patients with CRP 11 to 30 mg/l and 47 days in patients with CRP ≥30 mg/l (325).

In stage I to IV CRC patients, both GPS and mGPS showed significantly worse survival outcomes for higher GPS/mGPS than did lower GPS/mGPS. (303). The 5-year survival rates for GPS 2, GPS 1, and GPS 0 were 35%, 75%, and 93%. The 5-year survival rate for mGPS 2, mGPS 1, and mGPS 0 were 35%, 73%, and 92%. Both GPS and mGPS could prove useful in predicting the outcome of CRC patients.

Stage I to IV colon cancer patients with pre-operatively elevated CRP (>30 mg/l) were more than twice as likely to die from colon cancer than were patients with CRP<10 (326). An elevated CRP was of prognostic significance for DSS in surgically treated colon cancer patients regardless of stage.

In one meta-analysis, of 48 studies with CRC patients, 36 (75%) showed that high CRP strongly predicted survival. High CRP was an independent prognostic indicator in 31 reports (65%) (327). In another meta-analysis, the GPS/mGPS was an independent and promising indicator for predicting the prognostic outcome of CRC in both a curative and a palliative setting (328), and in yet another meta-analysis, high mGPS was associated with poor OS in CRC (329).
Post-operative CRP
Among stage I to III curatively resected CRC patients with post-operatively elevated CRP (CRP ≥ 90 mg/l), they suffered significantly worse RFS than did patients with CRP < 90 mg/l (330). In multivariate analysis, elevated CRP was an independent prognostic factor for RFS (HR 2.07). However, in another study on CRC patients with liver metastases, their CRP levels had no prognostic value regarding survival after surgery for liver metastases (331).

Pre- and post-operative CRP
In a rare study on pre- and post-operative CRP in CRC, the combination of pre- and post-operative CRP was predictive of prognosis (332). These patients were divided into three groups: A (high CRP both pre- and post-operatively), B (either high CRP pre-operatively and low CRP post-operatively or low CRP pre-operatively and high CRP post-operatively) and C (low CRP both pre- and post-operatively). The disease-specific 5-year survival rates were 54%, 73%, and 87% in Groups A, B, and C, differences that were statistically significant.

No guideline recommendations cover CRP and its use in CRC (3,4).

2.13.2.3 IL-6
Interleukin-6 (IL-6) is produced by various types of cells such as monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells, B cells, T cells, and various tumour cells (333,334). During acute inflammation, IL-6 is predominantly produced by monocytes and macrophages, whereas T cells produce IL-6 during chronic inflammation. IL-6, which stimulates YKL-40 production and angiogenesis through its receptors IL-6R and sIL-6R (313–315), is regarded an important tumour-promoting factor in various types of human cancers including glioma, lymphoma, melanoma, breast, ovarian, pancreatic, prostate, renal, and CRC (333).

In a review article by Knüpfer and colleagues, assessing 31 studies, colon cancer patients had higher IL-6 levels than did healthy controls, and higher IL-6 levels were associated with increased tumour stage and size, with metastases, and with shorter survival (334). In another review, circulating IL-6 appeared to be an independent prognostic marker in gastrointestinal cancer patients, and high IL-6 levels were associated with short OS (300). Regarding CRC, the results were inconclusive.

Pre-operative IL-6
CRC patients with high IL-6 (≥6.3 pg/ml) had significantly shorter DFS and OS than did CRC patients with low IL-6 (<6.3 pg/ml) (335). In stage I to III CRC patients with a high IL-6 level (>10 pg/ml), the 5-year survival rate was 56% and with a low IL-6 level (≤10 pg/ml), 5-year survival was 83% (336). High IL-6 level was an independent prognostic factor for survival (RR = 1.820). Similarly, Yeh and colleagues found that CRC patients with a high IL-6 (>10 pg/ml) had significantly shorter survival times and lower 3-, 5-, and 10-year survival rates than did those with low IL-6 (<10 pg/ml) (337).
Chung showed that serum IL-6 levels correlated with disease status of CRC and with survival, but this was not an independent prognostic indicator (338). Liu and colleagues also failed to show that IL-6 could serve as an independent prognostic indicator (339).

**mCRC and prognosis**

Patients with mCRC who had a high post-operative IL-6 (>5.6 pg/ml) had a mPFS of 7.7 months compared to 8.9 months for those with a low IL-6 level (≤5.6 pg/ml) (HR 1.54, P<0.001) (340). The mOS for those mCRC patients with high IL-6 vs. low IL-6 was 16.6 months vs. 26.0 months (HR= 1.92, P<0.001).

As for now, no guideline recommendations are available regarding IL-6 and its use in CRC (3,4).
3 AIMS OF THE THESIS

To study

- whether YKL-40, IL-6, CA19-9, or CRP in addition to CEA better predict recurrence and survival in radically operated stage II-IV CRC patients.

- whether adverse events (haematological or non-haematological) in stage II-III CRC patients receiving 5-FU-based adjuvant chemotherapy predict improved patient outcome.

- how CEA fluctuates during one chemotherapy cycle in mCRC patients receiving fluoropyrimidine-based chemotherapy, and to determine the best timing for CEA measurement and assess whether CEA alteration during one chemotherapy cycle is predictive of treatment response.

- whether CEA can replace CT in response evaluation of mCRC, and to study the optimal cut-off value for CEA in response evaluation.
4 MATERIALS AND METHODS

4.1 Patients and methods

All four studies took place at the Department of Oncology at Helsinki University Hospital, Helsinki, Finland and for Study II, patients from a French study were also included. The protocol of the studies was approved by the local Ethics Committee and the National Agency for Medicines, Helsinki, Finland, with informed consent required from all Finnish patients. For Study II, in addition, The Ethics Review Board at Saint-Antoine Hospital, Paris, France, reviewed and approved the original protocols, with written informed consent from all French patients. The studies were conducted according to good clinical practice (GCP) and according to the declaration of Helsinki.

<table>
<thead>
<tr>
<th>Trial</th>
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<td>MEPSYT TNF</td>
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<td>1. Raltitrexed and carmofur (n=20) 2. Raltitrexed (n=20) 3.5-FU-based therapy (n=20)</td>
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Table 9 Summary of the trials in this thesis
4.1.1 Study I

The LIPSYT trial (http://www.controlled trials.com/ISRCTN98405441), consisted of a total of 150 stage II to IV radically operated CRC patients who were enrolled in an open prospective randomised trial between November 1997 and August 2001 with its primary endpoint being treatment tolerability and secondary endpoint being predictive and prognostic biomarker evaluation. Primary results have appeared previously (341,342)

Biomarker samples were available in 147 of 150 cases post-operatively before the initiation of adjuvant chemotherapy. Median follow-up was 11.9 (range 8.9-12.7) years from operation. Blood sampling was performed every three months up to two years and every six months for up to five years. The final check-up was at 10 years. Radiology took place annually for the first three years and at five years.

Biomarkers (YKL-40 and IL-6) were analysed from frozen serum samples taken post-operatively before initiation of adjuvant chemotherapy. Post-operative CEA, CA19-9, and CRP were assessed as routine praxis. The median time from surgery to biomarker sampling was 48 days (range 19-124).

