Chapter 1

General Introduction and Outline of the Thesis
**Colorectal Cancer – General**

Colorectal cancer (CRC) is one of the most prevalent epithelial cancers, with an annual worldwide incidence of over 1 million cases. In the Netherlands, 15,000 new patients are diagnosed each year. Annually a total of 5,000 patients ultimately succumb to the disease. This is largely due to spread of the disease to distant sites such as liver or lung. In addition to the haematogenous spread of CRC to the aforementioned sites, it is also known to spread locally to the peritoneal surface.

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<th>TNM</th>
<th>Stage at first diagnosis</th>
<th>Five year Survival</th>
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<td>Tis</td>
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<td>T1-2</td>
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<td>T1-4</td>
<td>N0-2 M1*</td>
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Adapted from [www.oncoline.nl](http://www.oncoline.nl) & Siegel et al. 1-3

Tis= carcinoma in situ; T1=tumour invades submucosa; T2=tumour invades muscularis propria; T3=tumour invades through muscularis propria into subserosa or into non-peritonealised pericolic or perirectal tissues; T4=tumour penetrates visceral peritoneum or directly invades other organs and/or structures; N0=no metastases in regional lymph nodes; N1-2= regional lymph node metastases present; M0= no distant metastases; M1=distant metastases present

* The M1 category includes patients with peritoneal metastases.

**Colorectal Cancer – Peritoneal Metastases**

**Epidemiology**

Metastatic disease (Stage IV) is present in approximately 20% of patients at time of diagnosis (Table 1). Additionally, metastases develop in up to half of all CRC patients during the course of the disease. Roughly 10-25% of CRC patients develop peritoneal metastases (PM), of which 25% present with the peritoneum as the sole sight of distant metastases. The peritoneum is a composed of a single layer of mesothelial cells on top of a basal membrane underneath of which there is loose connective tissue, which cover the abdominal wall and internal organs. Peritoneal metastases (PM) are believed to be the result of tumour cell shedding into the peritoneal cavity, either spontaneously or as a result of spill during surgical procedures (Fig.1). These tumour cells are believed to subsequently spread through the abdominal cavity by means of the physiological flow of peritoneal fluid and gravitational forces. The process of attachment of tumour cells to the mesothelial cells of the peritoneum is believed to involve several steps and multiple molecules, ultimately
resulting in the development of solid tumour deposits on the peritoneal surface thus known as peritoneal metastases (PM) or peritoneal carcinomatosis. 10,11

**Figure 1.** Schematic overview of peritoneal dissemination of colorectal cancer, showing in a sagittal section, the proposed mechanism of transcoelomic spread of tumour cells which can ultimately lead to the formation of peritoneal metastases.

**Clinical Course of the patient presenting with Peritoneal Metastases**

**Clinical features**

The deposition and growth of the tumour cells on the surface of the peritoneum can lead to tumour deposits which can ultimately involve any intra-abdominal organ or structure which is covered by the peritoneum. Preferential sites for intra-abdominal tumour implantation on the peritoneal surface include: the omentum, the mesentery, the surface of the bowel, Douglas’ pouch, the right paracolic gutter and the diaphragm. 12-14 Patients initially present either with no or aspecific symptoms, which are often indistinguishable from the symptoms seen in malignant disease in general. Ultimately, the invasion of malignant cells and growth of peritoneal implants on the bowel surface can lead to bowel obstruction and ascites. 15 However, it should be noted that a considerable proportion of patients do not report any symptoms at the time of diagnosis. This is especially the case when the peritoneal implants are of limited size and little to no ascites is present. 16
Diagnosis

The adequate assessment of intra-abdominal tumour burden is essential for the initial diagnosis of PM. The diagnosis is made by visualization of the PM, either by imaging or by laparotomy or laparoscopy. Firstly, it is crucial to assess the extent of disease spread in order to assess whether further treatment is prudent. In addition it is also crucial for the evaluation of treatment response and follow-up. Notwithstanding, the currently available diagnostic tools are lacking for proper assessment in this sub-group of CRC patients.

