Prognostic and Predictive Factors in Metastatic Colorectal Cancer

EMERIK ÖSTERLUND
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Abstract

The outcome for metastatic colorectal cancer (mCRC) patients has improved substantially in recent decades. This has chiefly been observed in study populations, and predominantly in left-sided primary tumours, which is why we wanted to study if and how survival has improved in the background population. It has also been seen that certain molecular subtypes are more common in population-based materials, and, thus, we studied the prevalence and effects of different molecular alterations.

Paper I is a national population-based material of all 19,566 Swedish patients with a diagnosis of mCRC 2007-2016, 55% were male and 70% had synchronous metastases. Median overall survival (OS) for all patients was 14.0 months. An improvement could be seen over time, also in stratified analyses. OS was influenced by presentation of metastases, age, primary tumour location, and sex. All except sex remained statistically significant in a multivariable analysis. Differences of about one month in median OS were seen between healthcare regions, but these diminished over time.

Paper II included all 765 patients from the Uppsala Region with a mCRC diagnosis 2010-2020. Right colon primary tumours were seen in 38%, left colon in 27% and rectum in 34%. BRAF-V600E mutations (mt) and deficient mismatch repair (dMMR) had a poor OS and were more common in right colon primary tumours. Primary tumour location did not affect OS in subgroups according to mutations in RAS or BRAF, nor in a multivariable analysis. Molecular alterations seem to be more important than primary tumour location for prognosis.

Paper III studied KRAS-G12Cmt in three population-based and one real-world material. KRAS-G12C was seen in 2-4% of all tested and in 4-8% of all KRASmt. No differences in patient characteristics were observed between KRAS-G12C and other KRASmt. No differences in OS were seen between KRAS-G12C and other KRASmt, neither for all patients, nor in different treatment groups.

Paper IV studied atypical BRAFmt (aBRAFmt) in two population-based and one real-world cohort. aBRAFmt was seen in 1-4% of the adequately tested patients in the different cohorts. aBRAFmt patients were predominantly male, had dMMR less often, more rectal primary tumours, and less peritoneal metastases compared with BRAF-V600Emt. Serrated adenocarcinomas were seen in about half of the aBRAFmt. OS was significantly better for aBRAFmt than in BRAF-V600Emt, but worse than for RASmt and RAS&BRAFwt patients. Nine aBRAFmt received epidermal growth factor receptor inhibitors without responses.

Keywords: metastatic colorectal cancer, RAS mutations, BRAF mutations, biomarkers, outcome

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To God
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


II. **Osterlund E**, Hammarström K, Nunes L, Mathot L, Mezh eyeuski A, Sjöblom T, Glimelius B. Primary tumor location, molecular alterations, treatment, and outcome in a population-based metastatic colorectal cancer cohort. Manuscript


Reprints were made with permission from the respective publishers.
Other papers by the PhD candidate not included in this thesis:


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<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>aBRAF</td>
<td>atypical v-raf murine sarcoma viral oncogene homolog B</td>
</tr>
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<td>APC</td>
<td>adenomatous polyposis coli</td>
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<tr>
<td>BRAF</td>
<td>v-raf murine sarcoma viral oncogene homolog B</td>
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<tr>
<td>BSC</td>
<td>best supportive care</td>
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<td>CMS</td>
<td>consensus molecular subtypes</td>
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<td>CRC</td>
<td>colorectal cancer</td>
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<tr>
<td>CRT</td>
<td>chemoradiotherapy</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>cTNM</td>
<td>clinical Tumour-Node-Metastasis</td>
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<td>DFS</td>
<td>disease free survival</td>
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<td>dMMR</td>
<td>deficient mismatch repair</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
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<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
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<td>HR</td>
<td>hazard ratio</td>
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<td>KRAS</td>
<td>Kirsten rat sarcoma viral oncogene</td>
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<td>LAT</td>
<td>local ablative therapy</td>
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<tr>
<td>mCRC</td>
<td>metastatic colorectal cancer</td>
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<td>MDT</td>
<td>multidisciplinary team</td>
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<td>MMR</td>
<td>mismatch repair</td>
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<td>mOS</td>
<td>median overall survival</td>
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<tr>
<td>mt</td>
<td>mutation/mutant</td>
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<td>NGS</td>
<td>next generation sequencing</td>
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<td>N-RAS</td>
<td>neuroblastoma RAS viral oncogene homolog</td>
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<td>OS</td>
<td>overall survival</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PFS</td>
<td>progression free survival</td>
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<tr>
<td>PIK3CA</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha</td>
</tr>
<tr>
<td>pMMR</td>
<td>proficient mismatch repair</td>
</tr>
<tr>
<td>pTNM</td>
<td>pathologic Tumour-Node-Metastasis</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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<tr>
<td>RAS</td>
<td>rat sarcoma virus</td>
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<td>scRT</td>
<td>short course radiotherapy</td>
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<tr>
<td>TNM</td>
<td>Tumour-Node-Metastasis</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>WGS</td>
<td>whole-genome sequencing</td>
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<td>wt</td>
<td>wildtype</td>
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Introduction

Cancer and tumorigenesis

Cancer is one of the leading causes of morbidity and mortality worldwide, with an estimated 18.1 million new cases and 9.9 million deaths in 2020.\textsuperscript{1} The term cancer encompasses many different malignancies. Cancer is a disease of genetic origin that leads to uncontrolled proliferation and the ability to invade nearby tissues and form distant metastases. Cancers arise from an ancestral cell that at one point acquired a mutation that initiated a first step towards inappropriate reproduction. Daughter cells can then in turn acquire other mutations leading to further loss of controlled cell growth. Before the cancer has broken through boundaries between tissues it is called cancer in situ, but further mutations can allow the tumour to invade underlying tissue and shed tumour cells into blood or lymph. At this point the tumour is considered malignant.\textsuperscript{2} The biological capabilities of tumours have been classified by Hanahan and Weinberg, for making it easier to understand the diversity of malignant diseases. The first version included six hallmarks: sustaining of proliferative signalling, evasion of growth suppressors, resisting cell death, angiogenesis, activation of invasion, and enabling replicative mortality, and was proposed in 2000. Since then, two updates with a total of eight additional characteristics have been proposed.\textsuperscript{3-5}

There are three types of genes that play a major role in the triggering of the cancer: oncogenes, tumour suppressor genes, and stability genes. Mutations in oncogenes lead to a constitutively activated protein or an altered activity of the protein, which promotes growth. Tumour suppressor genes can in turn lead to cancer when mutated because they can no longer arrest the cell-cycle or cause cell-death. Since we have two copies of each gene usually both need to be mutated for a tumour suppressor to drive cancer. Mutations in stability genes lead to an accumulated number of mutations due to defect repair mechanisms, the whole genome is affected by this, but only mutations in oncogenes or tumour suppressor genes affect cell growth.\textsuperscript{6}

Mutations that promote development of cancer, so called “driver-genes” have been found in around 140 genes. These “driver-genes” can be classified into 12 different signalling pathways. Two to eight of these genes are usually
mutated in a typical cancer. The rest of the acquired mutations outside of these genes give no growth advantage and are so called passenger mutations.\textsuperscript{7}

Colorectal cancer tumorigenesis and classification

The tumorigenesis in colorectal cancer (CRC) has since long been thought to be a multistep process. Fearon and Vogelstein proposed a model (sometimes called adenoma-adenocarcinoma pathway) for the basis of the genetics behind CRC in 1990.\textsuperscript{8} The genetic properties have then been further studied and refined, and at present there are three major different sorts of genomic instability leading to CRC: chromosomal instability (CIN), deficient mismatch repair (dMMR) and CpG island methylator phenotype (CIMP),\textsuperscript{9} these and their genetic alterations are presented below. A simplified model of the adenoma to adenocarcinoma sequence is shown in Figure 1.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Adenoma-adenocarcinoma sequence in colorectal cancer}
\end{figure}

The most common form of genetic instability in CRC is CIN, seen in 85\%, which is characterized by an accelerated rate of gains or losses of whole chromosomes or parts of them, resulting in an altered karyotype.\textsuperscript{10,11} The exact reason for this is unknown, but inactivation of the tumour suppressor $APC$ has been proposed as a mechanism.\textsuperscript{12} Most CRCs have a loss of function in the adenomatous polyposis coli ($APC$) gene leading to a precursor adenomatous polyp. Mutations in other pathways, e.g. rat sarcoma virus ($RAS$), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha ($PIK3CA$), $SMAD2/4$, and $TP53$ will eventually lead to a malignant adenocarcinoma.\textsuperscript{7,13}

dMMR is caused by mutations or hypermethylation of DNA-mismatch repair (MMR) genes (chiefly MLH1, MSH2, MSH6, and PMS2).\textsuperscript{14} The MMR system is responsible for correcting small defects overlooked by the
deoxyribonucleic acid (DNA)-polymerase, such as mismatches, deletions, and insertions. Microsatellites are short repeating segments in which the DNA-polymerase can stutter. dMMR can lead to shorter or longer microsatellite sequences. If this occurs within a coding region it can lead to an altered expression or function of the gene.\textsuperscript{15} Commonly affected genes are \textit{TGFBR2} and \textit{BAX}.\textsuperscript{13}

Methylation in turn changes the activity of a gene in areas rich in CpG-islands by silencing the promoter region, in CRC it is usually the MLH1 gene that is methylated. CIMP can therefore inactivate tumour suppressor genes and be involved in the formation of cancer this way. V-raf murine sarcoma viral oncogene homolog B (\textit{BRAF}) mutations (mt) are usually seen in association with CIMP.\textsuperscript{13,16}

Another way of classifying CRC is according to the consensus molecular subtypes (CMS) classification system, proposed by Guinney et al. CMS1 is characterised by hypermutation, dMMR, and a strong immune activation. CMS2 and CMS3 are both epithelial and CMS2 is further characterised by WNT and MYC signalling, whereas CMS3 has metabolic dysregulation. CMS4 is mesenchymal and is further characterised by prominent transforming growth factor-\(\beta\) activation, stromal invasion, and angiogenesis.\textsuperscript{17}

**Colorectal cancer epidemiology, risk factors, and heredity**

CRC is the third most common cancer type and the second most common reason for cancer death, with an estimated 1.9 million new cases and 916 000 cancer deaths worldwide in 2020.\textsuperscript{1} CRC is more common in countries with a high or very high human development index. An increase in incidence has been observed in rapidly transitioning countries. In countries with a very high human developmental index the incidence is increasing but concomitantly the mortality is decreasing, and in countries with the highest human developmental index both incidence and mortality are decreasing.\textsuperscript{18} The risk of CRC is affected by age (with almost 90\% of the cases found in persons \(\geq\)50 years of age),\textsuperscript{19} and sex (with higher prevalence and mortality rates among males than females).\textsuperscript{20} Even if CRC generally is a disease of the elderly a rising problem is early onset CRC, in patients <50 years old, which is increasing in prevalence in contrast to CRC in general.\textsuperscript{21} The risk for CRC is also increased by smoking, obesity, salt intake, red meat intake, low intake of fruits and vegetables, and inflammatory bowel diseases.\textsuperscript{22-25} Factors protecting against CRC include removal of polyps, aspirin, menopausal hormone replacement therapy, and exercise (colon only).\textsuperscript{26-29}
A large proportion of CRC cases are familial and approximately 30% are inherited, however, only 5% have a highly penetrant and well characterised gene and the mechanism for the rest are not fully understood.\textsuperscript{30,31} Inherited mutations in the MMR genes cause Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer).\textsuperscript{32} Another hereditary disease is familial adenomatous polyposis, which is caused by a mutation in the \textit{APC} gene, which leads to tens to thousands of polyps in colon and rectum, in which cancer usually develops around a decade after the polyps appear.\textsuperscript{33}