Commercially available enzyme-linked immunosorbent assays (ELISAs) served for YKL-40 and IL-6 according to the manufacturers’ instructions. YKL-40: MicroVue YKL-40 ELISA (Catalog #8020), Quidel Corporation, San Diego, CA, USA, and IL-6: Quantikine HS600B, R&D Systems, Abingdon, OX, UK. The detection limit was 20 ng/ml for YKL-40, and the intra- and inter-assay coefficients of variation were (CVs) <5% and <6% (343). The detection limit was 0.01 pg/ml for IL-6, and the intra- and inter-assay were CVs ≤8% and ≤11% (344).

Serum levels of CRP, CEA and CA19-9 were determined as a part of the routine laboratory tests with automatic analysers as follows: CRP: immunoturbidimetric method (1998-2013) at HUSLAB laboratories, Helsinki University Hospital; CEA and CA19-9: immunoenzymatic assay, Bayer Immuno 1 (CEA: October 1998 – October 2005; and CA19-9: January 1998 – January 2006), or immunochemiluminometric assay, Abbott Architect (CEA: October 2005 and onward and CA19-9: January 2006 and onward). All measurements were performed by technicians blinded to the study endpoints.

An age-corrected percentile for YKL-40 was calculated according to the formula (343): \text{percentile}=100 / (1+ (YKL-40 ^{-3}) * (1.062 ^{\text{age}}) ^{5000})

Cut-off values were as follows: the ROC corrected cut-off for age-corrected YKL-40 level was the 70.7th percentile of normal controls YKL-40; 4.5 pg/ml for IL-6; 10 mg/l for CRP; 5 μg/l for CEA; and 26 kU/l for CA19-9.
4.1.2 Study II

Databases from two open, prospective, randomised studies, the French GERCOR C96.1 trial (880 patients) (345) and the Finnish LIPSYT trial (153 patients), were combined in order to increase the statistical power of the study, resulting in 1033 radically operated stage II and stage III CRC patients treated with 5-FU based adjuvant chemotherapy. The median follow-up time was 6.05 years (range 0.16–8.18).

Haematological (leucopenia, neutropenia, thrombocytopenia) and non-haematological (mucositis, diarrhoea, nausea/vomiting, hand-foot syndrome, or other toxicity) adverse events were assessed regarding their association with DFS and OS. The adverse events (AEs) were recorded and graded according to The National Cancer Institute of Canada Common Toxicity Criteria version 1 (from 1991) in the French study, and version 2 (from 1997) in the Finnish study. The worst toxicity grade during chemotherapy was taken into account in the analysis.

4.1.3 Study III

The MEPSYT-TNF trial evaluated systemic inflammatory parameters, clinical parameters, full blood count, liver function tests, and tumour markers weekly during one cycle of chemotherapy for mCRC.

Sixty patients were divided into three groups, 20 patients per group. Each group received a different combination of chemotherapy. Group 1 received a combination of raltitrexed and carmofur, group 2 received raltitrexed as a single-agent therapy, and group 3 received a 5-FU-based therapy (oral carmofur, i.v.bolus 5-FU, and i.v. continuous infusion 5-FU).

CEA fluctuation during one chemotherapy cycle was assessed. The laboratory tests were run at baseline (day 0, before initiation of chemotherapy), day 7, day 14, and after the treatment cycle (day 21 or day 28). The following laboratory tests were run at HUSLAB, Helsinki University Hospital, Helsinki: CEA, CRP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), gamma-glutamyltransferase (GT), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-α (TNF-α). CEA was determined by the AutoDELFIAVR assay (Wallac, Turku, Finland) at HUSLAB, Helsinki University Hospital, Helsinki.

Computed tomography (CT) of the thorax and the abdomen as well as CEA took place before the chemotherapy treatment cycle and after 8 to 12 weeks. CT response was evaluated prospectively by a senior radiologist blinded to the outcome according to the WHO criteria used at the time (60). For evaluation of treatment response, CT served as the gold standard. A 20% or greater increase in CEA level was regarded as tumour marker progression.
4.1.4 Study IV

This treatment response evaluation study included 66 patients with mCRC who had at least two CEA values and CT scans available at baseline and at 2 months. The aims were to find disease-control patients and also to find progressive-disease (PD) patients. High negative predictive value (NPV) and high sensitivity were the main criteria for finding optimal CEA cut-off values. CEA and CT were taken at baseline (before initiation of chemotherapy) and then every 2 months up to 8 months. The CT scans were re-reviewed by one experienced gastrointestinal radiologist according to RECIST 1.1 criteria blinded to the clinical outcome. The CT response served as the gold standard for evaluation of treatment response. CEA was determined by the AutoDELFIA^{VR} assay (Wallac, Turku, Finland) at HUSLAB, Helsinki University Hospital, Helsinki, with CEA level \( \leq 5 \mu g/l \) considered normal.

4.2 Statistical analysis

In all four studies, analyses were performed by StatView software, version 5.0.1 (SAS Institute, Abacus Concepts Inc., Berkeley, CA, USA) or SPSS (PASW statistics version 18.0 Inc., Chicago, IL, USA) or IBM SPSS (statistics version 21.0, SPSS Inc., Chicago, IL, USA) or IBM SPSS Statistics for Windows (version 23.0, IBM Corp., Armonk, NY, USA) or SPSS version 24.0 (IBM SPSS Statistics, version 22.0 for Mac, SPSS, Inc., Chicago, IL, USA, an IBM Company).

Descriptive data were median (range) or skewed distributions. The chi-squared test served for analysis of categoric data. The Wilcoxon signed-rank test served for paired comparisons and the Mann Whitney U-test or the Kruskall Wallis test for non-paired comparisons in non-parametric settings. Non-parametric correlation was calculated with Spearman’s rho. The Kaplan–Meier method served to estimate survival curves and median survival times with 95% confidence intervals (CI95%). Cox proportional hazards models and Log rank tests served to compare survival probabilities between patients. Receiver operating characteristic (ROC) and waterfall plot curves served to evaluate the cut-off values for different CEA values. The Pearson correlation coefficient served to evaluate the linear correlation between CEA and the target sum. The significance level was set at \( P < 0.05 \).

4.3 Ethical considerations

The protocol of the studies upon which this thesis is based was approved by the local Ethics Committee and the National Agency for Medicines, Helsinki, Finland, and informed consent was required from all Finnish patients. For Study II, the Ethical Review Board at Saint-Antoine Hospital, Paris, France also reviewed and approved the original protocols, and written informed consent was obtained from all French patients.
5 RESULTS

5.1 Study I

Baseline characteristics and biomarker levels
This study included 147 patients, median age 60 years (range 31-76). Most patients (88%) had locoregional disease (stages II to III), and 80 (54%) were still alive after 10 years. Of the 147, 65 (44%) had a relapse, with no new relapses found after 6.3 years. The cause of death was as follows: mCRC for 58 (84%), cardiovascular for 7 (10%), second cancer for 2 (3%), and other in 2 (3%). As for primary location, no difference emerged in levels of CEA, CA19-9, YKL-40, or CRP, but IL-6 was higher in patients with rectal cancer.

