Adverse events in the treatment of colorectal cancer:

Their use as predictive markers and impact on quality of life

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II. *Helicobacter pylori* related gastrointestinal symptoms in diagnostics and adjuvant chemotherapy of colorectal cancer; Soveri L-M, Österlund P, Ruotsalainen T, Poussa T, Rautelin H, Bono P. Oncol Lett 7: 553-559, 2012

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2. ABBREVIATIONS

Abbreviations for treatment regimens are not included in abbreviations

BSA	Body surface area
C. elegans	*Caenorhabditis elegans*
CI	Continuous infusion
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CIPN	Chemotherapy induced peripheral neuropathy
c-KIT	Cathepsin K
CMS	Consensus molecular subtypes
CRC	Colorectal cancer
DFS	Disease free survival
DPD	Dihydropyrimidine dehydrogenase
EGFR	Epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer
ERCC1	Excision repair cross-complementation group 1
ESMO	European Society for Medical Oncology
5-FU	5-Fluorouracil
GIST	Gastrointestinal stromal tumour
GLOBOCAN	Global Cancer Observatory
HFS	Hand-foot syndrome
HNPCC	Hereditary non-polyposis colorectal carcinoma
HRQOL	Health related quality of life
H. pylori	*Helicobacter pylori*
HTN	Hypertension
HUCH	Helsinki University Central Hospital
mCRC	Metastatic colorectal cancer
mRCC	Metastatic renal cell carcinoma
MMR	Mismatch repair
MSI	Microsatellite instability

mOS	Median overall survival
mPFS	Median progression-free survival
NCCN	National Comprehensive Cancer Network
NCCTG	North Central Cancer Treatment Group
NCI-C CTC	National cancer institute of Canada, common toxicity criteria
NOGGO	North-Eastern German Society of Gynecological Oncology
NSCLC	Non-small cell lung cancer
LV	Leucovorin
RR	Response rate
OS	Overall survival
PFS	Progression-free survival
PROM(s)	Patient recorded outcome measure(s)
ROS	Reactive oxygen species
RFS	Relapse free survival
RR	Response rate
SEER	Surveillance, Epidemiology, and End Results
TGF-β	Transforming growth factor-β
TNM	Classification of Malignant Tumours (Tumour, Nodes, Metastasis)
VEGF	Vascular endothelial growth factor
QOL	Quality of life

3. ABSTRACT

Colorectal cancer (CRC) is a common disease in all western countries. In Finland, 3360 new CRC patients were diagnosed in the year 2016. The incidence of CRC continues to rise in countries in which screening has not been launched; the diagnostic delay for CRC could be longer than that for other common malignancies. The CRC treatment process has been revolutionised over the past years, and the prognosis of CRC has improved, but we lack information regarding the prognostic and predictive factors that would help us to optimise the therapy for an individual patient. In addition, with improved survival rates, both acute and long-term toxicities require more attention.

Hypertension (HTN) is a well-recognised adverse event that is associated with all drugs involved in anti-VEGF (vascular endothelial growth factor) inhibition. The first study discussed in this thesis investigated whether HTN could act as a surrogate marker for efficacy during bevacizumab-containing therapy. We had 101 consecutive patients with mCRC, who had been treated with bevacizumab-containing palliative chemotherapy. We observed that patients who developed any grade of HTN showed a significantly improved response rate (RR) (30 *vs.* 20%; *P*= .025), median progression-free survival (mPFS) (10.5 *vs.* 5.3 months; *P*= .008), and median overall survival (mOS) (25.8 *vs.* 11.7 months; *P*< .001), compared to normotensive patients. In a multivariate landmark survival analysis, the development of HTN within 3 months after the start of therapy was an independent predictor of survival (HR 0.53; *P*= .007), along with the presence of other known mCRC prognostic factors. The significant association between HTN and treatment outcome was independent of the treatment line. According to our results, treatment-associated HTN might predict the outcome of bevacizumab-containing chemotherapy in mCRC patients and could potentially be utilized as a biomarker for continued care. However, our data require validation in large prospective studies.

Though the prevalence of *Helicobacter pylori* (*H. pylori*) has decreased in Finland, it continues to be a major public health problem worldwide. The role of the *H. pylori* infection as a confounding gastrointestinal comorbidity in the diagnosis of CRC is not well known, and it is not established whether *H. pylori* infection worsens chemotherapy-induced gastrointestinal toxicity. It is known that the diagnosis of CRC can be delayed owing to the misinterpretation of symptoms by both a patient and doctor and owing to the comorbidities present. In the second sub-study, we studied the role of different symptoms and *H. pylori* for diagnostics in seventy-nine radically operated stage II-IV CRC patients. Of these, thirty-seven patients (47%) were *H. pylori*-seronegative at the baseline and forty-two (53%) were seropositive. We observed that diagnosis was significantly delayed in patients who presented with functional dyspeptic symptoms (7.5 *vs.* 5 months; *P*= .035), but it was not delayed in patients with anaemia, bowel symptoms, occlusion, blood in the stool, infection, and hypolactasia. Likewise, the *H. pylori* infection was associated with a delay in diagnostics. The median time from CRC symptom onset to surgery in *H. pylori*-infected patients was significantly longer, as compared to that in non-infected individuals (6 *vs.* 5 months; *P*= .012). All patients were treated with 5-fluorouracil (5-FU)-based adjuvant chemotherapy. *H. pylori* seropositivity at the baseline was not associated with oro-gastrointestinal toxicity during chemotherapy. In conclusion, dyspeptic symptoms and the presence of *H. pylori* infection at the baseline delayed the initial diagnosis of CRC, which highlights the importance of thorough diagnostics. However, there is no association

between *H. pylori* infections and gastrointestinal adverse events during 5-FU-based adjuvant chemotherapy. Therefore, the eradication of *H. pylori* infections before providing 5-FU-based adjuvant chemotherapy cannot be routinely recommended.

The association between survival and adverse events in several types of cancer and especially those related to targeted therapies has been established, but data for CRC patients, and notably for those with early-stage CRC, are limited. In the third sub-study, we assessed whether adverse events could predict disease-free survival (DFS) or OS in stage II-III CRC patients. We pooled material from two prospective clinical trials and studied 1033 stage II-III CRC patients treated with 5-FU-based adjuvant chemotherapy. Adverse events of interest included haematological (leukopenia, neutropenia, thrombocytopenia) and non-haematological (e.g., mucositis, diarrhoea, nausea/vomiting, hand-foot syndrome) events. Any grade of neutropenia was associated with improved DFS (HR 0.76 $CI_{95\%}$ 0.61-0.95), while any grade of nausea/vomiting was associated with improved DFS (HR 0.74 $CI_{95\%}$ 0.59-0.92) and OS (HR 0.58 $CI_{95\%}$ 0.45-0.75), and mucositis was associated with improved DFS (HR 0.70 $CI_{95\%}$ 0.56-0.88) and OS (HR 0.67 $CI_{95\%}$ 0.52-0.88). Patients experiencing all three of these adverse events had the best outcome, whereas patients reporting no adverse events had the worst survival rates. According to our results, adverse events related to treatment with adjuvant 5-FU chemotherapy in early stage CRC patients, especially non-haematological adverse events, are strongly associated with the prediction of improved DFS and OS. Our findings require validation in large prospective trials, but if established, adverse events could guide the clinician in bringing about dose modifications, to maximize the treatment efficacy, while ensuring a lesser level of toxicity.

The use of oxaliplatin in the adjuvant setting reduces the risk of death 20% in stage III CRC patients. The major adverse event associated with oxaliplatin-based regimens is peripheral neuropathy, but the prevalence of acute and long-term neuropathy is not well established, especially in a subarctic climate, such as that observed in Finland. It is not well known whether there is a difference in the neuropathy observed with two standard regimens, i.e., oxaliplatin with capecitabine (CAPOX) and infusional 5-FU (FOLFOX), and whether long-term neuropathy influences the quality of life (QOL). In the fourth sub-study, we analysed the prevalence of oxaliplatin-induced acute and long-term neuropathy during and after treatment with CAPOX and FOLFOX and studied the effect of long-term neuropathy on QOL in a real-life patient population. One hundred and forty-four early stage CRC patients (72 CAPOX patients and 72 matched FOLFOX controls) were identified and evaluated for acute neuropathy (according to NCI-CTCAEv3.0) during adjuvant treatment. Ninety-two long-term survivors responded to QOL (EORTC QLQ-C30) and chemotherapy-induced peripheral neuropathy (EORTC CIPN-20) questionnaires and were prospectively evaluated for long-term neuropathy. Any grade of neuropathy was found to be present in 69% of patients at 4.2 years, at the median follow-up. Though neuropathy grade 2-4 did not influence the global health status, it was associated with decreased physical functioning (P= .031), role functioning (P= .040), and more diarrhoea (P= .021) in QLQ-C30 items. There were no differences in acute or long-term neuropathy between CAPOX- and FOLFOX-treated patients, and no seasonal variation was observed. We noted a significant association between acute and long-term neuropathy. Grade 0-1 acute neurotoxicity was significantly worsened to grade 2-4 long-term neuropathy in 36%, or 5 out of 14 patients. The ECOG 1 performance status at the baseline was a significant risk factor for long-term neuropathy. According

to our results, long-term neuropathy is observed after therapy in a significant proportion of patients and is severe in some patients, but it does not impair global health status. At least in a subarctic climate such as that in Finland, long-term neuropathy is not preventable in all patients with a reduction in the duration of therapy, but the performance status might predict the risk of long-term neuropathy. However, this observation needs to be validated further.

4. INTRODUCTION

CRC is the third most common cancer worldwide after lung- and breast cancer, and is the second most common cancer in Europe (Bray et al., 2018). In addition, it is the second most common cause of cancer-related death after lung cancer in the western world and Europe. In 2018, there were 1.8 million new CRC patients diagnosed worldwide, of which 499 667 were diagnosed in Europe and 694 000 died worldwide due to CRC; of these, 242 483 were from Europe. There has been an increase in the awareness about CRC in the general population, along with screening programmes that have been launched in several countries (Jones et al., 2010a). Nevertheless, the patient- or doctor-related diagnostic delays for CRC could sometimes be long (Korsgaard et al., 2008). Symptoms of CRC can be vague, and diagnostics can be confounded by other comorbidities, because of which the clinician needs to be vigilant (Walter et al., 2016).

The prognosis for CRC has constantly improved during the past two decades and overall 5-year survival rates currently exceed 60% (Allemani et al., 2018). Concerning early stage CRC, prognosis has improved due to the use of improved surgical techniques and 5-FU and oxaliplatin based adjuvant therapies in high-risk stage II and stage III patients (Andre et al., 2004b; Labianca et al., 2013). However, biological agents such as bevacizumab and epidermal growth factor receptor inhibitors (EGFR-inhibitors) that improved outcomes in mCRC patients have failed in adjuvant trials (Alberts et al., 2012; Allegra et al., 2011; de Gramont et al., 2012; Taieb et al., 2014). Recent studies in the adjuvant setting have mainly focused on optimizing the delivery and duration of the therapy, but we need tools to develop a specialised adjuvant therapy for each individual. The majority, i.e., 70-75% of patients, have local, early-stage disease at diagnosis. Despite the improvement in survival rates, there is still room for improvement, as it has been estimated that around 30%, even up to 50% of patients treated with a curative intent have ultimately relapsed, which emphasizes the need for improvements to be made in adjuvant therapy (Schmoll et al., 2012).

Evidence of an underlying, multifactorial biological background, which leads to the development of different CRC subtypes that are associated with different clinical courses and treatment responses, is rapidly increasing. From a historical perspective, 5-FU-based chemotherapy alone was the treatment for mCRC for three decades (Machover, 1997). The prognosis of the untreated disease is approximately 2-6 months (Sorbye et al., 2009), and with the use of the single agent 5-FU, survival improved up to 12 months. After irinotecan and oxaliplatin were introduced for clinical use, combination chemotherapies improved the mOS up to 20 months (Goldberg, 2005; Tournigand et al., 2004). Today, the mOS exceeds 30 months in clinical trials, with the use of biological agents (Van Cutsem et al., 2016). Approximately 10-15% of patients with metastatic disease survive for more than five years (Ahmed et al., 2013; Cronin et al., 2018a).

Almost two-thirds of CRC patients treated with curative intent were alive five years after the diagnosis, and they represent the third most common group of cancer survivors after prostate and breast cancer patients (Bray et al., 2018). Because of the improved prognosis, the prevalence of CRC survivors is likely to increase over the coming decades. Therefore, though it is important to study

acute adverse events, we also need to study long-term toxicities and what kind of impact those may have on the QOL in CRC survivors.

CRC is a heterogeneous malignancy and the course of the disease and treatment outcome can vary widely between individuals. We still have very few tools for optimizing treatments on an individual basis. In clinical practice, the TNM mainly guide the decision regarding the adjuvant therapy. In mCRC patients, only RAS mutations act as negative predictive markers for therapy by excluding patients not benefitting from EGFR- inhibitors. We need validated biomarkers that would be reliable in everyday clinical practice, to select precision medicines for each patient. We also need to understand which patient would benefit more from continuous treatment, as we are currently exposing some patients to toxicities without any benefit.

Even though we live in the era of tailored individual cancer therapies, we estimate the dose of available drugs based on the body-surface-area (BSA) (Gurney, 2002). It is especially well recognised that during treatment with 5-FU, significant level of variation is observed in the metabolism of individuals (Saif et al., 2009); thus, we currently treat some patients without sufficient dose-intensity, thereby compromising the optimal outcome, while exposing other patients to excessive toxicity. It is questionable whether we are optimally exploiting the standard drugs available at present. Instead of carrying out intensive research on new agents, it might be worth to shift some focus towards toxicity-based and pharmacokinetically guided dose escalation.

This thesis was conducted to investigate some clinically relevant issues in this field. Since we lack knowledge about predictive factors in the treatment of CRC, we were interested in studying whether typical, treatment-related adverse events were associated with treatment outcomes and if they could act as useful predictive factors. We were also interested in studying if evidence regarding *H. Pylori* colonisation interferes with CRC diagnostics and if it increases acute gastrointestinal toxicity during 5-FU-based adjuvant chemotherapy. Finally, long-term neuropathy after oxaliplatin-based adjuvant chemotherapy and its impact on QOL in CRC survivors has also been focused upon in this study.

5. REVIEW OF THE LITERATURE

5.1 Epidemiology

CRC is a considerable health issue that contributes to a remarkable cancer burden globally, especially in the western world, as it mostly affects countries with a high standard of living (Bray et al., 2018). In the western world, CRC is the third most common cancer in men, after lung and prostate cancer, and the second most common cancer in women after breast cancer, with approximately 1.8 million new CRC cases observed worldwide in the year 2018. The statistics are the same for Finland; CRC is the third most common cancer in Finland as well, as 3360 patients were diagnosed with CRC in the year 2016 (Cancer_registry, 2018).

In six years, the number of CRC patients has increased by 0.6 million; statistics reported by GLOBOCAN from the year 2012 showed that 1.2 million new patients had been diagnosed (Ferlay et al., 2014). It is predicted that 3.1 million new CRC cases would be reported globally in 2040 (Bray et al., 2018). A significant increase in the incidence of CRC is attributable to the rapidly increasing CRC incidence rates in many low- and middle-income countries, especially in Eastern Europe, Asia, and South America, as they are adapting western lifestyles (Center et al., 2009). Some stabilising or decreasing trends in incidence have been observed in highly developed countries, especially in the United States, probably due to systematic screening and early prevention (Ansa et al., 2018). In Finland, the incidence of CRC continues to increase (Cancer_registry, 2018).

With regard to mortality, CRC is the second leading cause of cancer death, both in the United States and Europe, including in Finland after lung cancer (Bray et al., 2018; Cronin et al., 2018a). There were 0.9 million deaths due to CRC in 2018, and it is predicted that 1.6 million deaths would be reported in 2040. In Finland, 1303 individuals died due to CRC in 2016 (Cancer_registry, 2018). Mortality trends are showing a decrease in most developed countries, such as Finland, because of an improvement in therapeutic options, and because of screening and early detection in some countries (Bray et al., 2018; Cancer_registry, 2018; Van Cutsem et al., 2016).

Prognosis for both early-stage and metastatic CRC has improved during the past twenty years, and especially over the past ten years (Allemani et al., 2018; Van Cutsem et al., 2016). The current 5-year overall survival rates in Europe exceed 60%, and are among the highest in Finland; 5-year survival rates of 64.9 % ($CI_{95\%}$ 63.7–66.2) and 64.4 % ($CI_{95\%}$ 62.6–66.1) have been observed for rectal and colon cancers, respectively, according to recently published statistics (Allemani et al., 2018). Thus, the number of CRC survivors has increased globally and in Finland, and represents the third largest group of cancer survivors, after the groups of prostate and breast cancer survivors; the prevalence is likely to continue to increase in the future (DeSantis et al., 2014). In patients with mCRC, the median survival duration in randomized trials exceeds 30 months (Van Cutsem et al., 2016) and approximately 10-15% of patients with mCRC survive beyond five years (Ahmed et al., 2013; Cronin et al., 2018b).

5.2 Risk factors

Age is a major risk factor for CRC. It is uncommon for the disease to occur before the age of 40, after which the risk begins to rise and increases significantly after the age of 50 (Cancersociety_fi, 2018). The median age at diagnosis is about 70 years (Jarvinen et al., 2013) . However, according to recent data, there has been a steady increase in CRC incidence in groups of individuals with age below 50, and even in those with an age of 20, for unexplained reasons (Austin et al., 2014). There is a strong association between CRC and the western lifestyle, which is characterized by the high consumption of red and processed meat, low dietary intake of fruit, vegetable, and fibre, obesity, smoking, excessive use of alcohol, and low physical activity (Grosso et al., 2017; Tuan and Chen, 2016). Inflammatory bowel disease is an independent risk factor for CRC, but due to improvements in treatments and surveillance, only a minority of patients with inflammatory bowel disease develop CRC (Annese et al., 2015).

It is estimated that about 70-75% of newly diagnosed CRC cases are sporadic without any predisposing factors or positive family history, about 5% are attributable to a known, predisposing genetic condition and in the rest of the cases there is an underlying, hereditary contribution by not yet identified genes. (Brandão and Lage, 2015). The lifetime risk of development of CRC in an "average" western individual is about 5-6%, but the risk increases up to 20%, if first- and/or second-degree relatives have a history of CRC (Jasperson et al., 2010; Rustgi, 2007).

The most common genetic condition associated with CRC is the Lynch syndrome (hereditary non-polyposis colorectal carcinoma, HNPCC), which accounts for about 3-4% of all CRC cases. In a patient with Lynch syndrome, the lifetime risk for developing CRC without appropriate follow-up is up to 90%, and there is also an increased risk of development of other malignancies, such as gynaecological and urogenital (Lynch et al., 2009; Watson et al., 2008). Other genetic conditions occur very rarely (Syngal et al., 2015). Only approximately 1% of CRC cases are attributable to familial adenomatous polyposis (FAP), and less than 1% of cases occur due to rare polyposis syndromes such as *MYH*-associated polyposis.

5.3 Pathways in the carcinogenesis of CRC

CRC is a heterogeneous disease that develops through multiple genetic and epigenetic alterations, which can be activated by environmental, inherited, or both factors (Bardhan and Liu, 2013). It is thought that CRC develops through two different morphological pathways. The first pathway involves the so-called classical adenoma-carcinoma sequence, which begins with premalignant lesions that are comprised of conventional adenomas, including tubular or tubulovillous adenomas (Fearon and Vogelstein, 1990; Tannapfel et al., 2010). Secondly, there is the so-called serrated neoplasia pathway, which begins with the development of sessile or traditional serrated adenomas or hyperplastic polyps. These two morphologic pathways are driven by different, partly overlapping molecular pathways that can be divided into three groups, depending on the mechanism by which the tumour develops (Bae et al., 2016; Nazemalhosseini Mojarad et al., 2013; Worthley and Leggett, 2010). The first group consists of germline or sporadic mutations in DNA mismatch repair genes,

which lead to the development of a DNA microsatellite instability (MSI) phenotype. The second group consists of mutations in *APC* or other genes activating the Wnt pathway, which leads to the development of the chromosomal instability (CIN) phenotype. Tumours in the third group develop as result of global genome hypermethylation, which lead to the silencing of the tumour suppressor gene (CIMP) phenotype. CIMP is associated with the sporadic MSI phenotype (Nazemalhosseini Mojarad et al., 2013).

All the above-mentioned groups have distinct genetic, pathologic, and clinical characteristics. The classification of CRC tumours into six different gene expression-based groups has been described, and there are three to six subgroups in each group, to classify CRC tumours into subtypes (Guinney et al., 2015). To resolve the inconsistencies among these classifications and facilitate their clinical translation and utility, an international consortium was formed. Based on a large set of gene expression and molecular, mutational, histological, and clinical data, it was possible to identify four different consensus molecular subtypes (CMS) of CRC tumours (Guinney et al., 2015). These subtypes are: CMS1 (MSI immune subtype including MSI, CIMP-high, hypermutated, and BRAF positive subtypes), CMS2 (canonical subtype, WNT/MYC pathway activation, high in somatic copy number alterations), CMS3 (metabolic subtype: SCNA/CIMP-low, KRAS mutations, and metabolic dysregulation), and CMS4 (mesenchymal subtype with high somatic copy number alterations and with prominent transforming growth factor-β, TGF-β, activation, stromal invasion and angiogenesis). According to Guinney et al., 14%, 37%, 13%, and 23% of tumours are of the CMS1, CMS2, CMS3, and CMS4 type (Guinney et al., 2015). The rest of the tumours are so-called mixed or intermediate tumours, and it is not possible to classify them effectively into any of the subtypes. The CMS classification system will be further discussed in section 5.11.

5.4   Symptoms and diagnostics

The symptoms and prognosis of CRC are directly associated with the growth of the tumour into the lumen of the bowel and the stage of the disease, which is determined by the extent of tumour growth through the bowel wall into adjacent organs and lymph nodes, and by the presence of distant metastases (Brenner et al., 2014; Greene et al., 2002). Classical symptoms that are alarming with regard to a CRC diagnosis include changes in bowel habits, especially in combination with an increase in the age and tumour bleeding (Glynne-Jones et al., 2017; Labianca et al., 2013). Bleeding could be either visible in the stool, especially in distal cancer, or can be microscopic, especially in proximal tumours that lead to iron deficiency and microcytic anaemia (John et al., 2011). In some instances, CRC presents with bowel obstruction, but weight loss, abdominal pain, and weakness are often associated with a more advanced stage. Symptoms and signs of CRC are often vague, non-specific, and long lasting, and their gradual increase makes the diagnostics challenging. CRC was observed to mimic even functional dyspepsia, and demonstrate a variety of symptoms that it might present with (O'Reilly and Long, 1987).

In cancer, early diagnosis is always important. It is important to improve prognosis and diminish psychological distress. Since CRC develops from recognizable, precancerous polyps, which can be detected and removed before they become cancerous, it has been of interest to screen CRC to detect

early-stage disease. Screening was shown to reduce mortality and be cost-effective (Issa and Noureddine, 2017). Screening programmes have been launched in some countries, but not in all. In Finland, screening was launched, but faecal occult blood screening did not reduce mortality in a pilot analysis (Pitkäniemi et al., 2015). The recommended screening method for the average-risk population is either an annual or biennial faecal immunochemical test (FIT), sigmoidoscopy every 5 years, or colonoscopy every 10 years (Bénard et al., 2018; Schoen et al., 2012). The participation rate for screening has varied significantly between nations and was 70% in Finland (Pitkäniemi et al., 2015). As long there is a lack of a nationwide screening program and participation rates are not satisfactory, it is not possible to detect pre-cancerous lesions and very early stages of CRC.

Even though screening programmes and media campaigns have increased general awareness regarding CRC (Jones et al., 2010a; Jones et al., 2010b), patient or health care system-related diagnostic delays are seen more often in CRC than in other common cancers (Korsgaard et al., 2008; Van Hout et al., 2011). One third of patients diagnosed with CRC have three or more consultations with a general practitioner before referral, as compared with that of only 17.9% for other cancers in a British patient material (Lyratzopoulos et al., 2013). This is mostly due to the vague nature of CRC symptoms, but it was shown that even rectal bleeding did not always lead to colonoscopy, if bleeding had already been long lasting (Walter et al., 2016).

The diagnostic delay of CRC can also be associated with other abdominal cancers and gastrointestinal comorbidities, and the diagnostic work-up can be confused by those, or terminated too early, due to another condition being diagnosed first (Walter et al., 2016). *H. pylori*, discussed in detail in section 5.5.1, is most commonly associated with dyspepsia (Malfertheiner et al., 2012). Its prevalence in Finland has remarkably decreased over the past decades and the same trend is seen in other western countries, as its prevalence is associated with socioeconomic status (Hooi et al., 2017). Globally, more than half of the world's population is infected with *H. pylori*. Another common gastrointestinal complaint is lactose intolerance. Finnish individuals tolerate lactose well, but globally, lactose intolerance affects most of the world's population (Storhaug et al., 2017). Data showing *H. pylori* and lactose intolerance to be confounding comorbidities in CRC diagnostics are scarce.

5.5   Gut microbiota

The entire gastrointestinal tract and especially the large intestine harbours an enormous number of bacteria, viruses, and fungi (Proctor, 2011). The composition of microbiota is highly dependent on lifestyle and environmental factors, and is affected by age. Therefore, there is great variation in microbiota, both intra-individually and inter-individually (D'Argenio and Salvatore, 2015; David et al., 2014; Perez-Cobas et al., 2013; Yatsunenko et al., 2012). Gut microbiota has a huge metabolic capability and has several important metabolic functions, including the production of vitamins, synthesis of amino acids, absorption of ions, participation in the conversion of dietary polyphenolic compounds, and it is involved in the biotransformation of bile acids (Boleij and Tjalsma, 2012; Jandhyala et al., 2015). Together with these metabolic functions, the intestinal microbiota plays a fundamental role in the induction, education and function of the immune system (Belkaid and Hand,

2014). It maintains the intestinal barrier and is essential in assisting in the generation of an adequate immune response to pathogens.

