Prognostic and predictive biomarkers in metastatic colorectal cancer constant and evolutionary perspectives

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CHRISTINA SIESING
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

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Christina Siesing

LUND
UNIVERSITY

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Abstract

Colorectal cancer (CRC) affects nearly 2 million people each year and accounts for 900 000 deaths worldwide. The main prognostic factor is disease stage at diagnosis and around 40% of the patients presents with or develop metastatic CRC (mCRC). Even if the disease has disseminated, cure is sometimes possible, but despite thorough selection of patients for metastasectomy, most patients will suffer relapse of the disease. Hence, there is a great need for new prognostic and predictive biomarkers in order to better select the appropriate treatment for each patient. The aim of this thesis was to study the prognostic and predictive impact of selected biomarkers, with particular focus on RNA-binding motif protein 3 (RBM3), in mCRC and to perform an extensive mapping of the spatial heterogeneity in mCRC with peritoneal carcinomatosis.

In paper I, RBM3 expression was assessed by immunohistochemistry (IHC) in primary tumours from 455 patients with mCRC. High RBM3 expression was an independent predictor of prolonged survival, and in the group with high RBM3 expression, a longer progression-free survival was seen in patients treated with oxaliplatin compared to patients treated with irinotecan in first line.

In paper II, RBM3 expression was assessed by IHC in 211 resected lung metastases and 164 paired primary tumours. High RBM3 expression in the lung metastases was an independent predictor of prolonged survival, in particular in patients treated with oxaliplatin at any time point. Other prognostic factors for prolonged survival were age ≤ 60 years, one metastasis, a lung metastasis <3 cm in size, disease free interval >24 months and adjuvant treatment.

In paper III, the spatial molecular heterogeneity was delineated in seven curatively treated patients with mCRC disseminated to the peritoneum. Multiregional targeted sequencing and IHC analysis of RBM3, special AT-rich sequence-binding protein 2 (SATB2) and mismatch repair (MMR) proteins were performed. The expression of RBM3 and SATB2 was allover low. Mutations in key CRC driver genes, i.e *KRAS, APC* and *TP53,* were homogenous across samples from individual patients, whereas less common mutations were more heterogenous. In some cases, a higher similarity was seen between PC and lymph node metastases than between PC and the primary tumour.

Paper IV is a study protocol for the planned On-treatment biomarkers in metastatic Colorectal Cancer for Life (On-CALL) study. The aim of this prospective, observational study is to follow up on relevant findings from the present thesis and to generate further knowledge on the spatial and temporal tumour heterogeneity and evolution of mCRC during curative treatment.

Key words Metastatic colorectal cancer, CRC, mCRC, RBM3, SATB2, NGS, Lung metastases, Peritoneal carcinomatosis, tumour heterogeneity

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Prognostic and predictive biomarkers in metastatic colorectal cancer

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To my mother and all other patients

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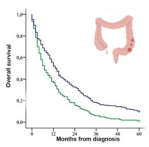
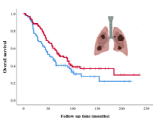
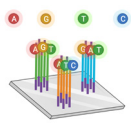
Thesis at Glance

Paper	Study question	Methods	Results	Conclusions
I 	Is RBM3 a prognostic factor in CRC, overall and in relation to first line chemotherapy?	IHC staining of RBM3 was evaluated in tumours from 455 patients with mCRC.	High RBM3 expression was an independent prognostic factor for a prolonged OS, and associated with a longer PFS in patients treated with first line oxaliplatin compared to those receiving irinotecan.	High RBM3 expression is an independent predictor of prolonged survival in mCRC, in particular in patients treated with first line oxaliplatin.
II 	Does RBM3 carry any prognostic value in patients with resected colorectal lung metastases?	RBM3 expression was evaluated by IHC in 211 resected pulmonary metasases and 164 paired primary tumours from patients with mCRC.	High RBM3 expression in the pulmonary metastases was associated with prolonged OS and RFS after PM and the prognostic value was particularly evident in patients treated with oxaliplatin.	High RBM3 expression is an independent prognostic factor for a prolonged survival after PM in patients with mCRC.
III 	Is CRC disseminated to the peritoneum a heterogenous disease?	TDS was performed on tumour samples (n=88) from multiple regions in seven curatively treated patients with PC from CRC. The expression of MMR proteins, RBM3 and SATB2 was evaluted by IHC.	Mutations in key CRC driver genes, i.e *KRAS, APC* and *TP53,* were homogenous across the samples, wheras less common mutations were more heterogenous. In some cases, a higher similarity was seen between PC and lymph node metastases than between PC and the primary tumour.	mCRC disseminated to the peritoneum is a complex disease that might well be a distinct entity from other mCRC.
IV 	How does the spatial and temporal molecular tumour heterogeneity affect treatment response and survival in patients with curatively treated mCRC?	A prospective observational study planned to enrol 100 patients with mCRC treated with curative intent. Multi-region TDS will be performed on resected tumours and on ctDNA from serial on-treatment blood samples.		

Abbrevations: CRC: Colorectal cancer, CtDNA: Circulating tumour DNA, IHC: Immunohistochemistry, mCRC: metastatic CRC, OS: Overall survival, PC: Peritoneal carcinomatosis, PFS: Progression-free survival, PM: Pulmonary metastasectomy, RBM3: RNA-binding motif protein 3, RFS: Recurrence free survival, TDS: Targeted deep sequencing, SATB2: Special AT-rich sequence-binding protein protein 2

List of Papers

The thesis is based on studies reported in the following papers, and are referred to in the text by their respective Roman numerals:

I. **Siesing C**, Sorbye H, Dragomir A, Pfeiffer P, Qvortrup C, Pontén F, Jirström K, Glimelius B, Eberhard J. High RBM3 expression is associated with an improved survival and oxaliplatin response in patients with metastatic colorectal cancer. *PLoS One* 2017;12:e0182512

II. Vidarsdottir H[#], **Siesing C**[#], Nodin B, Jönsson P, Eberhard J, Jirström K, Brunnström H. Clinical significance of RBM3 expression in surgically treated colorectal lung metastases and paired primary tumours. *Journal of Surgical Oncology* 2021;123:1144-1156

# These authors contributed equally to this paper

III. **Siesing C**, Petersson A, Ulfardsdottir T, Chattopadhyay S, Nodin B, Eberhard J, Brändstedt J, Syk I, Gisselsson D, Jirström K. Delineating the intra-patient heterogeneity of molecular alterations in treatment-naïve colorectal cancer with peritoneal carcinomatosis. *Manuscript*

IV. **Siesing C**, Petersson A, Olsson Hau S, Gisselsson D, Eberhard J, Jirström K. On-treatment biomarkers in metastatic Colorectal Cancer for Life: the On-CALL Study. *Manuscript*

Abbreviations

5-FU	5-fluorouracil
APC	Adenomatous polyposis coli
ASCO	American Society of Clinical Oncology
BRAF	Vraf Murine Sarcoma Viral Oncogene Homologue B1
CIMP	CpG island methylator phenotype
CIN	Chromosomal instability
CMS	Consensus molecular subtypes
CNA	Copy number alterations
CRC	Colorectal cancer
CRS	Cytoreductive surgery
CRT	Chemoradiotherapy
CRT	Classification and regression tree
ctDNA	Circulating tumour DNA
dMMR	Deficient MMR
DNA	Deoxribonucleic acid
EGFR	Epidermal growth factor receptor
EGFRi	EGFR-inhibitors
EMT	Epithelial-mesenchymal transition
EPIC	Early post-operative intraperitoneal chemotherapy
ESMO	European Society for Medical Oncology
FAP	Familial adenomatous polyposis
FFPE	Formalin-fixed paraffin-embedded
GCP	Good clinical practice
Gy	Gray

HC	Hierarchical clustering
HIPEC	Hyperthermic intraperitoneal chemotherapy
HR	Hazard ratio
HRAS	Harvey Rat Sarcoma Viral Oncogene Homologue
IBD	Inflammatory bowel disease
IHC	Immunohistochemistry
InDel	Insertion-deletion
KM	Kaplan-Meier
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homologue
LOH	Loss of heterozygosity
M	Distant Metastatsis
MAR	Matrix-attachment regions
mCRC	Metastatic colorectal cancer
miRNA	MicroRNA
MMR	Mismatch repair
mRNA	Messenger RNA
MSI	Microsatellite instability
MSI-H	MSI-High
MSI-L	MSI-Low
MSS	Microsatellite stable
MUTYH	MutY DNA glycosylase
N	Regional Lymph Nodes
NGS	Next-generation sequencing
NRAS	Neuroblastoma Rat Sarcoma Viral Oncogene Homologue
On-CALL	On-treatment biomarkers in metastatic Colorectal Cancer for Life
OS	Overall survival
PC	Peritoneal carcinomatosis
PCI	Peritoneal carcinomatosis index
PCR	Polymerase chain reaction

PD-1	Programmed death -1
PD-L1	Programmed death binding ligand 1
PD-L2	Programmed death binding ligand 2
PFS	Progression-free survival
PM	Pulmonary metastasectomy
pMMR	Proficient MMR
RBM3	RNA-binding motif protein 3
RBP	RNA-binding proteins
RNA	Ribonucleic acid
RT	Radiotherapy
SATB2	Special AT-rich sequence-binding protein 2
SBRT	Stereotactic body radiation therapy
SNV	Single-nucleotide variation
SPTC	Single patient tissue chip
T	Primary Tumour
TDS	Targeted deep sequencing
TGF-β	Transforming growth factor-β
TMA	Tissue microarray
TMB	Tumour mutational burden
TME	Total mesorectal excision
UICC	Union for International Cancer Controll
VEGF	Vascular endothelial growth factor
WES	Whole exome sequencing
WGS	Whole genome sequencing

Introduction

The word cancer originates from the Greek word for crab and is often accredited to Hippocrates, who thought that tumours, with their numerous blood vessels, reminded of a crab crawling in the sand. The oldest known portrayals of cancer tell about superficial tumours, easy to see with the eye, and one of the oldest dates back to the ancient Egypt, 2500 BC, describing a breast cancer as a bulging tumour of the breast for which there was no treatment[1].

Surgery of the bowel has been performed throughout history, often with high mortality rates. Disseminated disease was not curable and with the surgical techniques used, locally advanced tumours were not available for surgery. In the 1860s, the Austrian professor Theodor Billroth started to systemize cancer procedures in the abdomen, leading to better outcomes for the patients. He was also the first surgeon to perform anastomosis, making it possible to remove locally advanced tumors[2]. But even if all of the macroscopic tumour mass was removed, some patients still relapsed, and once dissemination was a fact, no cure was available. In 1882, however, Weinlechner published a report of a pulmonary metastasectomy (PM) performed when metastases were incidentally found in the lung of the patient during surgery of a primary chest wall sarcoma, reviewed in Cheung et al.[3]. In 1889, Keen published a report on a liver resection for removal of a neoplasm, and this report also contains a summary of 76 liver resections of hepatic tumours. Seventeen of the reported neoplasms were carcinomas, and out of the 76 patients, 63 recovered after surgery, a mortality rate of 14,9%[4]. Hence, surgery of metastases from the liver and lung was already performed in the 19th century, and the first reports of debulking surgery of peritoneal metastases are from 1930 by Dr Meign, as reviewed in Neuwirth et al[5]. The intention of the cytoreductive surgery (CRS) was not to cure the patient, but to enhance the palliation by reducing symptoms and preventing complications. Eventually, CRS developed towards a more aggressive cytoreduction, and in the 1970s, thoughts of intraperitoneal chemotherapy against peritoneal carcinomatosis (PC) started to grow[6 7].

During World War I, mustard gas was used as a chemical weapon and those who did not die immediately were affected by bone marrow suppression with consequences such as anemia and leukopenia[8]. This discovery was the starting point of chemotherapy development. However, the chemotherapy agents first discovered were inefficient against colorectal cancer (CRC). Heidelberger et al. reported on the

synthesis of fluorinated pyrimidines in 1957, and stated that *"It is evident from these results that this class of compounds exhibits a high order of tumour-inhibitory activity, which warrants further exploration",* thus laying the foundation of medical colorectal oncology as we know it today[9].

Colorectal cancer

Epidemiology and risk factors

Colorectal cancer affected 1.9 million people in 2020[10]. In the same year, 900 000 people died of CRC, making it the second deadliest cancer after lung cancer[10]. In Sweden, around 7000 people are diagnosed with CRC each year[11] and 3200 die of the disease[12]. The highest incidence globally is seen in Northern America, Europe and Oceania, but the incidence is rising in economically transitioning countries, for example Russia, China and Brazil[13], making the disease an indicator of socioeconomical development. CRC affects males to a greater extent than females, with a global incidence of 23.4/100000 and 16.2/100000, respectively. CRC is uncommon before the age of 40 and the majority of cases are over 70 years old[12 14]. There are however reports on a rising incidence in younger age groups and of younger people being diagnosed with more advanced tumours, indicating a true rise in incidence and not just a consequence of earlier diagnosis[15 16].

There are both genetic and environmental factors that can influence the risk of CRC. The most common hereditary condition predisposing for CRC is Lynch syndrome, a germline mutation in a mismatch repair (MMR) gene[17]. Lynch syndrome is inherited in an autosomal dominant manner and increases the risk of a number of different cancers, predominantly CRC, endometrial cancer and ovarian cancer[18]. Lynch syndrome is estimated to encompass approximately 3% of all CRC[19]. Another hereditary condition associated with colorectal cancer is Familial adenomatous polyposis (FAP), caused by mutations in the *adenomatous polyposis coli* (*APC)* gene which lead to numerous adenomas throughout the colon that can transform into cancer[20]. In families with a recessive inheritance of polyposis without the classical FAP mutations, genetic alterations in the mutY DNA glycosylase (*MUTYH*) gene have been found[21]. The gene encodes for proteins involved in the base excision repair, and the defect deoxyribonucleic acid (DNA) repair caused by *MUTYH* mutation generates an increased number of genetic alterations in the *APC* gene, leading to a FAP phenotype. The *MUTYH* associated polyposis accounts for approximately 1% of all CRC[22].