Post-operative CEA
Elevated CEA predicted increased hazard of relapse, with the HR adjusted for background variables being 7.91. For CEA, elevated vs. normal, specificity was 97% (CI95% 91-100), sensitivity 31% (CI95% 21-48), and PPV 89%, (CI95% 65-99%).

In adjusted univariate analysis, elevated vs. normal CEA was associated with impaired DFS (HR 7.23, CI95% 3.85-13.58; 10-year DFS rate 6% vs. 63%), and impaired OS (HR 7.16, CI95% 3.76-13.63; 10-year OS rate 6% vs. 68%). After adjusting for levels of CA19-9, YKL-40, CRP, and IL-6, elevated CEA was still associated with impaired DFS (HR 8.63, CI95% 3.82-19.50) and OS (HR 10.17, CI95% 4.35-23.79).

Post-operative CA19-9
Elevated vs. normal CA19-9 had an HR of 2.08 (95% CI .87-4.98) for relapse, a sensitivity of 16% (CI95% 8-27%), specificity of 89% (CI95% 79-95%), and PPV of 53% (CI95% 27-78%). No significant association appeared between elevated CA19-9 and DFS (HR 1.98, CI95% 0.99-3.97) or OS (HR 1.94, CI 95% 0.93-4.04). This was also the case in the subgroup with normal CEA.

In the small subgroup with concomitantly elevated CEA and CA19-9, all five patients relapsed (PPV 100%, CI95% 48-100%), and their numerically shorter DFS (0.41 vs. 0.76 years) and significantly shorter OS (1.8 vs. 2.9 years), were evident.

Post-operative YKL-40
More relapses occurred in the elevated YKL-40 group (HR 1.73; CI95% 1.02-2.93), and sensitivity was 56% (CI95% 43-69), specificity 69% (CI95% 58-79), and PPV 57% (CI95% 45-69%). In univariate analysis, elevated YKL-40 was associated with impaired DFS (HR 1.84, CI95% 1.14-2.96, 10-year DFS rate 37% vs. 59%) and OS (HR 1.97, CI95% 1.20-3.23, 10-year OS rate 43% vs. 64%). In adjusted multivariate analysis, elevated YKL-40 was still associated with OS (HR 2.24, CI95% 1.23-4.05), but not with DFS (HR 1.58, CI95% 0.88-2.86).

In the subgroup with normal CEA and elevated YKL-40, there was impaired DFS (HR 2.30, CI95% 1.27-4.16) and OS (HR 2.40, CI95% 1.28-4.52) compared
with concomitantly normal YKL-40 and CEA. The 10-year DFS rate was 49 vs. 71% and OS rate 55 vs. 77%.

In the small subgroup with concomitant elevation of both YKL-40 and CEA, of the 10 patients, 9 suffered a relapse (PPV 90%, CI95% 56-100%), had numerically shorter DFS (0.68 vs. 0.76 years), and a OS of 1.5 vs. 2.6 years.

**Post-operative CRP**

Elevated vs. normal CRP levels meant more relapses (HR of 1.95; CI95% 0.97-3.94), a sensitivity of 20% (CI95% 12-30%), a specificity of 96% (CI95% 89-99%), and PPV 77% (CI95% 44-93%). Elevated CRP was associated with impaired DFS (HR 2.31, CI95% 1.21-4.39; 10-year DFS rate 18% vs. 53%) and OS (HR 2.40, CI95% 1.20-4.80; 10-year OS rate 24% vs. 59%) in univariate analysis, but only DFS was significant (HR 2.53; CI95% 1.10-5.81; OS HR 2.49; CI95% 0.95-6.51).

In the subgroup with normal CEA and elevated CRP HR for relapse was 3.14 (95% CI 1.21-8.16) and impaired DFS (HR 3.54, CI95% 1.57-8.02) and OS (HR 3.10, CI95% 1.29-7.45) was noted. The 10-year DFS rate was 30% vs. 65% and OS rate 40% vs. 71%.

In the small subgroup with elevated CEA and CRP all of its four patients experienced a relapse (PPV 100%, CI95% 40-100%), and had numerically shorter DFS (0.39 vs. 0.76 years, HR 3.13) and OS (1.8 vs. 2.6 years, HR 1.52).

**Post-operative IL-6**

Elevated vs. normal IL-6 showed more relapses (HR 2.12; CI95% 1.11-4.03), a sensitivity of 28% (CI95% 17-40%), specificity of 91% (CI95% 84-97%), and PPV of 75% (CI95% 53-90%). In univariate analysis, elevated IL-6 was associated with impaired OS (HR 1.99, CI95% 1.05-3.76; 10-year OS rate 33% vs. 58%) but not significantly with DFS (HR 1.77, CI95% 0.95-3.30, 10-year DFS rate 25% vs. 53%) and no DFS or OS difference in multivariate analysis.

In the subgroup with normal CEA, elevated IL-6 was not significantly associated with DFS (HR 1.36, CI95% 0.53-3.51) nor with OS (HR 1.30, CI95% 0.48-3.51).

In the small subgroup with concomitantly elevated CEA and IL-6, all six patients had a relapse (PPV 100%; CI95% 54-100%) and shorter DFS (0.41 vs. 0.76, HR 2.16, CI95% 0.75-6.16) and OS (1.1 vs. 2.6, HR 1.96, CI95% 0.71-5.44).
5.2 Study II

Of all Study II patients, 61% developed any grade of toxicity, and 27% developed grade 3 to 4 toxicity.

Adverse events in univariate analysis
In univariate analysis, neutropenia, nausea/vomiting, mucositis, and worst-haematological toxicity were significant predictors for DFS or OS (Figure 6, Table 10). The median time for appearance of neutropenia of any grade was 2 months, for mucositis 1 month, for nausea/vomiting 1 month, for HFS 2 months, and for diarrhoea 2 months.

Figure 6  Kaplan-Meier curves for DFS for neutropenia, mucositis, nausea / vomiting and worst non-haematological toxicity (Study II)
Multivariate results for neutropenia, nausea/vomiting, mucositis, and combined toxicity

Increasing toxicity did not provide a better outcome, in other words, no linear correlation appeared between toxicity level and outcome (DFS or OS). Patients over the age of 70 with stage III disease and poor histologic differentiation had a significantly worse outcome. Patients who experienced no predefined toxicity had the worst outcome.

Neutropenia was evident in 47% of patients, nausea/vomiting in 54%, and mucositis in 43%. Neutropenia of any grade was associated with an improved DFS (Table 10) but not improved OS. Nausea/vomiting was associated with an improved DFS (HR 0.79 CI95% 0.63-0.99) and an improved OS (HR 0.61 CI95% 0.46-0.79) as was mucositis (DFS HR 0.71 CI95% 0.67-0.90 and OS 0.71 CI95% 0.54-0.93).