Several reports support the fact that dysbiosis, i.e., the imbalance in normal intestinal microbiota, which could be a consequence of environmental factors, infections, antibiotics, surgery, and chemotherapy, plays an essential role in several health conditions, such as obesity, diabetes, inflammatory bowel disease, and several autoimmune diseases (Belkaid and Hand, 2014; Blainey et al., 2012; Maloy and Powrie, 2011; Osborn and Olefsky, 2012; Turnbaugh et al., 2006; Wu et al., 2010). In addition, increasing amounts of evidence have shown that microbiota is a promoter and modifier in CRC carcinogenesis (Schwabe and Jobin, 2013). Inflammation is an important driver of carcinogenesis in CRC and the microbiota is important for modifying inflammatory responses (Belkaid and Hand, 2014). There is a complicated network between bacteria with the ability to shape a pro-tumorigenic environment, by driving the generation of pro-inflammatory responses and bacteria with protective, anti-inflammatory capabilities (Chow et al., 2010; Reinoso Webb et al., 2016). The microbiota shared by healthy adults most abundantly includes *Firmicutes*, *Bacteroidetes,* and *Actinobacteria,* while *Proteobacteria*, *Fusobacteria*, *Cyanobacteria,* and *Verrucomicrobia* are usually less well represented (D'Argenio and Salvatore, 2015). No single carcinogenic pathogen is identified in CRC, but increasing amounts of evidence suggest that some bacteria play an especially important role in colorectal carcinogenesis, such as *Streptococcus bovis*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Clostridium septicum*, *Fusobacterium* spp., and *Escherichia coli* (Chen et al., 2012; Gagniere et al., 2016; Gao et al., 2015).

The incidence of CRC is strongly associated with a western lifestyle. Gut microbiota is unfavourably modified by the lack of physical activity, obesity, and a western diet (Gagniere et al., 2016). CRC is associated with the reduced abundance of protective bacteria, such as *Clostridium* and *Roseburia,* which are important producers of butyrate (Ríos-Covián et al., 2016). Butyrate is the most important of all the short chain fatty acids, which are energy sources for colonocytes, and are released by the fermentation of complex carbohydrates using colonic bacteria. Butyrate plays a protective role against carcinogenesis, by promoting colon motility, improving visceral blood flow, preventing the overgrowth of pathogens, reducing inflammation, inducing apoptosis, and inhibiting tumour cell progression. The presence of fibre significantly induces the production of short chain fatty acids; therefore, a fibre-rich diet is shown to reduce CRC risk (Howe et al., 1992).

Studies demonstrate that there are differences in the drug metabolism of individuals due to differences in their microbiota (Gimenez-Bastida et al., 2018; Li et al., 2016; Scott et al., 2017). The amount of data available with regard to chemotherapeutic agents and microbiota is still scarce, but continues to increase. Microbiota can mediate treatment efficacy and toxicity by direct enzymatic activity and biochemical conversion of a drug (Alexander et al., 2017). Scott et al. explored whether the host response to 5-FU can be mediated by bacteria using the nematode *Caenorhabditis elegans* as a model (Scott et al., 2017). *C. elegans* were colonized with different strains of *Escherichia coli* and investigators observed up to 80-fold changes in the 5-FU efficacy, depending on the bacterial strain *C. elegans* was colonized with. Toxicity was measured in terms of nematode fertility. It was also

suggested that bacterial ribonucleotide metabolism is the primary mediator of 5-FU efficacy in *C. elegans*.

Irinotecan is activated by hydrolysis to form SN-38, an inhibitor of topoisomerase 1 (Mathijssen et al., 2001). It is deactivated in the liver by hepatic glucuronidation, producing SN-38G, which is then excreted into the gut with bile. Bacterial ß-glucuronidases in the gut lumen have the ability to reactivate SN-38G to its active, enterotoxic form, which leads to mucositis (Guthrie et al., 2017). There are numerous bacterial ß-glucuronidase isoforms that differ with regard to their substrate pharmacokinetics; therefore, there are differences between individuals with regard to the bacterial capability to reactivate SN-38G, depending on the presence of specific bacterial ß-glucuronidases and glucuronide membrane transporters (Wallace et al., 2015). The characterization of microbiota might identify patients at increased risk of developing irinotecan induced mucositis and diarrhoea. It was shown that ciprofloxacin and low doses of amoxapine were effective in the suppression of bacterial ß-glucuronidase activity and therefore decreased the risk for mucositis (Kodawara et al., 2016; Kong et al., 2014).

Microbiota can also mediate chemotherapeutic efficacy, by regulating the tumour microenvironment. In a study by Iida et al., three different cancer cell lines (including a CRC cell line) were transplanted under the skin of germ-free mice, mice treated with wide-spectrum antibiotics, and conventional mice (Iida et al., 2013). Germ-free mice and mice treated with wide-spectrum antibiotics lack normal commensal bacteria. After tumour growth, the mice were treated with immunotherapy or chemotherapy, using either oxaliplatin or cisplatin. Concerning oxaliplatin, reactive oxygen species (ROS) are essential for DNA damage and apoptosis in response to platinum compounds (Ozben, 2007). ROS are produced by tumour-infiltrating myeloid cells, but their production requires an induction signal from commensal bacteria. It was observed that in germ-free mice and mice treated with antibiotics, the tumour-infiltrating myeloid-derived cells produced lower levels of ROS (Iida et al., 2013). In these mice, the outcome of treatment with oxaliplatin was inferior, as compared to that in mice with an intact gut microbiota exhibiting normal microbiota mediated ROS production.

Even some fatal drug interactions during chemotherapy are probably mediated by microbiota metabolism, as observed in Japan, where sixteen patients treated with anti-viral drug sorivudine (1-β-D-arabinofuranosyl-5-(E)-(2-bromovinyl) uracil) and 5-FU died over a 40 day period due to the excessive toxicity of 5-FU (Diasio, 1998). It was noted that the *Bacteroides* species, *B. vulgatus*, *B. thetaiotaomicron*, *B. fragilis*, *B. uniformis,* and *B. eggerthii* secrete high concentrations of phosphorolytic enzymes hydrolysing sorivudine to (E)-5-(2-bromovinyl) uracil (BVU). BVU in turn inhibits the DPD enzyme, which leads to the inactivation of 5-FU and these patients died because of fatally high 5-FU concentrations.

5.5.1 *Helicobacter pylori*

Helicobacter pylori (H. pylori) is a gram-negative bacterium colonizing the gastric epithelium and is classified as a group I carcinogen since 1994 (Anonymous, 1994; Warren and Marshall, 1983). It

does not invade or reside in the colonic mucosa, but is known to move through the colonic lumen, as detected by immunohistochemistry in neoplastic colorectal tissue. A majority of infected patients are asymptomatic, but *H. pylori* is a known cofactor in some important gastrointestinal conditions (McColl, 2010). It plays a role in the development of duodenal and gastric ulcers. In *H. pylori* positive dyspeptic patients, eradication of the bacterium can lead to long-term relief of symptoms and dyspepsia therefore remains an accepted indication for *H. pylori* eradication (Malfertheiner et al., 2012). It is the most important risk factor for atrophic gastritis and gastric cancer, as is it estimated that the development of almost 90% of all gastric cancers could be attributable to *H. pylori* infections (Herrero et al., 2014). In addition, there is a strong association between gastric MALT lymphomas and *H. pylori* infections, and the eradication of *H. pylori* could be sufficient to cause the regression of most localized gastric MALT lymphomas (Fischbach et al., 2004). Even though the incidence of *H. pylori* infection has been decreasing in many countries, due to improved standards of living, *H. pylori* infection continues to be a major public health problem (Hooi et al., 2017). According to a global systematic review, approximately 4.4 billion individuals worldwide were estimated *H. pylori* carriers in 2015.

Ever since *H. pylori* was recognised as underlying pathogen in gastric cancer, there has been interest in investigating its role in other gastrointestinal malignancies, especially CRC. In 2013, three large meta-analyses were performed, and a positive association was observed between *H. pylori* infection and colorectal adenoma or CRC (Chen et al., 2013; Papastergiou et al., 2016; Rokkas et al., 2013; Wu et al., 2013). As an example, a study by Rokkas et al. observed a significant relationship between *H. pylori* and colon cancer (OR 1.3, CI$_{95\%}$ 1.07-1.59; *P*= .01) and colon polyps (OR 1.5; CI$_{95\%}$ 1.26-1.79; *P*= .000) (Rokkas et al., 2013). Sonnenberg et al. have performed the largest case-control study, in which biopsies from 156,000 subjects who underwent both colonoscopy and oesophago-gastro-duodenoscopy were included (Sonnenberg and Genta, 2013). *H. pylori*-related gastritis occurred more frequently among patients with colonic hyperplastic polyps (OR 1.24, CI$_{95\%}$ 1.18-1.30), adenomatous polyps (OR 1.52, CI$_{95\%}$ 1.46-1.57), advanced adenomas (OR 1.80, CI$_{95\%}$ 1.69-1.92), villous adenomas or adenomas with high-grade dysplasia (OR 1.97, CI$_{95\%}$ 1.82-2.14), and adenocarcinomas (OR 2.35, CI$_{95\%}$ 1.98-2.80). However, no large, randomised, longitudinal studies have been conducted. Cross-sectional and case-control studies can establish associations, but it is not possible to draw conclusions based on them, if causality between *H. pylori* and CRC exists.

Several hypotheses have been put forward about pathogenic mechanisms that could explain the possible link between a *H. pylori* infection and colorectal neoplasia (Papastergiou et al., 2016). Gastrin is a known trophic factor in the colorectal mucosa and a persistent *H. pylori* infection elicits hypergastrinemia (Renga et al., 1997). It was shown that gastrin was directly mitogenic in either normal or neoplastic colonic cells in vitro, and resulted in the hyperproliferation of colonic mucosa in transgenic mice. In addition, CRC tumour cells were shown to secrete gastrin (Finley et al., 1993). There are several studies reporting the association between elevated serum/plasma gastrin levels, an increased risk of colorectal adenoma, and/or CRC, but some studies have disputed these observations (Papastergiou et al., 2016). It has also been hypostatized that *H. Pylori* induced chronic atrophic gastritis might contribute to changes in the colorectal microflora, which would lead to dysbiosis (Zou et al., 2018).

5.6 Therapy options for CRC

5.6.1 *Early stage disease*

The treatment and prognosis of CRC depends on the stage of disease at diagnosis (Brenner et al., 2014). In early stage I-III CRC, radical surgery is a crucial mode treatment. In rectal cancer, radiotherapy might be provided preoperatively. The options include a long course of chemoradiation (50.4/1.8Gy) with capecitabine in patients with more locally advanced disease or a short course of radiotherapy (5x5Gy) without chemotherapy, to lower the risk of local recurrence (Benson and Venook, 2018b; Glynne-Jones et al., 2017). Palliative surgery of the primary tumour can be performed in advanced disease, to prevent complications that would occur later and/or relieve possible symptoms, but its impact on survival has not been established clearly.

The prognosis of colon cancer is most accurately predicted by the TNM stage. Based on the surveillance, epidemiology, and end results (SEER) database, which contained information about people diagnosed with colon cancer between 2004 and 2014, the 5-year survival rates by stage were: stage I 92%, stage IIa 87%, stage IIb 65%, stage IIIa 90%, stage IIIb 72%, stage IIIc 53% and for stage IV 12% (Anonymous, 2018). For rectal cancer patients 5-year survival rates were: stage I 88%, stage IIa 81%, stage IIb 50%, stage IIIa 83%, stage IIIb 72%, stage IIIc 58% and for stage IV 13%. As can be seen, the prognosis is better for stage IIIa patients than for IIb which can reflect the routine availability of adjuvant therapy for patients with stage III disease, in contrast to stage II.

Decisions about postoperative treatment after radical surgery are mainly taken after the assessment of the pathological tumour stage (Schmoll et al., 2012). In stage I CRC, no adjuvant chemotherapy is recommended due to favourable prognosis, but in stage II disease, the decision-making process becomes more complex. Treatment with single-agent 5-FU for six months was shown to result in a relatively small improvement in survival (absolute 3-5%) in stage II disease (Gray et al., 2004), with no additional OS benefit of oxaliplatin (Andre et al., 2004a; Andre et al., 2015a). It is therefore a question for which patient adjuvant chemotherapy is justified also considering toxicity and costs. According to guidelines in stage II patients presenting with risk factors such as histologically poor differentiation, vascular/lymphatic/perineural invasion, tumour obstruction or perforation, T4 tumour and less than 12 examined lymph nodes, risk for recurrence is considered increased and in these patients adjuvant therapy should be considered (Benson and Venook, 2018a; Benson and Venook, 2018b; Labianca et al., 2013). Single agent 5-FU is the standard therapy for stage II, but oxaliplatin can be considered, based on assessment of individual risk, i.e., in case of a T4 colon tumour.

Adjuvant chemotherapy after curative resection is recommended for all stage III patients without contraindications for therapy (Benson and Venook, 2018a; Labianca et al., 2013). The 5-FU-based regimens result in an improvement in DFS to 67%, as compared to that of 55%, observed with surgery alone (corresponding to a 30% proportional reduction in risk of recurrence [HR, 0.70; CI$_{95\%}$ 0.63-0.78]); the OS improved from 64% to 71% at 5 years with adjuvant chemotherapy (a 26% proportional reduction in risk of death [HR 0.74, CI$_{95\%}$ 0.66-0.83]) (Eisenhauer et al., 2009) (Gill et

al., 2004). The addition of oxaliplatin increased the absolute 5-year DFS from 6.2 to 7.5% and the overall survival from 2.7 to 4.2% in patients with stage III colon cancer (Brenner et al., 2014).

For rectal cancer patients, the optimal treatment option after surgery has not been well established (Benson and Venook, 2018b; Schmoll et al., 2012). It has not been indisputably proven that adjuvant chemotherapy results in benefits to the patient, and it is debatable whether it should be given with or without oxaliplatin.

Several multigene assays have been developed to provide prognostic and predictive information to support the decision regarding the stage II or III patients to be treated with adjuvant therapy (Benson and Venook, 2018a). These assays quantify the expression of tumour genes associated with a risk of recurrence, but are not predictive of the efficacy of adjuvant chemotherapy. Thus, data required are considered to be insufficient for supporting the use of these assays to guide decisions about adjuvant therapy. (Benson and Venook, 2018a).

5.6.2 *Metastatic disease*

At diagnosis, approximately 20% of patients present with synchronous metastatic disease and according to estimations at least 30% of patients treated with curative intent develop metachronous distant metastasis (Adam et al., 2015; Van Cutsem et al., 2016). The liver and lung are the most common and dominant sites of metastasis in colon cancer and rectal cancer patients, respectively. The next most common metastatic sites include the peritoneum, central nervous system, and bone (Van Cutsem et al., 2016).

In the metastatic setting, several approaches and therapeutic options could be considered. The most important factors in decision making are the ECOG performance status, liver and kidney function, tumour burden, and most importantly, the location of metastasis (Van Cutsem et al., 2016).

The possibility of achieving curation after the resection of metastasis in oligometastatic disease is a crucial therapeutic consideration (Van Cutsem et al., 2016). More than 50% of patients with mCRC will develop liver metastasis during the course of the disease, and surgery of patients with liver metastasis has remarkably contributed to the improved prognosis of mCRC patients (Van Cutsem et al., 2016). The 5-year OS rate for patients after liver resection exceeds 50%, as compared to that of 10-15% with palliative chemotherapy (Adam et al., 2015; Goldsbury et al., 2018). For patients with lung metastasis, 5-year OS rates after resection are 30-50% (Petrella et al., 2017). Peritoneal metastases occur in about 20% of patients and are associated with poor prognosis, but some of these patients are suitable for cytoreductive surgery. In addition to surgery, hyperthermic intraperitoneal chemotherapy (HIPEC) is usually delivered peri-operatively into the abdomen (Cashin et al., 2016; Ceelen, 2018; Maillet et al., 2016; Quenet et al., 2018; Verwaal et al., 2008). In ASCO 2018, the Prodige 7 trial was presented, in which the results with surgery alone were compared with those of surgery with HIPEC (Quenet et al., 2018). There were no differences in survival (OS 41.2 months in the non-HIPEC group *vs.* 41.7 months in the HIPEC group) or relapse-free survival (RFS) (11.1 months in the non-HIPEC group *vs.* 13.1 months in the HIPEC group). Hence, it is not established

whether HIPEC should be a part of treatment protocols in the future for these patients. In patients with non-resectable liver-limited disease, there are several loco-ablative methods available such as thermoablation, microwave ablation, stereotactic radiotherapy, and IRE (Van Cutsem et al., 2016). These can be curative in part of patients but can be used as a part of palliative therapy as well.

5.7 Chemotherapeutic agents in treatment of CRC

5.7.1 *Fluorouracil (5-FU)*

5.7.1.1 *Mechanism of action*

The drug 5-FU is an antimetabolite, anuracil analogue, which is the cornerstone in the treatment of CRC, both in the adjuvant and metastatic settings (Labianca et al., 2013; Longley et al., 2003; Van Cutsem et al., 2016). Its use was first based on the observation that rat hepatomas use uracil more rapidly than the normal cells (Miura et al., 2010). 5-FU is intracellularly converted into three active metabolites, i.e., fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP), which disrupt RNA and DNA synthesis, thus leading to cell death (Longley et al., 2003). However, the main mechanism of action of 5-FU is the irreversible inhibition of thymidylate synthase, which is an essential enzyme required for the synthesis of thymidine, a nucleoside required for DNA and RNA replication and repair, the lack of which leads to cell death (Longley et al., 2003). The 5-FU metabolite FdUMP binds to the nucleotide-binding site of thymidylate synthase, forming a stable ternary complex with the enzyme and reduced folinate, thereby blocking the binding of the substrate dUMP, which normally binds to it, and inhibits the synthesis of pyrimidine thymidine. High intracellular concentrations of reduced folate are necessary for the optimal inhibition of thymidylate synthesis; therefore, 5-FU is administered together with calcium folinate (leucovorin, LV). LV is an exogenous source of reduced folinate that enhances the cytotoxicity of 5-FU. It was demonstrated in several randomised trials with mCRC patients that LV caused a significant improvement in the RR, as compared to that observed with single-agent 5-FU (FU/LV 23% *vs.* 5-FU 11%, *P*< .000), but it had no impact on survival. (Anonymous, 1992). Before LV, 5-FU was administered with several other less active agents such as levamisole, to improve the cytotoxic effects.

5.7.1.2 *Bolus 5-FU*

5-FU is active only in the S-phase of the cell cycle and has a short half-life of 10-20 minutes (de Gramont and Thirion, 1994). Several ways of dosing, scheduling, and administering of 5-FU have been studied, to maximize the cytotoxic effect. Initially, two major treatment strategies were developed with 5-FU administered as a bolus shot, but with different dosing and scheduling (Machover et al., 1986; Petrelli et al., 1987). A traditional arm in several studies is the so-called Mayo regimen, put forward by the North Central Cancer Treatment Group (NCTTG), which consists of the

administration of an intravenous bolus containing 370-425 mg/m^2 of 5-FU for 3-5 minutes and infusion of 10-20 mg/m^2 of low-dose LV for five consecutive days within a four week cycle (O'Connell, 1989). Another traditional bolus regimen is the Roswell Park regimen, which consists of the administration of 500 mg/m^2 of a 5-FU bolus with 500 mg/m^2 of high-dose LV six times weekly for six weeks, after every eight weeks (Haller et al., 2005). Other bolus regimens have also been developed to improve tolerability, such as the Nordic FLv regimen, (bolus 5-FU 500 mg/m2 and bolus LV 60 mg/m2 days 1 and 2 every 2 weeks), which is traditionally mostly used in Nordic countries (Glimelius, 1993).

Due to toxicity issues that are discussed later, Mayo and Roswell Park are no longer as widely used as they were in the beginning of the 5-FU era, but they still are adequate therapy options that have been included both in the guidelines of the European Society for Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) guidelines (Benson and Venook, 2018a; Benson and Venook, 2018b; Van Cutsem et al., 2016). In developing countries, bolus regimens can be of importance, since there is no need for central intravenous catheters and infusion pumps for their administration, and the costs are lower, as compared to the costs associated with oral fluoropyrimidines.

5.7.1.3 *Continuous infusion of 5-FU*

Protracted and continuous infusion (CI) regimens were developed to increase the dose intensity of 5-FU therapy (de Gramont et al., 1997a). It was noted that it was possible to deliver four to five times higher doses of 5-FU by infusions, as compared to that delivered in the bolus form. In addition, it was shown that an infusional high-dose and bolus 5-FU shot act through different mechanisms that enhance each other in vitro. This observation led to the development of combination regimens of bolus and infusional 5-FU (Sobrero et al., 1993).

Several CI regimens have been studied and used. Today, the standard method of delivering infusional 5-FU can be considered as the so-called de Gramont regimen (LV5FU2), developed by the French GERCOD (Groupe d'Etude et de Recherchesur les Cancers de l'Ovaire et Digestifs) group. The de Gramont regimen consists of the administration of 400 mg/m^2 of a 5-FU bolus and infusion of leucovorin 200 mg/m^2 for two hours, followed by a 22-hour infusion of 600 mg/m^2 of 5-FU for two consecutive days. This regimen was further modified in order to improve outcome, toxicity profile, and patient convenience (de Gramont et al., 1997b). Today, a widely accepted standard regimen and a backbone for combination chemotherapy is the so-called modified or simplified de Gramont regimen involving the infusion of 400 mg/m^2 of LV, followed by infusion of a 400 mg/m^2 bolus of 5-FU on day 1, after which a continuous infusion of 2400 mg/m^2 of 5-FU is performed over 46 hours.

5.7.1.4 *Efficacy of 5-FU in mCRC*

Survival in patients with mCRC without any anti-tumoural treatment is expected to be approximately two to eight months (Scheithauer et al., 1993; Sorbye et al., 2009). Single agent 5-FU improved survival up to approximately 11 months, but survival rates of up to 15 months were reported in some studies (de Gramont et al., 1997a; Hansen et al., 1996; Scheithauer et al., 1993). Response rates

reported with the 5-FU/LV bolus were modest, but improved significantly with CI regimens, as demonstrated in a meta-analysis of nine trials (RR 14% *vs* 22%, respectively, *P*= .0002) (Anonymous, 1998). Another trial compared single agent 5-FU as a protracted infusion or bolus, and in this study, RR was 30% in the CI arm and 7% in the bolus arm (*P* < .001), but there was no difference in survival (mOS 11 *vs*. 10 months, CI and bolus arms, respectively) (de Gramont et al., 1997a). A study of 348 patients treated with the Mayo regimen or LV5FU2 resulted in a RR of 32.6% in LV5FU2, 14.4% with the Mayo regimen (*P*= .0004) (de Gramont et al., 1997a), and the mPFS was 27.6 weeks for patients receiving LV5FU2 and 22.0 for patients receiving Mayo (*P*= .0012). The mOS was not statistically significant between treatment regimens (mOS 62.0 weeks in the LV5FU arm and 56.8 weeks in the Mayo arm, respectively, *P*= .067). Thus, CI regimens improve the RR and PFS, but since the superiority of either bolus or CI regimens in terms of survival is not clearly proven, the most significant difference is the toxicity profile, which favours CI regimens.

5.7.1.5 *5-FU in the adjuvant treatment of CRC*

The year 1989 was a turning point in the treatment of CRC, as the North Central Cancer Treatment Group (NCCTG) reported that 12 months of treatment with 5-FU combined with levamisole after surgery reduced the risk of recurrence by 40% in stage III patients, as compared with that observed after surgery alone (Laurie et al., 1989). Another pivotal trial, the Intergroup 0035, studied the use of 5-FU plus levamisole, levamisole alone, or observation in stage II and III patients demonstrating a 41% relative reduction in the recurrence rate (*P* < .0001) (Moertel et al., 1990). It also demonstrated a significantly improved survival in patients on the 5-FU/levamisole arm; the median 5-year OS was 60% for adjuvant chemotherapy, versus that of 46.7% and 49% for the use of observations and levamisole alone, respectively (*P* = .0007). After these results were obtained, postoperative treatment with 5-FU and levamisole in stage II and III CRC patients became a new standard in the year 1990.

In addition to these pivotal trials of the adjuvant era, several trials were performed in the adjuvant setting in stage II and III patients, after combining different modulating agents with 5-FU to enhance the efficacy of the therapy. For example, the NSABP C-03 trial studied the efficacy of 5-FU/LV, as compared to that observed with a combination of methotrexate, alkylating nitrosourea lomustine, and 5-FU (MOF). The 3-year DFS rate was 73% for patients receiving 5-FU/LV, as compared to that of 64% for patients receiving MOF (*P*= .0004), which supports the use of LV in the adjuvant setting (Wolmark et al., 1993).

The NSABP C-04 trial randomized stage II and III patients to receive 5-FU/LV, 5-FU/levamisole, or 5-FU/LV/levamisole. For stage II patients, the use of 5-FU/LV reduced the risk of recurrence, as compared to that observed with 5-FU/levamisole (5-year DFS 75% *vs*. 71%) and enhanced survival (5-year OS 84% *vs*. 81%) (Wolmark et al., 1999). Stage III patients treated with 5-FU/LV experienced a 13% and 10% relative risk reduction, in accordance with the 5-year DFS (57% *vs*. 53%) and 5-year OS (67% *vs*. 63%), respectively. The combination of LV and levamisole added no further benefits. Thus, these findings demonstrated the superiority of 5-FU/LV over that of 5-FU/levamisole as the standard of care for stage III patients, also supporting a potential role of 5-FU/LV in stage II disease.

The Intergroup study 0089 investigated the effect of biochemical modulation of 5-FU, by comparing the 5-FU + low-dose LV, 5-FU + high-dose LV, 5-FU + levamisole, and 5-FU + levamisole + low-dose LV with each other (Haller et al., 2005). This study included both high-risk stage II and stage III patients. There were no statistically significant differences between regimens, in terms of DFS and OS. According to these results, the inclusion of levamisole does not improve outcome and high dose LV adds no extra benefit, as compared to the benefits observed with the low dose. In addition, this study proved that six months of therapy is equal to that for twelve months.

The GERCOR C96.1 study compared the Mayo regimen with LV5FU2 in stage II and III patients, as well as the results obtained with a treatment duration of 24 weeks versus those obtained with a treatment duration of 36 weeks (André et al., 2007). The Mayo regimen and LV5FU2 were equal in terms of the DFS (HR 1.01 CI$_{95\%}$, 0.81-1.27) and OS (HR 1.02 CI$_{95\%}$ 0.77-1.34). There were no statistically significant differences in the DFS or OS on providing treatment for a duration of 24 or 36 weeks

The PETACC-2 (Pan European trial in adjuvant colon cancer 2 study) study investigated whether infusional high dose 5-FU regimens were superior to bolus 5-FU/LV regimens (Kohne et al., 2013). Patients were randomised to receive either the Mayo regimen, high dose 5-FU alone (the Spanish TTD regimen; day 1, 5-FU, 3500 mg/m^2 continuous infusion for 48 h, given weekly during an 8-week cycle for 3-cycles), high dose 5-FU plus LV (the German AIO regimen; day 1, LV 500 mg/m^2 i.v. 2-h infusion, followed by 5-FU, 2600 mg/m^2 i.v. 24-h infusion, given weekly during a 6-week cycle for 3-cycles), or the LV5FU2 regimen. There were no significant differences in terms of RFS and OS between infusional high dose 5-FU and the Mayo regimens, but the Mayo regimen was the most toxic (Kohne et al., 2013).