Inflammatory bowel disease (IBD), i.e., Crohn´s disease and ulcerative colitis, increases the risk of CRC. IBD patients are now in colonoscopic surveillance programs and the mortality rate of CRC in IBD patients is decreasing[23].

The World Cancer Research Fund and American Institute for Cancer Research published the report *Diet, nutrition, physical activity and colorectal cance*r in 2018, in which they have reviewed published research concerning lifestyle factors and CRC. They state that intake of red and processed meat increases the risk of CRC, and so does an intake of two or more alcohol units per day. They also state that obesity as well as taller stature increase the risk of CRC[24]. Further on, cigarette smoking increases the risk of CRC and this risk has been shown to be higher for rectal than for colon cancer[25].

Studies on the relationship between the gut microbiome and development of CRC have been conducted in recent years and show a connection between alterations in the microbiome and colorectal carcinogenesis[26]. Patients with CRC have been found to have increased levels of for example *Bacteroides fragilis* and *Enterococcaceae* compared to healthy controls[27]. Pathogenic bacteria and microbiome suppression by antibiotics can also play a role in colorectal carcinogenesis[26]. The mechanism of dysbiosis and CRC development probably involves local inflammation in the gut[28].

Anatomy of the colon and rectum

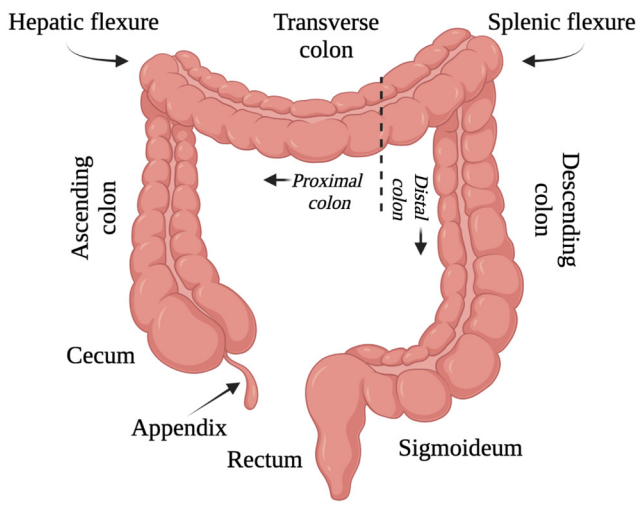


Figure 1. Anatomy of the colon and rectum. Created with BioRender.com.

The human colon is about 1.5 meters in length and extends from the caecum to the rectum. The large intestine arises embryologically from different entities, with the ascending colon, the hepatic flexure and 2/3 of the transverse colon, called the proximal colon, originating from the midgut, and the last 1/3 of the transverse colon,

the splenic flexure, the descending colon and rectum, called the distal colon, originating from the hindgut (Figure 1). There are also differences in the blood supply in that the proximal colon is supplied by the superior mesenteric artery and the distal colon receives arterial supply from the inferior mesenteric artery. The venous drainage occurs through veins that follow the mesenteric arteries and finally drain into the portal vein, however the most distal part of the rectum drains via the internal iliac vein and then to the inferior vena cava, not passing the portal vein[29].

Colorectal carcinogenesis

For many years, the development from normal intestinal epithelium to dysplastic adenoma and further on to carcinoma, driven by a series of genetic alterations, served as the model of colorectal carcinogenesis (Figure 2)[30]. However, enhancement in molecular pathology has deepened the understanding of CRC as a heterogenous disease, that can develop from classical adenomas, but also from serrated adenomas, with diverse molecular drivers.

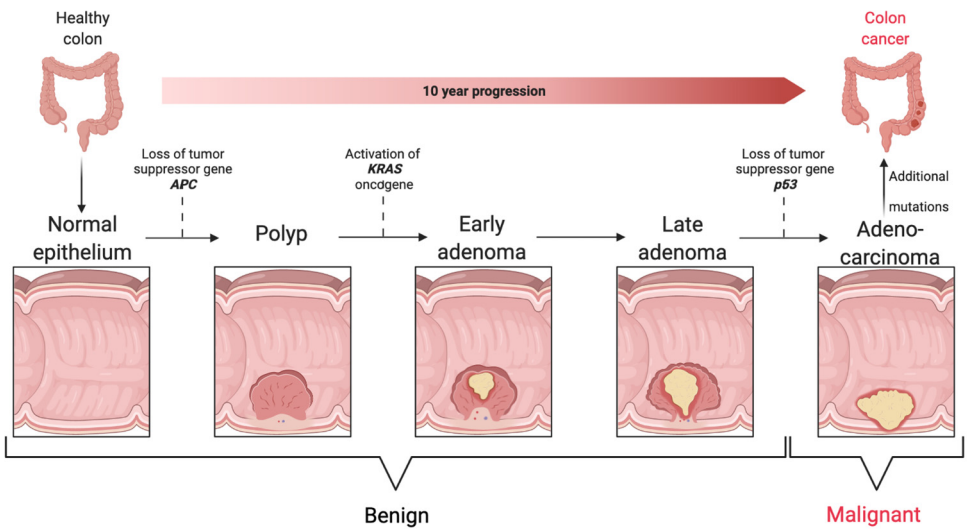


Figure 2. Colorectal carcinogenesis model according to Vogelstein et al.[30]. Created with BioRender.com

According to current knowledge, CRC can derive from one or a combination of three different pathways: chromosomal instability (CIN), CpG island methylator phenotype (CIMP) and microsatellite instability (MSI)[31].

A model of the CIN pathway, or the conventional pathway, was already described in 1990 by Fearon et al., who presented a genetic model that starts with a mutation in the *APC* gene, continues with genetic alterations in the *Kirsten-ras (KRAS)* gene, and then loss of *TP53*[32]. However, later studies have shown that all these three events only rarely occur in the same tumour[33]. Nevertheless, the genetic model suggested by Fearon et al. still points out important findings; that numerous genetic alterations are required, that the carcinogenesis occurs stepwise and that the temporal aspects of the genetic events are important[34]. The term chromosomal instability refers to the multiple losses of chromosomes or alleles that befall in this pathway[35], leading to aneuploidy and loss of heterozygosity (LOH).

The CIMP phenotype is an epigenetic pathway characterised by hypermethylation in promoter regions and silencing of tumour suppressor genes. CpG islands, short sequences rich in CpG dinucleotides, are found in the 5´region of most genes in many vertebrates[36]. Hypermethylation of these promotor areas, especially in tumour suppressor genes, leads to a deficient transcription even though the coding region of the gene is mutation free[37]. As aforementioned, there are two known precursor lesions to CRC, where the conventional adenoma was the first to be described. The serrated adenoma as a precursor was proposed in 2003 by Jass et al[38], and there are associations between the CIMP pathway, Vraf Murine Sarcoma Viral Oncogene Homologue B1 (BRAF) mutations, sporadic MSI and sessile serrated adenomas[39] [40].

MSI implies a defect in the MMR system. In Lynch syndrome, a germline defect in either of the genes *MLH1*, *MSH2*, *MSH6* or *PMS2* causes loss of expression of the corresponding protein[17]. These are all proteins involved in the repair system of DNA inaccuracies, and if the mismatch repair system is deficient, the cells are unable to overhaul replication errors appearing in the DNA strand, leading to an accumulation of, predominantly, frameshift mutations[41]. In sporadic MSI, hypermethylation of MMR proteins, mainly MLH1, leads to the same phenotype as in Lynch syndrome, i.e. a deficient MMR (dMMR) system and an accumulation of mutations[42].

Molecular characterization

Consensus molecular subtypes

In order to create consensus and to facilitate comparison of research results, The CRC Subtyping Consortium has put forward a molecular classification system for the heterogenous disease of CRC, consisting of four consensus molecular subtypes

(CMS): CMS1(MSI, immune), CMS2 (canonical), CMS3 (metabolic) and CMS4 (mesenchymal).

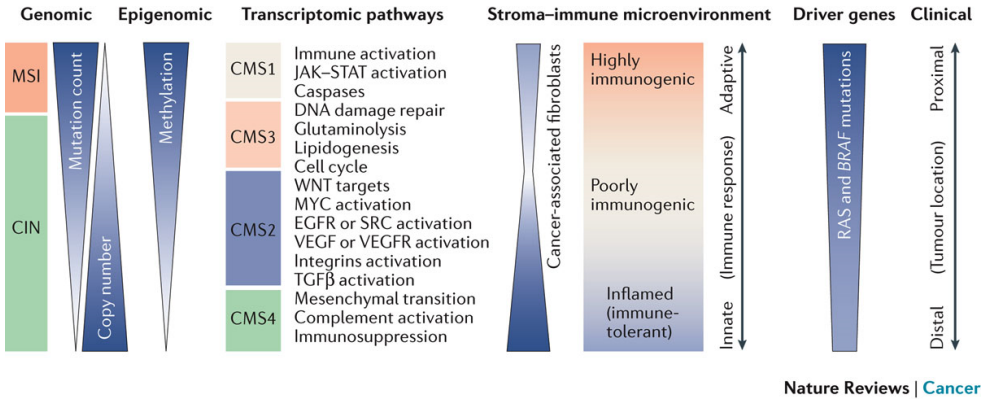


Figure 3. Schematic presentation of the consensus molecular subtypes[43]. Reproduced with permission of Springer Nature.

As seen in Figure 3, CMS1 is characterized by MSI/dMMR and therefore contains tumours with a high number of mutations and a high methylation level. The tumours are often situated in the proximal colon and are highly immunogenic. CMS1 includes approximately 15% of all CRC and the tumours are often BRAF-mutated[44]. CMS1 is associated with a shorter overall survival (OS) in the palliative setting, compared to the other subgroups[45]. However, if found early, patients with CMS1 tumours have a better prognosis than patients with tumours of other subtypes[46]. CMS2, also called *canonical*, includes tumours that develop through the earlier described classical pathway, with an adenoma developing to a carcinoma through stepwise occurring genetic events and activation of the WNT/β-catenin pathway. CMS2 represents approximately 40% of all CRC, and patients with CMS2 tumours have the best OS of all subtypes, regardless of tumour stage[44]. CMS2 tumours are often located in the distal colon and have higher levels of copy number alterations (CNA)[44]. The third CMS group, the *metabolic* subtype, contains around 15% of all CRC and is characterized by metabolic dysregulation in for example fatty acid and glutamine pathways. CMS3 is the subtype with the highest proportion of *KRAS* mutations, leading to epidermal growth factor receptor inhibitor (EGFRi) treatment resistance[44]. The metabolic dysregulation may, however, become a novel target for therapy, not only *per se*, but also as a path to overcome chemoresistance[47]. CMS4, the mesenchymal subtype, is characterized by a dense stromal infiltration and transforming growth factor-β (TGF-β) activation. These tumours often have high numbers of CNA but a low mutational burden. They develop from serrated adenomas and are often located in the distal colon[48]. CMS4 is the subtype with the worst 5-year OS, regardless of tumour stage[44].

Kirsten Rat Sarcoma Viral Oncogene Homologue

KRAS is a proto-oncogene located on chromosome 12[49]. The KRAS protein is a GTPase that in its activated state triggers the RAF/MEK/ERK/MAPK cascade involved in cell proliferation[50]. As seen in Figure 4, it also affects the PIK3A/PTEN/AKT pathway, that is involved in cell survival[51]. Point mutations in *KRAS*, predominantly in codon 12 and 13, are seen in around 35% of CRC[52], leading to a permanent activation of KRAS and persistent signalling of downstream pathways. *Neuroblastoma RAS* (*NRAS*) and *Harvey RAS* (*HRAS*) are two other genes in the RAS family. Mutations in *NRAS* and *HRAS* are rather uncommon in CRC and are seen in 3-5% and 2% of CRC cases, respectively[53 54], While *KRAS* mutations do not seem to be a prognostic biomarker in CRC in general, a prognostic value has been denoted in certain subgroups[55 56]. *RAS* mutation is, on the other hand, considered a negative predictive biomarker for EGFRi therapy, and extended *RAS* testing, including *KRAS* and *NRAS*, is recommended for CRC patients who are under consideration for anti-EGFR (epidermal growth factor receptor) treatment[57].

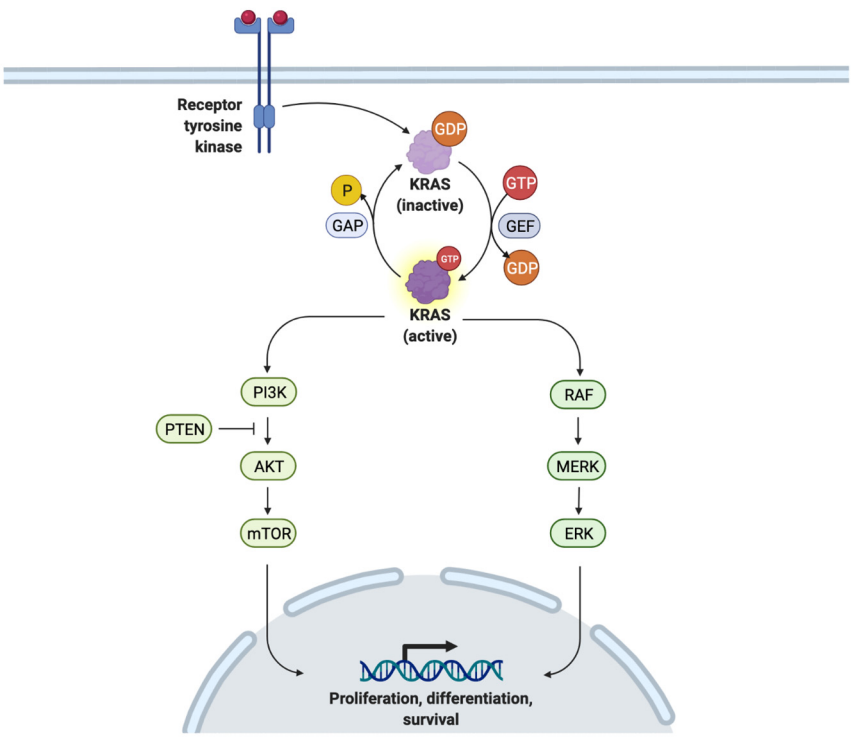


Figure 4. Schematic illustration of KRAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathway. Created with Biorender.com

Vraf Murine Sarcoma Viral Oncogene Homologue B1

The product of the proto-oncogene *BRAF* is also a part of the RAS/RAF/MEK/ERK/MAPK cascade, but acts downstream of *RAS*. Hence, it plays an important role in cell proliferation. *BRAF* is reported to be mutated in approximately 10% of CRC[58], though there are reports of a higher prevalence in unselected cohorts of patients with metastatic CRC[59]. The most common alteration in *BRAF* is a somatic point mutation leading to a V600E substitution, and activation of the MEK/ERK/MAPK cascade and downstream signalling [60]. *BRAF* mutation is associated with poor prognosis in stage IV CRC[61], especially in patients with microsatellite stable (MSS) disease[62]. *BRAF* mutations are also more prevalent in right-sided tumours and in female patients[63]. *BRAF* and *KRAS* mutations are most often mutually exclusive[64].