### Screening for and diagnosis of colorectal cancer

As the progress of CRC from a detectable precancerous lesion to a cancer is a slow process and since the outcome in patients is better in patients diagnosed at an earlier stage, there is much to be won by early detection through screening.\textsuperscript{34} Screening can be classified into non-invasive and invasive. Non-invasive methods include stool tests, blood tests, and radiology. The stool-based tests detect blood or shredded cell debris in the stool, whereas blood tests can detect for example certain genetic changes. Radiologic methods include for example computed tomography (CT) colonography. Flexible sigmoidoscopy and colonoscopy in turn are invasive methods. The invasive methods have the advantage of being able to directly visualize the tumour and take pathology specimens for further evaluation and, at the same time, remove small suspect lesions.\textsuperscript{34,35}

Symptoms of CRC include anaemia, weight loss, changes in bowel habit, rectal bleeding, and abdominal pain. These symptoms are however not specific and some can have no symptoms at all.\textsuperscript{36} Some patients can also present with more acute symptoms like bowel obstruction, perforation, or bleeding. Depending on the situation, location of the tumour and the state of the patient the acute presentations can be handled with emergency surgery, a bridge to surgery procedure or a palliative procedure.\textsuperscript{37}

The gold standard for diagnosis of CRC is colonoscopy. It makes it possible to assess the location of the tumour and take a biopsy from the tumour. The diagnostic accuracy is high and during the procedure it is also possible to remove adenomas that are found in the bowel. An alternative for endoscopy if it is not possible is CT colonography. This investigation can give information on the site of the tumour and on other synchronous lesions, but a tumour sample is not possible to obtain.\textsuperscript{34}
Staging of colorectal cancer

CRC staging is done using the Tumour-Node-Metastasis (TNM) system, presently either the American Joint Committee on Cancer 8th edition or the Union for International Cancer Control 8th edition. If the staging is done without examining a surgical specimen it is considered a clinical evaluation (cTNM), whereas if done after surgery, it is called pathologic (pTNM). The tumour (T) classification of CRC is based on the depth of the invasion, where T1 tumours invade the submucosa, T2 grow into the muscle, T3 grow through the muscle and into the subserosa and T4 grow through the subserosa to the peritoneum or onto/into other organs.38,39

Node (N) classification is based on tumour spread to regional lymph nodes. N1a means spread to one regional lymph node, N1b to 2-3, N2a to 4-6, and N2b to 7 or more regional lymph nodes. N1c means that nearby lymph nodes do not contain cancer, but that there is cancer in the tissue near the tumour (tumour deposits). The metastasis (M) classification is based on if distant metastases can be found, M1 means that there are distant metastases. M1 can further be divided into three groups. M1a means that cancer has spread to one distant site (but not to the peritoneum), M1b means that the cancer has spread to two or more distant sites (but not to the peritoneum), and M1c means that the cancer has spread either to the peritoneum alone, or to the peritoneum and other distant sites.38,39

Once all the three parts of the TNM have been classified, the tumour stage can be determined. A tumour with T1-2 and no regional lymph nodes with cancer or distant metastases is classified as stage I, and a tumour with T3-4 with no regional lymph nodes with cancer or distant metastases is classified as stage II. If there are positive regional lymph nodes but no distant metastases it is classified as stage III, and if distant metastases are seen it is classified as stage IV.38,39

Metastases in colorectal cancer

Metastatic colorectal cancer (mCRC) can either be present at diagnosis (synchronous metastases) or develop later (metachronous metastases), however, according to some definitions, metastases appearing within up to 12 months from the primary tumour can be considered synchronous.40 In Sweden metastases appearing before primary tumour resection are considered synchronous and those appearing after metachronous. Up to about 25% have metastases at diagnosis and another again up to 25% will develop metastases later.41 The most common metastatic site is the liver (seen in 70% of mCRC), followed by lungs (24%), lymph nodes (16%), and peritoneum (15%).42 Primary tumour location affects the pattern of metastasis, and liver metastases are less common
for right colon primary tumours compared with left colon and rectum. Lung metastases are most common among rectal primary tumours, whereas peritoneal metastases are most common for right colon primary tumours.

**Primary tumour location in mCRC**

Tumours in right colon are associated with higher age, female sex, synchronous presentation of metastases, and poor differentiation. The metastatic pattern also differs according to primary tumour location (see Metastases in colorectal cancer). The mutational pattern and MMR-status differ between primary tumour locations. BRAF-V600Emt and dMMR are more common among right-colon tumours, this is also the case for CIMP high tumours. Right sided tumours are less often treated with chemotherapy or liver resections. They might also affect the response to targeted therapy with EGFR-inhibitors even if they are RAS wildtype (wt). Furthermore, primary tumour location also affects outcome in mCRC, many studies have reported that right colon primary tumours do significantly worse than left colon and rectal primary tumours.

**Molecular testing of colorectal cancer**

Before selecting the tumour specimen for molecular testing it should be assured that the tumour cell content is sufficient, to minimize the risk for false negative results. Generally, specimens from both primary tumours (biopsy or surgical specimen), or metastases can be used since the concordance is high for most alternations presently analysed in the clinic, which is the case both for synchronous and metachronous metastases. It is recommended to test for molecular alteration in Kirsten rat sarcoma viral oncogene (KRAS) exon 2-4, neuroblastoma RAS viral oncogene homolog (NRAS) exon 2-4, and BRAF-V600Emt, as well as for MMR-status before initiation of first line treatment for mCRC, since these factors affect the choice of therapy and the prognosis (explained below). Testing for dihydropyrimidine dehydrogenase deficiency should also be carried out in patients planned for treatment with a fluoropyrimidine, such as 5-fluorouracil (5-FU) or capecitabine, since deficiencies can result in severe toxicities. There are several different methods used for molecular testing, these have evolved over time to be more effective and cheaper. The first generations of sequencing were based on synthesis, where a new complementary DNA strand is built and then investigated by electrophoresis. These are, however, too slow and expensive to be used more extensively why they have been replaced by
more modern methods. In the second generation, next generation sequencing (NGS), the sequencing reaction is split into numerous lanes which allows billions of processes to happen at the same time, making the capacity higher and costs lower, there are numerous different methods used for NGS-analyses. These methods have improved and whole genome sequencing (WGS) can be done. The advancement in molecular analysis methods and increased knowledge about cancer biomarkers and a larger number of actionable genetic alterations have led to more extensive sequencing methods being used more, these will most likely be part of clinical routine in the future. There are two main principles for MMR testing. The first is immunohistochemistry where staining for proteins encoded relating to the MMR genes MLH1, MSH2, MSH6, and PMS2, whereas the second method uses sequencing of microsatellite loci, for example using PCR or NGS.

**RAS mutations**

*RAS*mt are known oncogenic drivers. *KRAS*mt are seen in around 40% of CRCs and in *NRAS*mt in 2.5-4.5% of CRCs. *RAS*mt are a known negative predictive markers for EGFR-inhibitor efficacy. Patients with *KRAS*mt have worse overall survival (OS) and progression-free survival (PFS) compared with *KRAS*wt. Development of targeted therapy against *KRAS* has been challenging due to the many different activating mutations in the gene. *KRAS*-G12C was the first *KRAS*mt for which targeted treatment was developed, sotorasib is the first compound approved by the United States Food and Drug Administration, for treatment in non-small cell lung cancer. *KRAS*-G12C inhibitors have shown limited effect on mCRC as single agent. *KRAS*-G12C frequency varies widely in different cohorts. Some differences in sex, age, and metastatic patterns have been observed between different *KRAS*mt, however, non-consistent. Survival for patients with *KRAS*-G12C compared with other *KRAS*mt has been studied, however, also with conflicting results.

**BRAF mutations**

*BRAF*-V600Emt are seen in 4-10% of mCRC patients in trials, whereas higher frequencies of up to 20% have been observed in population-based materials. *BRAF*-V600Emt are most probably also resistant to EGFR-inhibitors due to downstream signalling regardless of medication given. *BRAF*-V600Emt are associated with more right-sided primary tumours, dMMR, peritoneal metastases, lymph node metastases, and fewer lung metastases compared with *BRAF*wt. *BRAF*-V600Emt are also associated with worse OS compared with *BRAF*wt patients (10-20 months vs 35-47 months) in clinical trials in patients with mCRC. In primary colon cancer, *BRAF*-V600Emt are
associated with shorter OS and time to recurrence among pMMR tumours,\(^8^6\) this, however, remains controversial regarding recurrence as another study saw no difference in RFS.\(^8^7\)

With the increased use of NGS other rarer, atypical BRAF\(^{mt}\) (aBRAF\(^{mt}\)) have also been found. These occur outside the most common V600E position, they are thus sometimes also called non-V600E BRAF\(^{mt}\). These are seen in around 2% of mCRC.\(^8^8,8^9\) BRAF\(^{mt}\) can be divided into three different classes: class 1 consists of the BRAF-V600Emt, that is able to signal as a monomer, class 2 is made up of mutations with an intermediate or high kinase activity that are RAS independent, and class 3 mutations have a low or absent kinase activity and are RAS dependent.\(^8^9\) Compared with BRAF-V600Emt, these patients are claimed to be younger, more often male, and have less peritoneal metastases.\(^8^8\) In one study aBRAF\(^{mt}\) had a significantly better survival than BRAF\(^{wt}\), and BRAF-V600Emt patients,\(^8^8\) whereas it in another study was similar to BRAF\(^{wt}\) and better than for BRAF-V600Emt.\(^8^9\)

**Deficient mismatch repair**

The function of the MMR system and the role of dMMR system in the tumorigenesis of CRC has been described above (see Tumorigenesis of colorectal cancer). In colon cancer, dMMR is associated with a reduced 5-year recurrence risk (22% vs 33%, \(p<0.001\)) compared with proficient mismatch repair (pMMR).\(^9^0\) In mCRC, dMMR is, however, a poor prognostic factor.\(^4^5,7^9\) It is also more common in population-based materials compared with clinical trials.\(^7^9\) CRC tumours with dMMR have a higher density of many types of immune cells.\(^9^1\) Patients with dMMR also show good response to PD-1/PD-L1 antibody treatments and they are presently the only subgroup of mCRC that has an indication for immune checkpoint inhibitors.\(^9^2\)

**Treatment**

**Multidisciplinary teams**

Multidisciplinary teams (MDT)s are recommended when planning the treatment. An improved outcome has been observed for discussed patients.\(^9^3\) In the core MDT it is recommended to have healthcare professionals within at least the following fields (and others when needed): pathology, radiology, surgery, radiation- and medical oncology (in the Nordic countries clinical oncology), and nursing. If this cannot be achieved at smaller centres, virtual MDTs should be available instead.\(^9^4\)
Surgery and adjuvant therapy for primary colon cancer

Before surgery synchronous cancers should be ruled out, if the preoperative endoscopy was inconclusive, it is recommended to either do a CT colonography or an endoscopy within three months from surgery. A CT of the abdomen and chest should also be done to determine if metastases are present. When the primary tumour is in the rectum it is recommended to do magnetic resonance imaging of the pelvis, since it gives valuable information for the final treatment decision. If the patient has an asymptomatic primary tumour and synchronous metastases that are not possible to resect it is today generally recommended not to remove the primary tumour.