5.7.1.6 *Treatment related adverse events during 5-FU based chemotherapy*

The toxicity of 5-FU is significantly associated with the type of regimen, dose, scheduling, and way of administration (Macdonald, 1999). In general, bolus regimens are more toxic than CI regimens. Especially higher rates of grade 3-4 leukopenia and stomatitis are associated with the 5-FU bolus, as compared with the CI regimen, but the occurrence of the hand foot syndrome (HFS) is significantly more frequent with CI regimens (Piedbois et al., 1998). In addition, different dosing and scheduling methods used for bolus administration lead to different kinds of toxicity profiles. The Mayo regimen is associated with higher rates of any grade 3-4 toxicity than the Roswell Park regimen (55.6% *vs.* 40.3%, respectively). Haematological toxicity and stomatitis occur especially more frequently with Mayo, but the use of Roswell Park results in more gastrointestinal toxicity (Haller et al., 2005). In the PETACC-2 trial, it was observed that grade 3-4 leukopenia (6.9% *vs.* 2.1%) and stomatitis (9.7% *vs.* 3.1%) were significantly more common with Mayo regimens, as compared to those of CI regimens, but grade 3-4 HFS was significantly more common in patients receiving a CI regimen (4.2% *vs.* 0.4%) (Köhne et al., 2013). In a subgroup analysis, the occurrence of grade 3-4 diarrhoea was more common with TTD (23.3%) and AIO (20.1%), as compared with that for LV5FU2 (3.5%). Thus, LV5FU2 had the most favourable toxicity profile, according to this study.

Table 1. Toxicity related to 5-fluorouracil

Study Regimen	Mayo	LV5FU2	Mayo	PVI 5-FU	Mayo	Roswell Park
Grade ¾ (%)						
Neutropenia	7.3	9.6	55.6	0.9	20	4
Thrombocytopenia	0.5	0.48	1.5	0.6	<1	-
Infection	3.9	5.3	5.5	3.3	5	1
Nausea	3.4	38.5	2.6	1.8	15	5
Diarrhoea	7.3	28.4	16.0	5.4	16	29
Mucositis	12.7	20.2	19.6	3.6	12	-
Cutaneous	0	14.9	3.5	6.3	<1	<1
Alopecia	1.5	12.0	14.3	0.3		
	(de Gramont et al., 1997a)		(Saini et al., 2003)		(Haller et al., 2005)	

5.7.2 *Capecitabine*

5.7.2.1 *Mechanism of action and efficacy*

Capecitabine is an oral fluoropyrimidine, an oral prodrug of 5-FU that presents with a pharmacokinetic profile that mimics continuous 5-FU infusion (Miwa et al., 1998; Walko and Lindley, 2005). It is orally administered twice daily for 2 weeks, followed by a one-week rest period in 3-week cycles (1250 mg/m^2 as a monotherapy). The prodrug is absorbed in an intact manner into the gastrointestinal tract, because of which it therefore has a bioavailability of nearly 100%. Capecitabine is first converted to 5′-deoxy-5-fluorocytidine by hepatic carboxylesterase, predominantly in the liver, and then converted to 5′-deoxy-5-fluorouridine by cytosine deaminase, both in tumour cells and in the liver; after this enzymatic cascade, it is finally activated to cytotoxic 5-FU, by thymidine phosphorylase. Thymidine phosphorylase is expressed in the liver and in several tumours at higher concentrations than that observed in normal tissues, and because metabolic conversion occurs with more specificity in tumour cells, systemic exposure to 5-FU is reduced, but the dose-intensity in the tumour cells is improved (Budman et al., 1998; Miwa et al., 1998; Walko and Lindley, 2005).

It was first shown by two large randomised trials in the metastatic setting that there were no significant differences in PFS or OS after treatment with capecitabine and the traditional bolus 5-FU (Hoff et al., 2001; Van Cutsem et al., 2001). However, an integrated analysis of these two trials demonstrated that the RR was significantly improved with capecitabine (26% *vs.* 17%, *P* < .002) (Van Cutsem et al., 2004).

In the adjuvant setting the use of, capecitabine was approved after results from the X-ACT (Xeloda in Adjuvant Colon Cancer Therapy) trial were published (Cassidy et al., 2004). In the X-ACT (n=1987) trial, patients were randomised to either capecitabine or the traditional Mayo regimen. A significant improvement in RFS (HR 0.86, $CI_{95\%}$ 0.74–0.99) was noted, but there were no significant differences in the DFS (HR 0.87, $CI_{95\%}$ 0.75–1.00) or OS (HR 0.84, $CI_{95\%}$ 0.69–1.01).

5.7.2.2 *Treatment related adverse events during capecitabine containing chemotherapy*

There are no direct head-to-head trials comparing the monotherapy of capecitabine and LV5FU2, but capecitabine is demonstrated to have a more improved safety profile than bolus 5-FU/LV, as shown in Table 2. A significantly lesser level of diarrhoea, stomatitis, nausea, alopecia, and grade 3-4 neutropenia were observed, resulting in a lower incidence of neutropenic fever and sepsis being reported with capecitabine (Cassidy et al., 2002). HFS was the only adverse event occurring significantly more frequently with the use of capecitabine. The X-ACT trial in the adjuvant setting reported that any grade of HFS occurred in 62% of patients in the capecitabine arm versus 10% of patients in the 5-FU/LV arm (*P*< .001), grade 1-2 HFS was observed in 44% versus 9% (*P*< .001) of patients, and grade 3 HFS was observed in 17% versus 0.6% (*P*< .001) of patients receiving capecitabine and 5-FU/LV, respectively (Cassidy et al., 2004). The number of dose reductions due to adverse events (33.9% *vs.* 42.2%, *P*= .0037) was significantly higher in the 5-FU/LV arm and there was a significant increase in the number of hospitalizations due to neutropenic infections (11.6% *vs.* 18.0%, *P*< .005) in the 5-FU/LV arm, as compared with that in capecitabine arm.

Table 2. Toxicities reported, Capecitabine versus FU/LV as a monotherapy

Study	(Van Cutsem et al., 2004)		(Hoff et al., 2001)		(Twelves et al., 2005)	
Regimen	Capecitabine	Mayo	Capecitabine	Mayo	Capecitabine	Mayo
	%		%		%	
Grade ¾						
Neutropenia	2.0	19.8	2.6	25.9	2	26
Diarrhoea	10.7	10.4	15.4	13.9	11	13
Stomatitis	1.3	13.3	3.0	16.0	2	14
Hand-foot syndrome	16.2	0.3	18.1	0.7	17	0.6

5.7.2.3 *S-1*

S-1 is like capecitabine an oral 5-FU derivate containing a combination of tegafur, gimeracil, and oteracil (Shirasaka, 2009). Gimeracil is a potent inhibitor of dihydropyrimidine dehydrogenase (DPD) and oteracil inhibits the conversion of 5-FU to active metabolites in the gastrointestinal tract. S1 is widely used and studied in the Asian population, but there are remarkable differences in the metabolism and toxicity profile of Asian and western populations (Kwakman and Punt, 2016). The Dutch Colorectal Cancer Group conducted a randomized, phase III SALTO trial, which had used the

primary end point to compare the incidence of HFS between S-1 and capecitabine in the first-line treatment of mCRC in the western population (Kwakman et al., 2017). S-1 was associated with a significantly lower incidence of HFS, as compared with that of capecitabine, but the level of efficacy was equal. The Nordic 9 trial demonstrated that the use of S-1 dose-reduced combination therapy with S-1 and oxaliplatin was well-tolerated in older and frail western patients (Winther et al., 2018). However, data with regard to S-1 in the treatment of CRC in western population is limited and S-1 is therefore not reimbursed in the treatment of CRC in Finland.

5.7.2.4 *Dihydropyrimidine dehydrogenase (DPD)*

Less than 15% of administered 5-FU is converted to active metabolites of 5-FU (van Kuilenburg, 2004). More than 80% of the drug is metabolized and eliminated by an enzyme, dihydropyrimidine dehydrogenase (DPD), which is encoded by the *DPYD* gene. Approximately 2-5% of the white population in North America and Europe present with a major mutation in *DPYD,* leading to the partial inactivation of DPD (Henricks et al., 2018; Morel et al., 2006). The complete loss of DPD is estimated to be much rarer, encountered only in 0.01-0.1% of the population. Patients with a DPD deficiency are at risk of excessive, severe toxicity, in the form of myelosuppression, diarrhoea, mucositis, and neurotoxicity if 5-FU is administered; especially in patients with a total loss of DPD, treatment can be lethal.

DPYD is highly polymorphic and several genetic variants have been identified, of which some have significant functional consequences with regard to enzymatic activity (van Kuilenburg, 2004). The most well studied gene variant is *DPD*2A*, a single-nucleotide variant at the intron boundary of exon 14, which results in the production of a truncated protein with virtually no enzyme activity (Wei et al., 1996). Other recognised variants that are significantly associated with 5-FU related haematological and gastrointestinal toxicity are c.2846A>T, c.1679T>G and c.1236G>A, according to a recent meta-analysis (Meulendijks et al., 2015). In patients with a known partial DPD deficiency, the dose should be adapted to avoid severe toxicity and in patients with complete DPD deficiency, fluoropyrimidines should not be used (Van Cutsem et al., 2016). According to guidelines, if known prior to treatment, in patients presenting with *DPD*2A* or c.1679T>G the 5-FU dose should be reduced 50% and in patients with c.2846A>T or c.1236G>A, the dose should be reduced at least 25% from the full dose (Caudle et al., 2013; Henricks et al., 2018).

DPYD gene variants are the best recognised predictive factors for 5-FU related toxicity, but do not account for all the observed cases of DPD deficiency or 5-FU toxicity, as other heterozygotic variants might play a role in toxicity (Caudle et al., 2013; Rosmarin et al., 2014). In addition, the DPD genotype does not faithfully reflect the phenotype, because of the post-transcriptional regulation of DPD through gene promoter hypermethylation (van Kuilenburg, 2004). DPD deficiency has been detected in 39-61% of patients with severe 5-FU related toxicity, which means that not all of the cases with severe toxicity can be explained by *DPYD* polymorphism. Bocci et al. reported three patients who displayed a low drug clearance of 5-FU, but in all three individuals, the DPD activity was intact (Bocci et al., 2006).

In addition to DPD, several other factors that predicted the sensitivity and toxicity to 5-FU have been studied (Van Cutsem et al., 2016). For example, data suggests that decreased levels of thymidylate synthase are associated with an improved response and survival with 5-FU (Fukushima et al., 2001; Peters et al., 2002). However, according to the guidelines, only DPD is established as a predictor for toxicity and efficacy, and DPD testing can be considered in routine clinical practice (Van Cutsem et al., 2016).

5.7.3 *Oxaliplatin*

5.7.3.1 *Mechanism of action and efficacy*

Oxaliplatin is a diaminocyclohexane platinum compound, which acts by impairing DNA replication, leading to cellular apoptosis (Misset et al., 2000). It is widely used in the treatment of mCRC since 2000, when De Gramont et al published a study with 420 previously untreated mCRC patients randomised to LV5FU2 with or without oxaliplatin (de Gramont et al., 2000). Oxaliplatin significantly improved the mPFS (9.0 *vs*. 6.2 months; *P*= .0003) and RR (50.7% *vs*. 22.3%; *P*= .0001). Improved mOS was further demonstrated by the NCCTG trial, after comparing the following three regimens: FOLFOX, IROX (irinotecan together with oxaliplatin), and the standard IFL (bolus 5-FU together with irinotecan) (Goldberg et al., 2004). FOLFOX was superior to IFL with regard to a significantly improved RR (45 *vs*. 31%), time to progression (8.7 *vs*. 6.9 months), and mOS (19.5 *vs*. 15 months). Thus, this study established the efficacy of oxaliplatin in the treatment of mCRC, after which it has been studied in various trials using different dosing. The combination of capecitabine and oxaliplatin (CAPOX) showed similar activity to FOLFOX in mCRC, in terms of PFS, OS, and RR, with a good level of tolerability (Ducreux et al., 2011).

The use of oxaliplatin in the adjuvant setting is well established by three pivotal trials, i.e., MOSAIC, NSABP-C07, and XELOXA. (Andre et al., 2004b). The MOSAIC study, published in 2004, randomised 2246 stage II or III CRC patients to receive LV5FU2 alone or with oxaliplatin (FOLFOX). The values for DFS at five years were 73.3% and 67.4% in the FOLFOX and LV5FU2 groups, respectively (*P*= .003); six-year OS rates were 78.5% and 76.0% in the FOLFOX and LV5FU2 groups, respectively (*P*= .046). Ten-year OS results were published in 2015, which demonstrated an even larger benefit in favour of FOLFOX; 10-year OS among all 2,246 patients was 71.7% in the FOLFOX and 67.1% in the LV5FU2 arm (*P*= .043), thereby increasing the absolute survival benefit in the FOLFOX arm from 2.2% at 6 years to 4.76% at 10 years (Andre et al., 2015a). However, no significant OS benefit of oxaliplatin was observed in stage II patients, not even with high-risk disease (defined as T4, tumour perforation, or fewer than 10 lymph nodes examined). Ten-year OS rates for FOLFOX and LV5FU were 75.4% *vs*. 71.7% (*P*= .058), respectively, in stage II high-risk patients (Andre et al., 2015a). The risk of death was significantly reduced in stage III patients (OS 67.1% for FOLFOX and 59.0% for LV5FU, *P*= .016).

The NSABP C-07 trial evaluated the addition of oxaliplatin to a weekly Roswell Park regimen, i.e. FLOX regimen, in 2409 patients with stage II or III disease (Yothers et al., 2011). The associated 3- and 4-year DFS rates were 71.8% and 67.0% for 5-FU/LV and 76.1% and 73.2% for FLOX,

respectively. The HR for DFS was 0.80 (CI$_{95\%}$ 0.69-0.93), signifying a 20% reduction in the risk of recurrence, in favour of FLOX ($P < .004$).

The XELOXA trial comparing the CAPOX and Mayo and Roswell Park regimens in 1886 stage III patients demonstrated the benefit of oxaliplatin being independent of the fluoropyrimidine backbone (Haller et al., 2011a). No stage II patients were included in this trial. As observed in the MOSAIC and NSABP-C07 studies, CAPOX demonstrated DFS benefit in favour of oxaliplatin at 3 years; DFS rate was 70.9% with CAPOX and 66.5% with Mayo (HR 0.80 CI$_{95\%}$ 0.69-0.93)). The HR for the OS for CAPOX, as compared to that for Mayo was 0.87 (CI$_{95\%}$ 0.69-0.93). The 5-year OS for CAPOX and Mayo were 77.6% and 74.2%, respectively (HR=0.87, CI$_{95\%}$ 0.72-1.05).

The IDEA collaboration investigated whether three months of oxaliplatin based adjuvant therapy is non-inferior to that provided for six months (Grothey et al., 2018). The major idea behind the study design was to reduce side effects, especially the neuropathy associated with oxaliplatin-containing adjuvant therapy. The IDEA collaboration was established in 2007 as a worldwide consortium in North America, Europe, and Asia, and it included six individual studies (TOSCA, SCOT, HORG, the IDEA France trial, ACHIEVE, CALGB/SWOG 80702). Data from all the six substudies were pooled for the final analysis. From a statistical perspective, the study did not meet its end point, as the boundary of non-inferiority was not reached. From the clinical perspective, the absolute difference between three and six month arms was only 0.9% (74.6% for 3 months *vs.* 75.5% for 6 months; HR=1.07, CI$_{95\%}$ 1.00–1.15). Differences were noted in the 3-year DFS between FOLFOX and CAPOX regimens, and between patients with T1–3N1 (low-risk) versus T4 or N2 (high-risk) disease. In the subset of low-risk patients, the DFS observed with 3 months of CAPOX treatment was non-inferior to that with 6 months (HR=0.85, CI$_{95\%}$ 0.71–1.01), but with regard to FOLFOX, its non-inferiority could not be proven (HR=1.10, CI$_{95\%}$ 0.96–1.26). In high-risk patients with T4 or N2 disease, DFS after 3 months of FOLFOX treatment was inferior to that of 6 months (HR=1.20, CI$_{95\%}$ 1.07–1.35), but non-inferiority was not proven in the CAPOX arm (HR=1.02, CI$_{95\%}$ 0.89–1.17) (Grothey et al., 2018).

According to the current NCCN guidelines, in low-risk patients, the recommended duration of adjuvant therapy is 3 months (Benson and Venook, 2018a). In high-risk patients, a duration of 3 to 6 months can be considered with CAPOX. If FOLFOX is chosen for low-risk patients, the recommended duration of therapy is 3 to 6 months, but in high-risk patients, the duration is always 6 months. ESMO guidelines are awaited.

5.7.3.2 *Treatment related adverse events during oxaliplatin containing chemotherapy*

In the MOSAIC trial, FOLFOX was associated with an increased rate of diarrhoea, vomiting, neurosensory toxicity, and neutropenia, as compared with that for LV5FU2 (Andre et al., 2004a). The NSABPC-07 trial reported higher rates of diarrhoea and nausea with FLOX, as compared to those with FU/LV, while the reported rates of vomiting and neutropenia were similar in both regimens (Kuebler et al., 2007).

The IDEA trial reported a significantly less grade 2 neuropathy in the three month arm, as compared to that for six months (17% *vs*. 48% for FOLFOX; 12% *vs*. 36% for CAPOX; *P*< .0001), as well as lower rates of any grade 3-4 adverse events (3% *vs*. 16% for FOLFOX; 3% *vs*. 9% for CAPOX; *P*< .0001) (Grothey et al., 2018). Grade 2-4 diarrhoea was also reported less often with a shorter duration of therapy (*P*< .0001 for FOLFOX; *P*= .01 for CAPOX).

5.7.3.3 *Oxaliplatin induced neuropathy*

Oxaliplatin and fluoropyrimidine combination therapy related toxicities are presented in Table 3. The most common dose-limiting adverse event associated with the use of oxaliplatin is peripheral neuropathy, which is classified into the acute and chronic forms (de Gramont et al., 2000; Giacchetti et al., 2000). The underlying mechanism of acute neuropathy involves the dysfunction of nodal axonal voltage-gated sodium (Na+) channels resulting from the oxalate chelating effect on both calcium (Ca2+) and magnesium (Mg2+) (Beijers et al., 2014). Chronic neuropathy is caused by the accumulation of oxaliplatin in the dorsal root ganglia cells, leading to morphological and functional changes in the nerves.

Acute neurotoxicity occurs in nearly all oxaliplatin treated patients and is mostly transient and reversible at the beginning of the therapy (Saif and Reardon, 2005). It appears during or shortly after oxaliplatin infusion and manifests typically with cold-allodynias, as dysesthesias and/or paraesthesia in the distal extremities, with a "stocking-glove" pattern. Acute oxaliplatin-induced neuropathy is unique and peculiar, since the sensory symptoms are commonly induced by exposure to cold. Some patients present with pharyngo-laryngeal dysesthesia, which causesa feeling of difficulty in breathing. Motor nerve function consistently remains unchanged during treatment and it is rare to observe motor symptoms, including tetanic spasms, fasciculations, and prolonged muscular contractions. The duration of acute neurotoxicity is typically from a day or two up to a week at the beginning of treatment, after which it becomes prolonged (Pachman et al., 2015). The intensity of the symptoms typically increases from the first cycle to the second cycle, after which the intensity stabilizes, but the duration of neuropathic symptoms is extended.

The most typical presentation of long-term neuropathy involves sensory symptoms, continuous tingling or impaired sensations, leading in the worst case to total numbness with a glove-sock-like pattern (Pachman et al., 2015; Saif and Reardon, 2005). Sensory ataxia and deficits in fine sensory-motor coordination are common. Chronic symptoms are not triggered by cold, as much as by the symptoms in the acute phase. In a manner similar to that of acute toxicity, chronic toxicity affects distal parts of extremities to a different extent, depending on the severity of the symptoms (Saif and Reardon, 2005).

Table 3. Toxicities reported with oxaliplatin-based regimens

Study Regimen	De Gramont et al, 2000 LV5FU2	FOLFOX	Andre et al, 2004 LV5FU2	FOLFOX	Ducreux et al, 2004 XELOX	FOLFOX	Kuebler et al, 2007 FU/LV	FLOX
Grade 3-4 (%)								
Leukopenia/ Neutropenia	5.3	41.7	4.7	41.1	5	47		
Infection			0.2	1.8	0	6		
Nausea/vomiting			3.2	10.9	5	14	19.4	28.3
Mucositis			2.4	2.7	0	<1		
Diarrhoea	5.3	11.9	6.6	10.8	14	7	32.2	38
Hand-foot sdr					14	7		
Neurotoxicity	0	18.2	0.2	12.4	11	26	0.7	9

5.7.3.4 *Excision repair cross-complementation group 1 (ERCC1)*

There is no predictive factor for oxaliplatin that has been proven to predict the toxicity or efficacious oxaliplatin treatments. The high excision repair cross-complementation group 1 (ERCC1) protein expression levels in immunohistochemistry were shown to be a negative predictive factor for platinum-based therapy in lung cancer patients (Olaussen et al., 2007). In CRC, ERCC1 has been studied and data suggest that a high expression level of ERRC1 is associated with poor prognosis and with a poor response to oxaliplatin based therapy (Qian et al., 2014). However, the data are insufficient to recommend the use of ERCC1 protein expression for treatment decisions involving the use of oxaliplatin in routine practice (Van Cutsem et al., 2016).

5.7.4 *Irinotecan*

5.7.4.1 *Mechanism of action and efficacy*

Irinotecan is a topoisomerase-I inhibitor that causes DNA damage and cell death through the active metabolite SN38 (Mathijssen et al., 2001). Two key trials were published with regard to mCRC in 2000 (Douillard et al., 2000; Saltz et al., 2000). Saltz et al. conducted a trial comparing the use of the 5-FU bolus either alone or in combination with irinotecan (IFL regimen), and the results revealed a significantly longer mPFS (7 months *vs*. 4.3, *P*= .004) and mOS (14.8 months *vs*. 12.6, *P*= .04) in the IFL arm. Similar results were reported by Douillard et al. and based on these trials, irinotecan was established as the first-line treatment option in CRC together with 5-FU (Goldberg, 2005).

IFL was associated with a greater level of toxicity and is no longer the first preference for a regimen. Irinotecan with infusional 5-FU/LV was shown to be better tolerated and FOLFIRI treatment is currently a widely accepted standard of care (Goldberg, 2005; Tournigand et al., 2001; Tournigand et al., 2004; Van Cutsem et al., 2016).

In the initial phase III randomised trials, a comparison of the use of irinotecan combined with capecitabine (CAPIRI) with that of FOLFIRI revealed various results. In the BICC-C study comparing FOLFIRI, IFL, and CAPIRI, both mPFS and mOS were inferior with the use of CAPIRI, as compared to that obtained with FOLFIRI (Fuchs et al., 2007). Median PFS was 7.6 months for FOLFIRI, 5.9 months for IFL, and 5.8 months for CAPIRI. Median OS was 23.1 months for FOLFIRI, 17.6 months for IFL, and 18.9 months for CAPIRI. CAPIRI arm was discontinued in this study due to toxicity. Similarly, EORTC 40015 reported inferior outcomes with CAPIRI, as compared with those obtained with FOLFIRI, and the study was discontinued following eight deaths unrelated to disease progression (Kohne et al., 2008). The higher toxicity associated with CAPIRI in these two trials could explain the inferior outcome, but consequently, CAPIRI is not an as widely accepted regimen as FOLFIRI. However, after studies by the AIO colorectal study group demonstrated that modified schedules of capecitabine and irinotecan regimen were feasible and effective, CAPIRI has been included as an option in the ESMO guidelines (Moosmann et al., 2011; Schmiegel et al., 2013; Van Cutsem et al., 2016).

In the adjuvant setting, irinotecan failed to improve DFS and OS, and its use is therefore not recommended in the adjuvant setting (Papadimitriou et al., 2011; Saltz et al., 2007; Van Cutsem et al., 2005; Ychou et al., 2009).

5.7.4.2 *Treatment related adverse events during irinotecan containing chemotherapy*

Typical toxic effects attributable to the use of irinotecan include diarrhoea, nausea, neutropenia, alopecia, and cholinergic symptoms (Cunningham et al., 1998). Of these, diarrhoea and neutropenia are the most common grade 3–4 toxicities, as detailed in Table 4. Diarrhoea associated with irinotecan can be especially severe, even fatal, but is well managed at most times, by following the antidiarrheal guidelines and with the intensive and early use of loperamide (Stein et al., 2010). Irinotecan associated toxicity is dependent on the underlying fluoropyrimidine backbone, and as mentioned, the IFL regimen was first the standard of care after irinotecan was introduced, but it was associated with notable toxicity levels; cases with fatal diarrhoea were also reported (Rothenberg et al., 2001). The increased risk of toxic death appeared to be confined to cumulative weekly bolus dosing, due to higher rates of neutropenia, as compared with that observed for the infusional irinotecan regimens (Rothenberg et al., 2001). The phase III randomised BICC-C trial comparing the bolus, infusional, and oral fluoropyrimidine backbone with irinotecan was the first to conduct a head-to-head comparison of IFL and FOLFIRI, which demonstrated the superior safety and efficacy of FOLFIRI (Fuchs et al., 2007). The combination of irinotecan and capecitabine turned out to be more complicated, as initial trials reported higher toxicity rates with CAPIRI, as compared with those observed with irinotecan plus intravenous 5-FU/LV treatment (Fuchs et al., 2007; Kohne et al., 2008). EORTC phase III study 40015 was discontinued prematurely because of excessive toxicity (39% of patients experienced grade 3–4 diarrhoea) and mortality (11% treatment related deaths) in patients undergoing CAPIRI treatment (Kohne et al., 2008). The BICC-C trial reported higher rates of grade 3-4 toxicities with CAPIRI, as compared with those observed with FOLFIRI (nausea/vomiting 34% vs 17.6%, diarrhoea 47.5% vs 13.9% and dehydration 19.1% vs 5.8%, respectively) (Fuchs et al., 2007). More recently, CAPIRI studies have reported lower rates of serious toxicities, probably due to

increased experience with the regimen and use of modified doses. A phase III study by the Hellenic Cooperative Oncology Group that compared CAPIRI plus bevacizumab and FOLFIRI plus bevacizumab demonstrated the efficacy of both treatment arms was similar, and there was a generally lower incidence of grade 3-4 events and no significant differences in toxicity profiles between the two regimens (Pectasides et al., 2012). CAPIRI combination therapy is an option included in the ESMO guidelines, if it is ensured that dose adjustment and proper management of side effects would be carried out (Van Cutsem et al., 2016).