Microsatellite instability

Microsatellites are short repetitive sequences of DNA, less than 10 base pairs in length, that are found in clusters in non-coding regions throughout the DNA strands[65]. Microsatellites are thought to arise through mistakes made by DNA polymerases[66] and these errors can usually be corrected by proteins in the MMR system. However, as forementioned, Lynch syndrome, or downregulation of MMR proteins by gene promoter methylation, causes deficiency in the MMR system, leading to an accumulation of mutations. Tumours with a functioning MMR system are denoted as MMR proficient (pMMR), or MSS.

MSI status can be determined in a number of different ways. Immunohistochemistry (IHC) can be used to determine the expression of the four MMR proteins MLH1, PMS2, MSH2 and MSH6[67]. dMMR tumours often show a complete loss of at least one of the MMR proteins. Another way to determine MMR/MSI status is through the polymerase chain reaction (PCR) technique. Five different satellites are used as markers and the tumour is denoted as MSS if it shows stability in all markers, MSI-Low (MSI-L) if instability is found in one of the markers and MSI-High (MSI-H) if instability is found in two or more of the markers[67]. MSI-L tumours are denoted as MSS and are, in contrast to MSI/MSI-H tumours, not associated with a better prognosis in early stages of CRC[68 69].

Staging

The stage of the disease is the most important prognostic factor in CRC. In the curative setting, treatment decisions are mainly based on disease stage, with support by other factors such as vascular and lymphatic invasion[70]. The TNM system describes the anatomical extent of the disease based on three components: tumour extension (T), affection of regional lymph nodes (N) and presence of distant metastases (M), as seen in Table 1. The T, N and M can then be combined into disease stages (I-IV), as seen in Table 2.

Table 1. The TNM staging system according to the Union for International Cancer Controll (UICC), 8th edition[71]. Reproduced with permission from John Wiley and Sons.

Primary Tumour (T)		**Regional Lymh Nodes (N)**	
TX	Primary tumour cannot be assessed	NX	Regional lymph nodes cannot be assessed
T0	No evidence of primary tumour	N0	No Regional lymp node metastasis
Tis	Carcinoma in situ: invasion of lamina propria	N1	Metastasis in 1 to 3 regional lymph nodes
T1	Tumour invades submucosa	N1a	Metastasis of 1 regional lymph node
T2	Tumour invades muscularis propria	N1b	Metastasis in 2 to 3 regional lymph nodes
T3	Tumour invades subserosa or into non-peritonealized pericolic or perirectal tissue	N1c	Tumour deposits, without regional lymph node metastasis
T4a	Tumur perforates visceral peritoneum	N2	Metastasis in 4 or more regional lymph nodes
T4b	Tumour directly invades other organs or structures	N2a	Metastasis in 4-6 regional lymph nodes
		N2b	Metastasis in 7 or more regional lymph nodes
Distant Metastasis			
M0	No distant metastasis		
M1	Distant metastasis		
M1a	Metastasis confined to one organ		
M1b	Metastasis to more than one organ		
M1c	Metastasis to the peritoneum with or without other organ involvement		

Table 2. Stages according to The UICC TNM staging system UICC, 8th edition[71]. Reproduced with permission from John Wiley and Sons.

Stage	**T**	**N**	**M**
O	Tis	N0	M0
I	T1, T2	N0	M0
II	T3, T4	N0	M0
III	Any T	N1, N2	M0
IV	Any T	Any N	M1

The prognosis in early stages of the disease is good with a 5-year OS of 99% in stage I, decreasing to 68-83% for stage II and 45-65% for stage III without adjuvant treatment[70]. The outcome for patients with stage IV disease, i.e. metastatic CRC (mCRC), has improved in the last decades and the median OS in randomised trials is now around 30 months[57].

Metastasation

Invasion and metastasis are one of the *Hallmarks of Cancer*, first introduced in the year 2000 by Hanahan et al.[72]. It is also a great clinical problem since metastases are responsible for the vast majority of cancer deaths. Around 20% of the patients diagnosed with CRC have disseminated disease at the time of diagnosis and another 30% develop metastatic disease over time[57 73]. In mCRC, the median OS is approximately 20 months[74]

Metastasation befalls mainly through lymphatic or hematogenous spread. The most common target organs for metastasis vary between different cancer types[75]. An

explanation for this variation was suggested already in 1889 by Stephan Paget[76]. In short, he stated that, similar to plants, different seeds (tumour cells), prefer different soils (microenvironments). This hypothesis was however opposed by James Ewing, who suggested that the main factor determining the pattern of metastasis was the anatomy of blood and lymphatic vessels around the primary tumour, as reviewed in Langley et al.[77]. The truth, as we know it today, includes both these routes and much more. The exact mechanism behind the process of metastasis is not known, but the transformation of cells from an epithelial to a mesenchymal phenotype, also called epithelial-mesenchymal transition (EMT), seems to be of importance for initiation of the process. EMT can be triggered though changes in several different pathways, e.g. the WNT pathway, the RAS/RAF/MEK/ERK pathway, the PIK3A/AKT pathway or through downregulation of TGFβ[78]. When the cells have intravasated they are protected by platelets and immune cells when traveling through the body, to avoid attack from for example natural killer cells[79]. When the cells have extravasated, angiogenesis is of importance, among other components, in order for the cells to colonise the new environment[79].

The liver is the most common metastatic site in CRC and around 25% of CRC patients develop liver metastases over time[80 81]. Liver metastases have been reported to be more common in distal CRC, and those originating from proximal colon cancer to be associated with worse outcome[81].

Lung metastases affect approximately 10-15% of patients with CRC, and the risk of developing lung metastases is higher in rectal than in colon cancer[82]. This is probably due to that the venous drainage of the lower part of the rectum goes directly into the common iliac vein and then to vena cava inferior, whereas the venous blood drained from the colon goes through the portal vein before entering the vena cava, hence passing the liver before it reaches the lungs. Among CRC patients with metastases in a single site, lung metastasis has been shown be associated with a superior survival compared to all other sites[83].

Synchronous PC has been reported in approximately 5-10% of primary CRC[84-86]. Many patients with uncurable disease develop PC over time, but the absolute number is not known since it is often not reported when the disease has already disseminated to other sites. The median OS for patients with PC varies between 6-24 months in different reports, depending on the type of systemic treatment given[87-89]. The dissemination route to the peritoneum has been suggested to be of another nature than the usual lymphogenic or hematogenic routes. *The peritoneal metastatic cascade* starts with cells detaching from the primary tumour, either spontaneously or mechanically, e.g. upon surgery. When in the peritoneal cavity, the cells are subjected to the regular fluid transport occurring between the peritoneal layers. This transport befalls clockwise and is driven by changes in the abdominal pressure, gravity, and peristaltic movements of the intestine. The cells will then adhere to the peritoneum, either through attachment to the mesothelium, the inner layer of the

peritoneum, or through connection to the lymphatic system through lymphatic stromata. After adherence, the cells invade the sub-peritoneal space and start to produce growth and angiogenic factors[90 91].

Tumour heterogeneity and evolution

Tumour heterogeneity is a well-known phenomenon that is linked to tumour progression and treatment resistance[92]. The heterogeneity on a population level, interpatient heterogeneity, implies differences between tumours in different individuals, even though the tumours are of the same histological type[92], for example mutations in *KRAS,* seen in 35% of all CRC[52]. At the individual level, tumour heterogeneity can be investigated at one time point in different locations, i.e. spatial heterogeneity, or over time in one location, i.e. temporal heterogeneity[92]. Temporal heterogeneity is a consequence of evolution as well as of the evolutionary pressure of systemic treatment, leading to treatment resistance when all treatment-responsive cancer cells have died, allowing for clones with resistant cells to expand[92]. Spatial heterogeneity can be seen within the primary tumour (intratumour heterogeneity), but also within metastases (intrametastatic heterogeneity) and between metastases (intermetastatic heterogeneity). According to current clinical practice, treatment decisions are often based on a single biopsy taken at the time of diagnosis, that can be seen as a snapshot of the cancer in time and place. It is evident that this procedure does not accurately reflect the extent of tumour heterogeneity, which might lead to inaccurate use of targeted therapy. Intratumour heterogeneity has also been suggested to be a prognostic marker *per se,* and a high level of intermetastatic heterogeneity in colorectal cancer has been shown to be associated with shorter survival[93]. *KRAS* mutations have been reported to be homogenous within the primary tumour[94 95], but some studies have shown an intra- and intertumour heterogeneity regarding *KRAS* mutations[96 97]. The heterogeneity between primary tumours and distant metastases in CRC has mainly been investigated in liver metastases and the results have been incongruous. Many studies report a high concordance rate between the primary tumour and metastases regarding driver genes such as *KRAS*, *BRAF* and *PIK3CA*[98-100]. Nonetheless, there are also reports of a higher degree of heterogeneity of *KRAS* mutations in synchronous liver metastases and of private mutations occurring in metachronous lung metastases[101 102]. One study investigated the intertumour heterogeneity in peritoneal carcinomatosis and reported a high concordance regarding *KRAS* and *BRAF* mutations as well as MSI-status[103]. In summary, genetic alterations known to occur early in the colorectal carcinogenesis, i.e. *KRAS* mutations, display a low degree of heterogeneity, but the subject merits further investigation, not least in relation to the potential selective pressure of oncological treatment and the temporal evolution of resistant clones.

Treatment

The treatment of CRC has improved over the last decades, mainly due to refinement of surgical techniques, neoadjuvant radiotherapy and neoadjuvant and adjuvant chemotherapy, leading to enhanced survival in high income countires[13]. Hence, CRC treatment is today a multidisciplinary team effort, and both the European Society for Medical Oncology (ESMO) and the American Society of Clinical Oncology (ASCO) recommend multidisciplinary discussions, especially for patients with disseminated disease[57 104].

Surgery

Surgery is the foundation of CRC treatment. For shallow pedunculated tumours, endoscopic resection with proper follow-up is a good treatment option[105]. More infiltrative tumours require surgery. The resection aims to remove the tumour and adjacent lymph nodes. In colon cancer, the extent of the resection depends on the lymphovascular drainage in the tumour area, but should encompass a segment of at least 5 cm of the colon on each side of the tumour[70]. It is also important to inspect, and if possible palpate, the abdominal and pelvic organs and the peritoneal cavity to ensure absence of metastases. Partial colectomy can, by virtue, be performed laparoscopically, with preserved oncological outcome but faster post-surgery recovery compared to open surgery[106 107]. In patients with intermediately advanced rectal cancers, total mesorectal excision (TME) is preferred, including excision of the entire mesorectal fat and lymph nodes, preceded by radiotherapy if necessary[108]. Patients with locally advanced rectal tumours should always receive neoadjuvant treatment with chemoradiotherapy (CRT). The surgical procedure depends on the extension of the tumour growth, but should at least include TME[108].

In about 15-20% of all CRC cases, surgery needs to be performed ahead of scheduled procedure due to obstruction, perforation, or bleeding[109 110]. Acute surgery has been associated with reduced OS compared to elective surgery and is often considered in decisions regarding adjuvant treatment[70 111]. An increased in-hospital mortality has also been shown for patients having undergone emergency surgery compared to patients who had an elective resection, although there was no difference in 5-year OS between the groups[110].

Cure is possible in patients with stage IV disease, but the selection of patients for metastasectomy is of great importance. The approach to patients with metastases should be multidisciplinary, and both technical and prognostic factors should be considered.

There are several factors being proposed as prognostic regarding metastasectomy in the liver. Fong et al. introduced a scoring system including node-positive primary tumour, synchronous disease (<12 months), more than one liver metastasis, liver metastasis > 5 cm and CEA level >200ng/ml, showing a 60% OS in the group with 0 points and 14% in the group with 5 points[112]. Hence, a thorough selection of

patients for liver surgery of CRC metastases is important. Yet, the disease specific 10-year survival has been reported to be 35%[113]. The arsenal for treatment of liver metastases includes neoadjuvant chemotherapy, surgical resection, and ablative treatment. Neoadjuvant treatment can be given to shrink the metastases to enable liver surgery (conversion treatment), or to evaluate the chemosensitivity of the disease, which is another strong prognostic factor[57]. For a patient with an upfront resectable disease, and favorable prognostic criteria, resection can be made without preoperative treatment[57]. Ablative treatments, for example radiofrequency ablation, microwave ablation and irreversible electroporation, can be combined with surgery to achieve local ablation of the metastases[57]. Liver transplantation for patients with non-resectable disease but without extrahepatic metastases can be a future treatment option, and in the ongoing SOULMATE study (NTC04161092), patients with non-resectable and non-ablatable liver metastases are randomized between liver transplantation and best alternative care.

Pulmonary metastasectomy is indicated if the patient is in good general condition, the primary disease is under control, any extrapulmonary metastases can be remedied and the pulmonary metastases are thought to be completely resectable[114]. There is one randomized study comparing metastasectomy with systemic treatment, however the study was closed in advance due to poor recruitment. The analysis encompasses 93 patients, and the median survival was 3.5 years in the group that underwent metastasectomy and 3.8 years in the control group[115]. As PM is established in clinical practice, randomized studies to evaluate the efficacy of the procedure are hard to conduct.

Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) is an established curative treatment for patients with peritoneal carcinomatosis. The procedure includes resection of the peritoneum followed by rinsing of the peritoneal cavity with heated oxaliplatin for 30 minutes. The procedure is extensive, often takes several hours and intensive care is often required after surgery. Two prognostic factors have emerged as important; the completeness of the cytoreduction and the extensiveness of the carcinomatosis calculated by the standardized peritoneal carcinomatosis index (PCI) score[116]. The index ranges from 0-39 and takes into account the location and the extension of the carcinomatosis[117]. A PCI score >20 is often considered a contraindication for CRS and HIPEC[118]. One randomized trial compared CRS and HIPEC with oxaliplatin to CRS alone[119]. The addition of chemotherapy did not enhance the median OS, that was 41.7 months in the HIPEC arm and 41.2 months in the arm with CRS only. However, the group with a PCI score ranging between 11-15 had a survival benefit from HIPEC[119].

Radiotherapy

Radiotherapy (RT) is an important part of rectal cancer treatment. Preoperative short-course RT of 5 Gray (Gy) x 5, followed by surgery the week after, has been shown to reduce the risk for local recurrence by half and has been standard since the

1990s for intermediate tumours [120]. Locally advanced tumours, associated with a higher risk of local and systemic recurrence, have been treated with CRT including 45-50 Gy in 25-28 fractions, accompanied by 5-fluorouracil (5-FU) intravenous infusion or oral capecitabine as radiosensitiser[108]. In the RAPIDO study, patients with locally advanced rectal cancer were randomized to short-course RT followed by CAPOXx6 or FOLFOXx9 accompanied by TME or 50.0-50.4 Gy on 25-28 fractions with concomitant capecitabine followed by TME[121]. The results showed a significantly decreased risk of disease-related treatment failure in favor of the experimental group, and short-course RT followed by systemic chemotherapy before surgery is now the new standard treatment for locally advanced rectal cancer.

For pulmonary metastases not suitable for surgery, stereotactic body radiation therapy (SBRT) might be an option. A systematic review of 18 studies by Cao et al. presented an estimated local control of 81% one year and 60% three years after SBRT of pulmonary metastases. The estimated 3-year progression-free survival (PFS) and OS , based on 6 and 11 of the studies, was 13% and 52% respectively[122]. Among the 18 studies included in the analysis, the dose of the given SBRT varied between 21 Gy in one fraction to 60 Gy in five fractions.

Chemotherapy

5-fluorouracil is an important component in medical oncological treatment of CRC. 5-FU is an anti-metabolite where a fluorine has been substituted with a hydrogen at the C-5 position of the nucleic acid uracil[123]. The working mechanisms of 5-FU are multitudinous and complex, and one mechanism of action is blocking of the enzyme thymidylate synthase, leading to reduced thymine formation and further on to inhibition of DNA synthesis[124]. 5-FU is potentiated by folinic acid, often given concomitantly[125]. 5-FU can be given through different administration routes; injection, bolus injection, continuous infusion over several days or orally in the form of the precursors capecitabine or tegafur. The most common side effects of 5-FU are mucositis and diarrhea

Oxaliplatin is a platinum derivative, discovered by Kidani et al. in 1978[126]. The mechanisms of action include formation of crosslinks within and between DNA strands as well as between DNA strands and proteins[127]. The crosslink causes inhibition of DNA replication and transcription, leading to cell cycle arrest and apoptosis[128]. Oxaliplatin alone has limited effect on CRC and is given in combination with 5-FU. This combination was first shown to give a significantly prolonged PFS in comparison with 5-FU alone (9.0 vs 6.2 months respectively) in mCRC[129] and is now a standard combination in adjuvant and palliative treatment. A clinical problem with oxaliplatin is the often dose-limiting side effect of peripheral neuropathy, and approximately 15% of the patients get grade III side effects according to WHO, i.e. intolerable paresthesia and/or marked motor loss, problems that can become chronic[130].

Irinotecan was first synthesized by Kunimoto et al. in 1987[131]. In the body, irinotecan is metabolized to its active metabolite SN-38, which in turn inhibits topoisomerase I leading to DNA strand breaks and apoptosis[132 133]. Irinotecan can be administered as a single agent but is often given in combination with 5-FU and occasionally oxaliplatin. Two common side effects are neutropenia and diarrhea. The diarrhea can be acute, as part of an acute cholinergic syndrome, which can be prevented by prophylactic administration of atropine sulphate[133].

TAS-102 is a cytotoxic combination of trifluridine and tipiracil hydrochloride that was authorised in the European Union in 2016[134]. Trifluridine is incorporated into the DNA, disturbing the DNA function and thereby preventing cell proliferation[135]. Tipiracil prevents the degradation of trifluridine, leading to an increased cytotoxic effect[136]. TAS-102 is authorised for patients with mCRC who have received former treatment and common side effects are bone marrow suppression and diarrhea[134].

Targeted treatment

There are several targeted therapies available for treatment of CRC. EGFRi are potent blockers of the tyrosine kinase receptor and its downstream signaling pathway RAS/RAF/MEK/ERK[137]. EGFRi have little effect on *KRAS* mutated tumours and are therefore only indicated for treatment of *KRAS* wild-type tumours[138]. Vascular endothelial growth factor (VEGF) is involved in angiogenesis, and plays an important role in tumour growth and metastasis[72]. Bevacizumab is a monoclonal antibody directed against VEGF, inhibiting its angiogenetic effect[139], that is indicated in patients with metastatic colorectal cancer. Side effects include hypertension, venous thromboembolism and hemorrhage, especially tumour associated[139]. There are other targeted therapies directed towards the vascularization of the tumour, such as aflibercept, directed towards VEGF, that is approved in combination with FOLFIRI for patients with mCRC who have shown resistance to oxaliplatin containing treatment. Ramucirumab is a monoclonal antibody directed towards the VEGF receptor, that is approved in combination with FOLFIRI for treatment of mCRC patients who have progressive disease after treatment with 5-FU, oxaliplatin and bevacizumab[140].

Pembrolizumab is a monoclonal antibody that binds to the programmed death -1 (PD-1) receptor, thus blocking binding of its ligands programmed death binding ligand 1 and 2 (PD-L1 and PD-L2), which in turn leads to an activated immune response. Treatment with pembrolizumab is indicated in patients with dMMR CRC[141]. In the Keynote-177 study, patients with MSH-H/dMMR metastatic disease were randomized to pembrolizumab or chemotherapy, and the median PFS was 16.5 months in the former compared to 8.2 months in the latter group[142].

Regorafenib is a multi-kinase inhibitor indicated for treatment of patients with mCRC who are not eligible for or have had disease progression on other available agents[143]. In the randomized double-blinded CORRECT trial, the Regorafenib arm

showed a median OS of 6.4 months compared to 5.0 months in the placebo group, a statistically significant difference of 1.4 months. The major side effects were reaction in the skin of the hands and feet, and fatigue[144].

Patients with tumours that are *BRAF*-mutated often have a poor prognosis and limited responses the therapy[61]. The BEACON trial investigated treatment options for patients with *BRAF*-mutated tumours[145]. The trial contained three groups: encorafenib and cetuximab with or without binimetinib or irinotecan/FOLFIRI in combination with cetuximab (control group). Median OS for patients receiving the triplet encorafenib/cetuximab/binimetinib and the doublet encorafenib/cetuximab was 9.3 months, whereas the median OS was 5.9 months in the control group[145]. The results led to a new standard of care for patients with *BRAF*-mutation.

Amplification of the *HER2*-gene is uncommon in CRC, with a prevalence of around 2%[146]. Phase II trials have reported response to dual HER2 blockade in CRC patients with *HER2* amplification, but treatment with HER2 blockade in mCRC must still be seen as experimental and should preferably be conducted within clinical trials[147] [148].

Neoadjuvant treatment

Neoadjuvant therapy is commonly used in a number of different cancer types, e.g. breast cancer, gastric cancer and oesophageal cancer. There is no tradition of neoadjuvant treatment in colon cancer, whereas it is often used in rectal cancer, as previously mentioned. The FOxTROT trial compared 6 weeks of neoadjuvant FOLFOX followed by surgery and 18 weeks of adjuvant FOLFOX with surgery and then 24 weeks of FOLFOX in patients with colon tumours that were staged to T3-4, N0-2 and M0 with computed tomography[149]. The number of incomplete resections was significantly lower in the group that had received neoadjuvant treatment. After two years of follow up, 14% of the patients in the group that had received neoadjuvant treatment and 18% in the control group had suffered a relapse, and the difference was not significant[149]. In the metastatic setting, a study comparing perioperative treatment with FOLFOX together with liver surgery versus liver surgery alone in patients with resectable liver metastases showed a prolonged PFS for the group that received perioperative chemotherapy[150]. No difference in OS was seen between the groups, however the study was designed with PFS as primary endpoint.

When a tumour or metastases are borderline resectable, down-sizing of the tumour is needed to enable surgery. The aim of such a treatment is to shrink the tumour. Since only a few studies have been conducted, the best chemotherapy regimen, or combination thereof, for conversion treatment remains to be established. However, guidelines recommend a cytotoxic doublet of 5-FU and oxaliplatin or irinotecan (FOLFOX/FOLFIRI) plus an EGFRi antibody for patients with *RAS* wild-type disease[57]. Another treatment option, and the primary choice for patients with *RAS*

mutations, is a cytotoxic doublet or triplet (FOLFOXIRI), sometimes given in combination with bevacizumab, even though the role of bevacizumab in the conversion setting is yet unclear[57].

Adjuvant treatment

The aim of adjuvant treatment is to reduce the risk of recurrence. A thorough discussion should be kept with the patient regarding risk of side effects, benefits of treatment and risk of recurrence before a treatment decision is made. TNM staging is still the most important factor in risk assessment after CRC surgery. The 5-year OS is reported to be 99% after surgery alone in patients with stage I disease, whereas it is 45-65% for patients with stage III disease[70]. MSI/MMR status is an important prognostic factor for patients with stage II disease since patients with MSI/dMMR disease have a much better survival compared to patients with MSS/pMMR tumours, and do not benefit from 5-FU treatment alone[151 152]. It is also important to retrieve at least 12 lymph nodes for assessment, given the risk of missed metastases if fewer nodes are evaluated[153]. For patients with stage II disease, additional factors such as lymphatic, venous or perineural growth or involvement of margins and serum CEA levels should be considered[70]. Guidelines divide stage II disease into three groups: low-risk with no pathological risk factors, intermediate-risk with single pathological risk factors, and high-risk including T4 tumours, <12 lymph nodes assessed or presence of multiple pathological risk factors. Patients with low-risk stage II disease are not recommended adjuvant treatment. Patients with intermediate risk whose tumours are MSI/dMMR are also appraised not to benefit from adjuvant treatment, whereas patients with intermediate risk and MSS tumours are recommended 6 months of 5-FU treatment. Patients with high-risk tumours should be offered 5-FU and oxaliplatin, either as FOLFOX for 6 months or as CAPOX for 3-6 months, and the same recommendation is given for patients with stage III disease[70]. Adjuvant treatment should start within 8 weeks after surgery in order to accomplish benefit[154]. The scientific evidence for adjuvant treatment after rectal cancer is not as strong as for colon cancer and the benefit is probably not as high[108].

Palliative treatment

When planning a palliative treatment, many factors should be considered, such as the disease dynamics, the patient´s attitude towards treatment and the toxicity of the treatment.

Standard first line treatment often consists of 5-FU, or capecitabine alone or in combination with either oxaliplatin or irinotecan. An improved response rate and PFS have been shown for cytotoxic doublet compared to 5-FU alone[129 155]. The anti-EGFR antibodies cetuximab and panitumumab as well as the anti-VEGF antibody bevacizumab have been shown to improve the outcome, either as prolonged PFS or OS[155-159]. However, and of note, patients with *KRAS*-wt tumours primarily located

in the proximal colon have been shown to respond more poorly to cetuximab than patients with *KRAS*-wt tumours located in the distal colon[160 161].

In recent years, maintenance therapy has been proposed to be an appealing concept after a period of induction therapy. Maintenance therapy refers to a de-escalation of treatment, especially for patients receiving oxaliplatin in first line, for example 5-FU together with bevacizumab[162]

For second line treatment, a switch to the cytotoxic agent not used in the first line should be made, e.g. first line FOLFOX should be followed by FOLFIRI, and vice versa [57]. If bevacizumab was not used in the first line, it should be considered in the second line[163]. Some patients might benefit from a third and fourth line of treatment with for example regorafenib, TAS-102 or EGFRi single or in combination with irinotecan[57].

As earlier mentioned, the KEYNOTE-177 study showed an improved PFS for patients with MSH-H/dMMR disease receiving pembrolizumab compared to those receiving chemotherapy in first line. Although not being included in all official guidelines yet, pembrolizumab will become a first line treatment for patients with MSI-H/dMMR disease[142].

Investigative biomarkers

RNA-binding motif protein 3

The transfer of information from gene to protein goes through ribonucleic acid (RNA). RNA is synthesized with DNA as a template, a process called transcription, in the cell nucleus. A 5´cap, a modified guanine nucleotide, is added to the first transcribed nucleotides of the RNA molecule directly after transcription. The cap aims to enable RNA recognition for the protein synthesis units, the ribosomes, and furthermore to ensure that the reading of the RNA molecule is made in the right direction. After its formation, the RNA molecule needs to undergo splicing, a process to remove RNA sequences not necessary for the creation of proteins. Since RNA is formed in the cell nucleus, it needs to be transported from the nucleus to the cytoplasm, where the protein synthesis takes place. In the cytoplasm, ribosomes translate the RNA code into proteins by deciphering the code and putting together the appropriate amino acids to the correct protein chain. The process from transcription to translation is regulated by RNA-binding proteins (RBP)[164]. To this day, several hundred RBPs are known, and the number keeps growing[165]. As abovementioned, RBPs are involved in the processing, modification, localization and translation of RNA, and they also increase the stability of the RNA molecule[165].