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>n (%)</th>
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<th>Overall Survival</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Frequency</td>
<td>HR</td>
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<tr>
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<td>0.61-0.95</td>
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<td>0.60-1.05</td>
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<td>0.60-1.29</td>
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<td></td>
<td>4</td>
<td>38 (4)</td>
<td>1.03</td>
<td>0.60-1.77</td>
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<td>0.61-0.95</td>
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<tr>
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<td>0.57-1.69</td>
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<td>0.56-0.88</td>
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</tr>
<tr>
<td>Worst non-haematological toxicity n=1031</td>
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</tr>
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<td>300 (29)</td>
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<td>1-4*</td>
<td>0.65</td>
<td>0.49-0.86</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 10 Frequency of significant adverse events and associations with DFS and OS for separate grades and for grades 0 versus 1-4 (Study II)

*Adjusted for age, stage, and histological differentiation which were significant in univariate analysis.
5.3 Study III

CEA fluctuation during one chemotherapy cycle
CEA showed a wavelike pattern, with lower values at day 7 and higher values at day 14, when compared with CEA on baseline day 0 or at day 21. CEA was significantly higher at day 21 than at day 0 (P=0.037). On cycle day 0, 84% of the patients had an elevated CEA level (median 60 µg/l, range 5.2-2960 µg/l). No significant CEA difference emerged between the differing chemotherapy regimens. We divided the evaluation cycles into an early group within the CEA surge period (chemistry cycles 1 to 2, n=38) and a late group (cycles 3 to 15, n=22), which showed no significant difference between their wavelike patterns.

CEA in relation to treatment efficacy
Patients with PD on CT had approximately four-fold higher median CEA levels (median CEA day 0 55.4 µg/l) than did patients with SD or PR (median CEA day 0 10.6-12.8 µg/l). We compared CEA before and at the end of the cycle according to treatment response and found a correlation in the PD group (P=0.024), but none in the PR (P=0.72) or SD groups (p=0.72). In this study, sensitivity for CEA was 53% and specificity for CEA was 72%.

Correlation of long-term CEA values with the one-cycle CEA value
Long-term CEA values and CT were available for 54 patients, performed at 8- to 12-week intervals. CEA increased significantly in patients with a PD response (77.0 µg/l vs. 190.0 µg/l, P=0.004), but no significant changes appeared in SD or PR patients. Sensitivity for CEA was 82% and specificity for CEA was 62%.

Correlation between CEA and liver-function tests and inflammatory parameters
Liver-function tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), and gamma-glutamyltransferase (GT) showed a wavelike pattern similar to that of CEA, with a decrease at day 7 and an increase at day 14 and normalization at the end of the cycle. None of the liver function tests predicted radiological response. Inflammatory parameters: C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-α (TNF-α) showed a wave-like pattern different from that of CEA, with an increase at day 7 and a decrease at day 14. None of the inflammatory parameters predicted radiological response.

5.4 Study IV

Correlation between CEA and target sum according to the RECIST
A significant linear correlation appeared between CEA and the target sum at all measurement points (P<0.001-0.01) except at 8 months (with its low number of cases). A positive correlation appeared between ΔCEA% and Δtarget sum% at all measurement points independently, whether the change was calculated from baseline (P<0.002) or from nadir value (the lowest value measured) (P<0.05).
Optimal CEA cut-off for finding patients with disease control

ROC curves at 2, 4, and 6 months showed that ΔCEA%, when compared with baseline, gave a greater area under the curve (AUC) than did the absolute CEA or ΔCEA values.

When CEA at 2 months was compared to baseline, the best combination of sensitivity and specificity was obtained by using a cut-off value of 31%. At time points 4, 6, and 8 months, the cut-off values were 57%, 23%, and 19% when compared only to baseline CEA level. For further analysis, a 20% increase served as the cut-off value.

The waterfall plot visualizes the relationship between ΔCEA% and Δtarget sum% (Figure 7). All patients with PR on CT had decreasing CEA values, and all PD patients had increasing CEA values. Of 36 patients, in 16 (44%) with SD according to RECIST, an increasing CEA level provided a lead time to PD on upcoming imaging. Thus, 0% of ΔCEA% was chosen as the arbitrary second cut-off for further testing.

Disease control on CT was apparent in all patients in the group with decreasing CEA, i.e. ΔCEA%≤0%, at 2, 4, and 6 months, compared with baseline or nadir value. Treatment could have continued without CT verification at 2, 4, and 6 months in 47%, 23%, and 3% of the cases.

None of the patients with decreasing CEA had PD. The highest NPV, 1.0, was evident when ΔCEA% was 0%. The ROC-curve-defined best cut-offs yielded NPV estimates of 0.98, 0.90, and 0.57.
**Increasing CEA and disease progression or lead time to progression**
Increasing CEA identified PD in 100% at 2, 4, 6, and 8 months. Increasing CEA without PD on CT was the lead time to progression on the next assessment 2 months later in 67%, 74%, 67%, and 50% of upcoming measuring points.

Sensitivity was 1.0 at 2, 4, and 6 months when the cut-off was 0% for $\Delta$CEA%. PPV was 0.31, 0.44, and 0.71 at the respective measuring points with a cut-off of 0%.

Neither the ROC-defined nor 20% cut-off showed any improved sensitivity.

**Progression-free and overall survival with raltitrexed and carmofur combination treatment**
For all 66 patients, median PFS was 5.9 (CI95% 5.0–6.7) months and median OS was 11.7 (CI95% 9.2–14.2) months. For the first-line patients, median PFS was 6.6 (CI95% 5.9–7.2) months and median OS was 13.4 (CI95% 9.2–17.6) months. For the second-line patients median PFS was 3.9 (CI95% 1.6–6.3) months, and the median OS was 8.0 (CI95% 3.1–13.0) months.

**Decreasing CEA as a prognostic marker**
Patients with a decreasing CEA at 2 months had significantly longer PFS, 6.6 vs. 4.3 months (HR 0.51 (CI95% 0.31–0.83), P=0.006) and longer OS, 15.5 vs. 10.9 months, (HR 0.58 (CI95% 0.35–0.97), P=0.036). No correlation with OS or PFS appeared at other time-points.
6 DISCUSSION

6.1 Post-operative prognostic biomarkers in stage II-IV CRC

In Study I, post-operatively elevated serum CEA, YKL-40, CRP, and IL-6 predicted recurrence and shorter survival, but in adjusted multivariate analysis, only serum CEA remained significantly associated with both DFS and OS. Patients with normal CEA and elevated YKL-40 or CRP had impaired DFS and OS. Patients with post-operatively elevated CEA in combination with elevated CA19-9, YKL-40, CRP, or IL-6 had a very high (90-100%) risk of relapse, and their OS was short.

6.1.1 Post-operative CEA

According to guidelines, the only recommended circulating biomarker to be used in CRC is CEA (4,346). Elevated pre-operative CEA has been an important prognostic factor in addition to TNM stage and other known prognostic factors such as perforation, high grade, and vascular invasion (6,8,9,145). However, both the sensitivity (50-80%) and specificity (80%) of CEA are limited, and a notable proportion of high-risk patients may remain undetected if CEA alone is measured (12,25,26,347). In the Cochrane review, CEA was insufficiently sensitive, even at low thresholds (<2.5 μg/l), leading to the conclusion that a rise in CEA never occurs in up to 20% of patients with a true recurrence (false negatives); multiple modalities are thus to be recommended (282).