Around two thirds of the patients will present with a primary CRC, for which radical surgery is possible. The best treatment leading to cure is a radical R0 (no tumour at border) resection of the primary tumour, including the whole involved bowel segment and its lymphatic drainage system. MDTs are recommended when planning treatment for primary CRCs, since many modalities are included. When removing an infiltrative tumour, it is recommended to remove enough lymph nodes, at least 12 lymph nodes need to be examined to accurately classify the tumour as stage II, which is important when deciding on adjuvant treatment.

The right colon (from caecum to two thirds of the transverse colon) arises from the midgut and is supplied by arterial blood from the superior mesenteric artery, whereas the remaining part of transversum to sigmoid colon is supplied by arterial blood from the inferior mesenteric artery. The resection of tumours arising in caecum, ascending colon, hepatic flexure or proximal transversum is a right-sided hemicolectomy, for tumours in the distal transversum an extended right-sided hemicolectomy is usually done. Tumours in the splenic flexure, descending colon and sigmoid colon are usually resected with a left-sided hemicolectomy, or for some sigmoid colon tumours with a sigmoid resection. The concept of complete mesocolic excision was introduced by Werner Hohenberger, Erlangen, Germany and is used for removing the colonic segment without breaking the different layers, which prevents spread of cancer during the operation, and enables an adequate lymph node harvest. If the colon tumour is locally advanced, a more extensive multivisceral resection can be performed, in this procedure the tumour and involved surrounded tissues are removed “en bloc”.

Neoadjuvant therapy is not as established in colon cancer as in rectal cancer, but it is an area being studied at present, tumour downstaging has been shown and an improvement in disease-free survival (DFS) reported from one study, whereas another saw no difference in DFS or OS. Adjuvant therapy with a fluoropyrimidine + oxaliplatin is by most international bodies recommended for all stage III tumours, and high-risk stage II colon tumours,
whereas fluoropyrimidine alone is recommended to intermediate risk stage II patients.\textsuperscript{101,108}

Surgery and adjuvant therapy for primary rectal cancer

Early tumours in the rectum (T1N0) can be removed through transanal endoscopic microsurgery or another endoscopic procedure. In low-risk tumours T1-T3 not suitable for local excision, the standard of care is either an anterior resection or an abdominoperineal resection with total mesorectal excision.\textsuperscript{97} Preoperative treatment for rectal cancer is decided based on chiefly radiologic (MRI) features. Very early and early cancers (T1N0 to T3a-bN1) can in most cases undergo resection without preoperative treatment. Intermediate risk tumours (low T3a-b or N1-2) can be operated directly if a good quality excision can be assured, otherwise short course radiotherapy (scRT) or chemoradiotherapy (CRT) are recommended before surgery. Locally advanced rectal cancers (T3c/d, very low tumours, or N1-2 with extranodal spread) need to receive preoperative scRT or CRT. In the case of advanced disease (T3 with involvement of the mesorectal fascia, T4a/b [especially if considered difficult to resect], or lateral node involvement) either scRT + CAPOX with delayed surgery, or CRT is usually recommended before surgery; in these cases extended surgery might also be needed.\textsuperscript{97}

The role of adjuvant chemotherapy for rectal cancer is not as established as for colon cancer.\textsuperscript{97} Some benefit has been shown of adjuvant chemotherapy after surgery alone,\textsuperscript{108-110} whereas this has not been seen after scRT or CRT.\textsuperscript{111-113} Addition of oxaliplatin to fluoropyrimidines as adjuvant treatment has in some studies improved DFS, but not OS, however, these studies have not used oxaliplatin as adjuvant therapy only and the control arm fluoropyrimidine treatment has differed.\textsuperscript{114,115} According to the most recent European Society for Medical Oncology (ESMO)-guidelines it is reasonable to consider chemotherapy for stage III and high-risk stage II rectal cancers,\textsuperscript{97} however, this is not generally recommended in guidelines. Since adjuvant chemotherapy is not proven to be effective in rectal cancer and compliance to this treatment is rather poor, many trials have explored the neoadjuvant chemotherapy together with the radiation, scRT or CRT, so called total neoadjuvant treatment. Several trials have reported more pathological responses and fewer distant metastases but not yet any survival gain.\textsuperscript{116,117} Tumours responding very well to any pretreatment may not need subsequent surgery and can be handled with a watch-and-wait procedure.\textsuperscript{118}

Resection and conversion therapy of mCRC

If it is possible to do an R0-resection (a resection leaving no tumour at the resection margin) this is a potentially curative treatment for mCRC with liver
or other metastases, generally resulting in 5-year OS-rates of 33-34% in population-based materials.\textsuperscript{119,120} Currently the consensus on technical resectability is if \( \geq 30\% \) of healthy liver is left after the surgery.\textsuperscript{121,122} Whether to give perioperative chemotherapy to patients with resectable liver metastases or not is still unclear. This has been investigated in one randomised study, that showed improved DFS, but not OS when comparing adjuvant fluoropyrimidine + oxaliplatin with surgery alone.\textsuperscript{123,124} The latest ESMO-guidelines recommend a combination of a fluoropyrimidine and oxaliplatin\textsuperscript{123,124} to patients with resectable liver metastases with unfavourable or unclear prognosis, whereas it might be unnecessary for patients with favourable prognosis.\textsuperscript{125}

Resection of lung metastases has also shown good results in selected patients, with 5-year OS rates of 40-53\%.\textsuperscript{126,127} In selected patients with peritoneal metastases cytoreductive surgery often combined with hyperthermic intraperitoneal chemotherapy can be utilised, which might result in long-term survival.\textsuperscript{128,129} The knowledge about the effects of resection of distant metastases is not based upon randomised trials comparing optimal medical treatment with or without metastasectomy but rather upon that long-term survivors are seen and that this appears considerably more often than after systemic therapies only.

If the CRC metastases are limited to the liver and/or the lungs, and are not resectable upfront but might become resectable, conversion therapy is recommended. Converted patients that undergo surgery have slightly worse survival than those that were upfront resectable, but still have an apparently better survival than patients that have not been operated at all.\textsuperscript{130} In the conversion setting a cytotoxic doublet or triplet in combination with a targeted agent is generally recommended.\textsuperscript{125} In \( RAS \text{wt} \) patients, addition of EGFR-inhibitors has shown better results than addition of bevacizumab and it is thus recommended for these patients, at least if the primary is left-sided.\textsuperscript{131} For patients with \( RAS \text{m}t \) tumours a cytotoxic triplet might be preferable over a doublet in this setting.\textsuperscript{132,133} A recent study was, however, not able to show an increased resection rate when comparing a chemotherapy triplet compared with a doublet in combination with biologic treatment.\textsuperscript{134} If the metastases become resectable it is recommended to operate them early to minimize toxicity.\textsuperscript{40} However, these patients also have a high risk of having disseminated systemic disease and a too short treatment may not kill all those tumour cells if present. Treatment may not always be possible after the metastasectomy.

Local ablative therapy

The term local ablative therapy (LAT) contains many different treatment modalities, such as stereotactic body radiotherapy, radiofrequency ablation, microwave ablation, laser ablation, cryoablation, and irreversible electroporation.\textsuperscript{135}
LATs are an alternative when there are a limited number of metastases, that are located unfavourably for surgery.\textsuperscript{125} LAT combined with chemotherapy has also shown superior OS and PFS compared with chemotherapy only in mCRC patients with unresectable liver metastases.\textsuperscript{136} LAT can also be utilized in combination with metastasectomy, when metastasectomy used alone would result in an insufficient liver remnant.\textsuperscript{137}

Treatment of mCRC without conversion potential

For most of the patients metastasectomies will not be possible,\textsuperscript{138} and for them the aim of treatment is disease control or palliation.\textsuperscript{125} Systemic treatment of mCRC has developed tremendously during several decades. Major benefits have been seen for the patients. Besides prolonged survival, the quality-of-life (QoL) has also improved since tumour related symptoms can be alleviated despite toxicities frequently seen during and after the treatments.\textsuperscript{139,140} Literature about the effects are extensive and multiple new trials are reported every year. It is beyond the scope of this thesis summary to make a comprehensive review of this literature. The following text brings-up some important aspects although not even these have been possible to cover entirely reflecting what knowledge is established and where controversies still exist.

Chemotherapy

5-FU or another fluoropyrimidine is the backbone in the treatment of mCRC and is used in most first- and second-line treatments regimens.\textsuperscript{141} Fluoropyrimidines can be given either as an infusion, a bolus injection, both bolus and infusion, or as an oral drug (capecitabine).\textsuperscript{142} 5-FU is since about 3 decades most often given with a biochemical modulator, today most often leucovorin.\textsuperscript{141} A better response rate and slightly better survival is achieved when oxaliplatin and/or irinotecan is added to the fluoropyrimidine and both can be considered as alternatives for first-line treatment combined with a fluoropyrimidine (today often named FOLFOX and FOLFIRI, although many variants exist).\textsuperscript{143,144} They can also be combined with capecitabine (named CAPOX and CAPIRI). CAPOX has shown similar efficacy as FOLFOX.\textsuperscript{145} CAPIRI however has a slightly worse toxicity profile compared with FOLFIRI.\textsuperscript{146} The combination of irinotecan and oxaliplatin can also be used together with a fluoropyrimidine (FOLFOXIRI), which has been superior to FOLFIRI, however, at the cost of toxicity.\textsuperscript{132} In an updated analysis of the TRIBE study an OS benefit could be seen for FOLFOXIRI + bevacizumab compared with FOLFIRI + bevacizumab.\textsuperscript{147} The TRIBE2 study compared FOLFOXIRI + bevacizumab (with re-introduction upon progression) with FOLFIRI + bevacizumab given until progression followed by FOLFOX + bevacizumab and saw a better OS in the FOLFOXIRI arm.\textsuperscript{148}
Biologic agents

There are also biologic agents that can be utilized in the treatment of mCRC: chiefly VEGF-inhibitors (e.g. bevacizumab), and EGFR-inhibitors (panitumumab and cetuximab). Addition of these to the chemotherapy regimen has improved outcome in the first line setting in many studies. VEGF-inhibitors have improved the results when combined with single fluoropyrimidine. VEGF-inhibitors improved PFS, but not OS when combined with 5-FU and oxaliplatin in a large trial, however, the addition of VEGF-inhibitors to FOLFOX or FOLFIRI in a smaller trial failed to show an effect.