RNA-binding motif protein 3 (RBM3) is an RBP with an RNA recognition motif, a structure known to enable RNA binding[166]. The *RBM3* gene is situated on the short arm of the X chromosome[166]. RBM3 expression increases in response to mild hypothermia, and hibernating animals have been shown to have increased levels of RBM3[167]. Increased expression of RBM3 has also been seen in response to hypoxia[168 169]. However, the underlying mechanism behind RBM3 upregulation is not known. RBM3 is known to be involved in global protein upregulation. For example, RBM3 can bind to the *COX-2* and *VEGF* genes and alter the translation of their messenger RNA (mRNA)[170 171]. RBM3 can also interact with the 60s subunit of ribosomes, leading to globally enhanced protein synthesis. Furthermore, RBM3 is involved in the Wnt/β-catenin signalling pathway, that is important during embryonic development and in cell differentiation, indicating an association between RBM3 and stemness[172 173]. The RBM3 protein is also involved in cell cycle progression, especially in the transition from the G2 to mitosis phase[171 174]. siRNA-mediated knockdown of RBM3 in colon cancer cells lines resulted in a decreased cell growth[171], and mice deficient in RBM3 showed a delayed proliferation and an increased population of cells in the G2-phase of the cell cycle[174].

With the molecular function of RBM3 and its response to hypoxia in mind, several studies have addressed the role of RBM3 in cancer. In several different cancer types, for example breast cancer, epithelial ovarian cancer, prostate cancer, and CRC high expression of RBM3 has been associated with an improved clinical outcome[175-178]. The mechanism behind the prognostic value of RBM3 is however not yet clear. The overexpression of RBM3 in cancer tumours might be driven by hypoxia, often seen in tumours, and, as previously mentioned, RBM3 has been shown to regulate the Wnt/β-catenin pathway and to induce stemness in CRC cells *in vitro*[169 172]. There are also suggestions that RBM3 expression is a predictor of response to treatment with platinum based chemotherapy in e.g. epithelial ovarian and pancreatic cancer[175 179], and an association between RBM3 and processes involved in DNA maintenance have also been demonstrated[180]. Another explanation for the relationship of RBM3 with chemotherapy sensitivity might be its involvement in cell cycle progression[181].

Special AT-rich sequence-binding protein 2

Special AT-rich sequence-binding protein 2 (SATB2) is a protein encoded by the *SATB2* gene located at the long arm of chromosome 2[182]. The protein has a molecular weight of 85.5 kDa and is preserved across different species of vertebrates[183 184]. SATB2 is a protein that is part of the nuclear matrix and binds to AT-rich sequences at the DNA strand, being referred to as matrix-attachment regions (MAR). MAR binding proteins are involved in chromatin organization, an important part of the transcription process[185]. Mice with homozygous knockout of *SATB2* die directly after birth[186]. Mutated mice show a shorter lower jaw compared to normal controls, and also present with multiple malformations in the facial

skeleton[186]. Similar findings have been reported for humans, where *SATB2* mutations have been associated with cleft palate[182]. *SATB2* also plays a role in differentiation of neurons and stem cells[187 188]. Regulation of *SATB2* occurs partly through microRNAs (miRNAs), short RNA sequences not translated into proteins, but involved in gene regulation[189].

SATB2 has been presented as a promising diagnostic biomarker for CRC since it is only expressed in glandular cells lining in the lower gastrointestinal tract[190-192]. In combination with cytokeratin 20, SATB2 has been shown to identify 95% of all colorectal carcinomas[192]. SATB2 has also been shown to be expressed to a limited extent in tumours of other origin, for instance in periampullary cancer and adenocarcinoma of the upper gastrointestinal tract[193 194]. Moreover, high SATB2 expression in CRC has been demonstrated to be an independent prognostic biomarker of improved prognosis and benefit from adjuvant treament[195]. High expression of SATB2 has also been shown to be a favourable prognostic marker in mCRC, and to be associated with a prolonged PFS in patients who received irinotecan-based chemotherapy in first line[196]. The mechanism behind the connection between SATB2 and enhanced survival is not known, but miRNA-31 expression can be a part of the explanation. Elevated miRNA-31 expression has been associated with poor prognosis in CRC, and it has also been shown to be involved in SATB2 regulation[197], leading to reduced SATB2 mRNA and protein levels[183].

Aims of the thesis

The general aim of this thesis was to study the prognostic and predictive impact of selected biomarkers, with particular focus on RBM3, in mCRC. Another aim was to perform an extensive mapping of the spatial molecular heterogeneity in CRC with peritoneal carcinomatosis.

The specific aims of each paper are listed below:

- To evaluate RBM3 as a prognostic factor in mCRC, overall an in relation to the choice of first-line chemotherapy (Paper I)
- To examine prognostic factors, including the expression of RBM3, in colorectal lung metastases and paired primary tumours (Paper II)
- To explore the degree of genetic spatial heterogeneity in CRC disseminated to the peritoneum (Paper III)
- To explore the spatial heterogeneity of MMR-proteins, RBM3 and SATB2 in CRC disseminated to the peritoneum (Paper III)
- To design a clinical study aimed at generating real-world data on the evolutionary progression of mCRC during treatment with curative intent, to gain further insight into the mechanisms underlying therapeutic failure (Paper IV)

Methodological considerations

The detailed methods are presented in the original papers. Therefore, the methods are only briefly presented herein and discussed.

Patient cohorts

This thesis is based on four different patient cohorts. The cohort in paper I consists of 798 patients and was initially created to study trial inclusion among patients with mCRC[198]. The cohort originally included all patients with mCRC referred to the oncology departments in Odense University Hospital, Uppsala University Hospital and Haukeland University Hospital between October 2003 and August 2006, but was later expanded, through regional cancer registries, to also include patients within the catchment areas who were diagnosed with mCRC but not seen at any oncology department. Sufficient tumour tissue for tissue microarray (TMA) construction was available in 462 of the 798 cases. This cohort is an attempt to mirror the true group of patients with disseminated CRC, since the patients included in trials are in general often a selection of patients with e.g. enhanced performance status and of younger age[199 200]. Among the 462 patients included in paper I, 35% had a performance status according to WHO of 2 or more, a patient group often excluded from clinical trials[199]. Out of the 462 cases, 75% had a primary tumour located in the colon and 25% in the rectum, which is in line with the numbers reported in the litterature[201]. The location of the primary tumour was in the proximal colon in 40% of the cases, and in other studies, this number varies between 20%-40%[202 203]. The prevalence of *BRAF* mutation in this cohort is 20%, which is considerably higher than in other studies, where the reported incidence is between 5%-12%[204 205]. However, the higher incidence of *BRAF* mutations in this cohort may well reflect the true incidence in mCRC, since *BRAF* mutations are associated with poor prognosis and these patients are probably underrepresented in clinical trials[206]. No interventions were conducted to this cohort, the original study was strictly observational. The treatment decisions were taken by the patients´ physician. There are many different factors to take into account when treatment decisions are made, such as the patient's will, performance status and symptoms. In order to compare two equivalent groups, the patients who had received combinational chemotherapy in first-line were dichotomised according to the agent given together with 5-FU i.e.

irinotecan or oxaliplatin. Out of the patients who received combination chemotherapy in first-line, 25% had received adjuvant chemotherapy prior to diagnosis of metastatic disease. Adjuvant chemotherapy for CRC often includes oxaliplatin, but for this cohort, there was no information regarding the agent given in the adjuvant setting. However, a majority of these patients received oxaliplatin as part of the first-line palliative chemotherapy, indicating that they had not received oxaliplatin as adjuvant treatment, or that a long period of time had elapsed since they received adjuvant therapy. Since irinotecan and oxaliplatin are considered comparable choices for first-line treatment, we have no reason to assume any obvious differences between these two groups[57].

The retrospective consecutive cohort in paper II consists of 216 patients with CRC metastasised to the lung, who underwent curative pulmonary surgery between 1st of January 2000 and 31st of December 2014 at Lund University Hospital. In this cohort, 57% of the patients had a primary tumour located in the rectum, a much higher incidence than in a cross-sectional cohort of mCRC. A higher incidence of rectal cancer is however in line with the expected in a cohort of patients with lung metastases since, as beforementioned, the lungs are the most common dissemination location for rectal cancer, probably due to the venous drainage of the rectum[82]. Out of the 216 patients, 40% were female, in comparison to 50% in cohort I. This might also be explained by the anatomical location of the primary tumour, since proximal tumours are more common among females[48]. The cohort in paper II also differs from the cohort in paper I, in that the patients are selected for curative treatment of their disseminated disease. In order to be subjected to curative surgery you need to have a good performance status and your disease has to be limited and under control.

Paper III is based on a cohort of seven patients who underwent CRS and early-post-operative intraperitoneal treatment (EPIC) or HIPEC for colorectal cancer disseminated to the peritoneum. None of the patients had received adjuvant or neoadjuvant chemotherapy, which was a selection criterion since we wanted to investigate the spatial heterogeneity in tumours unaffected by a potential selection pressure from treatment. Four of the patients had tumours originating in the proximal colon, which is in line with the reported location of the primary tumour in patients with PC[84]. Even though the cohort only consists of seven patients, the comprehensive sampling of tumour tissue from each patient (in total 88 samples, range 5-19) should provide a thorough map of the spatial heterogeneity in CRC patients with PC, even though no statistical conclusions can be drawn. As far as we know, this is the most extensive mapping of curatively treated patients with PC that has been conducted to date.

The study population in paper IV will consist of mCRC patients with synchronous disease selected for curative treatment regardless of the location of the metastases. The patients will be identified at a multidisciplinary tumour board meeting, where all potentially curable patients in the catchment area are discussed. We chose to include all curatively treated patients with mCRC in the study, even though the

cohort will be rather diverse, with a mix of neoadjuvant and adjuvant treatments given. Even though curative treatment of stage IV disease is now part of clinical guidelines, the scientific evidence, especially for pulmonary metastasectomy, is low and the optimal treatment regimen remains to be established. Even though the patients receiving curative treatment for stage IV CRC are thoroughly selected, only 20%-50% are cured[207], implicating that a deeper understanding of the diverse biology underlying metastatic disease is important in order to offer the best possible treatment for each patient. At first, we considered including all mCRC patients, no matter the treatment intention and timing of the metastasation. However, such a study population would be greatly divergent, and it would be hard to draw any conclusions from the results.

Tissue microarray

The TMA technique enables easy assessment of protein expression in multiple tumours. It was first described by Kononen et al. in 1998 and includes a gathering of tissue cores from different donor blocks into a recipient paraffin block that can be cut into thin slices and mounted on microscope slides (Figure 5)[208].

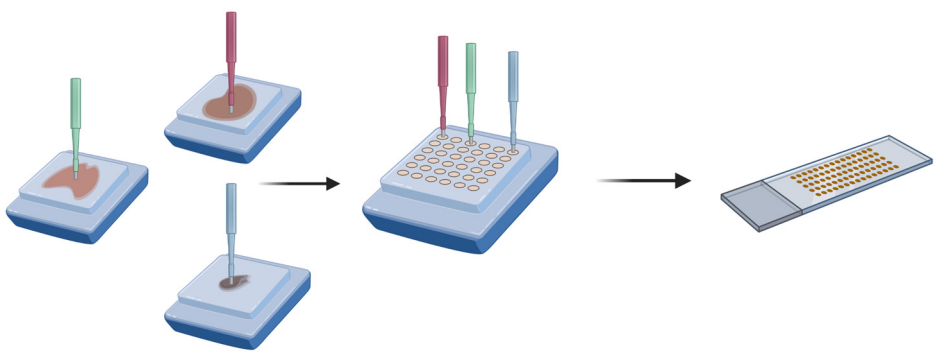


Figure 5. Schematic illustration of the TMA technique. Created with BioRender.com

This is an efficient method to facilitate assessment of protein expression, for example cancer biomarkers, in multiple tissue specimens, and the analyses are cheaper and less time and tissue consuming than staining and evaluation of whole tissue sections. Moreover, since all tissue samples are stained at the same time, the TMA technique minimises the potential intra-laboratory variation seen when a particular staining is conducted at different time points. One common criticism of the TMA technique is that the small samples do not accurately reflect the true

protein expression in the whole tumour, and that a heterogenous expression would be underestimated. However, comparisons of biomarker expression between TMA cores and whole tissue sections assessment have been conducted and show a good concordance[209 210]. It is however important to carry out a thorough evaluation of the morphology and quality of the donor tissue prior to TMA construction, in order to denote potential intra-tumour heterogeneity and exclude necrotic areas. Another way to minimise the risk of inaccurate biomarker expression assessment is to obtain more than one core from each donor block, and preferably also cores from multiple donor blocks. For construction of the TMA in paper I of this thesis, two cores were obtained from each case, and the cores were in the vast majority of the cases retrieved from the primary tumour. For construction of the TMA in paper II, two cores were taken from each metastasis and paired primary tumour, respectively. In paper III and in the prospective study described in paper IV, "single patient tissue chips" (SPTC) were or will be created, i.e. a compilation of multiple cores from different entities such as primary tumour, lymph node metastases and distant metastases from one patient into one TMA block. This enables a comprehensive evaluation of biomarkers and gives a good overview of the intra-patient tumour heterogeneity.

Immunohistochemistry

Immunohistochemistry is a tissue-based method widely used within biology to visualise and localise antigens with antibodies (Figure 6). The antigen is often a protein located in one or more compartments of the cell, e.g. the membrane, the cytoplasm or the nucleus. The antibody used to bind the antigen, i.e. the primary antibody, is usually of IgG class and can be either monoclonal or polyclonal[211]. Monoclonal antibodies bind only to one epitope of the antigen, making them specific, whereas polyclonal antibodies can bind to several epitopes, connoting higher sensitivity for the antigen[211]. Small changes in the epitope can impair the binding ability of a monoclonal antibody, while the binding capacity of a polyclonal antibody is less affected by changes in one epitope[212]. Monoclonal antibodies are produced in hybridomas, making the availability reliable once the hybrid cell line is in place. Polyclonal antibodies can differ over time since they are generated in different animals, and the availability of polyclonal antibodies depends on the size and lifespan of the animal used for its generation[212]. In order to detect the antigen and primary antibody complex, a secondary antibody can be used, that carries chromogen molecules effectuated by a polymer, making it possible to detect the antigen in a light microscope.