In this study, post-operatively elevated CEA had a PPV (of) 89%. Post-operatively elevated CEA was associated with impaired DFS (HR 7.23), which is in line with others’ findings (12,348,349). After curative resection of metastases, post-operative CEA has been a stronger marker (350–352).

6.1.2 Post-operative CA19-9

Serum CA19-9 is a gastrointestinal tumour marker also used in CRC. In metastatic disease, an elevated value has been a negative prognostic marker (284–286). In a study by Shin and colleagues with 4 794 curatively resected CRC patients, pre-operatively elevated CA19-9 was a strong predictor of poor overall survival (287) which was a finding in line with findings of others (288,348,349). After curative resection of metastases, post-operative CEA has been a stronger marker (350–352).

Post-operatively elevated CA19-9 was a significant predictor of early recurrence, whereas CEA was not (293). In our study, only a few patients showed post-operatively elevated CA19-9, with no statistically significant association with impaired DFS or OS noted.

Subgroup analysis showed that all patients with post-operatively and concomitantly elevated CEA and CA19-9 experienced a relapse and a short DFS and OS. This is in line with findings in radically resected stage IV patients in whom post-operatively elevated CEA and CA19-9 were associated with recurrences (280) and OS, and for OS, the only independent prognostic factor
was CA19-9 (350). Among radically resected CRC patients with recurrence, 7.8% had an elevated CA19-9, and no concomitantly elevated CEA was evident (290). A surveillance advantage of CA19-9 over CEA was evident in radically resected patients with pre-operatively elevated CA19-9 (354). Both these studies demonstrated the potential benefit of combining an elevated CA19-9 with an elevated CEA, which is in line with our results. More convincing data is, however, required before CA19-9 can be adopted as one clinical follow-up recommendation.

6.1.3 Post-operative YKL-40

Chronic inflammation plays an important role in colorectal carcinogenesis (355) and is associated with poor prognosis (323,356). YKL-40 and IL-6 are inflammatory biomarkers (301) and mediators for tumorigenesis, and are promoters of metastasis and of angiogenesis (298,313–316). Data is very limited regarding YKL-40 as a prognostic biomarker in CRC after radical surgery (320) and in metastatic disease prior to chemotherapy initiation (340). Serum YKL-40 can be elevated in CRC patients and is more sensitive than CEA and CA19-9, especially in non-metastatic disease (318,319,357). In mCRC, elevated prechemotherapy YKL-40 was associated with short OS (321), and in patients undergoing liver resection, the elevated pre-operative YKL-40 was associated with shorter OS (358).

We demonstrated that high post-operative YKL-40 in radically resected CRC patients was a predictor of relapses and of impaired DFS and OS. These findings are in line with those of Cintin and colleagues showing that post-operatively elevated YKL-40 in stage II to IV disease is an independent predictor of higher recurrence rates and impaired survival (320).

In this study, patients with normal post-operative CEA but with an elevated post-operative YKL-40 had more relapses, had impaired DFS (10-year DFS rate estimate 49% vs. 71%), and had worse survival (10-year survival rates 55% vs. 77%). Those patients showing concomitantly elevated CEA and YKL-40 had a 90% recurrence rate. This is in line with findings by Johansen and colleagues (318). YKL-40, combined with CEA whether normal or elevated, predicts survival and may improve risk stratification.

6.1.4 Post-operative CRP

CRP is a sensitive but non-specific inflammatory marker and is upregulated by cytokines such as IL-6, an important inflammatory mediator (322). Elevated serum CRP is associated with inflammation and cachexia in cancer patients including those with CRC, and with poor prognosis both in localized and metastatic disease (303,329,359,360). These studies mainly evaluated pre-operative CRP. We evaluated post-operative CRP and showed that CRP was associated with DFS and OS in univariate analysis. This is in line with findings of other series involving surgery for localized CRC (330,332) and after liver resection (331).
In subgroup analysis, a significantly worse 10-year DFS rate (52 vs. 73%) and OS rate (40 vs. 67%) appeared in patients with normal post-operative CEA and an elevated post-operative CRP, and all patients with elevated CEA and CRP post-operatively relapsed. This is in line with findings from other patient series (361).

### 6.1.5 Post-operative IL-6

Data regarding IL-6 as a prognostic inflammatory marker and mediator of impaired prognosis in CRC are ambiguous (300). Some studies show that IL-6 is an independent prognostic inflammatory marker in localized and mCRC (337,340), and others show that IL-6 is not (338,339). Our findings showed that IL-6 was not an independent marker for DFS or OS in multivariate analysis.

All patients with post-operatively elevated CEA and IL-6 relapsed. CRC patients with high pre-operative IL-6 had significantly shorter DFS in a study by Shiga and colleagues (335). IL-6 may cause increased expression of CEA-related adhesion molecules in CRC (362). IL-6 is associated with CEA and advanced stage, and is a negative prognostic marker of survival (336).

### 6.1.6 Strengths and limitations

All patients in this study were part of a randomised phase III clinical trial. Administration of the adjuvant chemotherapy and measurement of post-operative biomarkers took place in a prescheduled manner. The follow-up was standardized and thorough, which is a definite strength in this study. Another strength is its long follow-up of up to 12 years with reliable data about causes and dates of death from Statistics Finland (363). Adjuvant therapy comprised 5-FU and LV given as a bolus or a continuous infusion, which still is the mainstay in adjuvant treatment of high-risk stage II and stage III CRC as well as for the elderly patients. No combination chemotherapy with oxaliplatin was, however, included, the recommended therapy in very high-risk stage II and stage III CRC, and this must be regarded as a limitation (86). Fluoropyrimidines are still the cornerstone of oncological treatment, and they are still used both in the adjuvant and the metastatic setting of CRC (5,88,116,170).

We had no access to pre-operative CEA values because many patients were referred to our centre from other hospitals and we were not provided with that information. It would have been interesting to compare the pre-operative and post-operative CEA values, but that was impossible. The subgroups with concomitantly elevated CEA and a second marker: CA19-9, YKL-40, CRP, or IL-6, were small, numbering from 4 to 10, so drawing any firm conclusions is impossible.

### 6.1.7 What this means for the clinician

In univariate analysis in radically resected CRC patients, post-operatively elevated serum CEA, YKL-40, CRP, and IL-6 were associated with impaired
DFS or OS or both. Serum YKL-40 and CRP were independent predictors of poor prognosis, regardless of whether post-operative CEA was normal or elevated. CA19-9 and IL-6 added further information only when CEA was concomitantly elevated. If these findings can be validated, these biomarkers may prove helpful in decision-making regarding follow-up strategy and adjuvant therapy.

The post-operatively elevated CEA, or normal CEA in combination with concomitantly elevated YKL-40 or CRP, could prove useful in identifying high-risk patients who would benefit from more aggressive oncological treatments and more frequent follow-ups. In contrast, finding low-risk patients needing neither adjuvant therapy nor short-course chemotherapy would be meaningful, since oxaliplatin in particular is linked to long-term adverse events (364,365).