EGFR-inhibitors have only shown benefit in RASwt patients. In one study on RASwt patients an OS benefit of EGFR-inhibitor addition could only be seen in left-sided tumours, whereas right-sided RASwt patients only had a better response rate. This was further evaluated on left-sided RASwt patients in the randomized phase III PARADIGM trial where FOLFOX + panitumumab did significantly better than FOLFOX + bevacizumab. Combining the triplet FOLFOXIRI + panitumumab did not show any added benefit compared with FOLFOX + panitumumab, neither compared with FOLFOXIRI alone.

Treatment in molecular subgroups

BRAF-V600Emt are markers of poor prognosis in mCRC, and should be considered when choosing treatment, since a chemotherapy doublet might not be effective enough. FOLFOXIRI + bevacizumab has shown a nonsignificant trend of better OS compared with FOLFIRI + bevacizumab in the BRAF-V600Emt subgroup, this benefit could, however, not be observed in a later study. In fit patients a chemotherapy triplet can thus, based on the data mentioned before, be considered either alone or in combination with bevacizumab. Whether BRAF-V600Emt is a negative predictive marker for EGFR-inhibitors has been controversial, but generally EGFR-inhibitors are not recommended for BRAF-V600Emt (except when combined with encorafenib in second line, see below).

In patients with dMMR, immunotherapy is nowadays recommended. Pembrolizumab given to dMMR patients in first-line showed improved PFS in comparison with chemotherapy, OS was not significantly longer in the pembrolizumab group, which was most likely affected by the 60% crossover rate to pembrolizumab.
Treatment of older and frail patients

In frail or older patients not fit for combination therapy, but still suitable for less intensive treatment, feasible alternatives are a fluoropyrimidine, either alone, or in combination with bevacizumab. In the Nordic 9 study, a dose-reduced combination using S1 as fluoropyrimidine was superior to full-dose S1 only. For RASwt patients EGFR-inhibitors can also be used, either alone or combined with fluoropyrimidines. Patients not fit for any tumour-controlling treatment should receive best supportive care (BSC), see below.

Figure 2. Treatment algorithm for metastatic colorectal cancer, courtesy of Pia Österlund, ESMO World Congress on Gastrointestinal Cancer 2021

Treatment in different lines

Treatments according in different treatment lines according to mutation status are presented in Figure 2. The preferred choice in first line therapy according to ESMO is to give a chemotherapy doublet + EGFR-inhibitors to RASwt patients with left-sided primary tumours, whereas right-sided RASwt patients should receive doublet/triplet chemotherapy + bevacizumab. RASmt tumours should also receive doublet/triplet chemotherapy + bevacizumab. BRAF-V600Emt tumours are recommended a chemotherapy doublet/triplet + bevacizumab. Individual countries and other international groups have other recommendations although basically along the same lines (not further reviewed here).
The treatment is usually evaluated every two months with a CT-exam and is usually continued until disease progression. De-escalation to maintenance therapy can be an alternative in the case of doublet or triplet therapy. This is usually done with a fluoropyrimidine with or without biologic treatment.\textsuperscript{163-165} Another alternative is to take a break from treatment.\textsuperscript{166} There is, however, no clear consensus on when to do this and if maintenance therapy should be given or not. If the patient progresses on maintenance therapy or during a treatment break, re-introduction of the same line of chemotherapy is a feasible alternative.\textsuperscript{125,167}

The choice of second-line treatment regimen depends on the systemic therapy used in first line. If an oxaliplatin-based treatment was used in first line it is recommended to use irinotecan in the second line (either as single agent or presently in combination with 5-FU), whereas if an irinotecan based treatment was used in first line the recommendation is to give an oxaliplatin based doublet in second line.\textsuperscript{125} If bevacizumab was used in first line it is often recommended to continue it in second line as well\textsuperscript{168} for RAS\textsubscript{wt} patients that are EGFR-inhibitor naïve they can be used instead.\textsuperscript{125,169,170} If EGFR-inhibitors were used in first line a switch to bevacizumab is recommended.\textsuperscript{125,168} For BRAF-V600Emt an alternative in second line is the combination of encorafenib + cetuximab, which showed a significantly improved OS compared with irinotecan/FOLFIRI + cetuximab.\textsuperscript{56} For patients with dMMR the combination of nivolumab + ipilimumab is approved as second line treatment if regular chemotherapy was used in first line.\textsuperscript{171}

In third or further treatment lines EGFR-inhibitors can be used for RAS\textsubscript{wt} tumours with no previous EGFR-inhibitor treatment, sometimes in combination with irinotecan.\textsuperscript{125,172} Two other treatments that can be used regardless of mutational status are regorafenib (a tyrosine kinase inhibitor)\textsuperscript{125,173} and trifluridine/tipiracil (an antimetabolite), the latter often combined with bevacizumab.\textsuperscript{125,174}

It should be said that recommendations throughout the western world do not fully agree with the ESMO-guidelines even if they have been reached in consensus including many experts from several countries. Multiple trials have been performed, but they are not always conclusive in all details, and many clinical situations have not yet been subject to optimally designed clinical trials, and thus more trials are still needed for optimising treatment further.

**Best supportive care**

Best supportive care (BSC) is treatment that is given to relieve all types of symptoms of the patients, this treatment is given throughout the disease trajectory whenever needed but is especially important in the last phase of the treatment. Treatments can include for example pain treatment, antibiotics,
radiation for pain control, corticosteroids, transfusions, psychotherapy, palliative surgery, or any other symptomatic therapy.\textsuperscript{175}

Outcome in mCRC

In the past decades treatment for mCRC has developed substantially and in recent clinical trials median OS (mOS) of over 30 months have been reported, especially with the addition of biologic treatments.\textsuperscript{139,176,177} Survival has also improved thanks to surgeries for metastatic disease.\textsuperscript{178,179} In contrast, however, poorer survival of around 12 months in median has been reported in population-based materials.\textsuperscript{81,180-184} An improvement in outcome has also been seen in population-based materials, but not to the same extent as in study populations.\textsuperscript{179-184} Patients in clinical trials are selected and are thus usually younger, have a better Eastern Cooperative Oncology Group (ECOG) performance status and less comorbidity. Some metastatic sites and certain molecular alterations are often also underrepresented in study populations, and therefore they are not representative for the background population of mCRC patients.\textsuperscript{81,185,186}

Treatment in trial populations is for obvious reasons homogeneous even if alternatives have been compared. In populations, considerably greater heterogeneity exists because the background characteristics of patients differ and differences in opinions among treating physicians may exist. Finally, unmotivated differences may also exist. Lower resection rates are seen in population-based series compared with study populations,\textsuperscript{187} and there are many patients receiving no treatment at all in population-based materials.\textsuperscript{81} The differences in OS are probably largely explained by worse clinical characteristics (explained above),\textsuperscript{81,185,186} different molecular alterations.\textsuperscript{79,80} and differences in treatment,\textsuperscript{81,187} but perhaps also suboptimal usage of the treatment and care of the patients.

Higher age is also associated with worse OS,\textsuperscript{188,189} however, interestingly also the youngest patients have the worse OS in one study.\textsuperscript{188} Some differences in treatment have been observed between hospitals.\textsuperscript{190-192} Differences in metastasectomies have been observed between sexes,\textsuperscript{193} and some studies have reported OS differences between sexes, whereas others have not.\textsuperscript{193,194} Primary tumour location, \textit{RAS}mt, \textit{BRAF}mt, and dMMR also affect OS, details have been described above (see Primary tumour location, \textit{RAS} mutations, \textit{BRAF} mutations, and Deficient mismatch repair).
The Swedish Colorectal Cancer Registry

The Swedish Colorectal Cancer Registry (SCRCR) has included rectal cancers since 1995, and colon cancer since 2007. It covers around 99% of both colon and rectal cancers, respectively, and contains information on demographics, staging, and surgical treatments with a high accuracy. The accuracy of registration of recurrences after 5 years was also high when evaluated in two regions. Information about oncological treatments, particularly in mCRC is, however, still limited.

The Uppsala-Umeå Comprehensive Cancer Consortium

The Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN) is a collaborative biobank effort between Uppsala University, Umeå University, Stockholm University, and KTH Royal Institute of Technology. The aim of U-CAN is to develop a high-quality biobank of different cancer types, from different geographical areas. It includes longitudinal blood samples, tissue specimens, and patient data.
Present studies

Aims

Paper I: To explore if survival has improved and if differences can be seen according to baseline characteristics or geographically in a population-based nationwide Swedish cohort of patients with either synchronous or metachronous mCRC.

Paper II: To explore the frequency and effect of molecular alterations in a population-based cohort from one Swedish region and to study factors that affect treatment decision and outcome in mCRC.

Paper III: To study the prevalence of KRAS-G12C in population-based and real-world mCRC materials from the Nordic countries and the influence of this mutation on patient characteristics and outcome in a less selected material than clinical trials offer.

Paper IV: To study aBRAFmt in population-based and real-world mCRC materials from the Nordic countries to find out the prevalence and how these mutations affect baseline characteristics and outcome in a less selected material.
Patients

For paper I a dataset consisting of all 19,708 Swedish mCRC patients registered in the SCRCR in 2007-2016 was acquired. After extensive rectification 19,566 patients remained, of which 19,483 had available dates of diagnosis, MDT conference, or treatment initiation, and could be included in OS analyses.

The cohort in paper II is population-based and contains all 765 mCRC patients in the Uppsala region with a primary tumour diagnosis 2010-2020. About 60% of them were identified within U-CAN and the rest were acquired from SCRCR. In a validation study it could be shown that the coverage is close to 100%.

In paper III patients from four Nordic cohorts were combined. The first cohort was the Finnish prospective real-life RAXO-study, which included 1086 mCRC patients from all 21 Finnish hospitals treating cancer 2012-2018. In brief the inclusion criteria were that patients had to be over 18 years old, have a histologically confirmed diagnosis of mCRC, and be eligible for first-line treatment to be able to participate.

The second cohort was a population-based material that consisted of all patients from three Scandinavian regions around university hospitals (Odense, Denmark, Haukeland, Norway, and Uppsala, Sweden) with a mCRC diagnosis 2003-2006 (n=798).

The third cohort was a population-based cohort consisting of all mCRC patients with a diagnosis of primary CRC between 2010-2018 (n=626) in the Uppsala region in Sweden. It is based upon an earlier version of the Uppsala cohort in paper II, that has later been expanded.

The fourth cohort is a retrospective data-collection from four Finnish centres doing NGS testing (Helsinki, Jyväskylä, Tampere, and Turku), in this cohort an additional 49 patients with KRAS-G12C were identified and included.