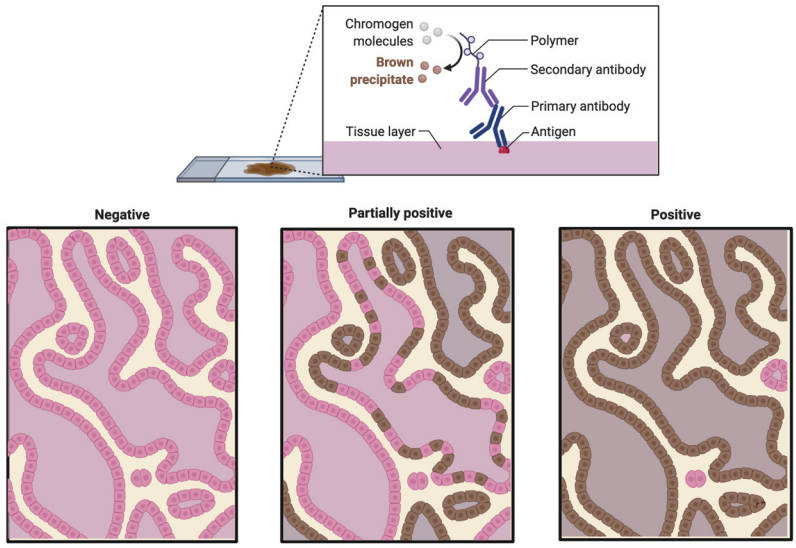


Figure 6. Schematic illustrations of IHC. Created with BioRender.com

The primary antibodies used in paper I, II and III were all monoclonal, well validated antibodies[177 213].

When interpretating IHC expression one should be familiar with the functionality of the studied antigen in order to enable a correct interpretation and minimise false positive results. It is also important to have external controls, e.g. cores from tissues for which the antigen expression is known, in the panel[214]. This could for example be mucosa from the lower gastrointestinal tract for assessment of SATB2[190]. If there are known internal controls it is also important to denote these during evaluation of IHC expression, in order to validate the staining[214]. For MMR-proteins, a nuclear staining reaction should be seen in e.g. lymphocytes and stromal cells[215].

For biomarkers not yet used in clinical practice, there are no established definitions of what is a positive or negative IHC result or an optimal cut off between high and low expression. This leads to difficulties when comparing results between different studies[216]. In order to implement a biomarker in the clinic, a standardized method for evaluation is of great importance. In paper I, we investigated both the nuclear and cytoplasmic expression of RBM3, since RBM3 can be expressed in both these cellular compartments[171]. We also dichotomized cytoplasmic and nuclear expression in two different ways, negative or positive expression and high or low expression, respectively. The cut off for high and low expression was determined with classification and regression tree (CRT) analysis. In paper II, we only validated the nuclear expression of RBM3, since the nuclear expression has more evidence as

a prognostic marker. The cut off used in paper II to dichotomize the expression into high and low was also determined with CRT analysis.

When evaluating IHC staining it is of importance to be blinded from group data, since knowledge about the patient outcome, for example, can lead to unintentional bias. It is also important to be aware of the so called "diagnostic drift" that may occur when one person evaluates a large cohort or the evaluation is performed during an extended period of time[217], leading to a gradual change in the assessment of the IHC expression over time. In paper I, II and III at least two persons have independently evaluated the IHC expression without knowledge of the clinical data. Differences in the scoring were discussed to reach consensus, in order to diminish inter-observer discrepancies.

Next-generation sequencing

Genetic sequencing is applied to determine the nucleotide sequence in DNA or RNA strands. The first generation of sequencing was the so called Sanger sequencing, described in 1977 by two time Noble Prize winner Frederick Sanger et al.[218]. Sanger sequencing was used when the human genome was first sequenced, through the Human Genome Project [219], that took 15 years and 3 billion US dollars to complete [220]. Next-generation sequencing (NGS) is a development of the Sanger technique that is much more efficient and less expensive. The human genome can now be sequenced within 24 hours and the cost is around 1000 US dollars per genome[221].

Sequencing, as seen in Figure 7, starts with DNA extraction followed by quantitation of the DNA to measure the amount of DNA available for sequencing. The extracted DNA is then prepared for sequencing through *library preparation* (Figure 7A*)*, a step including fragmentation of DNA and addition of adaptors to both ends of the fragments in order to make them compatible with the sequencer. The adaptors can be molecular barcodes used to identify fragments from a certain individual or sequences that bind to the surface of the platform used for the sequencing. The library preparation is often combined with target enrichment, a method used to select the DNA regions of interest[222]. The sequencing itself starts with a *cluster generation* (Figure 7B) to amplify the DNA fragments, thereby making the sequencing signal large enough to enable detection. In paper III, the NGS was carried out with an Illumina sequencer using *sequencing by synthesis* (Figure 7C), a method where fluorescently tagged nucleotides bind to the DNA template strand[223]. Apart from the fluorescent tag, each nucleotide contains a terminator ensuring that only one nucleotide at a time is added. The fluorescent signal indicates which nucleotide has been added, after which the terminator is cleaved, thus making it possible for the next nucleotide to bind[223]. During each round, one base pair per cluster is read. The DNA fragments can be read from both

ends, so called paired end sequencing[222]. The raw sequencing data are then aligned to a reference genome and data analysis is performed (Figure 7D), including quality analysis and deletion of PCR duplicates[222].

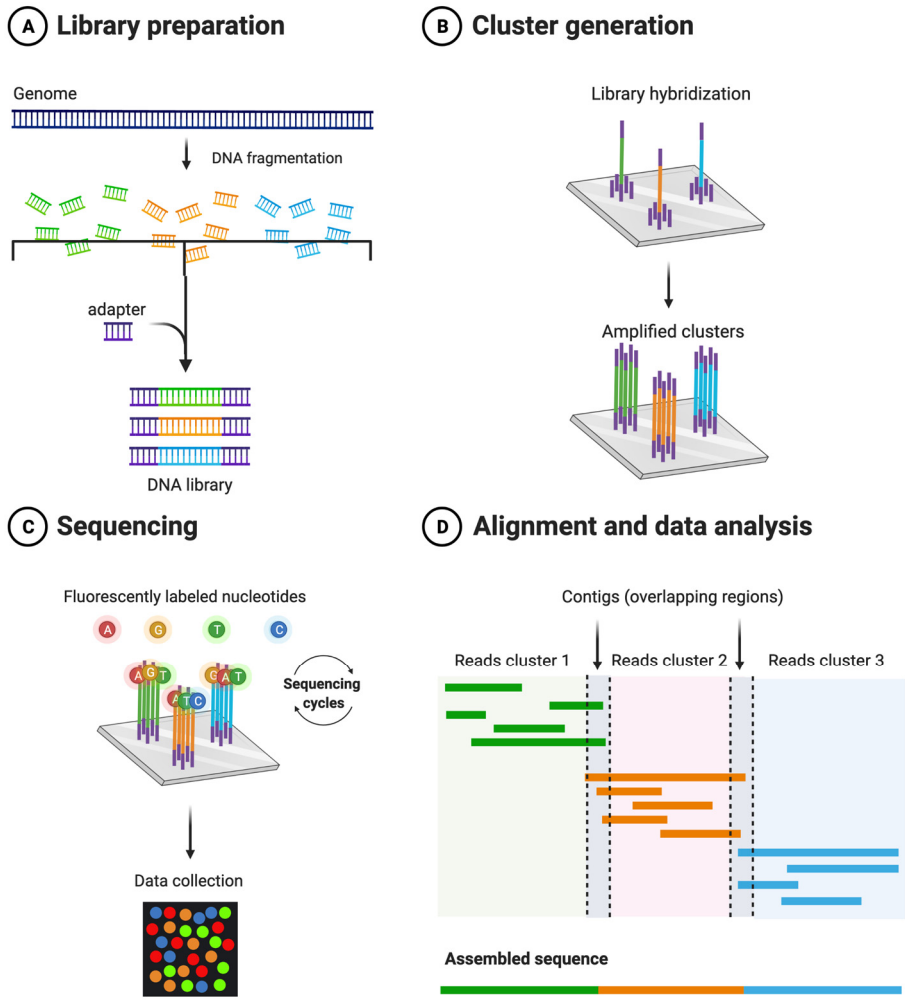


Figure 7. Schematic illustration of NGS with Illumina sequencer. Created with BioRender.com

The targeted NGS panel used in paper III is adjusted to suit formalin-fixed paraffin-embedded (FFPE) tissue samples[224]. Fresh frozen tissue is often not available for genetic sequencing, and FFPE samples are considered a good substitution, even though the DNA in FFPE samples is more degraded[225]. Out of our 88 samples analyzed in paper III, four were excluded from further analysis due to poor DNA

quality. Using a targeted panel instead of whole genome sequencing (WGS) or whole exome sequencing (WES) has both pros and cons. One of the advantages is the opportunity of an increased read depth, which can be 200-1000 reads compared to 30-60 reads with WGS, which is particularly advantageous when sequencing FFPE tissues or circulating tumour DNA (ctDNA) because of the, perhaps, poorer DNA quality or small fractions of malignant cells[226]. A targeted sequencing approach only finds aberrations in the targeted genes, and, hence, low pathogenic variants, or mutations previously not known to be pathogenic, are not detected. Sequencing with targeted panels also entails that the tumour mutational burden (TMB) must be calculated, in contrast to WGS that allows for a comprehensive measurement of TMB[227]. Furthermore, the panel used in paper III is focused on known alterations with clinical implication. When calculating TMB from such data, mutations in tumour suppressor genes must be excluded, since the panel is biased towards detecting these genes. The targeted panel used in paper III includes the entire exons of the 591 targeted genes, and 285 extra-exonic variants.

Statistical methods

In oncological research, comparison of survival between different groups is often essential to examine the effect of, for example, a new drug. One way to investigate differences in survival, or the time to an event, between different groups is the Kaplan-Meier (KM) estimate and log-rank test. The KM estimate uses the exact failure and censoring time and considers the number of individuals at risk for an event, and if you lose individuals to follow up, this does not affect the estimate of survival probability[228]. When few individuals remain at risk, the KM estimate should be interpreted with caution[228]. The log-rank test is a nonparametric test with the null hypothesis that there is no difference in survival between the groups[228]. Both the KM estimate and log-rank test assume that the hazard ratio remains constant over time[228]. In paper I and II, KM estimates and log-rank tests have been used to examine survival differences between groups. One can argue that the follow up time in some of the KM curves should have been reduced, since many patients had been censored. To facilitate the analysis of the curve and minimize the risk of misinterpretation, the KM graphs were combined with a table over the number of individuals at risk at certain time points.

A regression is a statistical model that investigates the relation between a variable, for example a risk factor, and an event[228]. A regression model can be used to predict an outcome, to perform a causal analysis or to adjust for confounders. Cox regression is the regression model most often used when working with survival data. Cox regression is interpreted using hazard ratios (HR), and with the Cox proportional hazards model, the HR estimates can be controlled for other covariates,

making it possible to identify confunders[229]. Similar to the KM and log-rank tests, Cox regression models assume a hazard that is constant over time[228].

As aforementioned, CRT has been used in paper I and II to dichotomize the cases into groups of high and low RBM3 expression. CRT clusters homogenous values of a dependent variable[230]. There are other data driven methods for dichotomization that create a cut off by maximizing or minimizing statistics, for example odds ratio, Youden´s index and Gini index[231]. One can argue that converting a continuous variable into a categorical is incorrect, as this might lead to, for example, loss of information and reduced power[232]. However, in the clinical setting, a dichotomization is often needed, as a physician must know whether a result is negative or positive for a certain aberration, depending on the definition, in order to make a decision to treat or not to treat the patient.

Hierarchical clustering (HC) is a method used to group homogenous clusters or subsets[233]. In paper III we have used an agglomerative approach, a method that starts from "the bottom", in this case the different samples, and successively groups the clusters together depending on similarities, ending up with one cluster at the "top"[234]. In order to determine the distance between the samples, the squared Euclidean method was chosen and for linkage we used the Ward´s method. The Ward´s method minimizes the variance within a cluster and is a method to be used when there is noise between the clusters. In paper III we have chosen to visualize the clusters in dendrograms. To test the fitness of the dendrograms and how well they keep the pairwise distances from the original data, the cophenetic correlation coefficient was used[235].

A hierarchical clustering does not say anything about when the subsets arose and not in which order the clusters appeared, it just presents similarities between clusters and the relative closeness between them. In order to visualise the timeline for the evolution of clusters or branches, a phylogenetic tree can be used. The data in paper III did not enable phylogenetic analysis, since there were samples that contained some variants that might not be pathological, even though they did not appear in the variant files of the normal samples. This was discovered when spot checks for comparisons with raw data files were conducted.

Ethics

The clinical data in paper I derive from a clinical study with ethical approval from the regional committee for medical and health research ethics – REC West in Norway, the regional ethical committee Uppsala in Sweden and the regional scientific ethical committees for southern Denmark, in Denmark[236]. The data in paper II and III are retrospectively gathered with ethical approval from the regional ethical review board in Lund, Sweden. None of these studies resulted in any change

in the medical treatment for the patients. All data have been anonymously analyzed and the results are not possible to track back to the original patient.

For paper IV, an ethical approval application has been written. The *On-treatment biomarkers in metastatic Colorectal Cancer for Life* – the On-CALL study is an observational study and enrolment in the study will not affect treatment decisions or the time to radiological or clinical follow-up. The study will be conducted in accordance with the good clinical practice (GCP) guidelines. Since the study includes analysis of tumour DNA, it is possible that somatic mutations, such as BRCA 1 or 2, will be found. If so, the patient will be contacted and informed about the finding, since it might have implications both in terms of treatment options and heredity. Therefore, the patient will, if he or she wishes, be referred to a genetic clinic for further counselling and testing, possibly also of family members.