Especially in stage II colon cancer patients, adjuvant therapy as single agent (11) and combination chemotherapy (366) have shown greater benefit. Follow-up aims at finding curatively resectable patients. In the FACS trial 2.3% of the patients in the minimal follow-up group were offered surgical treatment of recurrence with curative intent whereas the corresponding number for the more intensive follow-up groups was 6.6% to 8.0% (256). No survival benefit was detectable in the Colofol study, but resection rates were unmentioned (257).

6.1.8 Future prospects

In Finland, CRC treatment is centralised to high-volume centres and adding biomarkers to the follow-up protocols would offer the possibility of gathering large amounts of prospective information on biomarkers and learning more about them. Furthermore, adding biomarkers to the pre-operative and post-operative evaluation opens up the field for pre-operative vs. post-operative biomarker studies. These results should also be validated in large data sets containing both randomised and real-world data.

What would also prove interesting is to study right- and left-sided colon cancers with MSI testing, mutational analysis, and CMS subtyping, because these are distinct subgroups with highly variable prognoses, and little is known about tumour markers in these subgroups.

6.2 Adverse events as a clinical marker for treatment response

Study II was among the first big studies in showing that both haematological and non-haematological adverse events during adjuvant treatment were associated with survival in localized CRC. Earlier, studies had looked at the association between haematological toxicity and survival in breast cancer with adjuvant treatment and these showed the existence of an association between low leukocyte levels after chemotherapy and improved DFS (13,14).

The present study found that any grade of neutropenia was associated with improved DFS (HR 0.81). This is in line with a study by Sunaga and colleagues
revealing that more stage III CRC patients receiving adjuvant UFT (oral prodrug of 5-FU) and leucovorin had improved DFS with neutropenia than did those without neutropenia. In the literature are several studies showing that chemotherapy-induced neutropenia in CRC patients predict a better outcome (15,16,368). Shitara and colleagues showed that neutropenia among mCRC patients receiving FOLFOX treatment was associated with improved survival (15). Rambach and colleagues showed that any grade of neutropenia in patients receiving at least one line of chemotherapy was significantly associated with better OS (HR = 0.55) (368). One meta-analysis (eight studies, 2 745 patients) showed a significant survival benefit in mCRC patients with chemotherapy-induced neutropenia (HR=0.62) (16). In our study, neither leucopenia nor thrombocytopenia predicted survival. Full blood count was measured before the next chemotherapy cycle at day 28 and not at nadir (approximately 8-10 days, after bolus cycle initiation). It is possible that regular nadir counts would have noted more neutropenia.

Nausea/vomiting (any grade) was associated with improved DFS (HR 0.72) and OS (HR 0.62), and mucositis was associated with improved DFS (HR 0.74) and OS (HR 0.72). Patients who experienced no adverse events had the worst outcome. Oral and gastrointestinal mucositis are typical 5-FU-related toxicities, whereas nausea/vomiting are not (369). Our findings are in line with those involving osteosarcoma patients receiving chemotherapy, where grade 3 to 4 oral mucositis (HR 0.51, 95% CI 0.29–0.91), grade 1 to 2 nausea/vomiting (HR 0.37, 95% CI 0.16–0.85) and grade 1 to 2 thrombocytopenia (HR 0.49, 95% CI 0.27–0.87) were associated with improved overall survival (370). On the other hand, three phase II/III studies on recurrent ovarian cancer revealed that grade 3 to 4 nausea/vomiting was linked to significantly reduced PFS and OS (371).

In the analysis separated by grades, grade 1 to 2 neutropenia, mucositis, or nausea/vomiting showed the strongest association with survival. All patients with grade 3 to 4 toxicities had reduced chemotherapy intensity or dosing delays or both, meaning reduced drug exposure. Grade 1 to 2 toxicity, i.e. mild or moderate toxicity, seems to offer a possible target, assuming that chemotherapy exposure is sufficient and the toxicity manageable.

HFS is typical in continuous infusion of 5-FU and in peroral treatments (Tables 5 and 6). Here, no association emerged between HFS and survival outcome, possibly because of the low HFS rate (4% in grade 3) with our 5-FU regimen. Grade 3 to 4 HFS occurs in 10% to 20% of capecitabine-treated patients, and in other fluoropyrimidine regimens in 0 to 7% (372). The post hoc analysis of the X-ACT trial (comparing adjuvant capecitabine and FULV) revealed a non-significant improvement in DFS and OS for patients with any grade of HFS (373). This is in line with a study in mCRC showing that any grade of HFS during capecitabine improved the disease control rate (DCR), PFS, and OS (251), and in mCRC or locally advanced rectal cancer, where improved PFS/DFS and OS occurred (374). This HFS finding is therefore in line with the findings that mild or moderate toxicity is a measure of biologic activity, and that grade 3 to 4 is undesirable.
Patients with dihydropyrimidine dehydrogenase (DPD) deficiency caused by a mutation in their DPYD gene, are at risk for developing severe toxicity such as myelosuppression, diarrhoea, mucositis, and neurotoxicity when exposed to 5-FU (375). DPD is an established predictor for toxicity and efficacy, and according to guidelines, DPD testing can be considered in clinical practice (116).

6.2.1 Strengths and limitations

All the patients took part in randomised phase III clinical trials (a Finnish and a French trial) with rigorous toxicity assessment. Chemotherapy administration was standardized, and all follow-ups were prescheduled. And also, the long follow-up of up to 8 years is a definite strength. Being among the first to study adverse events and their association with survival in CRC patients and managing to include 1 033 patients in the study is definitely a strength. These results are still relevant, because fluoropyrimidines are still the cornerstone of adjuvant therapy both as single agent chemotherapy in the elderly and in stage II patients, and also as in combination with oxaliplatin (5,88,116,170).

Combination chemotherapy with oxaliplatin was not used in these trials which is a limitation. Mayo-like bolus regimens are no longer in use today and this must also be regarded as a limitation of this study. Baseline prognostic information was not comprehensive in the French (345), study such as pre- and post-operative CEA levels, and number of lymph nodes evaluated.

6.2.2 What this means for the clinician

Adverse events during adjuvant therapy could perhaps serve as clinical markers to evaluate the treatment’s biological chemotherapy intensity.

Prognosis is worst when there occurs neither haematological nor non-haematological toxicity. In the analysis separated by grades, grade 1 to 2 neutropenia, mucositis or nausea/vomiting had the strongest association with survival. All patients with grade 3 to 4 toxicities had prolonged infusion time, dose reductions, or dosing delays (or a combination of these) to reduce dose-intensity.

It seems as if grade 2 toxicity may prove to be a possible target, indicating when chemotherapy exposure is sufficient and toxicity manageable. In patients with no toxicity during the first cycles, intensification of their therapy may be a way to improve efficacy of adjuvant treatment (254,376).