Paper IV included patients from the RAXO- (n=1086) and PRCRC-studies (n=798, see Paper III) as well as 27 patients with aBRAFmta identified retrospectively from four Finnish hospitals doing NGS testing that includes testing for aBRAFmt (Helsinki, Jyväskylä, Tampere, and Turku).
Molecular testing

No molecular testing was done in **paper I**. Information about molecular properties in SCRCR has only recently been introduced and is still very incomplete.

In **paper II** the patients were molecularly tested in the clinical routine in 606 patients, in the beginning with pyrosequencing for *KRAS* and *BRAF*-V600E and from 2014 onwards with NGS for *KRAS*, *NRAS*, and *BRAF*-V600E mutations, from 2016 *PIK3CA* has also been reported. If sufficient tumour material or excess DNA from the clinical routine was available the Oncomine™ Tumor Mutation Load assay (ThermoFisher Scientific, MA) that covers 409 cancer genes was used in 451 patients. The libraries were sequenced on Ion S5 550 chips using the Ion S5 Sequencing Systems™ (ThermoFisher Scientific, MA). Variant calling was done using the Ion Reporter™ software (ThermoFisher Scientific, MA). Of these 397 had an adequate average read depth of ≥800 reads. An additional 23 could be assessed for hotspot mutations in *KRAS*, *NRAS*, *BRAF*, and *PIK3CA*. Patients with available fresh frozen tissue (n=187) were sequenced with WGS, within another project. MMR-testing was done either using immunohistochemistry for the four MMR proteins, with PCR for genomic regions with microsatellites, or using WGS.

**Paper III** included patients tested in four different cohorts. The testing for *RAS* and *BRAF*-V600E mutations and MMR-status was done in clinical routine in the RAXO-study, the Uppsala region cohort, and in the Finnish data-collection cohort. In the RAXO-study most were analysed using reverse transcriptase polymerase chain reaction, (except for a few that were sequenced using Sanger polymerase chain reaction or pyrosequencing) in the beginning of the inclusion period and later either using NGS or Illumina sequencing (in 123 patients). Patients in the Finnish data-collection cohort were tested using an NGS. In the Uppsala region cohort pyrosequencing was used in the first part of the inclusion period and from autumn 2014 an NGS was used. The composition of the NGS panels have varied, but have always included testing for *KRAS* exons 2-4, *NRAS* exons 2-4 and *BRAF*-V600E mutations. Testing in the PRCRC-study was done using an NGS testing for 44 cancer genes, described in detail earlier, and in some patients with pyrosequencing for *KRAS* and *BRAF*-V600E. MMR-testing was done either immunohistochemistry for the four MMR proteins or PCR for genomic regions with microsatellites.

Methods in **paper IV** are described above (Paper III) for the RAXO-study, the PRCRC-study, and the Finnish data-collection. One of the patients in PRCRC was sequenced using Illumina sequencing. Regarding testing for *aBRAF*mt this was done in 377 patients in the RAXO-study and 445 patients in the PRCRC-study.
Ethical considerations

The studies were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committees of Uppsala University (2003-303, 2004-M281, 2007-116, 2009-408, 2010-198, 2011-092, 2012-224, 2015-419 and 2018-490), Haukeland University Hospital (2009/2052), and Helsinki University Hospital (THL/2305/5.05.00/2019).

Statistical methods

Categorical variables were compared using Chi-square, logistic regression, or Fisher’s exact test. Continuous variables were compared using Mann-Whitney test if comparing two groups and Kruskal-Wallis test when comparing three or more groups. Median follow-up time was estimated using the reverse Kaplan-Meier method. OS and PFS was estimated using the Kaplan-Meier method. OS was defined as time from mCRC diagnosis to death or censored if alive at last follow-up. PFS was calculated from time of first line systemic therapy treatment initiation to disease progression or censored if no progression at last follow-up. Survival was compared using log-rank or Cox regression for univariable analyses and using Cox regression for multivariable analyses. Proportional hazard assumption was tested for using Schoenfeld residuals, the hazard ratio (HR) was interpreted as the average of a time varying hazard ratio.\textsuperscript{203} Conditional landmark analyses at two different time points were used for assessing the potential effect of guarantee-time bias in paper II. Two-tailed p-values <0.05 and 95% confidence intervals (CI)s not crossing 1 were considered statistically significant. All analyses were done using SPSS statistics versions 25, 27, and 29 (IBM corporation, Armonk, New York).
Results

The cohort in paper I consisted of 19,566 patients, of which 19,483 patients had registered dates of diagnosis and could be included in OS analyses. The median age for all patients was 72 years and 55% were male. In patients with synchronous disease the primary tumour was removed in 64%, and attempts were made in further 11% of the patients. Primary tumour resections or removal attempts were more common in the first time period (2007-2011) than in the second time period (2012-2016, 73% vs 66%, p<0.001). Among patients with metachronous disease 84% developed metastases/local recurrence within three years from the primary tumour diagnosis.

mOS was 14.0 months in all patients. It was longer in those with metachronous, than in those with synchronous disease (mOS 17.6 vs 13.1 months). Men also had longer mOS compared with women (mOS 15.0 vs 12.8 months). Furthermore, OS was affected by age, primary tumour location, and metastatic sites. An improvement could be seen when comparing those diagnosed 2007-2011 with those diagnosed 2012-2016 (mOS 13.1 vs 14.9 months, Figure 3), this was also seen in analyses stratified by presentation of metastases, age, sex, primary tumour location, degree of differentiation, and healthcare regions.

Regional differences were also studied. When comparing healthcare regions, one of the regions had a mOS about one month shorter compared with the other ones, this difference was slightly smaller in the second time period. When looking at individual regions the difference between them was larger (about 5 months) between the ones with the shortest and longest mOS. In

Figure 3. Overall survival according to time periods

Regional differences were also studied. When comparing healthcare regions, one of the regions had a mOS about one month shorter compared with the other ones, this difference was slightly smaller in the second time period. When looking at individual regions the difference between them was larger (about 5 months) between the ones with the shortest and longest mOS. In
multivariable analysis age, primary tumour location, presentation of metastases, healthcare regions and time periods all had effects on OS, whereas sex did not.

**Paper II** included all 765 patients in the Uppsala region with mCRC that had a primary tumour diagnosis 2010-2020, 510 had synchronous disease and 255 metachronous disease. Primary tumour location was right colon in 38%, left colon in 27%, and rectum in 34%, patient characteristics per primary tumour location are presented in Table 1. Median age was higher and female sex more common in right colon than in left colon and rectum.

### Table 1. Patient characteristics according to primary tumour location

<table>
<thead>
<tr>
<th></th>
<th>Right colon</th>
<th>Left colon</th>
<th>Rectum</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>74 (32-96)</td>
<td>72 (28-92)</td>
<td>70 (35-99)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>291 38%</td>
<td>207 27%</td>
<td>262 34%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>132 45%</td>
<td>114 55%</td>
<td>156 60%</td>
<td>0.003</td>
</tr>
<tr>
<td>Female</td>
<td>159 55%</td>
<td>93 45%</td>
<td>106 41%</td>
<td></td>
</tr>
<tr>
<td>Primary resection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>99 34%</td>
<td>93 45%</td>
<td>143 55%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>192 66%</td>
<td>114 55%</td>
<td>119 45%</td>
<td></td>
</tr>
<tr>
<td>Tumour grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>152 55%</td>
<td>138 74%</td>
<td>175 80%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High</td>
<td>122 45%</td>
<td>49 26%</td>
<td>44 20%</td>
<td></td>
</tr>
<tr>
<td>Presentation of metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>187 64%</td>
<td>153 74%</td>
<td>166 63%</td>
<td>0.031</td>
</tr>
<tr>
<td>Metachronous</td>
<td>104 36%</td>
<td>54 26%</td>
<td>96 37%</td>
<td></td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>143 49%</td>
<td>91 44%</td>
<td>129 49%</td>
<td>0.663</td>
</tr>
<tr>
<td>2</td>
<td>104 36%</td>
<td>76 37%</td>
<td>92 35%</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>44 15%</td>
<td>40 19%</td>
<td>41 16%</td>
<td></td>
</tr>
<tr>
<td>Metastatic sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>176 61%</td>
<td>149 72%</td>
<td>170 65%</td>
<td>0.029</td>
</tr>
<tr>
<td>Lung</td>
<td>86 30%</td>
<td>81 39%</td>
<td>138 53%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>115 40%</td>
<td>59 29%</td>
<td>30 12%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>82 28%</td>
<td>42 20%</td>
<td>71 27%</td>
<td>0.112</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>75 26%</td>
<td>70 34%</td>
<td>105 40%</td>
<td>0.003</td>
</tr>
<tr>
<td>1</td>
<td>102 35%</td>
<td>58 28%</td>
<td>83 32%</td>
<td></td>
</tr>
<tr>
<td>2-4</td>
<td>114 39%</td>
<td>78 38%</td>
<td>74 28%</td>
<td></td>
</tr>
<tr>
<td>Mutation status</td>
<td>BRAF-V600Emt</td>
<td>89 34%</td>
<td>21 11%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RASmt</td>
<td>117 45%</td>
<td>88 45%</td>
<td>158 64%</td>
<td></td>
</tr>
<tr>
<td>RAS&amp;BRAFwt</td>
<td>57 22%</td>
<td>86 44%</td>
<td>77 31%</td>
<td></td>
</tr>
<tr>
<td>Not tested</td>
<td>28 10%</td>
<td>12 4%</td>
<td>15 5%</td>
<td></td>
</tr>
<tr>
<td>MMR-status</td>
<td>pMMR</td>
<td>150 79%</td>
<td>130 96%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dMMR</td>
<td>41 21%</td>
<td>6 4%</td>
<td>3 2%</td>
<td></td>
</tr>
<tr>
<td>Not tested</td>
<td>100 100%</td>
<td>71 100%</td>
<td>102 100%</td>
<td></td>
</tr>
<tr>
<td>Type of treatment</td>
<td>Metastasectomy</td>
<td>62 21%</td>
<td>64 31%</td>
<td>87 33%</td>
</tr>
<tr>
<td>Systemic therapy only</td>
<td>129 44%</td>
<td>90 44%</td>
<td>113 43%</td>
<td></td>
</tr>
<tr>
<td>Best supportive care</td>
<td>100 34%</td>
<td>52 25%</td>
<td>62 24%</td>
<td></td>
</tr>
</tbody>
</table>

Patients with unknown primary tumour location (n=5) not presented separately here
Molecular analyses were available for 708 (93%) of the patients. Of these, 31% were \textit{RAS}\textit{&\textit{BRAF}}\textit{wt}, 52% \textit{RAS}\textit{mt}, and 17% \textit{BRAF}-V600Emt. \textit{BRAF}-V600Emt were more common in right colon, whereas \textit{RAS}\textit{mt} were more common in rectal primary tumours (Table 1). No difference in age, presentation of metastases or number of metastatic sites were seen between mutation status. \textit{BRAF}-V600Emt were female more often and had worse ECOG performance status compared with \textit{RAS}\textit{mt} and \textit{RAS}\textit{&\textit{BRAF}}\textit{wt}. Liver metastases were less common and peritoneal metastases more common in \textit{BRAF}-V600Emt compared with \textit{RAS}\textit{mt} and \textit{RAS}\textit{&\textit{BRAF}}\textit{wt}. Lung metastases were more common among \textit{RAS}\textit{mt} compared with \textit{RAS}\textit{&\textit{BRAF}}\textit{wt} and \textit{BRAF}-V600Emt.