Table 4. Toxicities reported with irinotecan-based regimens

Study Regimen Grade ¾ (%)	Salz et al, 2000		Douillard et al, 2000		Tournigand et al, 2004		Skof et al, 2009	
	IFL	FU/LV	FOLFIRI	LV5FU2	FOLFIRI	FOLFOX	CAPIRI	FOLFIRI
Leukop./Neutropenia	54	66	29	2	24	44	4	13
Infection	9	15	2	0	0	7	0	0
Nausea/vomiting	10	4	19	10	13	3	7	6
Mucositis	2	17	0	2	10	1	0	0
Diarrhea	23	13	44	26	14	11		
PPE			0	5	2	2	2	4
Cholinergic syndrome			2	0				

5.7.4.3 *UDP glucuronosyltransferase 1 family, polypeptide A1*

The UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1) is an enzyme involved in the glucuronidation pathway (Strassburg et al., 1998). The gene is a part of a locus encoding several UDP-glucuronosyltransferases, and its polymorphism is associated with an increased toxicity to irinotecan (nausea, diarrhoea, neutropenia). UGT1A1 is responsible for both bilirubin glucuronidation and the glucuronidation of SN-38, which is the active metabolite of irinotecan. In a manner similar to that for DPD and 5-FU, several genetic variants of UGT1A1 are associated with irinotecan related toxicities (Liu et al., 2014; Zhang et al., 2017). The UGT1A1*28 and UGT1A1*6 variants are especially associated with irinotecan induced neutropenia and diarrhoea.

Even though UGT1A1 gene polymorphisms are known to be predictive for irinotecan related toxicity, the UGT1A/UGT1A1 status is rarely used in clinical practice as a predictive biomarker of irinotecan toxicity and it is not routinely recommended in the NCCN or ESMO guidelines (Benson and Venook, 2018a; Benson and Venook, 2018b; Van Cutsem et al., 2016). It can be considered for patients with elevated bilirubin levels.

5.8 Biological agents in the treatment of CRC

5.8.1 *Anti-angiogenic therapy in colorectal cancer*

The formation of new blood vessels from the pre-existing blood vessels is known as angiogenesis (Ferrara et al., 2003). Physiological angiogenesis is required during embryogenesis and in normal tissue growth and repair, but angiogenesis is also a hallmark of cancer, since the supply of oxygen and nutrients by the vasculature is required for tumour growth (Ferrara et al., 2003). Pathological tumour angiogenesis has been in focus in cancer research for years since it can be therapeutically targeted. Vascular endothelial growth factors (VEGFs) comprise a large family of growth factors, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PLGF) (Carmeliet, 2003; Holmes and Zachary, 2005). Of these known growth factors driving angiogenesis, VEGF-A has emerged as the single most important regulator of neovascularization. The biological effects of VEGFs are mediated by the vascular endothelial growth factor receptors (VEGFRs), such as VEGFR-1, VEGFR-2, and VEGFR-3 (Jeltsch et al., 2013). This VEGF-VEGFR mediated route is the most utilized route for the treatment of CRC, but it is not the only one driving angiogenesis. There are several other pathways, such as the fibroblast growth factor (FGF-FGFR) and platelet-derived growth factor (PDGF-PDGFR) pathways (Zhao and Adjei, 2015).

There are several anti-angiogenic drugs that are in clinical use and under development in many types of malignancies, but CRC was one of the first solid tumours that benefitted from anti-angiogenic therapy. The first soluble VEGF-A ligand, bevacizumab, has been widely used in the treatment of CRC since 2004 (Hurwitz et al., 2004). In addition to VEGF/VEGFR ligands, angiogenesis can be targeted by oral tyrosine kinase inhibitors that block the intracellular domain of receptors involved in angiogenesis (Al-Abd et al., 2017).

5.8.1.1 *Bevacizumab*

Bevacizumab is an anti-angiogenic drug and a monoclonal humanized antibody that binds to circulating vascular endothelial growth factor A (VEGF-A) (Ferrara et al., 2003). It has been widely used in the treatment of mCRC since 2004, after Hurwitz et al had published the results from a trial comparing the use of IFL with or without bevacizumab (Hurwitz et al., 2004). In the bevacizumab arm, a significant improvement of 4.4 months in the PFS and 4.7 months in the OS were observed. Since then, bevacizumab has been studied in several randomised first- and second-line mCRC trials, and it has shown efficacy in terms of RR, PFS, and OS benefit in some in combinations with 5-FU, capecitabine, irinotecan, and oxaliplatin (Cunningham et al., 2013; Giantonio et al., 2007; Hurwitz et al., 2004; Kabbinavar et al., 2008; Saltz et al., 2008; Tebbutt et al., 2010).

The TML trial demonstrated that continuing with bevacizumab therapy while changing the cytotoxic backbone in second-line treatment after progression during a bevacizumab containing regimen improved the OS, as compared to that observed after continuing with chemotherapy alone (Bennouna et al., 2013). Patients continuing with bevacizumab showed a modest improvement in OS (11.2 *vs*. 9.8 months; HR=0.81, *P*= .0062) The BEBYP trial reported similar results, which suggested that

patients pre-treated with bevacizumab in first-line treatment can benefit from subsequent therapies that target VEGF (Masi et al., 2015).

Bevacizumab was studied in the adjuvant setting in AVANT and NSABP C-08 trials, but it failed to prolong DFS in both the trials, and is therefore not to be used in the adjuvant setting (Allegra et al., 2011; de Gramont et al., 2012).

5.8.1.2 *Aflibercept*

Aflibercept is a recombinant fusion protein containing VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2, fused to the Fc portion of human immunoglobulin (Ig)G1. It blocks the activity of VEGF-A, VEGF-B, and placental growth factor, (PlGF) by acting as a high-affinity ligand trap to prevent these ligands from binding to their endogenous receptors (Tseng et al., 2010). It was first shown in the VELOUR trial (n=1401) that OS improve from 12.1 months in the placebo arm to 13.5 months with aflibercept (HR=0.82, CI$_{95\%}$ 0.71-0.94) in the second line of treatment in patients pre-treated with oxaliplatin based chemotherapy, either with or without bevacizumab (Van Cutsem et al., 2012a). Aflibercept also significantly improved the mPFS, which was 4.7 with placebo to 6.9 months with the use of aflibercept (HR=0.76, CI$_{95\%}$ 0.66-0.87). The RR was also improved with aflibercept (11.1% *vs.* 19.8%, *P*= .0001). The outcome was not different between patients treated or not treated with bevacizumab in the first line.

5.8.1.3 *Ramucirumab*

Ramucirumab, a human IgG1 monoclonal antibody, binds specifically to the extracellular domain of VEGFR-2 with high affinity (Kong et al., 2017). It prevents the binding of the agonist ligands VEGF-A, VEGF-C, and VEGF-D, thereby blocking the activation of VEGFR-2. It was approved for second-line treatment with an irinotecan based regimen, after the RAISE trial (n=1072) demonstrated a statistically significant OS benefit for ramucirumab treated patients after first-line therapy with oxaliplatin based chemotherapy and bevacizumab (Tabernero et al., 2015). Median OS was 13.3 months for the ramucirumab group and 11.7 months for the placebo group (HR 0.84, CI$_{95\%}$ 0.73–0.98).

5.8.1.4 *Treatment related adverse events during bevacizumab containing therapy*

Typical adverse events reported in initial phase III trials with bevacizumab includedHTN, bleeding, arterial thromboembolic events (i.e., cerebral vascular events, myocardial infarction, transient ischemic attack, and angina), wound healing complications, proteinuria, and gastrointestinal perforations (Hurwitz et al., 2004; Kabbinavar et al., 2008; Strickler and Hurwitz, 2012). These toxicities have also been well established in real-life clinical patient populations by three large observational studies, i.e., BEAT, ARIES, and BRITE, and are well recognised in clinical practice (Bendell et al., 2012; Kozloff et al., 2009; Van Cutsem et al., 2009). BEAT, ARIES, and BRITE included almost 5,000 mCRC patients treated using the first or second line of treatment with bevacizumab containing chemotherapy, using different chemotherapy backbones. The main grade 3-5 toxicities observed included HTN (5.3-22%), bleeding (2.2-3.0%), arterial thromboembolic event

(1.0-2.3%), GI-perforation (2.0%), wound healing complications (1.0% in all and up to 3.5% in postoperative patients), and proteinuria (all grades, 1.0%). These rates are similar to those reported in randomised trials (Bendell et al., 2012; Kozloff et al., 2009; Van Cutsem et al., 2009).

HTN is the most common adverse event reported with the use of bevacizumab. The pathogenesis of bevacizumab induced HTN is not yet fully understood (de Jesus-Gonzalez et al., 2012; Syrigos et al., 2011). It is suggested that HTN is caused by impaired angiogenesis or endothelial dysfunction that is associated with bevacizumab. Another theory is that VEGF signal antagonism leads to the inhibition of nitric synthase, resulting in decreased levels of nitric oxide, which consequently leads to vasoconstriction and a decrease in the renal excretion of sodium resulting in HTN (Syrigos et al., 2011). HTN rates reportedly vary widely between studies due to the use of different measuring protocols (before treatments, between treatments, at home or in hospital) and different baseline characteristics of patients between studies (age, underlying HTN etc.). The BRITE trial reported about de novo HTN that required medication and the worsening of HTN in already hypertensive patients at the baseline in 22% of patients (Kozloff et al., 2009). The BEAT trial reported any grade of HTN in 30% and grade 3-4 in 5% of the patients (Van Cutsem et al., 2009); thus, less than in the previous phase III trials, which reported grade 3-5 HTN in up to 16% of patients (Hurwitz et al., 2004; Kabbinavar et al., 2008).

5.8.1.5 *Molecular Biomarkers for Anti-Angiogenic Therapy*

Despite intensive research, no biomarker is available yet for the identification of patients benefiting from anti-angiogenic therapy (Cidon et al., 2016). Genes involved in angiogenesis have been studied and they show a relatively high level of variation, ranging from a silent single nucleotide polymorphism to functional polymorphisms, but none of the genetic factors has turned out to be a reliable predictive factor. Several studies have also analysed circulating molecules, including different cytokines and angiogenic factors, such as angiopoietin-2 and VEGF levels (Goede et al., 2010; Jurgensmeier et al., 2013; Kopetz et al., 2010). The results are partly contradictory, as it was observed in biomarker analysis after the use of ramucirumab that high levels of VEGF-D were associated with improved outcomes (Tabernero et al., 2018), but data from the AGIT-MAX trial reported the opposite with bevacizumab (Weickhardt et al., 2015). Thus, in conclusion, there are no biomarkers or factors available for predicting the treatment outcome with anti-angiogenic therapy.

5.8.2 *Epidermal growth factor (EGFR) inhibitors*

5.8.2.1 *Cetuximab and panitumumab*

The EGF receptor, a transmembrane glycoprotein, is a member of the tyrosine kinase receptor family, which is known to be overexpressed in multiple malignancies, including CRC, and it contributes to cell proliferation and survival (Pines et al., 2010).

EGFR-directed antibodies are examples of a personalised, targeted therapy, the use of which has been based on the presence or absence of negative predictive RAS mutations in the tumour cells. In initial

trials, the administration of EGFR inhibitors was based on positive EGFR staining by immunohistochemistry, but its benefit was limited, as only around 40% of unselected, chemorefractory patients responded to it (Cunningham et al., 2004).

It was then demonstrated that approximately 40% of tumours harbour mutations in the exon 2 (codons 12 and 13) of the KRAS gene, which predicts the lack of benefit of administered EGFR inhibitors (Amado et al., 2008). Douillard et al reported in 2014 about a retrospective analysis of an expanded RAS analysis from the PRIME study, which was a trial that compared the use of FOLFOX alone and FOLFOX plus panitumumab in first-line treatment (n=1183) (Douillard et al., 2013). This study began the "new era" of expanded RAS analysis, the results of which revealed that mutations also occurred in KRAS exon 3 (codon 61) and exon 4 (codons 117 and 146), and NRAS gene exon 2 (codons 12 and 13), exon 3 (codon 61), and exon 4 (codons 117 and 146), which all predict the lack of benefit of EGFR-treatment. These other KRAS and NRAS mutations occur in ~10-15% of all patients (Dietel et al., 2015; Douillard et al., 2013). KRAS and NRAS constitutively activate EGFR downstream cell signalling, but this cascade can be blocked by inactivating EGFR in a wild-type tumour. Mutations in KRAS or NRAS genes lead to the continuous activation of downstream ERK signalling, regardless of EGFR blockade, and bypass EGFR inhibition (Misale et al., 2015). Thus, it is possible to exclude patients who do not benefit from treatment, but are exposed to toxicity. It was shown by the OPUS trial (n=315), which studied the use of FOLFOX versus FOLFOX and cetuximab in first-line treatment that detrimental effects were observed in cetuximab treated RAS mutated patients.(Bokemeyer et al., 2011; Bokemeyer et al., 2015). Thus, according to the ESMO and NCCN guidelines, it is mandatory to perform a RAS mutation analysis before the administration of EGFR inhibitors (Benson and Venook, 2018a; Van Cutsem et al., 2016).

The anti-EGFR antibodies cetuximab and panitumumab are active in different lines of treatment and as single agents or in combination with various chemotherapeutic agents (Benson and Venook, 2018a; Benson and Venook, 2018b; Van Cutsem et al., 2016). Cetuximab was first shown to significantly improve RR (13% *vs*. 0%), PFS (3.7 *vs*. 1.9 months, P<0.001) and OS (9.5 *vs*. 4.8 months; P<0.001), as compared with with the best supportive care (Jonker et al., 2007). The CRYSTAL trial explored the use of cetuximab in combination with FOLFIRI for first-line therapy, and demonstrated that there was a significant improvement in RR (57.3% *vs*. 39.7%, P< .001), PFS (9.9 *vs*. 8.4 months, *P*= .0012), and OS (23.5 *vs*. 20.0 months, *P*= .0093) in KRAS wild-type patients that were treated with cetuximab plus FOLFIRI, as compared to those treated with FOLFIRI alone (Van Cutsem et al., 2011). Cetuximab was approved for use in first-line therapy after these results were obtained. The results obtained with the use of panitumumab were first studied in comparison to those obtained with the best supportive care; it was demonstrated that in KRAS wild-type tumours, there was a significant improvement in RR (17% *vs*. 0%), PFS (12.3 *vs*. 7.3 weeks), and OS (8.1 *vs*. 7.6 months, HR 0.67, $CI_{95\%}$0.55-0.82) in panitumumab-treated patients, as compared to those obtained with the best supportive care (Amado et al., 2008). In the phase III PRIME study, FOLFOX was administered for first-line therapy with or without panitumumab, and it resulted in an improved RR in wild-type KRAS patients (57% *vs*. 48%, OR 1.47, $CI_{95\%}$ 1.07–2.04). Median PFS for wild-type KRAS patients was 10.0 *vs*. 8.6 months (HR=0.80 $CI_{95\%}$ 0.67-0.95) and an exploratory analysis of updated survival

(>80% OS events) demonstrated a significantly improved mOS in patients with wild-type KRAS of 23.8 months *vs.* 19.4 months (HR=0.83, CI$_{95\%}$0.70–0.98) (Douillard et al., 2014).

5.8.2.2   *Treatment related adverse events during therapy containing EGFR-inhibitors*

The major side effect associated with cetuximab treatment is skin toxicity, including acne-like skin rashes, dry skin, hair growth disorders, pruritus, and nail changes (Cunningham et al., 2004; Fakih and Vincent, 2010). Allergic and anaphylactic reactions are more common with the use of cetuximab than with panitumumab. Severe reactions are seen in approximately 3% of patients following cetuximab administration, with a fatal outcome observed in 0.1% of patients.

5.9   Additional drugs available for mCRC treatment

5.9.1   *Regorafenib*

Regorafenib, an oral multitarget tyrosine kinase inhibitor, targets a large set of kinases, including angiogenic and stromal receptor tyrosine kinases (VEGFR-1, -2, -3, TIE2, FGFR-1, PDGFR-β) (Crona et al., 2013). It was approved after results from the CORRECT trial (n=760) were published (Grothey et al., 2013). The mOS improved from 5.0 months using a placebo to 6.4 months with the use of regorafenib (HR= 0.77 CI$_{95\%}$ 0.64–0.94) as the last line of treatment in patients refractory to all available chemotherapy and biological agents.

The CORRECT trial reported any adverse event occurring in 93% of patients who were assigned to receive regorafenib and 61% of patients in the placebo arm (Grothey et al., 2013). The most commonly reported $\geq$ 3 adverse events included HFS, fatigue, diarrhoea, HTN, and rash or desquamation in 17%, 10%, 7%, 7%, and 6% of the patients, respectively. More grade 1-4 toxicities were reported in the placebo arm in this study, as compared to those reported for patients receiving regorafenib.

5.9.2   *TAS-102*

TAS-102 is a novel anti-metabolite that was first studied and used in Asia (Lenz et al., 2015). It is an oral drug and consists of a thymidine-based nucleic acid analogue, trifluridine, and a thymidine phosphorylase inhibitor, tipiracil hydrochloride (Mayer et al., 2015). TAS-102 acts in a manner similar to that of 5-FU, by inhibiting thymidylate synthase and incorporating it into DNA, but it was shown to be efficacious in 5-FU resistant cell lines in preclinical studies (Lenz et al., 2015). Tipiracil hydrochloride prevents the rapid degradation of trifluridine, allowing the maintenance of the adequate plasma levels of the active drug.

TAS-102 was approved in the United States and Europe after results from the RECOURSE trial (n=800) were published. The mOS improved from 5.3 months using a placebo to 7.1 months with the

use of TAS-102 (HR 0.68, CI$_{95\%}$ 0.58-0.81) in heavily pre-treated patients as the last line of treatment (Mayer et al., 2015).

The most frequently observed clinically significant adverse events associated with TAS-102 were neutropenia (38%) and leukopenia (21%) (Mayer et al., 2015). Febrile neutropenia was reported in 4% of the patients receiving TAS-102.

5.10 Personalized treatment of CRC

There are several clinically relevant genetic abnormalities that have been identified in many human malignancies, which can be therapeutically targeted, for example, BRAF in melanoma, c-KIT in GIST, and EGFR in lung cancer (Vogelstein et al., 2013). CRC is an exception, because although the genomic landscape of CRC has been extensively studied, there are no abnormalities identified that could be therapeutically targeted in every day clinical practice at present.

RAS mutation status is a negative predictive factor for EGFR-inhibition, but RAS is not a druggable mutation. In addition to KRAS and NRAS mutations, BRAF mutations have an impact on decision making, and MSI-H and HER2 are emerging as targets for the personalized treatment of CRC.

Personalized medicine should therefore widely encompass several aspects other than the mutational assessment of CRC patients.

5.10.1 *Individual metabolism*

The recommended doses of cytotoxic agents are determined in dose-finding phase I and phase II studies based on BSA, but sample sizes in these studies are small, and therefore insufficient for examining individual differences in drug metabolism (Gurney, 2002). In a real-life patient population, a standard dose might be insufficient to achieve the appropriate cytotoxic effect in individuals in whom the drug elimination process is faster, while in others, the same dose could lead to even fatal toxicity. As mentioned previously, mainly due to variations in the DPD enzyme activity, the area-under-the-curve can vary even up to 100-fold, which results in great variations in the response and treatment outcome for individuals treated with 5-FU (Saif et al., 2009). It was shown that with BSA-based standard dosing, only 20%–30% of patients are within the appropriate dose range, approximately 40%–60% of patients are below the dose range and therefore remain undertreated, and 10%–20% of patients are overdosed (Saif et al., 2009). In a randomised phase III study in mCRC patients, pharmacokinetically adjusted 5-FU dosing improved RR from 18.3% to 33.7% ($P = .004$) and numerically mOS from 16 months to 22 months ($P = .08$) as compared to the BSA dosing (Gamelin et al., 2008). Several other studies have also demonstrated that it is possible to reduce toxicities and improve treatment outcome if the dose of 5-FU is individually adjusted using pharmacokinetics (Saif et al., 2009).

5.10.2 *Microsatellite instability*

MSI tumours represent a high level of microsatellite instability and are recognised by the high frequency of their mutations, which is attributable to the silencing of different genes in the mismatch repair system (MLH1, MSH2, MSH6, PMS2) (Wei et al., 1996). About 80% of cases occur sporadically, most commonly due to the epigenetic hypermethylation and silencing of the MLH1 promoter area, but about 20% occur because of hereditary germline mutations (Lynch syndrome). MSI tumours can be divided into MSI-high (MSI-H) and MSI-low (MSI-L) tumours, depending on the extent of instability. MSI-H tumours represent a subset of CRCs with distinct clinical and pathological features and are often right-sided, poorly differentiated mucinous tumours exhibiting an increased number of tumour infiltrating lymphocytes (Alexander et al., 2001; Jass et al., 1998; Smyrk et al., 2001). Approximately 20% and 12% of stage II and stage III tumours, respectively, present with MSI (Roth et al., 2010), but in stage IV, a prevalence of 4-8% has been reported in the western population (Van Cutsem et al., 2016).

Increasing amounts of data support the fact that therapy should be tailored according to MSI status, both in the adjuvant and metastatic settings (Henricks et al., 2018; Yoshino et al., 2018) and both NCCN and Pan-Asian adapted ESMO consensus guidelines support routine MSI testing. Stage II MSI-H tumours are consistently associated with a more favourable prognosis, as compared to that of microsatellite stable (MSS) tumours with a low risk for recurrence (English et al., 2008; Lombardi et al., 2013; O'Reilly and Long, 1987; Ursem et al., 2018). An intact DNA replication error system known as the proficient MMR system appears to be necessary to mediate the cytotoxicity of 5-FU, and patients with MSI-H tumours do not derive a clear benefit from adjuvant 5-FU treatment (Cai et al., 2013). According to the guidelines, stage II MSI-H tumours should not be treated with single agent 5-FU in the adjuvant setting due to favourable prognosis, but also due to the limited efficacy of single agent 5-FU (Benson and Venook, 2018a; Benson and Venook, 2018b; Labianca et al., 2013). In stage III disease, MSI-H appears to have less prognostic value and adjuvant therapy should therefore be offered to patients with stage III MSI-H tumours (Benson and Venook, 2018a; Benson and Venook, 2018b). MSI-H tumours benefit from the inclusion of oxaliplatin, and if treated, an oxaliplatin-containing regimen should be chosen in case of an MMR deficiency (Andre et al., 2015a; Benson and Venook, 2018a; Benson and Venook, 2018b; Labianca et al., 2013). Updated 10-year results from the MOSAIC trial demonstrates that HRs for DFS and the OS benefit in the FOLFOX4 arm were 0.48 ($CI_{95\%}$ 0.20-1.12) and 0.41 ($CI_{95\%}$ 0.16-1.07), respectively, in patients with stage II to III dMMR CRC tumours (Andre et al., 2015b). The analysis of pooled data from NCCTG N0147 and PETACC8 also showed that MMR deficiency is a favourable prognostic factor for oxaliplatin-containing adjuvant chemotherapy in stage III CRC (Zaanan et al., 2018).

There is some contradictory data published with regard to irinotecan and MSI status. In a retrospective analysis of the CALGB 89803, which was a study of use of irinotecan in the adjuvant setting, the 5-year DFS of IFL-treated MSI-H patients was significantly improved, as compared to that for MSS patients (Meulendijks et al., 2015), but no benefit was observed in another trial, PETACC-3, which studied the use of irinotecan in adjuvant treatment (Klingbiel et al., 2015). The NSABP C-08 trial failed to show the benefit of including bevacizumab in the standard FOLFOX regimen for 1 year,

during the treatment of stage II/III colon cancer, but in the post hoc analyses, it was observed that MSI-H patients derived a statistically significant survival benefit from the addition of bevacizumab (Pogue-Geile et al., 2013). However, the data is currently contradictory and insufficient, because of which we cannot recommend VEGF-inhibitors in particular for patients with MSI-H tumours (Benson and Venook, 2018a; Benson and Venook, 2018b).

It has been postulated that the biological background for improved prognosis in early stage MSI tumours is the mutational load associated with these tumours and the increased lymphocyte infiltration, which enhances the host immune response (Smyrk et al., 2001). MSS tumours are immunologically "cold", but MSI-H tumours are "hot" due to a mutational high load being presented in an active immune microenvironment and the high expression of various checkpoint molecules (Le et al., 2015; Rokkas et al., 2013).

In the metastatic setting, MSI-H tumours represent a subset of CRCs that derive a benefit from immune checkpoint inhibitors (PD1/PD-L1 blockade) during late line treatment, according to phase II trials (Le et al., 2015; Overman et al., 2017; Overman et al., 2018), but phase III results are still awaited. Le et al. began a new era, as they conducted a phase II trial (n=41) that included both colorectal and non-colorectal tumours with or without mismatch-repair deficiencies. All patients were treated with pembrolizumab (PD1-inhibitor), after which significantly improved RR values and durable responses were seen in patients with MSI-H tumours (Le et al., 2015). The mPFS and mOS were not reached in CRC patients with MSI-H tumours, but the values were 2.2 and 5.0 months, respectively, for CRC patients with MSS tumours. The RR and PFS rates of CRC patients were 40% and 78%, respectively, for patients with MSI-H tumours (n=11), and 0% and 11% for patients with MSS (n=21) tumours at 20 weeks. All CRC patients had previously received at least two therapies.

In CheckMate-142 study 74 MSI-H CRC patients were treated with the PD1 inhibitor nivolumab (Overman et al., 2017). At a median follow-up time of 12 months, 23 out of 74 patients had achieved an objective response and 51 patients exhibited disease control that lasted for 12 or more weeks. The median duration of response was not reached. Further, improved results were even reported by the nivolumab plus ipilimumab cohort of CheckMate-142 (Overman et al., 2018).