6.2.3 Future prospects

An interesting approach would be to study adverse events with dose-intensified models. Dosing according to body-surface area is probably not the most appropriate, however, and fat-free mass-based dosing deserves further investigation (377).
6.3 CEA during one cycle of chemotherapy

In mCRC treatment response evaluation, two CEA values taken at least 2 months apart may indicate tumour progression, although the gold standard for determining tumour response is radiology. Response evaluation is usually planned for every 8 to 12 weeks, although very little is known about the correct or optimal timing for CEA measurement during a 3- to 4-week long chemotherapy cycle.

Often, in tumour-marker studies, measurement is repeated at 4- to 9-week intervals, with usually a decent correlation of CEA with radiologically evaluated treatment response (378). Patients with mCRC treated with oxaliplatin combined with bolus 5-FU took part in a study with CEA measurement every 14 days for the first two months of chemotherapy. Of 27 patients, 15% had a transient CEA increase. From the start of chemotherapy until CEA reached its peak value required 2 to 8 weeks. The CEA surge was not a sign of disease progression (17).

In another study, with 89 mCRC patients, 10 had a CEA surge. The period was 2 to 10 weeks (median 4 weeks) from the start of 5-FU-based chemotherapy for CEA to reach its peak value, and this was not linked to progression (18). This situation has also been described with irinotecan-based chemotherapy (379). Initial tumour-marker elevation, a surge, or a tumour flare, has been described for mCRC and also for other tumour types, such as non-seminomatous germ cell tumours (380), prostate (381), and breast cancers (382,383).

In Study III, the wavelike CEA pattern of variation, with decreased values at day 7 and increased values at day 14, was similar in all three chemotherapy groups when compared with CEA at baseline on day 0 or on day 21. The pattern was similar in the early treatment cycles to that in the later cycles (6-45 weeks). In other words, the initial surge does not explain the CEA fluctuation pattern. To my knowledge, no other researchers are looking at weekly CEA measurements. Fortnightly CEA measurements have been studied by the Sorbye and the Jia groups, involving palliative chemotherapy for mCRC (17,384) and the Yang group after surgery for the primary (385).

In this study, 60 patients with mCRC received three different types of chemotherapy regimens: 40 received fluoropyrimidine-based regimens (bolus, infusional, or an oral 5-FU prodrug), which is still the cornerstone of CRC treatment, and 20 received the antimetabolite raltitrexed. The fluctuation pattern was similar in all the different chemotherapy regimens. Modern combination regimens have not been under study regarding this CEA fluctuation phenomenon, but since modern regimens are based on fluoropyrimidines as well, one may find it probable that similar CEA fluctuations would occur with modern combinations. The CEA surge phenomenon has occurred with single-agent fluoropyrimidines and with oxaliplatin- and irinotecan-based therapy (17,18,379), and with these chemotherapies in combination with cetuximab (384).
Tumour markers are considered reflective of chemotherapy efficacy. This study correlated CEA alterations with treatment response according to radiology. When comparing the CEA level before and at the end of the cycle to treatment response, there was a statistically significant correlation only in the PD group (P=0.024). The sensitivity was 53% and the specificity 72%. CEA values at days 7 and 14 did not predict response. A CEA assessment with a 8- to 12-week interval was performed as well and showed a similar correlation in PD patients, with a sensitivity of 82% and specificity of 62%. Similar results have been shown by Jia and colleagues with CEA and CA19-9: sensitivity for CEA was 75% and for CA19-9 was 84% (384).

In many studies, baseline-elevations in both ALP and lactate dehydrogenase (LDH) are associated with worse prognosis (200,305,386). The present did not include analysis of LDH, but ALP was performed weekly, together with other liver-function tests. ALP was the only one of the liver-function tests showing a correlation with CEA in PD patients. None of the other liver-function test results correlated with treatment response or with CEA alterations, in line with an analysis by Sorbye and colleagues (302).

Inflammatory parameters, and CRP in particular, have been evaluated in response- and prognostic evaluations (300,331,332,358,387). IL-6, IL-8, and TNF-α reflect inflammation, liver damage, and tumour necrosis (388). No correlation with treatment response or with alterations in the CEA level was evident in our limited analysis. Some cytostatic agents like bleomycin, raltitrexed, oxaliplatin, and cytarabine, and especially biologic therapies are considered to be inflammatory-reaction inducers known as cytokine release syndrome (389,390), and these may affect biomarker levels, e.g. of IL-6 and CRP.

CEA is metabolized in the liver, so liver diseases may raise CEA levels (346). Chemotherapy frequently raises liver-function test levels. It is possible that a transient increase in CEA level reflects liver damage. Shedding of antigen or tumour necrosis is also a possible explanation for tumour marker fluctuation. The reason for CEA fluctuation during one chemotherapy cycle remains unknown.

6.3.1 Strengths and limitations

All patients in this study took part in the MEPSYT TNF trial. Administration of the chemotherapy and measurement of biomarkers was prescheduled. The response evaluation was done by CT of the thorax and the abdomen. CT images were re-evaluated by an experienced senior gastrointestinal radiologist blinded for the outcome. The fluoropyrimidine-based regimens are still the mainstay of mCRC treatment and as such are still relevant (88,116,170).

The chemotherapy regimens themselves constitute a limitation. Raltitrexed is only used in cardiotoxicity nowadays, and carmofur has been replaced in many western countries with capecitabine. The assessment period was limited to one cycle and this makes it difficult to relate the results to long-term efficacy. The patients were also assessed during different time-frames of their treatment,
which may be considered a strength or a limitation. This study had a relatively small number of patients, 60.

6.3.2 What this means for the clinician

In mCRC, the timing for tumour-marker assessment in response evaluation is crucial. Initial elevation of CEA during the first 4 to 6 weeks of a new therapy is possible, and this is also known as a tumour flare reaction or a surge reaction (17). In this study, CEA fluctuated during one chemotherapy cycle: it decreased on day 7 and increased on day 14 compared to baseline level. CEA should therefore not be measured during ongoing fluoropyrimidine-based oral chemotherapy nor within two weeks of intravenous chemotherapy administration. These findings have led to oncology and laboratory departments changing their routines for CEA measurement from mid-cycle to immediately before start of the chemotherapy cycle.

CEA elevation during a single 21- to 28-day cycle is indicative of treatment failure as is strongly supported in the study by Jia and colleagues (384). As combination therapies for mCRC are becoming increasingly costly, CEA measurement before every treatment cycle delivers a cost-effective means of targeting treatment, by revealing progressive disease earlier.

6.3.3 Future prospects

Timing of CEA measurement needs to be validated further in fortnightly cycling, as it has been studied in very small patient series thus far (17,18,384). Response evaluation for every treatment cycle requires further research, because our findings have been validated only by the Jia group (384) in their small retrospective patient series, and the clinical implications are substantial.

6.4 CEA could replace CT in response evaluation in mCRC

Approximately 60% to 70% of all CRC patients with recurrent disease can be offered oncological therapy, and the rest BSC (167). CEA and CT provide means of treatment response evaluation in mCRC. According to recommendations, CT should be performed every 2 to 3 months (62,391), together with CEA (3,4).