MMR-testing was done for 488 patients, 50 (10%) of them were dMMR. dMMR was more common in right colon than in left colon and rectum (21% vs 4% vs 2%, \(p<0.001\), Table 1). It was also more common in \textit{BRAF}-V600Emt compared with \textit{RAS}\textit{mt} and \textit{RAS}\textit{&\textit{BRAF}}\textit{wt} (33% vs 3% vs 9%, \(p<0.001\)). dMMR were older, more often female, more often high grade, and had more peritoneal metastases compared with pMMR, whereas no differences were seen regarding presentation of metastases, number of metastatic sites, or metastatic sites, other than peritoneum.

After diagnosis of mCRC, 508 (66%) received systemic therapy, of these 174 (23%) were treated with metastasectomy and/or LAT. Thirty-nine (5%) were treated with metastasectomy and/or LAT without systemic therapy and 217 (28%) with BSC only. Actively treated patients were generally younger and had better ECOG performance status. Metastasectomies and/or LATs were less common and BSC more common for right colon compared with left colon and rectum (Table 1), it was also less common for \textit{BRAF}-V600Emt compared with \textit{RAS}\textit{mt} and \textit{RAS}\textit{&\textit{BRAF}}\textit{wt}, and for dMMR compared with pMMR. EGFR-inhibitors were given to 44/156 (28%) actively treated \textit{RAS}\textit{&\textit{BRAF}}\textit{wt} patients in first line and to 68/156 (44%) in later lines.

mOS for all patients was 15.1 months. OS was inferior among right colon tumours compared with left colon and rectum tumours (median 10.8 vs 17.2 vs 21.6 months, Figure 4A), similar but non-significant trends were seen in analyses stratified by treatment. \textit{BRAF}-V600Emt had a worse OS than \textit{RAS}\textit{mt} and \textit{RAS}\textit{&\textit{BRAF}}\textit{wt} (median 6.9 vs 19.0 vs 24.4 months, Figure 4B), these remained significant also in analyses stratified by treatment. dMMR had a worse OS compared with pMMR among all patients (13.8 vs 22.9 months, Figure 4C), this was, however, not seen when stratified by treatment.

\textit{PIK3CA}mt were most common in right colon tumours, followed by left colon and least common in rectum (25% vs 19% vs 12%, \(p=0.002\)). \textit{PIK3CA}mt status did not affect survival, neither in all patients nor in analyses stratified by primary tumour, mutations status, or treatment group.
Figure 4A-C. Overall survival according to primary tumour location (A), mutation status (B), and MMR-status (C)
A multivariable model for OS was constructed using clinically relevant variables significant in univariable analyses, it was done using a backwards model. The following variables were included: age, presentation of metastases, number of metastatic sites, ECOG performance status, type of treatment, mutation status, MMR-status, and primary tumour location. All remained significant except for primary tumour location, that was removed. Age, presentation of metastases, and MMR-status were, however, significant in the opposite direction.

In paper III the final cohort consisted of 1871 patients with adequately molecularly classified tumours. KRAS-G12C was seen in 2-4% of all adequately tested patients and in 4-8% of all KRASmt in the different cohorts.

Table 2. Patient characteristics according to KRAS mutation

<table>
<thead>
<tr>
<th></th>
<th>KRAS-G12C</th>
<th>Other KRASmt</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>103</td>
<td>881</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 %</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>67 (35-86)</td>
<td>69 (23-99)</td>
<td>0.036</td>
</tr>
<tr>
<td>Median age (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46 45 %</td>
<td>57 55 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>378 43 %</td>
<td>503 57 %</td>
<td>0.734</td>
</tr>
<tr>
<td>ECOG performance</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>status</td>
<td>27 26 %</td>
<td>50 49 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>297 34 %</td>
<td>394 45 %</td>
<td>0.184</td>
</tr>
<tr>
<td>2-4</td>
<td>25 25 %</td>
<td>25 25 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>188 21 %</td>
<td>188 21 %</td>
<td>0.194</td>
</tr>
<tr>
<td>Primary tumour</td>
<td>Right colon</td>
<td>Left colon</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 28 %</td>
<td>36 35 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>295 34 %</td>
<td>266 30 %</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38 37 %</td>
<td>18 10 %</td>
<td></td>
</tr>
<tr>
<td>Tumour grade</td>
<td>Low</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74 85 %</td>
<td>13 15 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>588 64 %</td>
<td>111 67 %</td>
<td>0.821</td>
</tr>
<tr>
<td>Presentation of</td>
<td>Synchronous</td>
<td>Metachronous</td>
<td></td>
</tr>
<tr>
<td>metastases</td>
<td>61 59 %</td>
<td>42 41 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>555 63 %</td>
<td>326 37 %</td>
<td>0.454</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54 52 %</td>
<td>16 16 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>443 50 %</td>
<td>151 17 %</td>
<td>0.640</td>
</tr>
<tr>
<td></td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 32 %</td>
<td>16 16 %</td>
<td></td>
</tr>
<tr>
<td>Metastatic sites</td>
<td>Liver</td>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 64 %</td>
<td>40 39 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>615 70 %</td>
<td>321 36 %</td>
<td>0.633</td>
</tr>
<tr>
<td></td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 24 %</td>
<td>15 15 %</td>
<td></td>
</tr>
<tr>
<td>MMR-status</td>
<td>pMMR</td>
<td>dMMR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33 97 %</td>
<td>1 3 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>425 97 %</td>
<td>12 3 %</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
</tbody>
</table>

Patients with other mutations not presented separately here

KRAS-G12C were slightly younger than other KRASmt, but no other differences in clinical characteristics were seen between KRAS-G12C and other KRASmt (Table 3). Metastasectomies and/or LATs were done more often in KRAS-G12C compared with other KRASmt (38% vs 28%, p=0.040) and bevacizumab was added more often in any treatment line in KRAS-G12C compared with other KRASmt (74% vs 59%, p=0.007), similar but non-significant
trends were seen in analyses where retrospectively collected patients were excluded. No differences were seen between KRAS-G12C and other KRASmt in systemic therapies in first line treatment, between other systemic therapies in all lines, or in response to first line therapy.

Figure 5. Overall survival according to mutation status

OS was comparable between KRAS-G12C and other KRASmt (mOS 29.6 vs 22.0, Figure 5). It was also similar when comparing different treatment groups separately (systemic therapy only, metastasectomy and/or LAT, and BSC). This was also seen in verifying analyses excluding the 49 patients included retrospectively. A multivariable model correcting for relevant clinical factors was also constructed. The variables included were age groups, sex, primary tumour location, presentation of metastases, number of metastatic sites, ECOG performance status, treatment groups, and cohort. In the adjusted model HR was 1.03 (95% CI 0.75-1.43) for other KRASmt, with KRAS-G12C as reference.

Paper IV studied aBRAFmt in comparison with BRAF-V600Emt, RAS&BRAFwt, and RASmt patients in 1449 patients from four different cohorts. aBRAFmt were seen in 1-4% of adequately tested patients in the different cohorts, with slightly higher proportions in population-based materials. In total, 51 aBRAFmt were included. aBRAFmt were male more often, had more rectal primary tumours, and less peritoneal metastases compared with BRAF-V600Emt (Table 4). aBRAFmt also had concomitant RASmt more often but
were rarely dMMR in comparison with \textit{BRAF-V600Emt} (Table 4). Compared with \textit{RAS&BRAF\textsubscript{wt}} they had fewer primary tumours in the left colon, and less liver metastases. When compared with \textit{RAS\textsubscript{mt}}, \textit{aBRAF\textsubscript{mt}} had lymph node metastases more often, and worse ECOG performance status.

**Table 3. Patient characteristics according to \textit{BRAF} mutation**

<table>
<thead>
<tr>
<th></th>
<th>\textit{aBRAF\textsubscript{mt}}</th>
<th>\textit{BRAF-V600Emt}</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>69 (51-89)</td>
<td>69 (33-86)</td>
<td>0.533</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100%)</td>
<td>182 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20 (39%)</td>
<td>113 (62%)</td>
<td>ref</td>
</tr>
<tr>
<td>Male</td>
<td>31 (61%)</td>
<td>69 (38%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Primary tumour location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>17 (33%)</td>
<td>128 (71%)</td>
<td>ref</td>
</tr>
<tr>
<td>Left colon</td>
<td>11 (22%)</td>
<td>37 (21%)</td>
<td>0.061</td>
</tr>
<tr>
<td>Rectum</td>
<td>23 (45%)</td>
<td>15 (8%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Tumour grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>30 (70%)</td>
<td>92 (58%)</td>
<td>ref</td>
</tr>
<tr>
<td>High</td>
<td>13 (30%)</td>
<td>67 (42%)</td>
<td>0.159</td>
</tr>
<tr>
<td>Primary surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13 (25%)</td>
<td>35 (19%)</td>
<td>ref</td>
</tr>
<tr>
<td>Yes</td>
<td>38 (75%)</td>
<td>147 (81%)</td>
<td>0.330</td>
</tr>
<tr>
<td>Presentation of metastases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>30 (59%)</td>
<td>114 (63%)</td>
<td>ref</td>
</tr>
<tr>
<td>Metachronous</td>
<td>21 (41%)</td>
<td>68 (37%)</td>
<td>0.620</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22 (43%)</td>
<td>88 (48%)</td>
<td>ref</td>
</tr>
<tr>
<td>≥3</td>
<td>24 (47%)</td>
<td>56 (31%)</td>
<td>0.114</td>
</tr>
<tr>
<td>Metastatic sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>31 (61%)</td>
<td>99 (54%)</td>
<td>0.417</td>
</tr>
<tr>
<td>Lung</td>
<td>18 (35%)</td>
<td>42 (23%)</td>
<td>0.080</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>19 (37%)</td>
<td>73 (40%)</td>
<td>0.712</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>6 (12%)</td>
<td>58 (32%)</td>
<td>0.007</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12 (24%)</td>
<td>49 (27%)</td>
<td>ref</td>
</tr>
<tr>
<td>1</td>
<td>22 (43%)</td>
<td>71 (39%)</td>
<td>0.560</td>
</tr>
<tr>
<td>2-3</td>
<td>17 (33%)</td>
<td>62 (34%)</td>
<td>0.789</td>
</tr>
<tr>
<td>\textit{RAS}\textsubscript{-status}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>30 (59%)</td>
<td>178 (98%)</td>
<td>ref</td>
</tr>
<tr>
<td>Mutated</td>
<td>21 (41%)</td>
<td>4 (2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>\textit{MMR}\textsubscript{-status}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMMR</td>
<td>50 (98%)</td>
<td>93 (73%)</td>
<td>ref</td>
</tr>
<tr>
<td>dMMR</td>
<td>1 (2%)</td>
<td>34 (27%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Not tested</td>
<td>0 -</td>
<td>55 -</td>
<td>-</td>
</tr>
</tbody>
</table>

Patients with other mutations not presented separately here

Of the 51 included \textit{aBRAF\textsubscript{mt}}, class 2 mutations were seen in 11 (22\%), class 3 in 32 (63\%), class 1+2/3 in 4 (8\%), and 4 (8\%) could not be classified. Class 2 mutants had non-significant trends for higher age, more males, more rectal primary tumours, and worse ECOG performance status compared with class 3.