Based on the data presented, the Federal Drug Administration (FDA) in the United States has approved pembrolizumab, nivolumab, and a combination therapy of nivolumab with ipilimumab, for the treatment of MSI-H CRCs in patients that have progressed following fluoropyrimidine, oxaliplatin, and irinotecan containing therapy. Approval is awaited in Europe.

5.10.3 *KRAS and NRAS mutations*

KRAS and NRAS mutations are an example of personalised medicine, as they are negative predictive factors for the EGFR-inhibiting antibodies cetuximab and panitumumab in CRC patients (Douillard et al., 2013; Douillard et al., 2014; Heinemann et al., 2014; Van Cutsem et al., 2015; Venook et al., 2014). Patients with tumours that contain these mutations do not benefit from EGFR inhibitors and

RAS mutation analysis excludes approximately 50% of patients from this therapy. Data are discussed in detail in section 5.8.2.1.

5.10.4 *Mutation in BRAF[V600E]*

Another important finding from the study by Douillard et al. was the observation that approximately 8% of CRCs harbour a mutation in the BRAF gene, most often a point mutation in the V600E oncogene, but higher figures (10%) have been presented in a review by Ursem and in two Nordic studies (Algars et al., 2017; Douillard et al., 2013; Sorbye et al., 2015; Ursem et al., 2018). KRAS, NRAS, and BRAF[V600E] mutations are usually mutually exclusive, even though some rare exceptions have been reported. BRAF[V600E]-mutant mCRCs are a distinct biologic entity with a dismal prognosis that are associated with hypermutated tumours that frequently exhibit a CIMP phenotype and MSI (Cremolini et al., 2015b; English et al., 2008). BRAF[V600E]-mutated CRC tumours are commonly refractory to standard chemotherapy regimens, and unlike in melanomas, BRAF[V600E] mutated CRCs do not derive benefits from single agent BRAF-inhibitors (Cremolini et al., 2015b; Kopetz et al., 2015).

The TRIBE study evaluated the use of FOLFIRI plus bevacizumab versus FOLFOXIRI (5-FU in combination with irinotecan and oxaliplatin) plus bevacizumab as the first-line treatment in 508 patients, of which 28 patients presented with the BRAF[V600E] mutation (Cremolini et al., 2015b). Of these BRAF[V600E] mutated patients, 12 patients were assigned to the FOLFIRI arm and 16 patients were assigned to the FOLFOXIRI arm. In these BRAF[V600E] mutated patients, the mOS of patients treated with FOLFOXIRI plus bevacizumab was 19.0 months, as compared to that of 10.7 months in the FOLFIRI plus bevacizumab arm (HR 0.54 CI$_{95\%}$ 0.24–1.20). Although the number of patients with BRAF[V600E] mutations was small, based on this study and the patient series (Loupakis et al., 2014), FOLFOXIRI plus bevacizumab treatment is considered as a good first-line therapy option for patients with BRAF[V600E] mutations (Van Cutsem et al., 2016).

To improve the prognosis of BRAF[V600E] mutated CRCs, several trials have been conducted using the EGFR, MEK and BRAF inhibitors. FDA approved the use of encorafenib in combination with binimetinib and cetuximab for the treatment of BRAF[V600E] mutated CRCs in second or later lines of treatment, after results from the phase III BEACON trial were published (Cutsem et al., 2018).

Data indicate that the use of EGFR-inhibitors in BRAF[V600E] mutated patients is controversial, but evidence supporting the fact that these patients do not benefit from anti-EGFR antibodies has been accumulating (Pietrantonio et al., 2015; Rowland et al., 2015); both NCCN and ESMO guidelines recommend that EGFR inhibitors should not be used in BRAF[V600E] mutated patients (Benson and Venook, 2018a; Benson and Venook, 2018b; Van Cutsem et al., 2016).

Data suggest that the BRAF[V600E] mutation is associated with an inferior outcome in stage II and III disease as well, especially in MSS tumours, but there is no evidence proving that it can predict whether patients receiving adjuvant chemotherapy can benefit from it (Roth et al., 2010; Ursem et al., 2018).

5.10.5 *Non- BRAF^{V600E} mutations*

Jones at al. described using a dataset of nearly 10,000 patients that 2% of all CRCs and 22% of all BRAF mutated patients harboured non- BRAF^{V600E} mutations that can be detected in next generation sequencing platforms (Jones et al., 2017b). In their study, non- BRAF^{V600E} mutated tumours were associated with a younger age, male gender, and lower-grade tumours, and were more often left-sided, as compared to BRAF^{V600E} mutated tumours. In the dataset described by Jones et al., patients with non-BRAF^{V600E} mutated tumours had a mOS of 60 months, as compared to an mOS of 43 months in all RAS wild-type patients, and a mOS of 11 months in the BRAF^{V600E} mutated patients. Another small study of 10 patients with BRAF mutations at codons 594 and 596 also found that these mutations were associated with an improved prognosis, as compared to that of individuals with the BRAF^{V600E} mutation (Cremolini et al., 2015a). According to Jones et al. (Jones et al., 2017a), these patients should not be treated as aggressively as BRAF^{V600E} mutated patients, but data regarding non-BRAF^{V600E} mutations is limited at the moment.

5.10.6 *Human epidermal growth factor receptor 2 (HER2)*

The overexpression or amplification of HER2 has been reported in around 20% of breast cancers (Zhang et al., 2003) and in 7%–34% of gastric cancers (Reichelt et al., 2006). HER2 targeted therapies are established treatment strategies used for the treatment of these cancers, and show a significant improvement in survival; therefore, HER2 has been of interest also in CRC (Bang et al., 2010; Swain et al., 2015).

HER2 amplifications or mutations are detected in approximately 5% of all CRCs, but its role as a biomarker for prognosis remains debatable (Sartore-Bianchi et al., 2016). Instead, HER2 is emerging not only as a negative predictor of response to EGFR-inhibitors, but also as a target for therapy (Bertotti et al., 2011; Yonesaka et al., 2011). The clinical benefit of dual HER2-inhibition using trastuzumab and lapatinib in CRC was first demonstrated in the Italian HERACLES trial (Sartore-Bianchi et al., 2016). In previous pre-clinical studies, it was demonstrated that HER2 targeted monotherapy was inferior to dual inhibition in CRC patients (Leto et al., 2015). In the HERACLES trial of 27 eligible patients, 1, 7, and 12 patients achieved CR, PR, and SD, respectively, and this was the best response observed in this heavily pre-treated patient population (Sartore-Bianchi et al., 2016).

5.10.7 *PIK3CA*

The PI3K signalling pathway is involved in the pathogenesis of CRC. |Mutations in PIK3CA exons 9 and/or 20 are present in 10-20% of CRC patients and are associated with other molecular alterations, including the *KRAS* mutation and CIMP phenotype (Inamura, 2018; Karakas et al., 2006). The possible prognostic and predictive value of the PIK3CA mutation in mCRC is not yet established, but data suggest that the PIK3CA mutation is a negative predictive factor for EGFR-inhibitors (Jhawer et al., 2008).

Nonsteroidal anti-inflammatory drugs might supress colorectal cancer progression through the inhibition of cyclooxygenase-2 and synthesis of prostaglandin E2, which are enhanced by activated PI3K signalling (Domingo et al., 2013). The use of aspirin for the chemoprevention of CRC has been studied in several trials (Algra and Rothwell, 2012; Rothwell et al., 2012) and prospective randomised trials are ongoing to define the true impact of aspirin in PIK3CA mutated tumours (www.clinicaltrials.gov).

5.10.8 *Tumour sidedness in first-line therapy*

Two randomised phase III trials investigated the use of the biological agents, cetuximab and bevacizumab with chemotherapy in first line treatment. The FIRE-3 study (n=493 KRAS exon 2 wild-type patients) compared the use of FOLFIRI plus cetuximab and that of FOLFIRI plus bevacizumab; the primary endpoint of the study was RR, and PFS and OS were the secondary endpoints. No differences were observed in RR or mPFS (10.0 *vs*. 10.3 months, respectively, *P*= .55) between arms, but the mOS was significantly longer in the cetuximab arm (28.7 *vs* 25.0 months, HR 0.77, CI$_{95\%}$ 0.62–0.96) (Heinemann et al., 2014). In a following analysis that was limited to all the RAS wild-type patients, the OS benefit was more pronounced with the use of cetuximab.

In the CALGB 80405 study (n=1137 KRAS exon 2 wild-type patients), first-line treatments with FOLFOX/FOLFIRI with bevacizumab or cetuximab were compared. There were no statistically significant differences in PFS or OS that were observed between treatment regimens. The mOS was 30.0 months in the cetuximab-chemotherapy arm and 29.0 months in the bevacizumab-chemotherapy arm (HR=0.88 CI$_{95\%}$ 0.77–1.01) (Venook et al., 2017). The mPFS was 10.5 months in the cetuximab-chemotherapy arm and 10.6 months in the bevacizumab-chemotherapy arm (HR CI$_{95\%}$ 0.84–1.08). The RR in the cetuximab and bevacizumab arms was 59.6% and 55.2%, respectively; thus, the values were similar (difference, 4.4%, CI$_{95\%}$ 1.0%-9.0%, *P* = .13). In patients treated with FOLFOX and cetuximab, the mOS was 30.1 months and in patients treated with FOLFOX and bevacizumab, the mOS was 26.9 months (*P* = .09). In FOLFIRI-treated patients, the trend was reversed; the mOS was 33.4 months with the use of bevacizumab and 28.9 months with that of cetuximab (*P* = .28). Based on results from these two trials both ESMO and NCCN guidelines concluded that bevacizumab and EGFR-inhibitors are equally effective options that can be used for first-line therapy in mCRC patients.

The general recommendations regarding targeted therapy were changed after retrospective analyses of the primary tumour location had showed that sidedness is a significant, independent prognostic and predictive factor (Tejpar et al., 2016). Tumours that originate in the splenic flexure, descending colon, sigmoid colon, rectum, or distal one-third of the transverse colon are classified as left-sided, distal tumours (Stintzing et al., 2017a). They are derived from the embryonic hindgut, whereas tumours that originate in the appendix, cecum, ascending colon, hepatic flexure, or two-thirds of the transverse colon are derived from the embryonic midgut and are classified as right-sided, proximal tumours. There are several differences in the epidemiology, pathology, mutation profile, and clinical presentation of proximal and distal tumours, probably due to their distinct embryologic origins. Proximal tumours account for approximately 30% of CRCs and are more common in older females who often present with a higher TNM stage, as compared to that observed for distal tumours (Mik et

al., 2017). Proximal tumours exhibit microsatellite instability, poor differentiation, mucinous histology, CIMP-high status, serrated pathway signature, CMS1 (immune) and CMS3 (metabolic) subtypes, and BRAF[V600E] mutations more often than distal tumours. Distal tumours are observed more often in men with CIMP-low status, classical chromosomal instability, and CMS2 (canonical) and CMS4 (mesenchymal) subtypes. Regardless of the treatment used, recent meta-analyses have consistently demonstrated that OS is significantly worse in patients with proximal tumours, as compared to that of those with distal tumours (Arnold et al., 2017; Holch et al., 2017; Petrelli et al., 2016). It was reported in CALGB 80405 that in all KRAS wild-type patients with distal tumours, the OS was significantly longer, as compared with that observed for proximal tumours (33.3 *vs.* 19.4 months; HR, 1.55; *P* < .0001) (Venook et al., 2017).

The strongest evidence of sidedness was obtained from the CALGB 80405 trial, conducted with the largest number of patients (n=1139) (Venook et al., 2017). In all RAS wild-type patients with left-sided tumours, the mOS was 39.3 months in the cetuximab arm, as compared to that of 32.6 months in the bevacizumab arm (HR 0.77, $CI_{95\%}$ 0.59-0.99), regardless of the chemotherapy backbone. In contrast, in all RAS wild-type patients with right-sided tumours, the mOS was 13.6 months in the cetuximab arm, and 29.2 months in the bevacizumab arm (HR 1.36, $CI_{95\%}$ 0.93-1.99). Two meta-analyses conducted after pooling data from several trials confirmed this; RAS wild-type left-sided tumours have a significantly greater survival benefit from anti-EGFR treatment, as compared to that observed with anti-VEGF treatment, when included along with standard chemotherapy (Arnold et al., 2017; Tejpar et al., 2016). Patients with tumours originating from the right side of the colon seem to benefit from bevacizumab treatment, but EGFR inhibitors might be associated with even detrimental effect and according to recent guidelines EGFR-inhibitors should not be used in first-line treatment in proximal tumours (Arnold et al., 2017; Benson and Venook, 2018a; Yoshino et al., 2018).

5.11 Consensus molecular subtypes

CMS was first described in early-stage CRC cohorts, but the association between CMS and treatment outcome in mCRC patients is not established yet (Mooi et al., 2018). It is now being intensively researched and validated in large patient cohorts, but increasing amounts of evidence already suggest that CMS classification is both a prognostic and predictive factor in CRC (Lenz et al., 2017; Mooi et al., 2018; Okita et al., 2018; Stintzing et al., 2017b).

In the original study by Guinney et al., important associations between CMS groups and clinical variables were reported (Guinney et al., 2015). CMS1 (immune subtype) tumours were frequently right-sided and diagnosed more frequently in females, and had a higher histopathological grade. The CMS1 population showed very poor survival after relapse, which is associated with a number of BRAF mutations and patients with MSI in this subtype. CMS2 (canonical subtype) tumours were mainly left-sided, showed superior survival after relapse and this subtype was observed in a larger proportion of long-term survivors. CMS4 (mesenchymal) tumours tended to be diagnosed at more advanced stages and displayed the worst OS and RFS, both in univariate and multivariate analyses (adjustment for clinicopathological features, MSI, and BRAF and KRAS mutations).

The clinical relevance of the CMS classifications in the adjuvant setting was evaluated in the PETACC-8 cohort (Marisa et al., 2017), which was a phase III trial that compared adjuvant FOLFOX with or without cetuximab in stage III CRC patients. Samples from PETACC-8 were assigned to different consensus molecular subtypes; 17%, 34%, 4%, and 45% of samples were assigned to CMS1, CMS2, CMS3, and CMS4 groups, respectively. It was observed that the subtype was significantly associated with treatment outcome in multivariate analysis. Individuals whose samples were included in the CMS4 group had the shortest OS (HR=1.7, P= .021), which is in line with the findings of the study by Guinney et al. The effect of treatment with cetuximab was worse than that of treatment with FOLFOX alone in CMS1 tumours, which is probably associated with the large number of right-sided tumours in this subtype.

With regard to metastatic disease, the CALGB 80405 trial reported that the patients with the CMS2 subtype had the best outcome and those with the CMS1 subtype showed the worst outcome (Lenz et al., 2017). Patients with CMS1 tumours who were treated with bevacizumab had a significantly longer OS, as compared to that of those treated with cetuximab, but patients with CMS2 tumours who received bevacizumab treatment tended to have a shorter OS than those who received cetuximab. A study by Okita et al. reported that the effects of irinotecan based chemotherapy were significantly superior to those associated with the use of oxaliplatin, in terms of PFS and OS, in patients with the CMS4 tumour subtype (Okita et al., 2018). In patients treated with EGFR inhibitors, patients who had the CMS1 subtype showed a worse PFS and OS, but those with CMS2 showed a particularly good efficacy, as compared to that of other subtypes. It was previously reported after the AGITG MAX trial that there was an improvement in PFS, after the addition of bevacizumab to capecitabine +/- mitomycin (Tebbutt et al., 2010). A subset of samples from this study were classified according to CMS groups, and it was observed that CMS subtypes were prognostic of survival, and that while CMS2 was associated with the best outcome, CMS1 was associated with the worst outcome (Mooi et al., 2018). According to these results, patients with CMS2 and possibly those with CMS3 tumours could derive benefits from the addition of bevacizumab to first-line capecitabine-based chemotherapy, but no such benefit was seen for those with CMS1 and CMS4 tumours in this study.

5.12 Liquid biopsies

Cell-free DNA (cfDNA) has gained attention in the field of oncology, after it was discovered that a part of it originates from tumour cells and can be isolated from any of the body fluids; isolation is mostly performed using the peripheral blood and urine (Babayan and Pantel, 2018). The blood-derived circulating tumour DNA (ctDNA) contains tumour-specific genetic and epigenetic alterations that reflect the intra- and inter-tumoural heterogeneity, which are not detected by tissue biopsies (Diaz et al., 2012; Katsiampoura and Kopetz, 2014; Misale et al., 2012). This method has gained increased interest and might mark the start of a new era in personalized medicine. If the liquid biopsy method would be established in further studies to be clinically feasible and reliable, it can represent a modality for improving personalized medicine. It is studied as a tool for screening, and for monitoring the risk of relapse after surgery, treatment efficacy, clonal evolution, and emerging therapy resistance in real-time (Babayan and Pantel, 2018).

Several studies suggest that liquid biopsies could be utilized for the management of CRC at different stages (Babayan and Pantel, 2018). There are studies demonstrating that ctDNA can predict recurrence after surgery in early stage CRC (Tie et al., 2016). The detection of ctDNA after the resection of stage II CRC was predictive for recurrence in a prospective study by Tie et al (n=230). CtDNA was detected post-operatively in 7.9% of the patients who were not treated with adjuvant chemotherapy. Of these, recurrence was observed in 79% of the patients, but recurrence was observed in only 9.8% of patients with negative ctDNA (HR=18 CI$_{95\%}$ 7.9-40). In patients treated with adjuvant therapy, the presence of ctDNA after the completion of chemotherapy was associated with an inferior RFS, as compared to that of patients in whom ctDNA was not detected (HR=11 CI$_{95\%}$, 1.8-68) (Tie et al., 2016).

The clinical decision to use EGFR-inhibitors is based on RAS analysis, conducted with a tissue biopsy. However, it is an invasive method and it is not always possible to retain a representative histological biopsy. In addition, its sensitivity is limited, as only a small portion of a tumour is commonly biopsied. It is not possible to detect genetic heterogeneity within or between lesions or the evolution of new mutations during therapy using tissue biopsies (Katsiampoura and Kopetz, 2014). It has been shown that in tumours that were initially considered to be RAS wild-type tumours, there are pre-existing RAS-mutant subclones, and resistance has been acquired towards EGFR-inhibitors, due to the presence of these resistant subclones (Diaz et al., 2012; Misale et al., 2012). There has been an increase in the level of interest in studying liquid biopsies for monitoring treatment efficacy and evolution of RAS-mutant clones during EGFR-containing therapy (Diaz et al., 2012; Katsiampoura and Kopetz, 2014). These RAS-mutant clones emerge during anti-EGFR treatment and the number of clones present declines when treatment is suspended; acquired resistance might therefore be delayed or avoided by taking breaks from the use of EGFR-inhibitors (Goldberg et al., 2018).

The phase II CRICKET trial (n=28) was designed to investigate the activity of the re-challenge with cetuximab and irinotecan in initially RAS/BRAF wild-type mCRC patients who had progressed during cetuximab and irinotecan containing first-line therapy (Rossini et al., 2018). The role of liquid biopsies as a tool for identifying patients that were more likely to benefit from the re-challenge strategy was investigated and liquid biopsies were performed. Patients with RAS wild-type ctDNA had a significantly longer PFS than those with RAS mutated ctDNA (3.9 *vs.* 1.9 months; HR 0.48 CI$_{95\%}$ 0.20-0.98) and in none of the patients with PR RAS mutations were detected in baseline liquid biopsies.

A phase III trial, FIRE-4 is ongoing to evaluate the significance of treatment holidays from EGFR-therapy and to investigate the use of liquid biopsies for tracking and identifying patients with emerging and acquired resistance (www.clinicaltrials.gov).

5.13 Quality of life (QOL)

Quality of life (QOL) assessment is a multidimensional concept that includes an evaluation of the physical, functional, social and emotional well-being of an individual (Anonymous, 1947; Post, 2014). As more patients treated with adjuvant therapy survive cancer and thus continue to live years

after cessation of therapy, and as patients with metastatic disease live longer with the aid of palliative therapies, the level of interest in measuring QOL both in cancer patients on treatment and in long-term survivors continues to increase. The incorporation of QOL measurements into clinical trials is therefore nowadays a well-established practice. At present, Patient-reported Outcome Measures (PROMS) are being focused upon, because it is established that there is often a discrepancy between the NCI-CTCAE grading of adverse events and PROMs (Van Cutsem et al., 2017).

5.13.1 *Assessment of QOL*

There are several validated, standardized, disease specific and general QOL questionnaires in use today. The University of Oxford has conducted a review that provided a list of the most promising generic and cancer-specific instruments that have been evaluated in CRC patients (http://phi.uhce.ox.ac.uk/pdf/CancerReviews). Four general questionnaires on the list were: Medical Outcomes Study 36-Item Health Survey (SF-36), Medical Outcomes Study 12-Item Health Survey (SF-12), European Quality of Life Questionnaire (EQ-5D), and EORTC Quality of Life Questionnaire (EORTC QLQ-C30). In addition, two CRC-specific instruments were identified, the Colorectal Cancer Specific Quality of Life Questionnaire (EORTC QLQ-CR38) and Functional Assessment of Cancer Therapy – Colorectal (FACT-C).

SF-36 is a questionnaire comprising of 36 items, and is used in a wide range of conditions and in the general population. SF-12 contains 12 items and was developed from SF-36, because of the need for a shorter, less intensive questionnaire that could be completed more rapidly (Sprangers et al., 1999). EQ-5D was developed to provide an instrument for economic evaluation (Anonymous, 1990)**.**

However, one drawback of these general questionnaires is the risk of ignoring cancer specific symptoms; therefore, QLQ-C30 was developed by EORTC, initially for evaluating the QOL of cancer patients participating in clinical trials (Bergman et al., 1994). Today, the EORTC QLQ-30 is probably the most used questionnaire in cancer studies. It is translated into several languages and has been validated in patients with several cancer types (http://qol.eortc.org/). It contains nine multi-item scales, as there are five functional scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea and vomiting) and a global health and quality-of-life scale. Several single-item symptom measures are also included (http://qol.eortc.org/).

In the same manner in which general questionnaires can ignore cancer specific symptoms, the general cancer specific questionnaire can ignore some disease specific aspects; therefore, QLQ-C30 contains cancer specific sub-scales. QLQ-C38 is a questionnaire for CRC patients that contains 38 CRC specific questions, to be delivered in addition to the QLQ-C30 questionnaire (Sprangers et al., 1999). The 38 items are added to the 30 core items of the QLQ-C30, resulting in a questionnaire with 68 items. Like QLQ-C30, QLQ-C38 consists of both multi-item scales and single-item measures. It includes two functional scales (body image and sexual function), seven symptom scales (micturition problems, gastrointestinal tract symptoms, chemotherapy side effects, defecation problems, stoma-related problems, and male and female sexual problems), and three single-item measures (sexual enjoyment, weight loss, and future perspective) (http://qol.eortc.org/).

The FACT-C is a 7 item colorectal cancer-specific module that supplements the functional assessment of cancer therapy – General (FACT-G) questionnaire and assesses the following four domains investigated by FACT-G: physical, emotional, functional, and social/family wellbeing (Ward et al., 1999). In addition, FACT-C assesses nine CRC-specific items: swelling/cramping in the stomach area, weight loss, control of bowels, digestion, diarrhoea, appetite, body image of all CRC patients, in addition to attitudes towards ostomy and difficulty with ostomy care.

5.13.2 *Assessment of neuropathy*

Peripheral neurotoxicity is a common side effect caused by several chemotherapeutic agents, especially vinca-alkaloids, taxanes, and platinum compounds, such as oxaliplatin, which is one of the most neurotoxic agents in oncology (Addington and Freimer, 2016; Mols et al., 2014). Interest towards oxaliplatin induced long-term neuropathy and its effect on QOL has gained increased interest, as it was observed that neuropathy seemed to be under-reported in clinical randomised trials (Beijers et al., 2014).

Several grading scales have been developed to assess the development and severity of the neuropathy induced by chemotherapeutic agents, but there is no consensus regarding the most preferable and appropriate scales that should be used for conducting an assessment of oxaliplatin induced neuropathy (Alberti et al., 2014; Lavoie Smith et al., 2013). Different scales have their strengths and weaknesses, but an increasing level of emphasis is placed on patient reported outcome measurements (Lavoie Smith et al., 2013; Postma et al., 2005). It is shown that physician reported outcome underestimates subjective adverse events (i.e. fatigue, nausea) and a patient reported outcome reveals the worsening of a symptom before a physician reported outcome (Mayor, 2015). A study by Alberti concluded that both subjective and objective measures should be combined to enhance the validity of the measurement (Alberti et al., 2014).

As with other adverse events, the most commonly used scale to assess neuropathy has been the NCIC-CTC grading scale (Postma and Heimans, 2000). It requires sensory and motor symptoms to be graded separately, even though motor function is rarely affected by oxaliplatin treatment. The NCIC-CTC sensory scale is based on the subjective evaluation of the presence of paraesthesia and sensory loss (grade 1), their interference with function (grade 2), interference with activities of daily living (grade 3) and toxicity including permanent symptoms with interference with function or activity of daily living (grade 4). As oxaliplatin has a unique toxicity profile that results in both cumulative peripheral sensory neuropathy and acute reversible cold-allodynia, a specific Levi grading scale was developed to assess oxaliplatin induced neurotoxicity (Levi et al., 1992). It assesses both the intensity and the duration of neuropathic symptoms. The widely used functional assessment of cancer-gynecologic oncology group-neurotoxicity (FACT/GOG-NTX) questionnaire (Huang et al., 2007) has also been used in the SCOT, a substudy of the IDEA collaboration, to assess both acute and long-term neuropathy (Iveson et al., 2018). The total neuropathy score (TNS) is a complex assessment of neuropathy that includes objective measures, such as pin pricks, vibration thresholds, and nerve conduction studies, in combination with a subjective report of sensory, motor, and autonomic items (Cavaletti et al., 2003). Due to the objective measurements, it is not feasible to use the TNS in every

day clinical practice; shorter versions have been developed to evaluate only the clinical signs and symptoms of neuropathy.

At present, the patient reported outcome that is used most commonly for evaluating neuropathy is probably the EORTC CIPN20 (Lavoie Smith et al., 2013; Postma et al., 2005). It contains 20 items assessing sensory (9 items), motor (8 items), and autonomic symptoms (3 items) and it uses a 4-point scale for assessing symptom severity during the past week (1 = "not at all," 2 = "a little," 3 = "quite a bit," and 4 = "very much") (http://qol.eortc.org/). As observed for QLQ-C30, all scale scores are linearly converted to a 0–100 scale, with higher scores indicating a greater symptom burden.