Results and Discussion

Paper I

In paper I the IHC expression of RBM3 and its possible prognostic and predictive value was examined in a cohort of 798 patients with mCRC. TMAs were constructed from 462 cases, and the tumour samples were mainly obtained from primary tumours. RBM3 expression could be evaluated in 455 cases, and the nuclear as well as cytoplasmic expression was denoted.

Out of the 455 cases, 46 (10%) and 67 (15%) were negative for nuclear and cytoplasmic expression, respectively. The evaluation score of RBM3 was calculated as intensity (0-3) x fraction (0-1), giving a range between 0-3. With a cut off at 0.550 for nuclear expression and 0.025 for cytoplasmic expression, determined with CRT analysis, 166 cases (36%) were denoted as having high nuclear expression and 128 (28%) as having high cytoplasmic expression.

Any positive and high RBM3 expression in both the nuclei and cytoplasm was associated with $BRAF_{wt}$ tumours, and nuclear positivity was more common in rectal cancer. *BRAF* mutations are more frequent in right-sided colon cancer, and in this paper, we did not consider sidedness other than colon vs rectal origin. Re-analysis of the cohort revealed that there were no associations between RBM3 expression, neither nuclear nor cytoplasmic, and the location of the primary tumour (right, left and rectal). This is in contrast to the study by Melling et al., wherein RBM3 expression was found to be higher in proximal tumours[237].

The prognostic value of RBM3 was examined in relation to OS in the entire cohort. KM analyses (Paper I, Figure 2) revealed that both high nuclear and cytoplasmic RBM3 expression was associated with a significantly longer OS. Median OS was 13 months for patients with high tumour-specific RBM3 expression and 7 months for patients with low tumour-specific RBM3 expression, and the differences in survival were similar for nuclear and cytoplasmic expression. These findings were confirmed in multivariable Cox regression analyses (HR=0.67, 95% CI=0.50-0.90 for nuclear RBM3 expression and HR=0.66, 95% CI=0.48-0.91 for cytoplasmic RBM3 expression) (Paper I, Table 2).

Among patients with high RBM3 expression, those who had received oxaliplatin-based chemotherapy in first line had a significantly longer PFS than those who had received irinotecan-based chemotherapy. The median PFS was 9.36 vs 8.80 months

(p=0.020) for nuclear expression, and 8.99 vs 8.77 months (p=0.022) for cytoplasmic expression. OS was also prolonged in patients with tumours displaying high nuclear or cytoplasmic RBM3 expression who received oxaliplatin based chemotherapy in first line, and these findings remained significant even after exclusion of patients who had previously received adjuvant treatment, even though the actual benefit in survival time was less than a month. The differences in PFS and OS might seem negligible, but, of note, the median survival in this cohort of 455 patients with mCRC was only 11 months. Hence, these differences might well be larger in a cohort with more fit patients.

As aforementioned, the association between an increased RBM3 expression and a prolonged survival has been shown in many other studies, both on CRC and other cancer types. Melling et al. showed that loss of RBM3 expression was an unfavourable prognostic marker associated with advanced tumour stage and poor prognosis in CRC[237]. Hjelm et al. also showed that high expression of RBM3 was associated with improved prognosis in CRC[177]. In both studies, only nuclear expression of RBM3 was evaluated. Melling et al. defined weak staining as an intensity of 1+ or 2+ in up to 50% of the tumour cells or 3+ in less than 20% of the tumour cells. If translated into the index we have used, it would correspond to a cut off of 0.6. Hjelm et al. dichotomized their cohorts into RBM3 negative or positive[177]. Since RBM3 is an RBP, it can be expressed both in the nucleus and in the cytoplasm. In paper I, 10% of the tumours lacked RBM3 expression in the nucleus whereas 15% lacked cytoplasmic expression. The optimal prognostic cut off was determined at 0.550 for nuclear expression and at 0.025 for cytoplasmic expression, most likely due to the higher nuclear than cytoplasmic expression. Moreover, in the multivariable Cox regression analysis in paper I, the continuous variable of nuclear, but not cytoplasmic, RBM3 expression was an independent predictor of a prolonged OS. Hence, assessment of nuclear RBM3 expression appears to be the preferrable method, but the optimal cut off value needs to be validated in additional studies.

The mechanism behind the prognostic and potential predictive value of RBM3 is not known. Perhaps it all comes down to the involvement of RBM3 in cell cycle progression, especially in the transition from the G2 to mitosis phase[171 174]. Oxaliplatin, on the other hand, has been shown to increase the number of colorectal cancer cells in G2/mitosis phase arrest of the cell cycle and to induce apoptosis[238]. If RBM3 drives the cells towards the mitosis-phase of the cell cycle and oxaliplatin mainly acts in the same phase, this might, at least in part, explain the prolonged survival seen in oxaliplatin-treated patients with tumours expressing higher levels of RBM3. The connection between RBM3 expression and improved response to platinum-based treatment has been seen in other studies. Ehlén et al. showed that both mRNA and protein expression of RBM3 were significantly higher in a cisplatin sensitive cell line of ovarian cancer compared to its cisplatin resistant derivative, and that silencing of RBM3 led to decreased cisplatin sensitivity[175]. Furthermore,

Karnevi et al. demonstrated that silencing of RBM3 rendered pancreatic cancer cell lines less sensitive to oxaliplatin[179].

Of note, in this study, the majority of analysed tumour samples were from the resected primary tumours, in some cases dating back several years. Yet, RBM3 expression carried a prognostic value in line with previous studies on CRC cohorts of mixed stages. This finding implies that RBM3 expression might be quite stable over time, but this hypothesis could not be tested, since none of the cases had paired samples from the primary tumour and metastases.

Paper II

In paper II, RBM3 expression was evaluated in pulmonary CRC metastases derived from 216 patients, as well as paired primary tumours from 174 cases. The majority of the primary tumours were located in the rectum (57). The 5-year OS was 56% and the median OS was 68 months. The median OS was significantly shorter for patients who received neoadjuvant chemotherapy (42 months vs 78 months, p=0.002), and significantly longer for patients who received adjuvant treatment compared with those who did not receive adjuvant treatment (92 months vs 57 months, p=0.004).

In this study, only nuclear expression of RBM3 was considered, which is henceforth referred to as "RBM3 expression". RBM3 expression could be evaluated in at least one lung metastasis from 211 patients and in the primary tumour from 164 patients. An evaluation score was calculated from the intensity (0-3) and fraction (four groups, 0-4, see paper II for details), giving a score with the range of 0-12. A prognostic cut off was determined through CRT analysis and was set to 6. This dichotomisation, based on the first resected lung metastasis, rendered one group with high RBM3 expression including 61 (29%) of the patients and one group with low RBM3 expression, including 150 (71%) patients. Among the primary tumours, 74 (45%) were denoted as having high and 90 (55%) were denoted as having low RBM3 expression. Low RBM3 expression in the lung metastases was significantly associated with some adverse clinicopathological characteristics, such as higher CRP levels before surgery and metastases larger than 3 cm. High expression of RBM3 in the lung metastases was significantly associated with prolonged survival (p=0.002) and RFS (p=0.013) after resection. Multivariable Cox regression analysis confirmed that low RBM3 expression in the lung metastases was an independent factor for shorter OS and RFS. No such association was seen regarding the expression of RBM3 in the primary tumour.

Patients who had received oxaliplatin at some point during their disease had a significantly prolonged OS (HR: 1.67, 95% CI: 1.17-2.37, p=0.004). Patients treated with oxaliplatin who had high expression of RBM3 in their lung metastases had a

significantly prolonged survival compared to patients with high RBM3 expression in their lung metastases who had not received oxaliplatin (p=0.008). This difference was not seen in patients with low expression of RBM3 in their lung metastases.

Comparison of the expression of RBM3 in the primary tumours and paired lung metastases showed significantly higher expression in the lung metastases (p<0.001). Subgroup analysis revealed that this difference was mainly seen in patients with metachronous disease and in patients who did not receive neoadjuvant treatment. Patients with high RBM3 expression in both the primary tumour and lung metastasis had the best OS, whereas patients with a retained low expression had the worst prognosis.

Among the 216 patients included in this study, 57% had a primary tumour located in the rectum. Among all CRC diagnosed, rectal cancer usually accounts for 1/3 of cases[239]. The high prevalence of rectal cancer in this cohort might reflect the higher risk of lung metastasis from rectal cancer compared to colon cancer[82].

The shorter OS seen in this study among patients who had received neoadjuvant treatment is notable. A patient who receives neoadjuvant treatment prior to surgery is likely in a situation where the lung metastases might not be suitable for surgery if not reduced in size or number, hence indicating a more unfavourable situation upfront, with a considerable risk of recurrence. In the light of these findings, PM should be given thorough consideration in cases where neoadjuvant treatment is needed to enable this procedure. Along this line, the finding of an association between adjuvant treatment and a prolonged OS might not only be due to the adjuvant treatment itself, one should also consider why some patients did not receive adjuvant treatment at all. This could, for instance, have been due to that their performance status was too poor, that they already had received adjuvant treatment in another context and therefore deemed unlikely to benefit from treatment, or that they might have received neoadjuvant treatment without responding. ESMO guidelines recommend oxaliplatin-containing adjuvant treatment after PM if the patient has not received any such treatment before[57].

In this study, 29% of the lung metastases and 45% of the primary tumours were denoted as having high RBM3 expression. In paper I, 36% of the cases, mainly primary tumours, were denoted as having high expression, using a lower cutoff. In paper I, the mean RBM3 score was 1.02 out of 3, and in paper II, the mean RBM3 scores were 8.53 and 6.73 out of 12 for lung metastases and primary tumours, respectively. Hence, the RBM3 expression was higher in the cohort in paper II compared to paper I. The patient characteristics also differ between the cohorts in paper I and II in that all patients in paper I have non-curable disease, whereas all patients in paper II have been treated with curative intent, thus having a more favorable prognosis.

Patients treated with oxaliplatin at any point during their disease had a significantly prolonged survival. This was however only true for patients who had high RBM3

expression in their pulmonary metastases, and not for patients with low RBM3 expression in their metastases. This finding further supports the connection between RBM3 and response to oxaliplatin seen in paper I, although in paper II, the potential predictive value was only seen for RBM3 expression in the metastases. Since the patients have received oxaliplatin at different time points during the disease course, the picture is somewhat more complicated when it comes to the evaluation of potential treatment effects in this cohort. Since all patients undergoing surgery for both a primary tumour and metastases should receive oxaliplatin at some time point, one can only speculate about the reasons why some patients had not been treated with oxaliplatin.

In paper II, we had an opportunity to compare the expression of RBM3 in primary tumours and lung metastases, and found that the expression was higher in the latter, especially in patients with metachronous disease and in patients who did not receive neoadjuvant treatment. One reason for this observation could be that tumour clones with high RBM3 expression are less aggressive, even in a disseminated state, hence growing more slowly once they have settled into the lungs. Tissue from liver metastases were available in 52 cases, and further analyses (not in the paper) showed that the expression of RBM3 did not differ significantly between liver and lung metastases. Furthermore, RBM3 expression in the liver metastases was not prognostic, possibly due to the small number of cases. Previous studies on malignant melanoma have shown a decreased RBM3 expression in metastases compared to the primary tumours, both in human tumours and *in vitro*[240 241], and an association of high RBM3 expression with improved outcome[240]. On the other hand, in pancreatobiliary-type periampullary adenocarcinoma, RBM3 expression was shown to be higher in metastases than in the primary tumours[179]. Notably, in the latter study, high RBM3 expression was an adverse prognostic factor in patients who did not receive adjuvant treatment, but a favorable prognostic factor in patients who received adjuvant treatment. With RBM3 mainly being a biomarker of good prognosis, a decreased expression in metastases would be expected. However, in light of its proposed ability as a predictive biomarker, an up-regulated expression in metastases could also be advantageous.

Paper III

Paper III encompasses a comprehensive multi-regional profiling of genomic alterations by targeted deep sequencing (TDS) in seven patients with PC deriving from CRC. In parallel, the expression of selected biomarkers SATB2, RBM3, and MMR proteins was mapped by IHC.

SATB2 expression was predominantly low in all cases (Figure 2, paper III). In five of the cases, the expression was completely negative. In one of the cases, the highest

expression was seen in lymph nodes and PC, and in another case, the expression was heterogenous, and the highest expression was seen in the primary tumour. RBM3 expression differed between the cases, with two having almost completely negative expression, one having a rather homogenous high expression and four having different degrees of heterogenous RBM3 expression. In one of the latter, the highest RBM3 expression was seen in the primary tumour and the PC, whereas the expression in the lymph node metastases was rather low. One of the cases lacked expression of the MMR-proteins MLH1 and PSM2, and all other cases showed different degrees of MMR-protein heterogeneity. Overall, MSH2 was the most homogenously expressed protein in all cases.

The spatial genomic profiling revealed the highest TMB in the patient with dMMR. The other patients could be divided into one group with medium TMB and one with low TMB. The patient with dMMR also showed an MSI genotype.

Mutations in the *KRAS* gene was the most common gene alteration seen in four of seven patients, followed by *TP53* (3/7) and *APC* (2/7). *KRAS* and *APC* were shared mutations between all samples, whereas one sample in one patient lacked the TP53 mutation. Examples of other shared mutations were *PTPRD*, *BRAF*, *FBXW7*, *PPP2R1A*, *ALK* and *PTEN* (Paper III, Figure 4C).

The most common copy number gains, among genes that also showed single-nucleotide variation (SNV) or insertion-deletions (InDel) mutations, were *CARD11, IKZF1* and *FLT4.* Among all genes in the panel, copy number gains were most often seen in *GATA3* and *EGFR*. Heterozygous loss of tumour suppressor genes was commonly seen in TP53 and PIK3CD (Paper III, Figure 4D). Two patients had a heterozygous loss of *TP53* and a gene alteration (SNV or InDel).

Hierarchical clustering visualized in dendrograms are shown in Figure 5, paper III. In most cases the HC revealed a relative closeness between PC samples, lymph nodes samples and certain samples from primary tumours. However, there were two cases where multiple carcinomatosis samples showed a higher similarity to lymph node samples than to samples from the primary tumour.