Histology of \textit{aBRAF\textsubscript{mt}} was assessed in 44 (86\%), of the evaluated samples 24 (59\%) were serrated adenomas, 15 (37\%) were adenocarcinoma not otherwise specified, 1 (2\%) was undifferentiated histology, and 1 (2\%) had mucinous
histology. There was a trend for more serrated adenomas in class 3 compared with class 2.

Patients with aBRAFmt and BRAF-V600Emt were treated with metastasectomies and/or LATs or systemic therapy less often than RAS&BRAFwt and RASmt. Nine aBRAFmt received EGFR-inhibitors, among these no responses were seen, and five had progression at the first response evaluation. Among aBRAFmt the addition of bevacizumab had a trend of better OS and PFS.

aBRAFmt had significantly better OS (median 14.4 months) than BRAF-V600Emt (11.2 months) and significantly worse OS than RASmt (23.4 months) and RAS&BRAFwt (30.5 months, Figure 6). Similar trends were seen in patients treated with systemic therapy only. Outcome after metastasectomy and/or LAT among aBRAFmt was like that of RAS&BRAFwt and RASmt and better than for BRAF-V600Emt. No difference in OS was seen between class 2 and class 3 aBRAFmt. RAS&BRAFwt patients with concomitant aBRAFmt had significantly worse OS, which could also be seen for RASmt with concomitant aBRAFmt.

Figure 6. Overall survival according to mutation status
Discussion

The results in paper I highlight that OS is shorter (median 14 months) in the background population than in clinical trials or at single hospitals reporting their results in literature.\textsuperscript{139,176,177} Patients in clinical trials and generally at single hospitals are selected and not presentable for the whole population.\textsuperscript{81,183} An improvement in OS was seen over time also in the background population, however, the improvement seen is far from the one in clinical trials. The difference presented seems to be true as it was seen also in stratified analyses and in a multivariable model. Data in the literature are consistent regarding that OS has improved since the 1980-1990s.\textsuperscript{179-182} Regarding the time period our study is from an improvement was seen in a cohort from the SEER database,\textsuperscript{204} whereas, a Dutch population-based study saw no improvement.\textsuperscript{205}

The study also shows that no apparent differences in OS exist, that could indicate that there are unmotivated differences between geographical regions or gender in Sweden. These latter conclusions are, however, uncertain due to the lack of knowledge about oncological treatment details and molecular alterations. In a similar study also based upon the Swedish national material from SCRCR between 2007-2016, gender inequality was reported.\textsuperscript{206} This study reported that liver surgery was performed less often in females, an aspect not studied by us. Although the differences according to sex were minor, they ought to be similar between two studies based upon the same patient material. It is hard to provide a good explanation for the slight differences noted that resulted in different conclusions. Our patient material was more extensively scrutinized and small patient groups not truly distant metastatic and where differences in registration during the period may have been present were identified and removed. Another aspect is that the other study only included patients with synchronous disease. It is not possible to exclude gender differences, but it is my belief that they are minor, if present at all. Marked differences in age, primary tumour location, and prevalence of the two molecular properties having the worst prognosis, \textit{BRAF}-V600Emt and dMMR, may be difficult to handle statistically in the regression analyses.

In paper II a large population-based cohort, with in practice all mCRC patients in one region were studied. We could confirm that \textit{BRAF}-V600Emt (seen in 17%) and dMMR (seen in 10%) are more common in population-based materials than in trial populations, where these are seen in 2-12% and
Both of these molecular alterations have a poor prognosis and they probably therefore less often make it to trials or hospitals reporting data in research publications. Right colon primary tumours were more common in this cohort (38%) than in recent clinical trials (25-30%). OS was worse in right colon tumours in our material, an aspect also seen by many others. When studying OS in homogenous subgroups by mutation status (among all tested and among pMMR only) we could not observe any OS difference between right colon and left colon primary tumours among *BRAF*-V600Emt, *RAS*mt, and *RAS&BRAF*wt. Earlier studies have reported no differences in OS between right- and left-sided tumours among *BRAF*mt and *RAS*mt. However, among *BRAF*wt patients OS was better in left-sided tumours compared with right-sided, as presently is believed. These findings are further supported by results from multivariable OS analyses where primary tumour location was removed from the model as non-significant; in another study this was, however, not seen. Based upon this it seems as if the poor prognosis among right colon tumours at least partially could be explained by molecular alterations with a poor prognosis being more prevalent. This work highlights the importance of knowing molecular alterations for as many patients as possible to be able to draw firmer conclusions.

In paper III *KRAS*-G12C was seen in 2-4% of all patients and in 4-8% of *KRAS*mt in the different cohorts. This is in line with results from large databases of molecularly tested patients. Regarding clinical characteristics we saw no differences between *KRAS*-G12C and other *KRAS*mt. Some differences in sex, age, and metastatic patterns have been observed between different *KRAS*mt in other studies, however, non-consistent. Metastasectomies were more common in *KRAS*-G12C compared with other *KRAS*mt, this was, however, only a trend when excluding patients retrospectively collected. Partially this could be explained by that the retrospectively collected patients were more recently collected, as metastasectomies have become more common over time. Regarding the use of chemotherapy no major differences were seen between *KRAS*-G12C and other *KRAS*mt. With similar prognosis and clinical presentation, differences in treatment may also be chance findings.

OS was similar for patients with *KRAS*-G12C and other *KRAS*mt. This has also been reported in an Italian study, whereas other studies have shown an inferior OS for *KRAS*-G12C compared with other *KRAS*mt. No difference in PFS was seen in our study, the Italian study also showed similar PFS, whereas other have shown inferior PFS for *KRAS*-G12C.
Since the publication of this study \textit{KRAS}-G12C inhibitors have been further studied in combination with cetuximab. In a phase 3 trial by Fakih et al a statistically significant improvement in PFS could be seen for two different doses of sotorasib combined with cetuximab when compared with standard of care (median 5.6 vs 3.9 vs 2.2 months). Differences in OS are yet to be shown as data mature.\textsuperscript{217} At present there is also an ongoing study comparing adagrasib + cetuximab in second line mCRC treatment.\textsuperscript{89,218} \textit{KRAS}-G12C therefore seem to be like other \textit{KRAS}\textsuperscript{mt} regarding patient characteristics, treatment, and outcome. Which contrasts to most earlier studies claiming differences. Even if \textit{KRAS}-G12C are rare and the effects of \textit{KRAS}-G12C inhibitors limited in clinical trials so far, it is step forward in the world of precision medicine and targeted therapy, that hopefully can change the outcome for this subgroup of patients in the future. It also seems like other \textit{KRAS}mt could be targeted, inhibitors of \textit{KRAS}-G12D have shown promising results in animal models,\textsuperscript{219,220} and are presently being studied in a phase 1/2 trial.\textsuperscript{221} Because these mutations are more common (about 1/3 of \textit{KRAS}mt) they are something that could benefit a larger proportion of the patients.\textsuperscript{65} If also other \textit{KRAS}mt can be targeted in the future up to 40% could have targeted therapies.\textsuperscript{63}

\textbf{Paper IV} shows a prevalence of 1-4\% for \textit{aBRAF}mt in the different cohorts studied, in line with previous studies showing a prevalence of around 2\%.\textsuperscript{88,89} Slightly higher proportions were seen in population-based materials where testing of all patients with sufficient tumour material was done. This fits with that \textit{aBRAF}mt have a worse outcome, similar to what we have earlier reported, namely that molecular alterations with a worse prognosis, such as \textit{BRAF}-V600Emt and dMMR are more common in population based materials compared with study populations.\textsuperscript{79,80} Similar results were also seen in paper II in this thesis.

In our material patients with \textit{aBRAF}mt were about as old as \textit{BRAF}-V600Emt, but older than \textit{RAS}\&\textit{BRAF}wt and \textit{RAS}mt, which contrasts to another study showing that \textit{aBRAF}mt patients were younger than \textit{BRAF}-V600Emt and about as old as \textit{RAS}\&\textit{BRAF}wt and \textit{RAS}mt.\textsuperscript{88,89} \textit{aBRAF}mt were more common in males in our study, which has also been seen in another large study.\textsuperscript{88} \textit{aBRAF}mt were more similar to \textit{RAS}\&\textit{BRAF}wt and \textit{RAS}mt in the sense that they have less right colon primary tumours than \textit{BRAF}-V600Emt, which is confirmed in other studies as well.\textsuperscript{88,89,222} Possibly related to this we could see that peritoneal metastases were less common among \textit{aBRAF}mt compared with \textit{BRAF}-V600Emt, as reported by others.\textsuperscript{88,222} Co-occurring \textit{RAS}mt were seen more often among \textit{aBRAF}mt compared with \textit{BRAF}-V600Emt (41\% vs 2\%) in our study, again reported also by others.\textsuperscript{88,89,223} For dMMR the opposite was seen, dMMR was seen more often
among \textit{BRAF-V600Emt} and in \textit{aBRAFmt} about as often as in \textit{RAS\&BRAFwt} and \textit{RASmt} (72% vs 2% vs 3% vs 4%), and similar to other studies.\cite{88,89,222,223}

Metastasectomies and/or LATs among \textit{aBRAFmt} were more frequent than among \textit{BRAF-V600Emt}, but less frequent than for \textit{RAS\&BRAFwt} and \textit{RASmt}. These frequencies go well in hand with the OS observed between mutational groups. Nine patients in total were treated with EGFR-inhibitors, none of them had responses to the therapy. Similar results have been observed in another study,\cite{89} whereas two other studies showed responses, mainly among class 3 \textit{aBRAFmt}.\cite{222,223}

OS was longest in \textit{RAS\&BRAFwt} followed by \textit{RASmt}, \textit{aBRAFmt}, and \textit{BRAF-V600Emt} (median OS 31 vs 23 vs 14 vs 11 months). \textit{aBRAFmt} had a significantly better OS than \textit{BRAF-V600Emt}, which has been seen in other studies as well.\cite{88,89} Compared with \textit{BRAFwt} patients (including both \textit{RAS\&BRAFwt} and \textit{RASmt}) another study has reported an even better outcome among \textit{aBRAFmt} contrary to our results,\cite{88} whereas others have reported worse or similar results more similar to ours.\cite{89,224} No difference in OS between class 2 and class 3 \textit{aBRAFmt} was seen, as in another study.\cite{89}

Our study shows that \textit{aBRAFmt} are rare and differ clinically from \textit{BRAF-V600Emt}. Regarding prognostic effect they do worse than \textit{RAS\&BRAFwt} and \textit{RASmt}, but better than \textit{BRAF-V600Emt}. As \textit{aBRAFmt} are prognostic and seem to affect efficacy of EGFR-inhibitors it should be recommended to include them in molecular analyses of mCRC in the clinics. It is also important that treating clinicians know about the different \textit{BRAF} mutation classes and that they differ from one another. Most studies to date on this mutation are small and often selected, why larger and preferably prospective studies are needed for firmer conclusions, particularly about sensitivity to EGFR-inhibition. There are also ongoing trials studying treatments specifically in this subgroup of patients.\cite{225} \textit{aBRAFmt} will probably be an important part of precision medicine in the future.