5.13.3 *Prediction and prevention of CIPN*

Despite intensive research, no factors that can predict the development or severity of CIPN have been identified (Pulvers and Marx, 2017). Data published are partly contradictory, but most studies have identified an association between acute and long-term neuropathy and the cumulative dose (Park et al., 2011; Stefansson and Nygren, 2016; Tournigand et al., 2006). The Southwest Oncology Group examined their database to identify patients that had been treated with taxane therapy (Hershman et al., 2016). They noted an association between age and neuropathy, and reported that significantly more neuropathy was observed in patients with complications because of diabetes, as compared with that in patients with no diabetes. However, this study included only patients with an age of over 65 years and other studies have reported about conflicting results with regard to both age and diabetes (Pulvers and Marx, 2017). Interestingly, patients with a history of autoimmune disease were half as likely to experience neuropathy (Hershman et al., 2016). However, in conclusion, no baseline factors that can act as predictors for neuropathy have been established.

OPTIMOX 1 study evaluated intermittent use of oxaliplatin, the "stop and go" strategy, which is currently a recommended treatment strategy with the administration of oxaliplatin (Benson and Venook, 2018a; Benson and Venook, 2018b; Van Cutsem et al., 2016). In OPTIMOX 1 (n=620), FOLFOX was provided until progression in one treatment arm and in another one FOLFOX was provided for 6 cycles, followed by maintenance with 5-FU/LV alone for 12 cycles, after which FOLFOX was reintroduced (Tournigand et al., 2006). The median PFS and OS were 9.0 and 19.3 months, respectively, in patients treated with FOLFOX until progression, as compared with those of 8.7 and 21.2 months, respectively, in patients treated using the stop-and-go strategy (P = not significant). Oxaliplatin was reintroduced in only 40.1% of the patients, but response or stabilization of the disease was achieved in 69.4% of patients in whom oxaliplatin was reintroduced. Results show that administering oxaliplatin using the the stop-and-go strategy did not compromise RR, PFS, and OS. Lower rates of grade 3 neuropathy were reported in patients treated using the stop-and-go strategy (13% vs 19%; P = .0017) (Tournigand et al., 2006).

The OPTIMOX 2 phase II trial (n=216) randomised patients to either 5-FU/LV maintenance or a treatment holiday between FOLFOX administration (Chibaudel et al., 2009). The primary endpoint, duration of disease control (calculated as the sum of the duration of PFS in the initial FOLFOX

therapy and with the subsequent reintroduction of FOLFOX), was better in the continued treatment arm (13.1 months vs 9.2 months, P=0.046), as was the PFS.

According to ESMO guidelines, patients receiving FOLFOX or CAPOX plus bevacizumab should be considered for maintenance therapy with fluoropyrimidine plus bevacizumab, after an induction phase of 8 or 6 cycles, respectively (Van Cutsem et al., 2016).

In addition to the use of the stop-and-go strategy, no agents have been recommended for the prevention or treatment of neuropathy. There were high expectations from the study of calcium-magnesium infusions, but it turned out negative. Some results were obtained with the use of venlafaxine in the prevention of CIPN, but data is insufficient to recommend any agent in the prevention of CIPN (Durand et al., 2012; Jordan et al., 2016). A phase III study is currently ongoing, after promising results were obtained with calmangafodipir (Karlsson et al., 2017). With regard to the treatment of chronic, painful neuropathy, the best data support was available for carrying out treatment with duloxetine (Smith et al., 2017).

6. AIMS OF THE THESIS

The present study, including its four substudies was conducted to find the answers for the following questions:

Study I: Is HTN associated with treatment outcome in mCRC patients treated with bevacizumab containing chemotherapy?

Study II: Is *H. Pylori* infection predictive of chemotherapy induced gastrointestinal toxicity during 5-FU based adjuvant chemotherapy in CRC patients?

Study II: Does *H. Pylori* interfere with primary CRC diagnostics?

Study III: Are haematological or non-haematological adverse events predictive of the efficacy of 5-FU based adjuvant chemotherapy in CRC patients?

Study IV: What is the incidence of acute and long-term neuropathy in CRC patients treated with adjuvants FOLFOX or CAPOX?

Study IV: Does long-term neuropathy have an impact on QOL in CRC survivors?

7. MATERIAL AND METHODS

7.1 Patient material and ethical aspects

An overview of study characteristics is presented in Table 5. Study I included 101 patients who had received palliative treatment for mCRC, and studies II-IV included 79, 1033, and 144 patients respectively, who received adjuvant treatment after radical resection for stage II-III CRC.

Study I was a retrospective study based on information in hospital charts, and it was performed with institutional approval from the department of Oncology at HUCH, without obtaining formal patient consent. In studies II-IV, the Ethical Review Board at HUCH had approved the protocols and written informed consent had been obtained from all patients.

Table 5. Study characteristics

Study characteristics	Study I (n=101)	Study II (n=79)	Study III (n=1033)	Study IV Retrospective (n=144) Prospective (n=92)
Radically operated stage II/III/IV	No	Yes	Yes	Yes
Non-resectable metastatic disease	Yes	No	No	No
Chemotherapy regimens	Bevacizumab in combination with: FOLFIRI (n=60) Irinotecan or CAPIRI (n=21) CAPOX or FOLFOX (n=12) 5-FU/LV or Capecitabine (n=7)	5-FU Mayo (n=41) Simplified LV5FU2 (n=38)	5-FU Mayo or modified Mayo (n=513) LV5FU2 or simplified LV5FU2 (n=520)	CAPOX (n=72) FOLFOX (n=72)
Toxicity assessment	NCI-C CTCv.2	NCI-C CTCv.2	NCI-C CTCv.1 in French NCI-C CTCv.2 in Finnish	NCI-C CTC v.3 EORTC CIPN20

Study I included all consecutive mCRC patients treated with bevacizumab containing chemotherapy between April 2004 and December 2005 at the Helsinki University Central Hospital (HUCH) Cancer clinic. There were 114 bevacizumab treated patients identified from the patient charts, of which 101 patients with evaluable disease had received treatment with bevacizumab. These 101 patients were eligible for efficacy assessment.

Study II included 79 radically operated CRC patients with *H. pylori* serology and lactose intolerance assessment (stage II-III n=73, stage IV n=6). These patients were the first patients from the randomised LIPSYT trial that included a total of 153 patients. The LIPSYT was an open, prospective, randomised single institution study conducted using radically operated CRC patients, and it is registered on http://www.controlled-trials.com/ISRCTN98405441. The primary endpoint of the LIPSYT was treatment tolerability and toxicity.
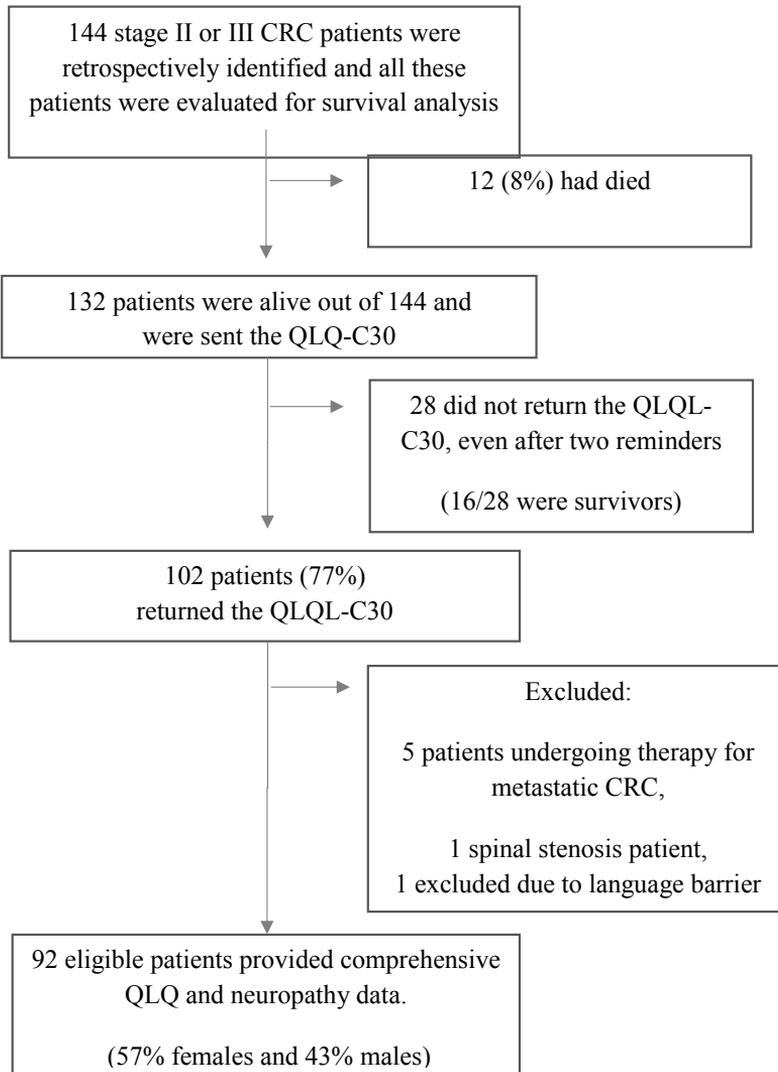
Concerning study II, there were 37 seronegative and 42 seropositive patients at diagnosis of CRC. Serum samples were collected to study the *H. pylori* status prior to adjuvant treatment, and during and after treatment (at 2, 4, and 6 months), and at 8 and 12 months from the date of initiation of treatment. In addition, 77 out 79 patients were evaluated for lactose intolerance, using an oral lactose tolerance test at the baseline and at 4 and 8 months after the initiation of adjuvant chemotherapy. With regard to the diagnostic delay, patients were asked about the duration for which they had had cancer related symptoms before surgery and the main symptom because of which the patient visited a doctor. The time to diagnosis was calculated as the time from the beginning of the symptoms to the date of surgery. All patients (n=79) were included in the toxicity analysis, but six patients were excluded from the analysis of diagnostic delay since it was not possibly to determine the time from the onset of symptoms to diagnosis in these patients. Only stage II-III patients were included in the efficacy analysis of DFS and OS.

Study III consisted of 1033 patients with radically operated stage II or III CRC. To increase the statistical power of the study we pooled data from two prospective, randomised trials; the Finnish LIPSYT (n=153) and French GERCOR C96.1 (n=880). In original GERCOR C96.1 primary end-point was DFS between bolus and infusional regimens in the adjuvant setting. All patients with complete data on toxicity and survival were included and therefore not all patients from the GERCOR C96.1 were eligible (905 patients in the original trial).

In study IV, we included 144 patients. All stage II and III CRC patients who were treated with the adjuvant CAPOX (n=72) at the HUCH department of oncology between 1.1.2000 - 31.9.2009 were identified from hospital files and matched with comparative FOLFOX controls from the same register. All patients who were alive were sent the EORTC QLQ-C30 questionnaires and two reminders. An extensive interview was performed with consenting patients (n=92). During the phone interview, the EORTC CIPN20 questionnaire was filled-in, to assess long-term neuropathy. By the time of the study, CIPN20 was not yet translated into Finnish or Swedish; therefore, instead of sending questionnaires in English, a phone interview was performed after obtaining permission from EORTC. Six patients were excluded during the interviews, due to different confounding factors and one was excluded, because he was not skilled enough to communicate effectively in English or Finnish. At

the end of the process, there were 92 patients who had consented, and for whom complete data, including both filled-in QLQC-30 and CIPN20 forms were available (Figure 1).

Figure 1.   Flowchart for patients in study IV

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┌─────────────────────────────────────────┐
│   144 stage II or III CRC patients were  │
│   retrospectively identified and all     │
│   these patients were evaluated for      │
│   survival analysis                      │
└─────────────────────────────────────────┘
              │         ┌──────────────────────────────┐
              │───────▶ │      12 (8%) had died         │
              │         └──────────────────────────────┘
              ▼
┌─────────────────────────────────────────┐
│   132 patients were alive out of 144 and │
│   were sent the QLQ-C30                   │
└─────────────────────────────────────────┘
              │         ┌──────────────────────────────┐
              │───────▶ │   28 did not return the QLQL- │
              │         │   C30, even after two reminders│
              │         │                               │
              │         │   (16/28 were survivors)      │
              │         └──────────────────────────────┘
              ▼
┌─────────────────────────────────────────┐
│   102 patients (77%)                      │
│   returned the QLQL-C30                    │
└─────────────────────────────────────────┘
              │         ┌──────────────────────────────┐
              │───────▶ │   Excluded:                   │
              │         │   5 patients undergoing therapy│
              │         │   for metastatic CRC,          │
              │         │   1 spinal stenosis patient,   │
              │         │   1 excluded due to language   │
              │         │   barrier                      │
              │         └──────────────────────────────┘
              ▼
┌─────────────────────────────────────────┐
│  92 eligible patients provided            │
│  comprehensive QLQ and neuropathy data.   │
│                                           │
│  (57% females and 43% males)              │
└─────────────────────────────────────────┘
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7.2 Treatment regimens

In study I, any chemotherapy regimen containing bevacizumab was allowed; the most commonly used regimens included those of FOLFIRI (n=60), CAPIRI, or irinotecan (n=21), CAPOX or FOLFOX (12), and 5-FU/LV or capecitabine (n=7) (Osterlund et al., 2011).

Adjuvant chemotherapy regimens in studies II and III included either the Mayo (or modified Mayo with 3- to 5-min intravenous 5-FU bolus 370–425 mg/m^2 and LV infusion 10–20 mg/m^2) or the LV5FU2 in France or simplified LV5FU2 in Finland (Andre et al., 2003; Osterlund et al., 2007).

In study IV, all patients were treated with either CAPOX or FOLFOX (Haller et al., 2011b; Soveri et al., 2018).

7.3 Assessment of adverse events

In study I, the recorded adverse event of interest was HTN, and it was graded retrospectively from the patient charts. Blood pressure was measured by a nurse at the baseline and before each bevacizumab infusion. Blood pressure values were recorded at every assessment and the highest grade was recorded every three months. Three months was chosen, because there were patients with both 2- and 3-week long dosing schedules, and all patients started a new treatment cycle every three months.

In study II, the adverse events of interest included functional dyspepsia, stomatitis, diarrhoea, constipation, flatulence, nausea, and the worst oro-gastrointestinal toxicity. All these toxicities were recorded in a patient diary and graded according to the NCI-C CTC version 2. The worst toxicity grade of each specific adverse event during adjuvant chemotherapy was considered in the analysis.

In study III, the adverse events studied included anaemia, neutropenia, thrombocytopenia, the category of worst haematological toxicity, HFS, diarrhoea, nausea/vomiting, mucositis, category of other toxicities (i.e. alopecia and fatigue) and the category of worst non-haematological toxicity. These adverse events were recorded for every cycle and the worst grade of all cycles was considered in the analysis. In the French study, toxicities were recorded using NCI-C CTC version 1, and they were recorded in the Finnish study using version 2, but there are no significant differences in the grading of the studied events between these two versions.

In study IV, the adverse events of interest were acute and long-term neuropathy. Both acute and long-term neuropathy were recorded and graded by experienced consultants according to NCI-CTCAE v 3.0. Data for acute toxicity were collected by one researcher (Ulrika Hänninen) and data for long-term neuropathy were collected by the author LMS. Both the sensory and motor neuropathy grades were denoted, but the sensory grade was worse than motor in all the cases, and the sensory neuropathy grade was used in analyses. In addition, CIPN20 was used to assess long-term neuropathy, as a patient reported outcome (PROM).

8. Statistical analysis

In all the four studies, we used Graph Pad Prism version 6.00 (GraphPad Software, La Jolla, California, USA), Sigmaplot version 11.0 (Systat software Inc, USA), or SPSS version 24.0 (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0 Armonk, NY, USA) for conducting statistical analyses.

Statistical analyses in study I and III were performed by the statistician Tuija Poussa, researchers Pia Österlund, and the author, and those for study II were performed by the statisticians Tuija Poussa and Emmanuel Quinaux, researchers Thierry Andre, Aimery de Gramont, Pia Österlund, and the author; those for study IV were performed by the statisticians Markku Karhunen and researchers Annamarja Lamminmäki, Pia Österlund, and the author.

In all studies, the significance level was set at $\alpha = 0.05$. DFS was defined as the time from diagnosis to the date of relapse, while PFS was defined as the time from the initiation of treatment to the time of progression or death because of any cause; OS was defined as the time from diagnosis to the date of death or date of last follow up. RR was used as the endpoint in study I, according to RECIST 1.1 criteria (Eisenhauer et al., 2009).

Power analyses were performed by the statistician for study II. For study I, III, and IV all patients were included and no retrospective power calculations were performed.

8.1   Study I

It was hypothesised that an association between HTN and treatment outcome in mCRC patients treated with bevacizumab containing chemotherapy might exist. The association between PFS, OS, and HTN was assessed by Kaplan-Meier analysis and the comparison between groups was done by the log-rank test. The landmark survival analysis was performed to eliminate the bias caused by the time-dependent definition of HTN and the landmark point was set at three months, when a majority of patients had developed HTN. Cox proportional hazard models were used to perform univariate analyses and the HTN status used was that of the landmark time. Crude models were first constructed using baseline characteristics (age, gender, primary tumour site, line of treatment, WHO performance status, number of metastatic sites), and the significant of these were added as covariates together with the HTN status in the multivariate Cox model. The variables with non-normal distributions were compared using the Mann–Whitney U-test, and the χ^2-test was used for categorical variables.

8.2   Study II

In the second study, it was hypothesised that the *H. Pylori* infection might increase chemotherapy induced gastrointestinal toxicity during 5-FU based adjuvant chemotherapy and that *H. Pylori* might interfere with primary CRC diagnostics. Binary logistic regression analysis was performed to compare the toxicities between groups. The chemotherapy regimen was included as a categorical

covariate due to randomization. The interaction between *H. pylori* positivity and chemotherapy treatment was tested in the beginning and in the case of interaction, the association between *H. pylori* and a symptom was assessed in both treatment groups separately but otherwise omitted. The results are presented as adjusted odds ratios with 95% confidence intervals (CI$_{95\%}$). DFS and OS were assessed by using Cox regression analysis. The log-rank test was used to study the diagnostic delays. For patient characteristics between *H. pylori*-seropositive vs. -seronegative patients the χ^2 and Mann-Whitney U tests were used.

8.3   Study III

The aim of the study was to determine if the occurrence of haematological or non-haematological adverse events could predict the efficacy of 5-FU based adjuvant chemotherapy in CRC patients. Two types of treatment regimens were used; therefore, possible interactions between the treatment regimen and adverse events were first examined, but as no interactions were observed ($P > 0.05$ for interactions for all adverse events), only the treatment regimen was included in the models. Cox proportional regression analysis was used to study the association between DFS, OS, and adverse events. In univariate crude analysis, the age, stage, and histologic differentiation were significant and therefore included further in multivariate Cox proportional hazards regression analyses. A stepwise multivariate Cox proportional regression analysis was performed using forward selection for assessing which of the significant adverse events was the most important prognostic factor (model was adjusted for age, stage, and histologic differentiation). Cox proportional regression analyses and Kaplan–Meier survival curves were used to evaluate how the number of different significant adverse events predicted DFS and OS. Survival curves were constructed using the Kaplan–Meier method and to estimate the survival rates at 5 years and at the end of 8.2 years (the longest follow-up time in GERCOR C96.1 study)

8.4   Study IV

The aim of the study was to assess the incidence of acute and long-term neuropathy in CRC patients treated with adjuvant FOLFOX or CAPOX and evaluate if long-term neuropathy had an impact on the QOL in CRC survivors. A neuropathy of grade ≥ 2 was considered clinically significant; therefore, grades 0-1 versus 2-4 were used as cut-offs for acute and long-term neuropathy. Chi-square and the non-parametric Mann–Whitney U tests were used for comparing the baseline characteristics and the outcome variables between the groups. Spearman's rho test was used to test the correlations between continuous variables. Logistic regression analysis was used to test the predictive value of different variables for acute neurotoxicity and long-term neuropathy. No adjustments were made for conducting multiple comparisons. Time to event distributions were estimated using Kaplan-Meier curves and compared using log-rank tests.

9. RESULTS

9.1 Study I: "Hypertension and overall survival in metastatic colorectal cancer patients treated with bevacizumab-containing chemotherapy"

9.1.1 *All patients*

In all the 101 patients, the median follow-up time was 63.5 months. By the time of analysis, 100 (99%) of these patients had progressed and 90 (89%) of them had died. The RR was 50% and disease control rate was 89%. In all patients, the median PFS was 8.8 months (range, 8.0–9.6) and OS was 18.9 months (range, 15.1–22.7). The median duration of bevacizumab-containing chemotherapy was 8.6 months (range, 1.2–70.5)

9.1.2 *Effect of HTN on outcome*

Patient characteristics (age, gender, tumour site, treatment line, WHO performance status, chemotherapy used) were well balanced and there were no significant differences except for the number of metastatic sites ($P=$ .002) between hypertensive and normotensive patients.

A total of 57 (56%) patients were diagnosed with any grade of HTN (n=26 grade 1, n=28 grade 2 and n= 3 grade 3), during bevacizumab treatment, while 44 (44%) patients remained normotensive (grade 0). Forty-two (74%) out of 57 patients that developed HTN while receiving the therapy, were normotensive at the baseline, and had no previous history of hypertension. Fifteen (26%) patients were normotensive with antihypertensive medication at the baseline, but HTN recurred during bevacizumab treatment.

The median time from the start of bevacizumab treatment to the onset of HTN was 1 month (range, 1–15) and the onset of HTN was observed within 6 months from the start of treatment in 95% of the patients. The mPFS (5.3 *vs.* 10.5, $P=$ .008), mOS (11.7 *vs.* 25.8, $P<$ .001), and RR (20 *vs.* 30%, $P=$ .025) were significantly higher in hypertensive patients.

9.1.3 *Significance of early HTN for survival*

To avoid the bias due to the time-dependent definition of HTN and determine whether early HTN was predictive of survival within three months of treatment, we performed the landmark analysis, in which the HTN status of each patient was determined at three months and survival was calculated from that time point onwards. One patient had already died and was excluded from the analysis. At three months, 48 patients were hypertensive and 53 were normotensive. At 3 months, the mOS in hypertensive patients was 19.9 (range, 15.7–24.1), compared to 12.3 months (range, 6.9–17.7, $P=$ .020) in normotensive patients.

Univariate analysis was performed using the Landmark model. The HTN within 3 months, performance status, line of treatment, number of metastatic sites, and type of chemotherapy were all statistically significant for survival. In multivariate analysis, HTN was an independent predictor of survival (HR 0.53; *P*= .007), together with the line of treatment and number of metastatic sites.

9.1.4   *HTN, OS, and treatment line*

All eligible patients (n=101) for whom bevacizumab treatment was started during April 2004 and December 2005 were included in the study. Thirty-three patients were treated in the first line, 39 in the second line and 29 patients were treated in the third or later line. The median duration of bevacizumab-containing chemotherapy in first-line treatment was 12.0 months (range, 1.2–68), and that in the second line of treatment was 7.5 (range, 1.3–70.5), while it was 6.6 months (range, 1.9–27) in the third or later lines of treatment.

An association between HTN and OS benefit was observed, regardless of the treatment line. After first-line treatment, the mOS for hypertensive patients was 28.8 months (range, 12.8–44.8) and that for normotensive was 15.3 months (range, 14.0–16.6) months, *P*= .291. In second-line treatment, there was an equally significant difference between hypertensive and normotensive patients with regard to the mOS, which were 30.3 (range, 19.8–40.9) and 10.9 (range, 0.36–21.5) months (*P*= .028), respectively. In the third or later lines of treatment, the mOS was 18.1 (range, 12.1–24.0) months for hypertensive and 11.4 months (range, 7.5–15.3) for normotensive patients (*P*= .007).

9.2   Study II: ""*Helicobacter pylori*-related gastrointestinal symptoms in diagnostics and adjuvant chemotherapy of colorectal cancer"

9.2.1   *Symptoms of CRC present at diagnosis and diagnostic delay*

The most common symptoms of CRC included altered bowel habits (diarrhoea, constipation, alternating function, or mucous faeces) in 53 (73%) patients, blood in the stool in 36 (49%) patients, functional dyspepsia (postprandial fullness, nausea, belching, early satiety, epigastric pain, and burning) in 26 (35%) patients, occlusion/perforation in 10 (14%) patients, and infectious symptoms in 10 (14%) patients. Regardless of the symptoms present, the median time from the onset of symptoms to surgery of the primary tumour in 73 patients was 6 months (range, 4–11) and the longest time was 42 months in one *H. pylori* seropositive patient.

In patients presenting with bowel symptoms, blood in the stool, occlusion/perforation or infectious symptoms, the median time to diagnosis for CRC from the onset of symptoms was 5 months (range, 2–8). In patients presenting with functional dyspepsia, the median time to diagnosis from the onset of symptoms was 7.5 months (range, 4–14). This difference of 2.5 months was statistically significant (*P*= .035).

Time to diagnosis was also significantly longer in 35 *H. pylori*-seropositive patients, as compared to that in 38 seronegative patients. The median time to diagnosis of CRC from the onset of symptoms was 6 months (range, 4–12) in *H. pylori*-seropositive, as compared to 5 months (range, 4–8) in *H. pylori*-seronegative patients (*P*= .012). In six (17%) seropositive patients, but in none of the seronegative ones, the time to diagnosis for CRC from the onset of symptoms was greater than 18 months.

Diagnostic delays did not turn into a statistically significant inferior survival at the minimum follow-up time of 120 months. The DFS rates in stage II and III patients (n=73) were 61% and 67% in *H. pylori*-seropositive and seronegative patients, while the corresponding OS rates for these patients were 61% and 69%, respectively. In the entire cohort of 79 patients, the DFS rate was 64% and OS was 65%.

We also assessed lactose intolerance using an oral test in 77 (97%) of the patients. There were 14 (18%) patients diagnosed with hypolactasia (blood glucose increase > 1.1 mmol/L and 12 patients (16%) had borderline hypolactasia (blood glucose increase 1.1-1.6). Hypolactasia and *H. pylori* seropositivity were not correlated (*P*= .20) with each other and hypolactasia or borderline hypolactasia did not interfere with diagnostics.