6.4.1 Optimal cut-off for CEA in treatment response evaluation

In Study IV, the aim was to identify patients with disease control by finding a cut-off value for CEA. We were the second to use nadir values with a RECIST 1.1 criteria for both CT and CEA evaluation (392).

After testing several cut-off values for a CEA increase, the optimal value turned out to be 0%. In other words, if CEA at a given measuring point was the same or lower than at baseline or nadir this corresponded with disease control. This cut-off identified all patients with disease control on CT. NPV was 1.0. This is
in line with previous findings in which NPV was 0.50 to 1.00, when using cut-offs from -50% to +50% compared with baseline (20,393–397).

No patient with decreasing CEA had PD; thus sensitivity is 1.0, in line with findings of Jia et al (384). The Hanke group showed a sensitivity of 76% and the Wang group a sensitivity of 70% to 81% (20,396,397).

PPV was 0.31 to 0.71 at various time points. The aim was not to identify patients with PD (with an increasing CEA), and therefore the low PPV was not a concern in this setting. For decision-making regarding possible further treatment, patients with PD needed a CT anyway.

Specificity was 0.56 to 0.75 with a cut-off of 0%. This is in line with previous findings: 0.47 to 1.00, with a cut-off off -30% to +50% compared with baseline (20,393,394,396,397). The low specificity was not a concern, since most patients with disease control on imaging and an increasing CEA (showing a lead time to progression) would require a CT anyway for further treatment evaluation.

6.4.2 CEA replacing imaging in response evaluation

Medical care is expensive, and the growing cost is a challenge for society. New, cheaper ways of caring for our patients is essential, but without reducing quality of care. Thus replacing CT with CEA should be of great interest. Several studies have compared CEA and CT in evaluation of treatment response in mCRC, but few have looked at the aspect of reducing imaging cost (20,392–404).

We showed that CEA can replace CT in evaluation of treatment response in 23% to 47% of the cases. No CT verification would have been necessary at 2 and 4 months, a finding in line with findings of Hanke and colleagues, who reported a figure of 55% (397), but the majority of other studies failed to report any percentages. According to the literature CEA can replace CT in limited settings, for instance in patients with peritoneal carcinosis (402), in patients with a CEA increase of >200% (403), or in patients with a CEA rise of at least 50% (397). De Haas and colleagues (393) claim that CEA may be sufficient for response evaluation in a palliative chemotherapy setting with disease control as the endpoint, as do Kim and colleagues (404), showing that CEA >20% could prove useful in monitoring tumour progression during palliative chemotherapy, as does the Wang group (20,396), with a sensitivity of elevated CEA for prediction of progressive disease of 70% to 81% and accuracy of 85% to 90%; none of these studies provides any figures for omitting CT, however.

6.4.3 CEA response in patients with normal baseline CEA

A decreasing CEA at 2 months correlated with longer OS and PFS; this was also in line with most earlier findings (392,396,398–401).
In the present study, normal post-operative CEA appeared in 11% of the patients, which was in line with the 10% to 27% in other studies (20,395,396,400,403,404). At progression, 57% of this study’s patients had a CEA above the reference range, a figure also in line with others’ figures (20,395,396,400,403,404).

6.4.4 Strengths and limitations

All patients in this study took part in the phase I-II MEPSYT study. The administration of the chemotherapy and measurement of biomarkers thus took place every two months in a pre-defined way. The response evaluation with external review of CT scans is a definitive strength. And also, replacement of CT by CEA is a strength because irradiation leads to increased cancer risk, especially in children (405,406).

The chemotherapy regimen assessed is comparable to fluoropyrimidine-based infusional regimens, but time in treatment is shorter than with modern combination chemotherapy and biologics. Raltitrexed can still be used in patients who have developed cardiotoxicity from previous chemotherapy (194,195) and previously it has been used in combination with fluoropyrimidines, oxaliplatin, and irinotecan (183,189,407), but use outside a cardiotoxicity setting is rare due to toxicity and efficacy issues, cytokine-release syndrome (193), and treatment related deaths (186). Carmofur is still used in Japan, but in many western countries it has been replaced with capecitabine. Thus, the regimens used in this study are not the most relevant and also the number of patients, only 66, was relatively small. Raltitrexed is rarely used nowadays, and carmofur is only used in Finland and Japan and in many western countries carmofur has been replaced with capecitabine.

6.4.5 What this means for the clinician

With a cut-off value for CEA at 0%, we identified all disease control- and PD patients. This cut-off worked well with normal CEA values within the reference range. Fluoropyrimidines, particularly in combination with oxaliplatin or to a lesser extent with irinotecan, cause an initial transient CEA rise, known as a flare or surge, in 11% to 18% (17,379,394,408,409). The peak is at 2 to 3 weeks, and the duration is 8 to 9 weeks (408,410). A surge does not reflect progression, whereas a constant rise in CEA does (409,410). Tumour-marker surge was not really a concern in this study, because a CEA increase would automatically lead to CT assessment, as recommended by Sorbye and colleagues (411). Secondly, a marker surge is mostly limited to the first weeks of first-line therapy.

CT, with its cancer risk for children (405,406), means that replacement of CT with CEA is clinically valuable. The cost for one serum CEA analysis is cheap compared with the cost for CT, the CEA price being approximately 2% that of CT allows for substantial savings.

These results are only applicable in patients with non-resectable mCRC, and therefore early response evaluation, with re-evaluation of resectability and
local ablative therapies, should be provided to patients with oligometastatic disease (116).

In clinical practice, treatment-response evaluation of mCRC patients receiving oncological treatment occurs every 1 to 3 months with CT and CEA. According to the findings of this present research, decreasing CEA values compared with baseline or nadir value opens up for the possibility of replacing CT with CEA in treatment-response evaluation. In other words, these findings could offer more convenience and less radiation for the patients in treatment-response evaluation, and also more savings for the health care system.

6.4.6 Future prospects

In Finland, CRC patients receive their oncologic treatment in high-volume centres, so it would be interesting to initiate a study to validate our findings in patients receiving modern combination chemotherapy.
7 CONCLUSIONS

- Radically operated stage II-IV CRC patients with increased post-operative CEA, YKL-40, and CRP had impaired DFS and OS, and those with normal post-operative CEA and elevated YKL-40 or CRP had impaired DFS and OS; those with elevated post-operative CEA concomitantly with elevated CA19-9, YKL-40, CRP, or IL-6 experienced relapses amounting to 90% to 100%.

- Mild to moderate haematological (neutropenia) and non-haematological (mucositis and nausea/vomiting) adverse events during adjuvant treatment of stage II to III CRC patients were prognostic biomarkers for improved DFS and OS; the worst survival was evident for patients without predefined toxicity.

- CEA fluctuates in a wavelike pattern during a fluoropyrimidine-based chemotherapy cycle in mCRC patients, and therefore CEA should not be measured during the ongoing cycle, but just before the next cycle is due; CEA increase during the cycle was indicative of treatment failure.

- Decreasing CEA compared with baseline or lowest measured value can replace CT in response evaluation of mCRC during ongoing chemotherapy.
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