The results from paper III-IV show that also less common molecular alterations are important to test for and that large, combined materials are needed for at least reasonably firm conclusions. Even if many materials are combined as done here it is difficult to get enough statistical power. One way of overcoming this is having large databases where data can be deposited by researchers. These types of databases have been created and, without question, they have created much knowledge. This is not entirely unproblematic and control over the selection of patients is needed as all knowledge gained from these may not always be clinically relevant. This is because often only a small, selected proportion of patients in a cohort or trial can be analysed. An example of this is a study by Innocenti et al where only 24% had molecular analyses done, and where the authors state that \textit{BRAF-V600Emt} are seen less often
among black patients than among white patients (5% vs 14%). This is, however, more likely explained by a differential inclusion of patients and not differences between ethnicities.

Several initiatives have tried to limit selection and analyse as many cases as possible, one such example is the U-CAN database in Uppsala/Umeå. In the cohort in paper II about 60% were included in U-CAN but even in a database/biobank as this one, selection will be present, as for example patients in poor condition may not make it to the doctor and/or there is not enough tumour material to perform molecular analyses. In paper II molecular analyses were completed for as many patients as possible and 93% could be analysed, which should make data more reliable. However, even in this dataset there were still non-completely tested tumours due to limited material and five patients only had a clinical diagnosis.

A major strength of the articles included in this thesis is that the cohorts are population-based or real-world, which makes them representable for the background population. The cohort studied in paper I includes all mCRC patients in Sweden over a 10-year period, which makes the material optimal to study the outcome and how it has changed and the effect of demographics factors on a nationwide level. There is always a risk of errors in the registration and that, e.g., some recurrences have not been registered. In a validation study in two regions, we note that these errors in SCRCR are limited or in the order of a few percentages. Potential errors were also reduced to a minimum by evaluation and rectification of the data. The cohort in paper II was also population-based and included all patients with mCRC in a Swedish region, here the data could be carefully rectified. One of the other materials in paper III and IV is also population-based. Another strength of population-based materials is that the natural course of the disease can be studied in non-treated patients. The second cohort included in papers III-IV is a real-world cohort where selection is evident, but not to the same extent as in clinical trials.

All cohorts used in papers II-IV have reliable clinical data which makes it possible to study different aspects of the disease. In all cohorts the aim has also been to make molecular analyses as complete as possible and in the combined material 73% of the patients could be adequately characterised, which is far from perfect but still better than in retrospective analyses of clinical trials. Only a handful of patients are lost to follow-up, which also make survival data reliable and robust.

A limitation for paper I is that information on molecular alterations and treatment of metastatic disease was not available, which makes it impossible to adjust for these factors. Regarding molecular testing it is a limitation that many different methods for testing were used as the analyses were performed in the clinical routine in the RAXO-study and for a large proportion of the
patients in the Uppsala region. Especially in the earlier years it is also a limitation that aBRAF\textsuperscript{mt} and some KRAS codons were not included in the molecular analyses. As aBRAF\textsuperscript{mt} and KRAS-G12C are rare, retrospectively collected patients without controls had to be included in paper III-IV to get enough patients for firmer conclusions on these subgroups. To control for this, sensitivity analyses excluding these patients were also done which did not alter the results. The PRCRC-study is older compared than the other cohorts in paper III-IV which can be seen as a limitation. This affects OS among all patients, but when stratifying by treatment group OS is similar between the cohorts included, and therefore this should not affect the results. Even if the aim has been to collect large patient materials it is still problematic that some subgroup analyses are based upon quite a small number of patients, this is, however, something that always will be a problem. Comparison of treatments is also hard to do as there is a selection for which treatment that is used. Treatment effects are best studied in randomised trials. The data here are on the other hand better when describing treatments used in the clinical routine.
Conclusions

Study populations differ from population-based and real-world materials regarding demographic factors, molecular alterations, and outcome. OS has improved also in population-based materials although the gain during the most recent decade was limited, and no major differences according to gender or geography could be seen. *BRAF*-V600Emt and dMMR are common in right colon primary tumours and this seems to at least partially explain the worse prognosis seen in right colon primary tumours. *KRAS*-G12C mutations are rare in mCRC and do not differ from other *KRAS*mt regarding demographics or outcome. *aBRAF*mt are also rare, affect demographics, and confer a worse prognosis compared with wildtype tumours. Large materials and combinations of materials are needed for studying rare molecular properties and future studies need to include other rare mutations as well since drugs against these can be developed.
Future investigations

In the future it continues to be important to further study clinical characteristics, treatments, and molecular properties of mCRC, to gain more knowledge about the disease and improve treatment and outcome for the patients.

Regarding clinical characteristics the cohorts included in this thesis have extensive and reliable clinical information which makes it possible to further study different aspects of mCRC. We plan to study the influence of regional lymph node metastases, as data on these in mCRC is sparse.\(^{227}\) This is planned to be studied in a combined material of the RAXO-study, PRCRC-study, and Uppsala region cohort, in a subgroup of patients with primary tumour resections. Preliminary results have been presented at the ESMO congress in Madrid 2023. Patients with regional lymph nodes differed in many aspects from those without regional lymph nodes, for example regarding primary tumour location, degree of differentiation, and metastatic sites.

In the national material used in paper I the information on oncological treatments given was limited and therefore not analysed. An updated file was acquired recently, which includes a total of 32 054 patients, here information of treatments is registered better. The plan is to analyse treatments more in depth in this project.

Molecular analyses are also important to study further. At present mainly mutations in KRAS, NRAS, and BRAF genes, and MMR-status are the main molecular alterations generally recommended to test for in mCRC.\(^{125}\) Broad genetic testing has been done in mCRC, but also other omics including proteomics have made their way into the field. All this research has generated a lot of new knowledge, but a large part is not clinically relevant and has not made it to the clinics. This highlights the need for translational research where the aim is to move knowledge gained from basic science to the clinics. Another challenge with these analyses is the large amount of data created. The interpretation can also be challenging as many parameters are included, one way of overcoming this could be to use artificial intelligence for data interpretation. Learning more about the biology of CRC will make it possible to implement precision medicine even more in the clinic. New targeted therapies could be developed, and treatments could be tailored for the patients, which in turn could improve outcome and quality of life for the patients.
Broad genetical analyses is an aspect that will be studied in the materials included here. In the Uppsala region cohort, a genetic panel consisting of 409 cancer genes was analysed in 397 patients and 187 patients that had frozen tissue available were sequenced with WGS. This will make it possible to analyse other molecular alterations as well.

There are also plans to study other \textit{KRaS} mt, which is of interest since inhibitors against other \textit{KRaS} mt than G12C have been developed. This will be analysed in the same combined material as in paper II. Plans are also to include patients from a Nordic clinical trial (Nordic-VII).\textsuperscript{228}

Since \textit{aBRAF} mt are rare and data on them sparse, larger combined materials are needed. There are plans on combing our data with data of researchers in the United States, Canada, and Italy to gain a better understanding of the behaviour and the most optimal treatment of \textit{aBRAF} mt.

The development within the field of CRC is something I want to be a part of also in the future besides working clinically with cancer.
Populärvetenskaplig sammanfattning

Tjock- och ändtarmscancer är tillsammans den tredje vanligaste cancerformen och den näst vanligaste orsaken till död i cancer. Omkring en fjärdedel av patienterna har metastaser redan vid diagnos och nästan lika många får metastaser senare. Överlevnaden vid spridd sjukdom har förbättrats märkbart de senaste årtiondena, dock främst i kliniska studier där individer med de bästa förutsättningarna har valts ut; de är ofta yngre, friskare och har en bättre funktionsnivå. I denna avhandling har patienter från populationsbaserade material där alla patienter ingår och så kallade ”real-world” material där patienterna inkluderas i den kliniska vardagssjukvården. Detta gör resultaten mer representativa för hela populationen.


I det andra delarbetet är ett populationsbaserat material från Uppsala där alla 765 patienter med spridd tjock- och ändtarmscancer 2010–2020 inkluderats. Primärtumörer i höger del av tjocktarmen sågs hos 38%, i vänster del av tjocktarmen hos 27% och i ändtarmen hos 34%. En sämre överlevnad sågs hos dem med primärtumör i höger del av tjocktarmen. BRAF-V600E mutationer och mikrosatellitinstabilitet var vanligare bland primärtumörer i höger del av tjocktarmen och dessa hade sämre överlevnad. Dessa faktorer verkar vara viktiga för prognosen än var primärtumören i sig startade.

I det tredje delarbetet studerades en specifik mutation i KRAS genen, nämligen KRAS-G12C. Denna är av särskilt intresse eftersom målinriktad behandling har utvecklats mot förändringen. För detta arbete kombinerades fyra nordiska patientmaterial. KRAS-G12C mutationer utgjorde 2–4% av all molekylärt testade och 4–8% av dem med KRAS mutationer. Vad gäller bakgrundskaraktäristika var KRAS-G12C väldigt lika övriga KRAS muterade och gällande behandling var operationer av metastaser lite vanligare bland KRAS-G12C än övriga KRAS muterade, medan användningen av kemoterapi var mer lika.
Avseende överlevnaden kunde ingen skillnad ses mellan KRAS-G12C och övriga KRAS mutationer. Detta skiljer sig från tidigare studier där man beskrivit skillnader dels i bakgrundskarakteristika och överlevnad mellan KRAS-G12C och övriga KRAS muterade.

Det fjärde delarbetet studerades atypiska BRAF mutationer, vilka är separata från de vanligaste BRAF-V600E mutationerna. Eftersom dessa mutationer är ovanliga kombinerades tre olika nordiska material för att kunna dra starkare slutsatser. Atypiska BRAF mutationer sågs hos 1–4% i de olika materialen. Dessa skiljde sig från BRAF-V600E mutationer i form av att de oftare var män, oftare hade primärtumörer i ändtarmen, mer sällan hade metastaser i bukhinnorna och mer sällan hade mikrosatellitinstabilitet. Överlevnaden för dessa var signifikant bättre än för BRAF-V600E muterade men sämre än för dem med RAS&BRAF vildtyp eller RAS mutationer. Dessa mutationer verkar således vara en egen undergrupp som inte bör grupperas ihop med andra undergrupper.

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