9.2.2   *Adverse events in* H. pylori-*seronegative and* H. pylori-*seropositive patients*

Adverse events during 5-FU adjuvant treatment were compared between *H. pylori*-seronegative and *H. pylori*-seropositive patients. The adverse events studied included stomatitis, functional dyspeptic symptoms (including postprandial fullness, belching, early satiety, epigastric pain, and burning), diarrhoea, constipation, flatulence, nausea, and the category of the worst oro-gastrointestinal toxicity. No statistically significant differences were noted in functional dyspeptic or gastrointestinal toxicities between *H. pylori*-seronegative and *H. pylori*-seropositive patients.

9.3   Study III: "Association of adverse events and survival in colorectal cancer patients treated with adjuvant 5-fluorouracil and leucovorin: Is efficacy an impact of toxicity?

9.3.1   *Univariate analysis*

DFS and OS were assessed in 1033 patients treated with 5-fluorouracil as bolus or LV5FU2. Median follow-up was 6.05 years. In univariate analysis significant predictors for DFS or OS were neutropenia, nausea/vomiting, mucositis and worst non-haematological toxicity and these were used in further analysis.

Patients presenting without any toxicity had the worst outcome, but there was no linear improvement in the outcome with an increase in the toxicity level. Patients with grade 1 nausea/vomiting, grade 2 neutropenia, grade 2 mucositis, or grade 2 of the worst non-haematological toxicity had the best

survival. Frequencies for predefined toxicities and the association with DFS and OS are presented in Table 2.

9.3.2 *Survival analysis*

Cox regression analysis was performed dichotomizing between grade 0 vs grade 1-4 toxicity. As observed in the univariate analysis, leukopenia, thrombocytopenia, the worst haematological toxicity, diarrhoea, HFS, or other toxicities were not associated with DFS or OS (all *P* values >0.10). Neutropenia, mucositis, nausea/vomiting, and the worst non-haematological toxicities were significant, both in unadjusted and adjusted cox regression analysis (adjusted for other significant predictors, age, stage, and histological differentiation). Any grade of neutropenia was associated with an improved DFS but not with OS, in both non-adjusted and adjusted models, whereas mucositis, nausea/vomiting and the worst non-haematological toxicity were significantly associated both with DFS and OS (Table 6).

Table 6. Frequency of significant adverse events and association with DFS and OS for separate grades, and for grades 0 versus grades 1-4

Adverse Event	Grade	Frequency n (%)	Disease-Free Survival HR	95% CI	P value	Overall Survival HR	95% CI	P value
Neutropenia	0	537 (53)	1.00			1.00		
n=1022	1	204 (20)	0.79	0.60-1.05	0.10	0.81	0.58-1.13	0.22
	2	153 (15)	**0.74**	0.54-1.02	0.06	**0.77**	0.53-1.13	0.19
	3	90 (9)	0.88	0.60-1.29	0.52	1.00	0.65-1.54	1.00
	4	38 (4)	1.03	0.60-1.77	0.92	1.26	0.70-2.27	0.44
	1-4*		**0.76**	**0.61-0.95**	**0.02**	**0.82**	**0.64-1.06**	**0.13**
Nausea/vomiting	0	474 (46)	1.00			1.00		
n=1032	1	379 (36)	**0.78**	0.62-0.99	0.04	**0.60**	0.45-0.79	<0.001
	2	141 (14)	0.76	0.54-1.06	0.10	0.59	0.39-0.88	0.01
	3	38 (4)	0.98	0.57-1.69	0.94	0.96	0.53-1.73	0.90
	4	0 (0)						
	1-4*		**0.74**	**0.59-0.92**	**0.008**	**0.58**	**0.45-0.75**	**<0.001**
Mucositis	0	587 (57)	1.00			1.00		
n=1031	1	229 (22)	0.84	0.65-1.09	0.19	0.78	0.57-1.06	0.12
	2	149 (15)	**0.52**	0.36-0.75	<0.001	**0.48**	0.31-0.75	0.001
	3	52 (5)	0.97	0.61-1.55	0.90	1.15	0.69-1.89	0.59
	4	14 (1)	0.93	0.38-2.26	0.88	0.89	0.33-2.40	0.81
	1-4*		**0.70**	**0.56-0.88**	**0.003**	**0.67**	**0.52-0.88**	**0.003**
Worst non-haematological toxicity	0	143 (14)	1.00			1.00		
	1	300 (29)	0.75	0.55-1.03	0.08	0.70	0.48-1.00	0.05
	2	371 (36)	**0.54**	0.39-0.75	<0.001	**0.48**	0.33-0.70	<0.001
n=1031	3	181 (18)	0.83	0.59-1.17	0.28	0.73	0.49-1.09	0.12
	4	36 (3)	0.73	0.39-1.36	0.33	0.77	0.39-1.52	0.44
	1-4*		**0.65**	**0.49-0.86**	**0.003**	**0.57**	**0.41-0.79**	**0.001**

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

The co-occurrence of all the significant adverse events (neutropenia, nausea/vomiting, and mucositis) was significantly associated with the best survival. In patients presenting with only one adverse event (seen in 33%), the HR for DFS was 0.86 (CI$_{95\%}$ 0.66–1.14, P= .30), while in patients presenting with two adverse events (seen in 28.2%), the HR was 0.64 (CI$_{95\%}$ 0.47–0.86, P= .003); in patients presenting with all three adverse events (seen in 18.5%), the HR was 0.61 (CI$_{95\%}$ 0.44–0.80, P= .004).

In patients presenting with only one adverse event, the HR for OS was 0.74 (CI$_{95\%}$ 0.53–1.01, P= .06), while in patients presenting with two adverse events, the HR was 0.59 (CI$_{95\%}$ 0.42–0.84, P= .003); in patients presenting with three adverse events, the HR was 0.49 (CI$_{95\%}$ 0.33–0.74, P= .001).

We also investigated which of the significant adverse events was the strongest predictor of survival, using a stepwise multivariate COX proportional regression analysis. Any grade of mucositis or nausea/vomiting was statistically significant in this model. With regard to mucositis, the HR for DFS was 0.71 ($CI_{95\%}$ 0.57–0.90, P = .004) and that for OS was 0.71 ($CI_{95\%}$ 0.54–0.93, P = .01). With regard to nausea/vomiting, the HR for improved DFS was 0.79 (0.63–0.99, P = 0.04) and that for OS was 0.61 (0.46–0.79, P < 0.001). Thus, nausea/vomiting was the strongest predictor of OS.

9.4 Study IV: "Long-term neuropathy and quality of life in colorectal cancer patients treated with oxaliplatin-containing adjuvant chemotherapy

9.4.1 *Acute and long-term neuropathy*

Sensory neuropathy of any grade was observed during chemotherapy in 136 (94%) out of 144 patients; grade 1, grade 2, grade 3, and grade 4 neuropathy was observed in 24%, 45%, 25%, and 1% of the patients, respectively. Long-term neuropathy was observed in in 69% (grade 1/2/3/4 in 36/24/8/1%) of patients at a median follow-up at 4.2 years. There was a significant association observed between acute neurotoxicity during treatment and long-term neuropathy during follow-up.There were no differences in acute or long-term neuropathy between treatment regimens (CAPOX vs FOLFOX) and no differences in CIPN20 scores were noted. No baseline factor was associated with long-term neuropathy, with the exception that a performance status of ECOG 1 at the baseline predicted the occurrence of long-term neuropathy (OR 3.9, $CI_{95\%}$ 1.3-12.2). No differences were observed in the long-term neuropathy between patients who had treatment durations of ≥3 months *vs.* < 3 months.

9.4.2 *Neuropathy and QOL*

Grade 2-4 long-term neuropathy associated with impaired physical and role functioning and with higher rates of diarrhoea, but it did not affect global health status. In patients with grade 3-4 long-term neuropathy QOL scores for role functioning and financial problems were affected, but the global health status was not affected in these patients either compared to patients with grade 0-2 neuropathy. There were no differences between QLQ-C30 subscales among treatment regimens.

9.4.3 *Seasonal variation*

As Finland has a sub-arctic climate, we studied whether the mean outside temperature has an effect on neuropathy. There were no seasonal variations, as treatment in coldest winter months was not significantly associated with grade 2-4 neuropathy and the severity of long-term neuropathy was not associated with the month during which oxaliplatin treatment was started or stopped.

9.4.4 *Acute neuropathy and survival*

We studied whether acute neuropathy predicted survival. There were no statistically significant differences in the DFS or OS of patients presenting with grade 0-1 neuropathy and those with 2-4 neuropathy, but the trend towards superior OS was observed in patients presenting with grade 2-4 neuropathy (Figure 2).

Figure 2.   Overall survival in 92 stage II or III colorectal cancer patients treated with FOLFOX or CAPOX, divided based on neuropathy grade 0-1 versus 2-4.
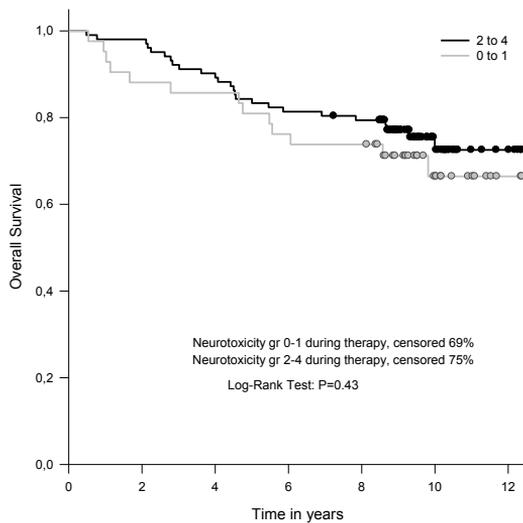
9.4.5 *Treatment regimen and survival*

There was numerical but not statistically significant difference in DFS (Figure 3A.) and OS (Figure 3B) favouring CAPOX treatment.

Figure 3. Disease-free survival (A. upper panel) and overall survival (B. lower panel) in 144 CAPOX and FOLFOX-treated stage II and III colorectal cancer patients.
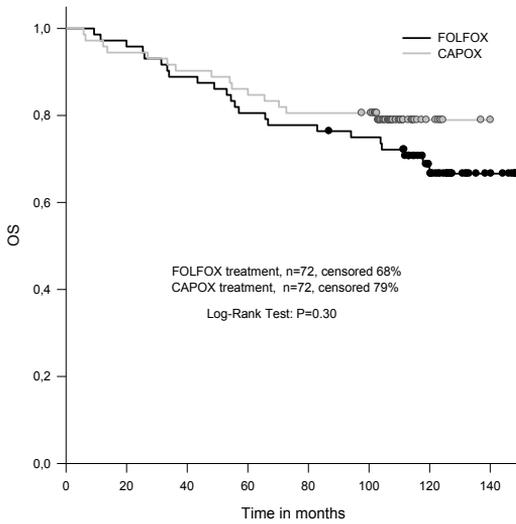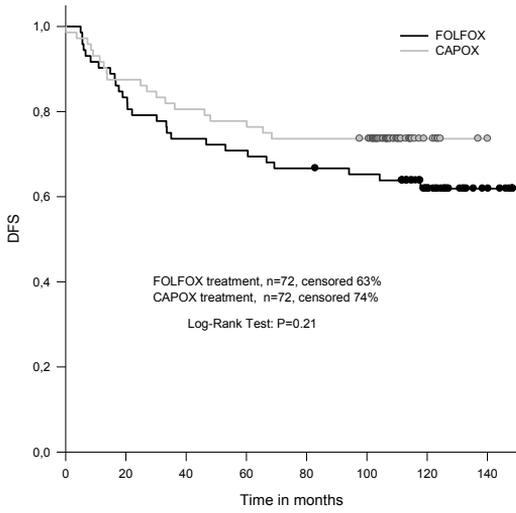
10. DISCUSSION

10.1 What does this thesis mean for a clinician?

This study was performed to answer the following clinically relevant questions:

- Does *H. pylori* interfere with CRC diagnostics?
- Does *H. pylori* increase acute oro-gastrointestinal toxicity during 5-FU-based adjuvant chemotherapy?
- Are common treatment-related adverse events associated with treatment outcome and could those be considered as clinically feasible predictive biomarkers?
- How common is long-term neuropathy after oxaliplatin-based adjuvant chemotherapy and does it impair QOL in CRC survivors?

10.2 Diagnostic delay in CRC

CRC is a major public health problem. If it is metastatic and incurable, it causes suffering and results in considerable economic burden for the individual and the health care system.

The major solution to this is early diagnosis. The role of the primary care physician cannot be emphasised enough and is essential in the early diagnosis and treatment of gastrointestinal diseases (Gikas and Triantafillidis, 2014). Unfortunately, it is also shown that the diagnostic delay in CRC due to physician and health care system issues (Walter et al., 2016) can be longer than that observed due to patient-related factors (Turunen and Peltokallio, 1982), and is also longer than that of other common cancers (Korsgaard et al., 2008; Mitchell et al., 2008; Van Hout et al., 2011).

Our study established in line with most previous studies the fact that clear, severe symptoms such as bowel obstruction, anaemia, and intestinal bleeding lead to colonoscopy and the diagnosis of CRC. However, signs of CRC can be vague and sometimes mimic those of upper GI-tract complaints (O'Reilly and Long, 1987). We observed that in *H. pylori* positive patients and in patients presenting with dyspepsia, colonoscopy was postponed and the diagnosis of CRC was significantly delayed. The delay was one month in *H. pylori* positive patients and 2.5 months in patients presenting with dyspepsia, as compared with that for patients presenting with bowel obstruction, anaemia, and intestinal bleeding. In contrast to *H. pylori infection*, we observed that lactose intolerance did not interfere with diagnostics. It can be speculated that this is due to small number of patients in our study; with a larger number of patients, the results would have been different, because of the high prevalence of lactose intolerance as a gastrointestinal comorbidity worldwide. To the best of our knowledge, our study is one of the few to explore the significance of *H. pylori* and lactose intolerance in CRC diagnostics, for which comparative data is scarcely available.

The diagnostic delay in the group of *H. pylori* positive patients did not result in inferior survival during the follow-up duration of 10 years. Even though it has been shown that the screening and early detection of CRC decreases mortality (Shaukat et al., 2013), the results of most other studies are in line with ours, and suggest that symptom duration has no apparent impact on survival in CRC patients (McDermott et al., 1981). Several studies have reported a U-shaped association between diagnostic interval and mortality in CRC patients, i.e., either very short or long diagnostic delays are associated with increased mortality (Torring et al., 2012). A prospective population-based Danish study by Tørring et al. showed that the mortality rates were significantly higher for patients presenting with alarming symptoms that suggested cancer occurrence, and with those showing the shortest and longest diagnostic intervals (Torring et al., 2011). However, there were no statistically significant associations between diagnostic interval and mortality in patients presenting with vague symptoms, which was in line with the results of our study. Similar results about survival and the nature of CRC symptoms have also been reported by another study that pooled data from three population-based CRC studies in Denmark and the United Kingdom (Torring et al., 2013).

The association of a short diagnostic delay with increased mortality can be explained by the fact that patients presenting with alarming symptoms showed an advanced stage of metastasis, probably in part because acute surgery, which is a known risk factor for impaired outcome, had been performed in these patients (Jeong et al., 2017). It can also be possible that these patients present with a more biologically aggressive disease. A diagnostic delay could be attributable to either a patient or doctor in patients with a long diagnostic interval and increased mortality, which leads to diagnosis being performed at a more advanced stage and an inferior treatment outcome.

In our study, diagnostic delays are modest in number, but clinically relevant. Most importantly, our observations highlight the importance of performing a thorough diagnostic work-up, even though comorbidities are present. The clinicians responsible for the diagnostics should be aware of the vague nature of CRC symptoms. In a patient presenting with anaemia or abdominal discomfort, gastroscopy is often the first examination to be performed, but in a high-income country such as Finland, CRC is much more common than gastric cancer (Bray et al., 2018; Cancer_registry, 2018). Therefore, according to our results, colonoscopy should always be considered for a patient presenting with gastrointestinal symptoms; regardless of the symptoms present and concern about limited resources, it might be more reasonable to recommend colonoscopy before gastroscopy. According to our results, in patients that are diagnosed with *H. pylori* infections, a colonoscopy should be performed despite a diagnosis of an H. pylori infection.

10.2.1 *H. pylori and survival*

We did not observe statistically significant differences in survival between *H. pylori* positive and negative patients, but we observed a trend towards inferior survival in *H. pylori* positive patients. The emergence of data has shown that the microbiota is a driver of CRC carcinogenesis and modulator of the toxicity and efficacy of chemotherapeutic agents. Data concerning *H. pylori* infections in the context of CRC are still contradictory and scarce. There are studies demonstrating the association between *H. pylori* infection and CRC (Chen et al., 2013; Rokkas et al., 2013; Wu et al., 2013), but

there is a lack of prospective longitudinal data that would thoroughly prove the causality. The sample size in our study is too small to draw conclusions or find significant differences in survival, but we can speculate whether the inferior trend in survival in *H. pylori* positive patients is truly due to the infection, diagnostic delay, or the possible modulation of the efficacy of chemotherapy by *H. pylori* and its toxins. It is unknown whether the antibiotics or proton pump inhibitors used to treat *H. pylori* infections modified the gastrointestinal microbiota, which lead to dysbiosis and the carcinogenesis of CRC. No such data exist, to the best of our knowledge.

Increasing amounts of evidence have suggested that oro-gastrointestinal microbiota are an important driver of carcinogenesis and play a significant role in modulating treatment efficacy and treatment related toxicity. *Mycoplasmas* are bacteria that abundantly express thymidine phosphorylase and are associated with several cancers (Bronckaers et al., 2008). As the efficacy of capecitabine is dependent on thymidine phosphorylase activity, the presence of *M. hyorhinis* was shown to enhance the cytotoxic effect of capecitabine. The presence of *M. hyorhinis* in the tissues might therefore modify the efficacy and toxicity of capecitabine. To the best of our knowledge, there is a limited availability of data about *H. pylori* and its capability to modify the effect of chemotherapy; it would be interesting to study this topic further.

Recently, it was demonstrated that gut microbiota play a significant role in the immunotherapy treatment response in melanoma patients (Gopalakrishnan et al., 2018). A study by Gopalakrishnan et al. observed that there were notable compositional differences in melanoma patients who responded to immunotherapy with PD1-blockade, as compared to those who were non-responsive. Patients with the highest diversity of gut microbiota showed the best outcome. The *Ruminococcaceae* family was enriched in responders and was responsible for a greater level of T cell penetration into tumours; higher levels of circulating T cells were observed in these patients. Patients with abundant levels of *Bacteriodales* spp. had higher levels of circulating regulatory T cells, myeloid-derived suppressor cells, and a blunted cytokine response, which resulted in the dampening of anti-tumour immune responses. A favourable microbiota was also associated with increased levels of antigen processing and presentation by the immune system at the tumour site. A study by Matson et al. showed as well, . that a significant association was observed between commensal microbial composition and clinical response in melanoma patients treated with immunotherapy with PD1-blockade (Matson et al., 2018).

A major drawback in the treatment of CRC has been the modest response of immunotherapy in MSS patients, as opposed to significant benefits noted in MSI-H tumours (Kalyan et al., 2018). Unfortunately, the number of patients exhibiting MSI-H represents less than 5% of patients with mCRC. Early phase data suggested that combination therapy with PD-L1 inhibitor atezolizumab and MEK inhibitor cobimetinib improved OS, as compared to that observed with regorafenib in heavily pretreated mCRC patients with MSS tumours (Bendell et al., 2018b). It is shown that cobimetinib favourably alters the tumour microenvironment and T-cell responses that enhance anti-tumour immune activity, by inhibiting MEK1/MEK2 in the MAPK pathway. It was therefore hypothesized that a combination of atezolizumab and cobimetinib would enhance immune recognition and contribute to greater anti-tumour activity, but no enhanced efficacy was noted in a

phase III trial (Bendell et al., 2018a). Since we now know that the microbiota plays a crucial role in modifying immune responses, it is interesting to speculate whether it would be possible to enhance the response generated after immunotherapy in MSS patients in the future, by altering the microbiota. Gut microbiota would probably present a target for personalized cancer therapy in the future, as discussed by Petrosino as well (Petrosino, 2018).

10.2.2 *Acute chemotherapy-related adverse events and H. pylori*

The association between acute chemotherapy-induced toxicity and *H. pylori* in CRC patients is a topic that has been rarely investigated, and to the best of our knowledge, our paper is still one of the few to focus on *H. pylori* and chemotherapy-related toxicity in CRC patients. Our study was based on the hypothesis that *H. pylori* might increase oro-gastrointestinal toxicity induced by 5-FU-based chemotherapy and that the eradication of *H. pylori* before therapy might be recommended. The use of 5-FU is associated with high rates of gastrointestinal toxicity (Macdonald, 1999) and *H. pylori* is equally associated with such symptoms (Malfertheiner et al., 2012), i.e., symptoms such as dyspepsia and nausea could be attributable to *H. pylori* and chemotherapy.

We showed that *H. pylori* seropositivity was not associated with treatment-related gastrointestinal toxicity during 5-FU-based adjuvant therapy. Since we included only 79 patients in our study, it is difficult to make definitive conclusions; results contradictory to ours have been published recently. A Chinese study reported significantly higher rates of nausea in *H. pylori*-infected patients with metastatic gastric cancer or mCRC patients treated with oxaliplatin and irinotecan-based combination chemotherapy, as compared to uninfected patients (Yao et al., 2017). As *H. pylori* is a significant carcinogen associated with gastric cancer (Herrero et al., 2014), it causes gastric complaints (Malfertheiner et al., 2012); in addition, because the toxicity attributable to combination chemotherapy differs from that to single agent 5-FU, it is not possible to directly compare our results with those of the Chinese study. Additionally, an essential difference between the studies is that we graded toxicities using NCI-CTCAE v2, but the Chinese study used PROMs. It is challenging to grade nausea objectively; therefore, it is interesting to speculate whether our results would have been different if we had used direct PROMs instead of a patient diary. In addition, a small study (n=79) reported that significantly more thrombocytopenia was observed in *H. pylori*-infected stage III CRC patients during chemotherapy, as compared to that observed in non-infected patients (Tanriverdi, 2014). We focused on oro-gastrointestinal toxicity in our study, but with regard to the results obtained by Tanriverdi et al., it would be interesting to analyse the haematological toxicity as well.

Based on our results, we cannot recommend the routine screening and eradication of *H. pylori* before 5-FU-based chemotherapy. However, due to the inclusion of a small number of patients in the study, it would be interesting to repeat our study design after the inclusion of a larger number of patients, because though *H. pylori* is not a major health problem in Finland, it is a major problem globally. There are also other interesting aspects that require further investigation. All our patients were treated intravenously with 5-FU. Because orally ingested drugs reach the gastrointestinal tract and are exposed to the microbiota in the stomach and gut, it is questionable whether the increased toxicity

in *H. pylori* seropositive patients had also been observed with the use of capecitabine. To the best of our knowledge, no such data has been published yet.

10.3 Adverse events as biomarkers of the outcome

10.3.1 *HTN as a biomarker in bevacizumab treatment*

HTN is a typical treatment-related adverse event of all drugs with VEGF/VEGFR inhibition (Launay-Vacher and Deray, 2009). Bevacizumab-induced HTN was first considered a harmful adverse event, but HTN and its association with outcome were widely investigated in several tumour types eventually. Increasing amounts of data suggest that HTN induced by the inhibition of VEGF/VEGFR could act as an easily monitored biomarker for efficacy in e.g. glioblastoma, breast, renal, ovarian and lung cancer (Gampenrieder et al., 2014; Lombardi et al., 2013; Zhong et al., 2015).

By the time our study was completed, the association between HTN and treatment outcome had been studied, mainly in renal cell carcinoma (mRCC) patients. Rini et al published a meta-analysis of sunitinib-treated patients, which demonstrated that patients who developed HTN after the first cycle of sunitinib treatment showed significantly improved mPFS, mOS, and RR, as compared to that in normotensive patients (Rini et al., 2011). Rini et al also showed that mRCC patients presenting with grade ≥ 2 HTN that were treated with interferon-α plus bevacizumab showed a significantly improved mPFS and mOS, as compared to normotensive patients (Rini et al., 2010). Bono et al. had also reported about similar results for bevacizumab-treated mRCC patients (Bono et al., 2009).

These findings enhanced our interest in the study of HTN and outcome in mCRC patients. By this time, Scartozzi et al had reported small, retrospective study of 39 mCRC patients treated with bevacizumab-containing chemotherapy. They noted that mCRC patients presenting with HTN (n=8) during therapy showed a significantly improved PFS and OS, as compared with that of normotensive patients (Scartozzi et al., 2009). Our study was the largest when it was published, and identified 57 out of 101 patients with HTN, enabling us to make a larger comparison. We observed that HTN was a significant, independent predictive factor for improved RR, PFS, and OS, despite the line of treatment. Tahover et al. observed significant improvement in the mPFS (29.9 *vs.* 17.2 months, *P*= .024) and OS (median not reached *vs.* 36.8 months, *P*= .029) in hypertensive bevacizumab-treated mCRC patients (Tahover et al., 2013), which was in line with the findings of our study. These findings by our and Tahover's group were further established in a meta-analysis, in which the occurrence of bevacizumab-induced HTN was associated with significant improvements in the PFS (HR$=0.57$, CI$_{95\%}$ 0.46–0.72), OS (HR$=0.50$, CI$_{95\%}$ 0.37–0.68) and RR (RR$=1.57$, CI$_{95\%}$ 1.07–2.30) (Cai et al., 2013). A retrospective Japanese study evaluated the effect of bevacizumab-induced HTN and prognosis in a large population of CRC patients (n=315) and non-small cell lung cancer patients (n=317) (Nakaya et al., 2016). Again, with this larger sample size, it was found that OS of both CRC and NSCLC patients was significantly prolonged if they developed HTN during early bevacizumab treatment, as compared to that of normotensive patients.

Thus, an increasing level of evidence suggests that HTN is a predictive biomarker, but large prospective trials that support this finding have not yet been conducted. Those would be required to conclusively establish that HNT is a surrogate marker for efficacy. It would be interesting to continue to conduct research on this topic, because it would be easy to monitor HTN, when it is considered to be a biomarker in every day clinical practice and the monitoring of HTN is a part of normal clinical practice. Because economic constraints need to be taken into account, the cost-effectiveness of bevacizumab has been questioned on some occasions (Goldstein et al., 2017; Lange et al., 2017). Effective patient selection is therefore of the utmost importance. If HTN is established as a predictive biomarker for efficacy, it would allow us to choose the correct patients for continued bevacizumab therapy. It would help us to reduce healthcare costs; most importantly, the risk of unnecessary adverse events for a patient would be reduced. In addition, when it is unclear whether VEGF inhibition should be continued during the second line of treatment, HTN monitoring could enable us to reach and support a decision, as according to our results, the level of HTN was significant, regardless of the line of treatment.