The low expression of SATB2 in this cohort of patients with CRC disseminated to the peritoneum corresponds to previous findings showing an association of reduced SATB2 expression with aggressive tumours and adverse clinical outcome[195 196]. Mezheyeuski et al. reported a lower SATB2 expression in mCRC with peritoneal metastasis compared to CRC disseminated to other locations[196]. These findings are further supported by our results, implying that low SATB2 expression could be a hallmark of peritoneal metastasis.

As aforementioned, high RBM3 expression has most often been associated with a favourable outcome. In paper III, the expression was overall low, which is in line with previous studies, since mCRC disseminated to the peritoneum must be considered an aggressive disease. However, despite the overall low RBM3

expression, it was often higher in lymph nodes or in peritoneal carcinomatosis compared to the primary tumours, which is in line with the findings in paper II, where the pulmonary metastases had a higher expression than the primary tumours. It is also noteworthy that the highest RBM3 expression in this study was seen in a patient with an adverse prognosis.

The patient with dMMR and MSI also showed the highest TMB and number of unshared mutations. In addition, this patient also had the lowest number of CNA. MSI has been suggested to impel clonal evolution in mCRC[242], an observation of interest that could however not be confirmed in our study, given the small number of cases. There were also three other patients with MSS diseaese who had a comparatively high TMB. TMB has been suggested to be a better marker for response to immunotherapy in mCRC than MSI status[243], but since there are no established guidelines of how TMB should be calculated or how the cut off should be set, MSI status is still the best predictive biomarker in this regard.

Mutations in known driver genes were shared among all samples from individual patients in our study, which is in line with previously published data[244 245]. This is particularly important regarding *KRAS* and *BRAF* status, since these genes are used as prognostic and predictive biomarkers in the clinic. Two of the patients in our study showed a mutation in *TP53* and LOH in the same gene, indicating a complete loss of the tumour suppressor gene *TP53*, which is often seen in tumour cells with mutated *TP53*[246].

The HC result, demonstrating a closer interrelation between lymph nodes and peritoneal carcinomatosis than between each of these entities with the primary tumour is noteworthy. The well-known fact that presence of tumour cells in the lymph nodes at the time for surgery is associated with a shorter OS is an integral part of the foundation of TNM as a staging system[70]. There are however studies showing that distant metastases and lymph node metastases can arise either from common or from independent subclones in the primary tumour[247]. Robert Weinberg stated in 2008 that "*the mechanisms of physical dissemination of a variety of tumour cells will come into clear view over the next 5 years*" [248], but as for many things, dissemination is a complex process and we still, 13 years later, have much more to learn.

Paper IV and future perspectives

The On-CALL study, is a prospective, single-arm, observational study. The study aims to deepen the knowledge on the evolutionary progression of mCRC during curative treatment. It further aims to investigate the reasons for treatment failure still seen in a considerable number of patients. All patients with synchronous mCRC treated with curative intent at Skåne University Hospital will be invited to

participate. The enrolment is planned to be up to 100 patients and will start in the beginning of 2022. An SPTC will be constructed for each patient, including tissue samples from primary tumours, lymph node metastases and distant metastases. Blood samples will also be drawn at multiple time points, and comprehensive DNA-sequencing of tumour tissue and ctDNA will be performed.

Even though metastasectomy of liver, lung as well as peritoneal metastases from colorectal cancer are now incorporated into clinical practice, much more is still to be learned about tumour and patient factors that influence treatment response and survival. In paper II, the 5-year OS after PM was 56%. After liver surgery and CRS in combination with HIPEC, the 5-year OS has been reported to be between 35%-60% and 35%-40% respectivley[112 113 119]. Hence, there is an evident need for improved strategies for curative treatment of mCRC.

Neoadjuvant treatment prior to surgery of metastases is today mainly given if there is a need for down-sizing, whereas adjuvant treatment is recommended if the patient has not received any prior treatment[57]. The role of perioperative treatment for patients with resectable liver metastases has been investigated in the EPOC trial, that compared perioperative FOLFOX plus surgery with surgery alone in patients with resectable liver metastases. No difference in OS was seen between the groups, but there was a prolonged PFS in the group that received perioperative chemotherapy[150 249]. A meta-analysis by Liu et al. included 18 studies that investigated the efficacy of neoadjuvant chemotherapy in patients with resectable liver metastases, and found support for an improved OS after neoadjuvant chemotherapy, even if the studies included were quite diverse[250]. For pulmonary and peritoneal metastases, even less is known regarding the efficacy of perioperative treatment. The On-CALL study will provide an excellent opportunity to comprehensively map the tumour heterogeneity and evolution in individual patients. In 2020, a CRC expert group from the United States National Cancer Institute published a summary of the current data on ctDNA in CRC care[251]. They presented four major areas where ctDNA can contribute to CRC care: detection of minimal residual disease, management of patients with rectal cancer, monitoring response to therapy, and tracking clonal dynamics in response to therapy. Thus, it is evident that the On-CALL study will contribute to an enhanced knowledge in several of these areas. Moreover, being an observational study with no extra hospital visits, the patients do not need to meet any particular inclusion criteria, which will give an opportunity to study the true group of patients with mCRC treated with curative intent. Hopefully, the On-CALL study can provide a deeper understanding of the influence of perioperative treatment on the clonal evolution in mCRC, leading to a more personalised treatment, possibly also including adaptive strategies, and, ultimately, to an improved outcome for this group of patients.

Main conclusions

- High RBM3 expression is an independent predictor of prolonged survival in patients with mCRC.
- High RBM3 expression is associated with prolonged PFS in mCRC patients treated with first-line oxaliplatin compared to irinotecan.
- RBM3 expression is higher in lung metastases than in primary tumours.
- High RBM3 expression in lung metastases is an independent predictor of prolonged survival after pulmonary metastasectomy in patients with mCRC.
- Peritoneal carcinomatosis originating from CRC is a complex disease that may be a distinct entity from other mCRC.
- Further knowledge about the evolution of mCRC during treatment is needed in order to personalize oncological treatment and to enhance survival for patients with mCRC.

Populärvetenskaplig sammanfattning

Ungefär 7000 personer drabbas varje år av tjock- och ändtarmscancer i Sverige, vilket gör det till en av de vanligaste cancerformerna. Om cancern hittas, och opereras bort, i ett tidigt stadium utan att ha spridit sig någon annanstans i kroppen är prognosen god. Om cancern har spridit sig, metastaserat, är det i vissa fall fortfarande möjligt att bli botad, men hos de allra flesta får man rikta in sig på att bromsa sjukdomen. I min doktorsavhandling har jag undersökt nya biomarkörer vid metastaserad tjock- och ändtarmscancer, deras koppling till sjukdomsprognosen och om de kan hjälpa till att förutsäga vilka som kommer svara på vissa typer av cancerbehandling.

En biomarkör kan sägas var en markör, eller en indikator, för en sjukdom och dess utveckling. Ett exempel är PSA-värdet som följs vid prostatacancer. I mina studier har jag framför allt tittat på en markör som heter RNA-binding motif protein 3, RBM3. RBM3 är ett protein som finns inne i cellen och som är involverat i celldelning. Ett högt uttryck av RBM3 i tumörceller har i tidigare studier visat sig vara kopplat till en god prognos i flera olika cancertyper, till exempel bröstcancer, äggstockscancer och tjock- och ändtarmscancer. Det finns också studier som visar att ett högt uttryck av RBM3 är förenat med en god effekt av behandling av en grupp cellgifter som är baserade på platinum och som ofta används vid behandling av tjock- och ändtarmscancer.

I min första studie undersökte vi uttrycket av RBM3 hos 455 patienter med spridd tjock- och ändtarmscancer, som alla var aktuella för bromsande behandling. De patienter som hade tumörer med ett högt RBM3-uttryck levde längre än de patienter vars tumörer hade ett lågt uttryck av RBM3. Vi såg också att bland de patienter som hade ett högt uttryck av RBM3 levde de patienter som fått behandling med det platniumbaserade cellgiftet oxaliplatin längre än de som hade fått behandling med ett annat cellgift, irinotekan. Resultaten i vår studie talar för att högt RBM3-uttryck i tumörcellerna är en biomarkör för god prognos vid spridd tjock- och ändtarmscancer och att det kan vara en biomarkör för svar på cellgiftsbehandling med oxaliplatin.

I min andra studie undersökte vi uttrycket av RBM3 hos 211 patienter vars tjock- och ändtarmscancer spridit sig till lungorna och där dottertumörerna, metastaserna, hade opererats bort. Även i denna studie såg vi att ett högt uttryck av RBM3 i tumörcellerna i lungmetastaserna var kopplat till en bättre prognos. Det var inte bara

överlevnaden som var bättre bland de patienter som hade ett högt RBM3-uttryck i lungmetastaserna; bland de som fick tillbaka metastaser tog det längre tid innan återfallet visade sig vid högt RBM3-uttryck. Vi såg också andra faktorer som var kopplade till bättre prognos, till exempel att patienten var yngre än 60 år, bara hade en lungmetastas, som dessutom var mindre än 3 centimeter, och om det hade gått mer än två år sedan man fick sin ursprungstumör. När vi jämförde uttrycket i lungmetastaserna och i ursprungstumörerna såg vi att uttrycket av RBM3 var högre i lungmetastaserna än i ursprungstumörerna.

I den tredje studien bestod studiegruppen av sju patienter vars tjock- och ändtarmscancer hade spridit sig till bukhinnan och som hade genomgått en omfattande operation för att få bort metastaserna i bukhinnan. Förutom att skala bort bukhinnan sköljde man under operationen med cellgifter i bukhålan för att försöka döda eventuella cancerceller som fanns kvar och på så sätt öka chansen att bota patienten från cancern. Vi har sammanställt flera vävnadsprover från varje patient, bland annat prov från ursprungstumören, lymfkörtelmetastaser och metastaser i bukhinnan, i särskilda vävnadsmatriser, s.k. tissue microarrays. Då antalet patienter som genomgår denna typ av operation är begränsat och kunskapen kring nyttan med den omfattande operationen är relativt låg valde vi att analysera flera biomarkörer samt även DNA från flera olika vävnadsprover. Vi valde att analysera bland annat RBM3-uttrycket, men även en annan biomarkör som heter SATB2. SATB2 är en markör som är specifik för cancer i tjock- och ändtarmen. Ett högt uttryck av SATB2 har i tidigare studier visar sig vara kopplat till en bättre prognos än ett lågt uttryck. Vi undersökte också uttrycket av fyra så kallade MMR-proteiner som är involverade i reparationen av DNA-strängen när den blivit skadad, till exempel vid delning av cellen. Utöver detta har vi sekvenserat DNA, det vill säga undersöka ordningen på olika molekyler i DNA-strängen, i vävnadsproverna. Syftet var att ta reda på vilka genförändringar, mutationer, som fanns i tumörerna, men också att se om mutationerna skiljde sig mellan till exempel ursprungstumören och metastaserna i bukhålan hos samma patient.

Uttrycket av RBM3 var generellt längre i denna studie jämfört med de grupper vi tidigare studerat och det överlag låga uttrycket av RBM3 i kombination med det begränsade antalet undersökta individer gjorde det inte möjligt att dra några slutsatser om dess prognostiska värde. Även SATB2-uttrycket var lägre än vad tidigare studier visat. Uttrycken av båda markörerna varierande något mellan ursprungstumörer, lymfkörtelmetastaser och metastaser i bukhinnan, men vi kunde inte se något mönster mellan de olika patienterna. Att uttrycken av både RBM3 och SATB2 var låga beror sannolikt på att en tjock- och ändtarmscancer som spridit sig till bukhinnan är en mer aggressiv tumör än en cancer som spridit sig till exempel till lungan.

Sekvenseringen av DNA-strängen hos tumörcellerna visade att välkända cancermutationer fanns hos alla patienter och uttrycktes i de flesta vävnadsproven. Mutationer i till exempel i *KRAS*-genen och *BRAF*-genen, som rutinmässigt används

som biomarkörer för vissa typer av behandlingar, sågs i samtliga vävnadsprover från enskilda patienter, vilket är bra, eftersom det talar för att man kan använda ett redan befintligt vävnadsprov och inte behöver ta nya för att bestämma om en viss behandling är lämplig. Vi såg också att mer ovanliga mutationer oftare förekom endast i enstaka vävnadsprover, som ett tecken på att tumörcellerna i de olika vävnadsproven utvecklats åt olika håll. Ibland såg vi stora likheter i mutationerna mellan ursprungstumören och metastaserna i bukhinnan, medan det i andra fall fanns större likheter mellan metastaserna i bukhinnan och lymfkörtelmetastaserna. Sammanfattningsvis kan vi konstatera att tjock- och ändtarmscancer som spridit sig till bukhinnan verkar skilja sig från annan tjock- och ändtarmscancer, både när det gäller biomarkörer och cancerutveckling.

Den sista delen av avhandlingen är ett studieprotokoll för en planerad studie kallad *On-treatment biomarkers in metastatic Colorectal Cancer for Life* - On-CALL, med planerad start 2022. Patienter med nyupptäckt spridd tjock- och ändtarmscancer som erbjuds botande behandling kommer att bjudas in att delta och syftet med studien är att skapa mer kunskap kring hur uttrycket av olika biomarkörer varierar under en botande behandling av en spridd sjukdom. Vävnadsprover från såväl ursprungstumör som metastas/-er kommer att samlas in från varje patient och blodprover kommer att tas före och efter operation samt under eventuell medicinsk behandling före och efter operationen, för att följa utvecklingen av uttrycket av olika biomarkörer över tid. Vår förhoppning är att resultaten av studien skall göra det möjligt att bättre förutsäga vilken behandling som bäst gagnar enskilda patienter, samt när denna skall ges. Kort och gott att bättre kunna individanpassa behandlingen för att slippa onödiga biverkningar och för att så många som möjligt skall kunna leva ett så långt och friskt liv som möjligt.

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Prognostic and predictive biomarkers in metastatic colorectal cancer

Christina Siesing is a clinical oncologist working at Skåne University Hospital in Sweden. During her doctoral studies she has investigated biomarkers, especially RBM3, in metastatic colorectal cancer both in a constant and evolutionary perspective.

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