The major problem with using HTN as a predictive factor might be the level of reliability for carrying out assessments. HTN is easy to grade objectively, according to the NCI-CTCAE grading scale, but environmental and psychological factors easily bias HTN, as it is affected by the timing and manner of measurement, which leads to wide variations in values (Vischer and Burkard, 2017). Currently there are no specific guidelines to treat bevacizumab-induced HTN (Syrigos et al., 2011). It is not known if a certain type of medication should be favoured; the impact that the medication might have on outcome over the long term remains unknown. Our study was too small for us to definitively conclude about this aspect; therefore, larger trials need to be conducted.

According to our results, HTN strongly supports the continuation of bevacizumab-containing therapy. HTN is not a reason to terminate therapy, but it should be treated according to common treatment practices for HTN treatment.

10.3.2 *Haematological toxicity as a biomarker for survival*

Saarto et al had already studied the association between haematological toxicity and survival in breast cancer patients in the 1990s (Saarto et al., 1997). They demonstrated the association between a low leucocyte nadir and improved survival in 211 breast cancer patients treated with doxorubicin-based adjuvant chemotherapy. This association was also shown by Poikonen et al. in a subsequent study in breast cancer patients treated with adjuvant CMF (cyclophosphamide, methotrexate, and fluorouracil) (Poikonen et al., 1999). These were one of the first studies investigating the topic, but since then, the association between haematological toxicity and survival has been widely studied and shown in several tumour types.

To the best of our knowledge, data for chemotherapy-treated CRC patients were scarcely available by the time we published our study, at least in the adjuvant setting, and the number of reports is still limited (Rambach et al., 2014; Shitara et al., 2009; Shitara et al., 2011; Sunaga et al., 2014). A meta-analysis that was performed with data obtained from 9,528 patients with different malignancies in

thirteen trials had previously demonstrated that the mortality was reduced (HR=0.69, $CI_{95\%}$, 0.64-0.75) in patients with grade 3-4 leucopoenia or neutropenia, as compared to that in patients with a lower-grade or complete lack of cytopenia (Shitara et al., 2011). This meta-analysis also included data from a study with 153 mCRC patients treated with FOLFOX during first-line treatment (Shitara et al., 2009). There was a significant level of association between neutropenia and OS; the risk of death was reduced by 45% in patients with grade 1-2 neutropenia (HR=0.55 $CI_{95\%}$ 0.31-0.98) and by 65% in patients with grade 3-4 neutropenia (HR=0.35 $CI_{95\%}$ 0.18-0.66).

Another study with 399 mCRC patients treated with at least one line of chemotherapy was published in concordance with our paper (Rambach et al., 2014). This was a retrospective analysis similar to ours, which demonstrated an association between any grade of neutropenia and OS (HR=0.55, $CI_{95\%}$ 0.43-0.70, *P*< .0001) and thrombocytopenia and OS (HR=0.70 $CI_{95\%}$ 0.56-0.88, *P*= .025) that occurred during the first two lines of chemotherapy. In contrast to the results of our study, anaemia during chemotherapy was significantly associated with inferior OS (HR=1.9 $CI_{95\%}$ 1.22-2.97, *P*= .005) in their study. In our study, thrombocytopenia and anaemia were insignificant.

Sunaga et al have also retrospectively investigated the association between neutropenia and survival in 123 CRC patients with stage III disease receiving uracil and tegafur/LV as adjuvant chemotherapy (Sunaga et al., 2014). They demonstrated a significant improvement in the DFS in neutropenic patients, as compared to that in non-neutropenic patients (3-year DFS 81.2% versus 57.3%, *P*= .0213), but there were no statistically significant differences in the OS; these results were in line with those obtained in our study. However, Rambach et al and Shitara et al demonstrated the association between neutropenia and OS in metastatic disease. It is possible that haematological toxicity acts as a predictive factor in patients with advanced disease, but not for OS in the adjuvant setting. A limitation of our study was the unavailability of nadir counts in the bolus arm. It can be speculated that with nadir values, the DFS benefit would have turned into a significant OS benefit as well. Since the use of 5-FU as a single therapy does not typically lead to a considerable level of cytopenia, non-complicated neutropenia is probably not detected. The strength of our study was its large sample size.

10.3.3 *Non-haematological toxicity as a biomarker for survival*

Most trials have focused on studying the association between haematological toxicity and survival, which is understandable, since haematological toxicity is easy to monitor objectively. We were interested in studying non-haematological toxicity, which is a comparatively less studied topic. According to our results, non-haematological toxicity is especially associated with improved survival in patients treated with 5-FU-based chemotherapy. To the best of our knowledge, our study was one of the first to report the association between non-haematological toxicity and survival in CRC patients.

Chemotherapy-induced gastrointestinal toxicity has mainly been attributed to the disruption of the mucosal barrier, which lines the entire alimentary tract (Lee et al., 2014). In our study, we refer to oro-gastrointestinal mucositis, including that occurring in the mucosal lining of the upper as well as the lower gastrointestinal tract. Today, it is increasingly recognised that the pathobiology of mucositis

is complex, and involves not only the mucosal barrier injury, but also the mucosal immune system and that an important role is played by the release of pro-inflammatory cytokines (Lee et al., 2014). It is increasingly being recognised that these inflammatory processes, which contribute to the oro-gastrointestinal toxicity of chemotherapeutic agents, play an essential role in the development of anti-tumour immunity, reduction of the risk of recurrence, and improvement of patient survival. With regard to these data, it is rational to consider mucositis as a surrogate marker for efficacy, especially since 5-FU is associated with a notable level of mucosal injury. Hence, we expected to discover an association between diarrhoea and outcome, but did not do so.

Instead, we saw a strong association between nausea/vomiting and outcome. The association was so strong that it is hard to preclude that it was a coincidence, but equally hard to explain; data regarding this association are scarcely available, but the symptom of nausea probably reflects the occurrence of mucosal injury throughout the gastrointestinal tract, and can therefore be considered a predictive factor. A report by the European Osteosarcoma Intergroup showed in line with our findings that in osteosarcoma patients treated with doxorubicin and cisplatin in addition to surgery for localised disease, grade 3-4 oral mucositis (HR=0.51, $CI_{95\%}$ 0.29-0.91), grade 1-2 nausea/vomiting (HR 0.37, $CI_{95\%}$ 0.16-0.85), and grade 1-2 thrombocytopenia (HR 0.49, $CI_{95\%}$ 0.27-0.87) were associated with 5- and 10-year survival rates in a multivariate analysis (McTiernan et al., 2012). On the other hand, a meta-analysis published as an abstract by the North-Eastern German Society of Gynecological Oncology (NOGGO) reported that grade 3-4 nausea/ vomiting significantly decreased both PFS and OS in patients with ovarian cancer (n=1213) (Woopen et al., 2017). Observations by NOGGO emphasize the importance of receiving supportive care along with chemotherapy. Grade 3-4 nausea/vomiting not only reflects the excessive toxicity of therapy, but compromises the well-being of a patient with a high risk of termination of the therapy.

Twelves et al. demonstrated in a post-hoc analysis of the X-ACT trial that HFS was associated with improved outcome in patients treated with capecitabine (Twelves et al., 2012).The association between HFS and survival was also established (Hofheinz et al., 2012) in a pooled dataset from AIO KRK-0104 and the Mannheim rectal cancer trials. In this study, patients with any grade of HFS showed a significantly improved PFS/DFS (29.0 *vs*. 11.4 months; HR=0.69, *P*= .015) as well as OS (75.8 *vs*. 41.0 months; HR=0.56, *P*= .001). No difference was noted between patients presenting with HFS at the beginning of therapy or those that developed HFS later. In patients presenting with HFS, a significantly higher rate of any grade of diarrhoea, stomatitis/mucositis, and fatigue was reported, but no similar correlation with haematological toxicity was found. It had been interesting to know if diarrhoea, stomatitis/mucositis, and fatigue were associated with outcome in capecitabine treated patients. With regard to our observations, it might be speculated that both the mucosal lining and the skin are more susceptible to injury from 5-FU than haematopoiesis. This could also explain why haematological toxicity, at least in the adjuvant setting, does not seem to be as strong a predictor of survival as non-haematological toxicity. Enhancing the effect of registered chemotherapeutic and biological agents

Despite the improved prognosis for patients with early stage CRC, disease recurrence occurs in 30-50% of patients (Koo et al., 2013). Therefore, tools to further improve the treatment outcome in the

adjuvant setting are needed. Irinotecan and the biological agents have failed to show efficacy in early-stage disease and no new drugs are in sight at least shortly. Thus, we need to investigate whether it might be possible to improve treatment outcome by enhancing the cytotoxic effect of 5-FU both in the adjuvant and metastatic settings.

In clinical practice, patients undergoing chemotherapy that do not experience adverse events sometimes enquire whether the treatment is beneficial at all, and based on our results and the published data, this question is highly relevant. We know that it is possible to improve outcome and decrease treatment-related toxicity using pharmacokinetically guided dose escalation, but pharmacokinetic measurements are cumbersome, and therefore cannot be performed easily in clinical practice (Saif et al., 2009). This explains why we do not use pharmacokinetically guided dose escalation in the clinic, but the author of this thesis wonders whether we have focused so intensively on the development of new agents that such a "simple" approach is forgotten.

Tumour sidedness has been a topic that has attracted great interest in CRC oncology lately, but it had already been reported in 1990 that there are major differences in the underlying pathogenesis of proximal and distal tumours (Bufill, 1990). It took 15 years for this topic to attract higher levels of interest, and our treatment practices have now drastically changed. Tumour sidedness does not require complicated measurements to guide the therapy. Likewise, though follow-up of adverse events is carried out in everyday clinical practice, treatments are currently modified only if the toxicity level is high. High toxicity is associated with poorer survival and consequently, the reduction in the dose should be performed in time. Probably the best outcomes are achieved when the toxicity level is mild or moderate, with or without dose reduction. As observed in our results, patients presenting with grade 2 neutropenia, mucositis, and the worst non-haematological toxicity had the most favourable outcomes. As discussed, HFS was shown to be associated with the outcome. HFS is a not a life-threating adverse event; it can easily be monitored and the inconvenience caused because of HFS can be relieved by supportive approaches, such as the use of appropriate creams. A difficulty encountered while obtaining our results is that adverse events such as mucositis and nausea/vomiting cannot be easily or objectively monitored, and even grade 1 nausea or mucositis can be highly inconvenient, compromise well-being, and decrease QOL. Therefore, it is difficult to set the dose based on the grade of nausea/vomiting, a higher grade of mucositis, or deeper neutropenia, but the capecitabine dose could be titrated to ensure at least mild HFS. Skin toxicity is a known predictive factor for EGFR-antibodies, and therefore the phase II EVEREST trial studied whether the skin toxicity-based dose-escalation of cetuximab improved outcome (Van Cutsem et al., 2012b). In this study, the cetuximab dose was escalated if the patient did not present with at least grade 2 skin toxicity during the first 21 days of treatment. Dose escalation was proven to result in an improved RR (30% *vs*. 16%) and disease control rate (70% *vs*. 58%), but did not significantly improve OS. To the best of our knowledge, there is scarcity of data with regard to toxicity-based dose escalation and chemotherapeutics in CRC patients.

Henricks et al. recently published results from the first prospective study conducted to investigate the effect of dose individualisation on fluoropyrimidine-related toxicity, based on the four best-recognised *DPYD* variants (Henricks et al., 2018). They observed that genotype-guided dosing was

feasible in clinical practice and dose individualisation reduced the risk of severe 5-FU-related toxicities, but they did not evaluate treatment outcome. They did perform pharmacokinetic measurements, and the findings suggested that applied dose reductions in *DPYD* variant allele carriers did not result in 5-FU underdosing, but it does not discussed about whether we should screen for fast metabolizers to avoid underdosing in these patients.

To establish the impact of results in study III, a prospective randomised trial using toxicity-based dose-escalation needs to be conducted.

10.4 Neuropathy and Quality of life

Nowadays, CRC survivors represent the third largest cohort of cancer survivors because of their improved survival, which would hopefully improve further (Amstutz et al., 2018). Acute toxicity during therapy has been of interest as long as chemotherapeutics have been in use, but the interest in long-term toxicities and QOL after therapy in cancer survivors is increasing. Data in the metastatic setting suggest that QOL is an independent predictor of improved survival and similar findings have currently been observed in early-stage disease (Braun et al., 2011; Maisey et al., 2002; Wong et al., 2014). The American Society of Clinical Oncology (ASCO) was the first to establish a Cancer Survivorship Task Force in 2004, to develop a coordinated strategy for providing follow-up care to the growing population of cancer survivors. ASCO has currently established its guidelines for survivorship care (El-Shami et al., 2015; McCabe et al., 2013) and ESMO has published a patient guide for survivors (www.esmo.org).

It is known that patients report higher grades of toxicities than doctors, and that patients start to report adverse events earlier than doctors (Mayor, 2015). HTN or haematological toxicity is easy to grade because the value is dependent on an objectively measured value. On the contrary, the grading of nausea, fatigue, diarrhoea, and neuropathy is more complicated, as we know that there is a variation between how patients interpret, tolerate, and report their symptoms and how they cope with them in everyday life (Bernhard et al., 1999b). Therefore, though PROMs are being focused on and commonly incorporated in clinical trials, in clinical practice, we still grade toxicities according to versions of NCI-CTCAE.

In general, the QOL in CRC survivors is reported to be relatively good (Adams et al., 2016; Jansen et al., 2010; Ramsey et al., 2000; Ramsey et al., 2002), but advanced age at diagnoses, higher BMI, lower education, smoking, and other co-morbidities are associated with a lower QOL (Adams et al., 2016; Jansen et al., 2010). However, these factors can cause the QOL to deteriorate independently, and might not be specifically related to CRC and CRC survivorship. Patients are often fearful about stomas in advance and some studies report an inferior QOL in stoma patients (Guren et al., 2005; Jansen et al., 2010); however, there are also studies that have shown contrary results (Smith-Gagen et al., 2010). The most critical aspect reportedly influenced by the presence of the stoma is the social component of QOL, which partially differs based on the gender (Krouse et al., 2009). Females are reported to experience more psychological and physical stress due to the stoma, but men report more sexual dysfunction (Guren et al., 2005).

What is reported about the impact of adjuvant therapy on QOL in CRC survivors? It is known that acute toxicity during 5-FU-based adjuvant therapy is reversible and short-lived, and QOL during therapy is dependent on the regimen and the acute toxicity profile of the treatment (Chau et al., 2005; Chen et al., 2015; Norum et al., 1997). Patients treated with single agent 5-FU do not experience long-term toxicities, and deterioration in the long-term QOL is minor, as compared to patients treated with surgery alone (Gray et al., 2007; Norum et al., 1997). On the contrary, in patients treated with oxaliplatin-based adjuvant chemotherapy, increasing amounts of data suggest that long-term neuropathy can be permanent (Grothey et al., 2018; Iveson et al., 2018), but it is not established whether it impairs the long-term QOL. An increasing number of studies have reported about neuropathy and QOL findings, but the methods to assess neuropathy and follow-up times between studies vary, which makes it difficult to conduct comparisons. In addition, the availability of data regarding the prevalence and course of neuropathy in a subarctic climate such as that of Finland is minimal.

We studied the prevalence of acute and long-term neuropathy, and impact of long-term neuropathy on QOL in CAPOX- and FOLFOX- treated patients in the Finnish sub-arctic climate. Any grade of acute neurotoxicity was observed in 96% of the patients included in our study, which is in line with the results of some previous studies, which have reported neurotoxicity rates of 92-95% (Andre et al., 2004b; Ducreux et al., 2011; Farhana et al., 2018). However, we have reported grade 3-4 neuropathy during adjuvant therapy in 26% of the patients, which is more than reported in large phase III adjuvant studies such as MOSAIC (FOLFOX, 12.5%), XELOXA (CAPOX, 11%), and NSABP C-07 (FLOX, 8.4%), but in line with that of smaller studies (Argyriou et al., 2012; Ducreux et al., 2011; Farhana et al., 2018). We have also reported about grade 2-4 acute toxicity in 71% of the patients, which is more than that reported by the IDEA study in the six-month arm (FOLFOX arm 48% and CAPOX arm 45%) (Grothey et al., 2018). We did not observe differences between treatment regimens with regard to the prevalence of acute neuropathy. Our observations might be attributed to the subarctic climate.

With regard to long-term neuropathy, we have reported any grade and grade 2-4 of long-term neuropathy in 68% and 33% of patients, respectively, at a median follow-up time of 4.2 years. MOSAIC has reported about any grade of neuropathy in 24% of patients becoming grade 3 in 0.7% of patients at 18 months (Andre et al., 2004a). Further, MOSAIC has reported neuropathy grade 1 in 12%, grade 2 in 3%, and grade 3 in 0.7% of patients at year 4 of follow-up (André et al., 2009). The NSABPC-07 trial has reported about persistent neuropathy at 27 months in 10% of patients (Land et al., 2007). Thus, the patients in our study demonstrate more long-term neuropathy than those in two large, randomised trials, but our results are in line with those of a small Australian study (n=25), which showed that oxaliplatin-induced nerve damage did not get resolved during a median follow-up of 2 years, and that significant clinical and neurophysiological deficits persisted in 79 % of patients (Park et al., 2011). Notably, the Australian and our studies specifically assessed neurotoxicity using neurophysiological tests or deep interviews, which can explain the differences observed, as compared to pure toxicity grading using NCI-CTCAE in clinical trials. We did not observe differences between treatment regimens with regard to the prevalence of long-term neuropathy or in CIPN20 subscales.

After the publication of the IDEA results (Grothey et al., 2018), the duration of adjuvant therapy in low-risk patients was reduced to three months, but the recommended duration for high-risk patients is still six months. Our study is of value, as it demonstrates that the development of neuropathy is hard to predict and long-term severe neuropathy can be observed during treatments conducted for a duration that is ≤ 3 months. There was statistically more long-term neuropathy observed in patients presenting with acute grade 2-4 neurotoxicity. We observed that an ECOG performance status that was > 0 was associated with long-term neuropathy and to the best of our knowledge, the availability of such data is scarce. Medicated diabetes was not associated with neuropathy in our patients. Apart from ECOG performance status, our findings are in line with most previous studies demonstrating that no baseline factor predicts long-term neuropathy (Brouwers et al., 2009; Uwah et al., 2012).

The risk of long-term neuropathy has been associated with a cumulative dose of oxaliplatin in most previous studies (Beijers et al., 2015; Park et al., 2011), though not by all (Brouwers et al., 2009; Stefansson and Nygren, 2016). Data supporting the association between cumulative dose and neuropathy come partly from pivotal metastatic trials, but a growing body of evidence from adjuvant trials has shown that lower cumulative doses of oxaliplatin result in less acute and long-term neuropathy. In the IDEA trial, grade 2-4 acute neurotoxicity was reported in 17% of the patients treated with FOLFOX and in 15% of patients treated with CAPOX in the three month arm, which is significantly less than that observed in the six month arm (48% *vs.* 45%, respectively) (Grothey et al., 2018). In addition, of the three pivotal adjuvant trials, the least long-term neuropathy was reported with NSAPB C-07, with the smallest cumulative dose of oxaliplatin (25% less in FLOX as compared to that in FOLFOX and CAPOX) (Andre et al., 2004a; André et al., 2009; Haller et al., 2011a; Land et al., 2007). We did not see a significant association between the cumulative dose of oxaliplatin and risk of acute or long-term neuropathy in our study. The high cumulative dose used in our study (770 mg/m^2) and the small patient number might explain this.

The strength of our study is the long follow-up and assessment time, for the objective NCI-CTCAEv3.0 and PROMs in the form of CIPN, as well as the assessment of both acute and long-term neuropathy. We also have data for all consecutive CAPOX patients with matched FOLFOX controls, which would enable us to perform a direct head-to-head comparison. Methodological challenges were encountered, as the CIPN20 was not available in Finnish or in Swedish and was therefore filled-in during the interview together with the patient, which affected the results either way. The interviews really gave us a deep insight into the phenomenon known as reframing means, during which the perception of QOL is affected by the life-threating disease, i.e., cancer and its treatment (Bernhard et al., 1999a). Most people think that it is of utmost importance to survive cancer; even though the cost of QOL would be high and they would remain symptomatic, they still considered the QOL to be relatively good.

Some studies have shown that neuropathy has a negative influence on QOL (Mols et al., 2014; Stefansson and Nygren, 2016; Tofthagen, 2010), but most studies have investigated QOL and neuropathy separately. Mols et al. conducted a systematic review of 14 studies, in which three of these studies did not find an association between neuropathy and QOL, while the other 11 concluded that more neuropathy is associated with a lower QOL. However, it is difficult to associate the meta-

analysis with our study, as it included many cytotoxins, different tumours, and treatment indications (Mols et al., 2014).

The biggest study conducted in CRC patients is a population-based Dutch study (n=1643), which reported about health-related QOL (HRQOL) and chemotherapy-induced neuropathy findings in CRC survivors, 2 to 11 years after diagnosis (Mols et al., 2013). HRQOL was assessed with QLQ-C30, while neuropathy was assessed with CIPN20. In all patients, the most common neuropathy-related symptoms reported in CIPN20 were erectile problems (42% of men), trouble hearing (11%), trouble opening jars or bottles (11%), tingling in the toes/feet (10%), and trouble walking on stairs or standing up (9%). Patients who received oxaliplatin reported significantly more tingling (29% *vs.* 8%; $P= .001$), numbness (17% *vs.* 5%; $P= .005$), and an aching or burning pain (13% *vs.* 6%; $P= .03$) in the toes/feet, as compared to that in patients not treated with chemotherapy at all. In addition, it was studied whether there was a difference between patients treated with chemotherapy, with or without oxaliplatin. Patients treated with oxaliplatin reported more frequent tingling in the toes/feet (29% *vs.* 14%; $P= .0127$), as compared with patients treated with fluoropyrimidines alone. As discussed by the authors, the most common symptoms reported are those types that can be commonly caused by several other conditions as well by cancer or its treatment. It was expected that patients treated with oxaliplatin would report significantly more tingling, numbness, and aching/burning pain, as compared with patients that were not treated with chemotherapy. Only tingling was observed to be significantly increased in a comparison between chemotherapy groups that did or did not receive oxaliplatin, which might be explained by the small number of chemotherapy patients (162 oxaliplatin-receiving, 118 other chemotherapy-receiving) included in the study, as discussed in the report. In addition, patients exhibiting the maximum number of neuropathy symptoms reported significantly and clinically relevant worse HRQOL scores on all EORTC QLQ-C30 subscales (all $P< .01$).

A cross-sectional patient series in 111 CRC survivors demonstrated that oxaliplatin-induced neuropathy impairs the emotional and physical well-being of patients, and thus affects the QOL (Tofthagen et al., 2013). This is in line with the impaired physical functioning noted in our study. In addition, our findings demonstrate that patients with long-term neuropathy exhibit impaired role functioning, which is in line with the findings of the Swedish study (Stefansson and Nygren, 2016). In our material, long-term neuropathy was associated with more diarrhoea, but in other subscales, we did not find any associations. An important observation is that the global health scale is not affected by long-term neuropathy, which is in line with the findings of the Swedish study (Stefansson and Nygren, 2016), but contrary to those of the studies by Tofthagen and Mols (Mols et al., 2013; Tofthagen et al., 2013). Even severe, grade 3-4 neuropathy did not impair the global health status in our study. This can be explained by the reframing process observed during the interviews. As a patient who was retired owing to impaired dexterity told us, "Neuropathy is a low price to pay for being alive."

Our study has a significant value because it was conducted in a sub-arctic climate. To the best of our knowledge, such data are scarcely available. Altaf et al. reported that in a Danish patient material , patients who received oxaliplatin during the coldest months of Danish winter (December-February) required more dose reductions during their first cycle, as compared to those required for patients

treated outside this period (Altaf et al., 2014). The Danish study did not provide long-term neuropathy data. As mentioned previously, a Swedish study reported that there is no association between the cumulative dose of oxaliplatin and long-term neuropathy, which is in line with our findings; this poses a question about whether acute and long-term neuropathy are triggered at lower doses in a subarctic climate (Stefansson and Nygren, 2016). We observed no difference in acute or long-term neuropathy in patients treated during the coldest months (January-February), and received the bulk of their treatment in winter, as compared to patients treated outside this period. Stefansson et al. do not report neuropathy grades, only CIPN20 scores, which makes direct comparison challenging. They have also not reported about any data related to acute toxicity, climate, and temperature. Therefore, our study is one of the few to report neuropathy findings in relation to temperature, in a subarctic climate. We noted no seasonal variation, but this might be attributable to the small number of patients and the fact that the mean temperature in a sub-arctic climate is low around the year.

To conclude the findings of the substudy III, it is notable that in a subarctic climate such as that of Finland, it is not possible to eliminate the risk of grade 2-4 long-term neuropathy by reducing the duration of therapy to three months. Even though the global health status was not deteriorated in patients suffering from grade 2-4 long-term neuropathy, our observations establish that long-term neuropathy is present in a significant number of patients, and because we cannot treat neuropathy, we must inform all patients about the potential risk of long-term neuropathy after oxaliplatin based adjuvant therapy.

11. SUMMARY

- The assessment and eradication of *H. pylori* before the initiation of adjuvant 5-FU-based treatment cannot be recommended.

- An important message to clinicians is that *H. pylori* and related symptoms might interfere with CRC diagnostics. Even if a diagnosis of *H. pylori* infection has been made, colonoscopy should always be considered in differential diagnostics.

- Patients who develop or require intensified treatment for HTN during bevacizumab treatment might survive longer and HTN might be a predictive surrogate marker for treatment efficacy and continued care.

- Neutropenia is associated with significantly improved DFS in patients treated with FU-based adjuvant chemotherapy.

- Mild or moderate non-haematological toxicity is a strong predictor of improved DFS and OS in patients who received FU-based adjuvant chemotherapy; mucositis and nausea/vomiting seem to be especially predictive. No treatment-related toxicity associated with the poorest survival.

- The prevalence of long-term neuropathy after oxaliplatin-based adjuvant chemotherapy seems to be higher than expected in real-life patient populations.

- Long-term neuropathy did not deteriorate the global QOL, but had an impact on physical and role functioning scales.

12. ACKNOWLEDGEMENTS

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Leena-Maija Soveri

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14.ORIGINAL PUBLICATIONS