Tumour marker testing, such as serum CEA levels, have been shown to be able to diagnose recurrent CRC, including PM. However, these raised levels only indicate recurrence of disease, not the volume or location of intra-abdominal tumour deposits. Both volume and location are critical factors in the selection of patients for aggressive surgical treatment.

Computed Tomography (CT)- and/or Positron Emission Tomography (PET) scanning also lack the sensitivity and specificity needed for the timely detection of, especially, smaller peritoneal tumour nodules (<1cm). The value of pre-operative imaging in PM has not only been described to be dependent on lesion size, but also dependent on the evaluating radiologist. It has been proposed that one difficulty in the interpretation is that the peritoneum follows the natural contours of the intra-abdominal organs, making it difficult to assess whether it is affected by disease spread by these modalities, especially in the case of limited peritoneal involvement and/or small (<1cm) nodules. Furthermore, one study also shows that the greatest inaccuracy of pre-operative CT imaging for PM is seen in the pelvis, one of the preferential sites for peritoneal implantation of tumour cells. Consequently, the extent of the peritoneal carcinomatosis is often underestimated.

The extent of intra-abdominal spread can only truly correctly be assessed and documented during either laparoscopy or laparotomy. Laparoscopy continues to be one of the most effective methods of PM detection, which also gives the treating physician the opportunity to collect tissue for definite pathological diagnosis. Yet, this remains an invasive procedure, which is accompanied by the risks associated with any operative procedure. Furthermore, adhesions from prior surgeries can potentially hinder adequate and complete inspection of the peritoneal surface.

However, pre-operative imaging by means of CT- and/or PET-scanning still remains essential. Firstly, for the detection of extra-peritoneal disease, such as liver- and/or lung metastases, in order to properly screen and select potential candidates for treatment, as CRS and HIPEC is largely reserved for patients with isolated PM. Secondly, in the case a patient presents with symptoms, logistically it is faster to perform imaging studies to assess disease burden than to immediately operate if the symptoms are not directly life-threatening.
Possible treatment strategies for peritoneal metastases of colorectal cancer

*Treatment – Systemic Therapies*

In the past, peritoneal metastases have been solely regarded as a condition only amenable to treatment with a palliative intent, due to the belief that the condition inevitably leads to a rapid demise of the patient. There is a considerable paucity of data concerning the outcome of systemic therapies in specifically patients with PM of CRC. One hurdle in the interpretation of the efficacy of treatment in this subgroup of metastatic colorectal cancer patients is the difficulty in assessing treatment response with imaging using standard RECIST criteria, as is customary with systemic metastases such as liver- and lung metastases. Due to this difficulty in objectifying treatment response this subgroup of metastatic CRC patients is underrepresented in randomized controlled trials assessing chemotherapeutic regimens in metastatic colorectal cancer. The few studies assessing the response to treatment in patients presenting with PM focus on regimens including 5-FU and only some on the addition of oxaliplatin and irinotecan in retrospective cohorts. On average, the survival of these patients ranges from six to 12 months. The general tendency observed in these studies is that patients with PM fare considerably less well than patients with metastases confined to either the liver or the lung. However, it should be mentioned that a substantial proportion of the patients analyzed in these studies do not present with isolated PM, but in combination with either liver or lung metastases. This makes it difficult, if not impossible, to assess the effect of systemic treatment in patients with isolated PM of CRC.

The advent of newer treatment strategies, including those with monoclonal antibodies against the Epidermal Growth-Factor Receptor (EGFR) and Vascular Endothelial Growth-Factor (VEGF), have shown promising results in patients with metastasized colorectal cancer in general. Yet, the effect of treatment with these targeted therapies in isolated PM of CRC is still largely unknown. Only one study in which patients were treated with an anti-VEGF agent (Bevacizumab) show that a subgroup of patients with PM, both isolated or in combination with haematogenous metastases, show an overall survival of about 15 months. Another cohort of patients with PM, isolated or in combination with other metastatic sites, were treated with Bevacizumab, Cetuximab or Panitumumab in palliative setting showed overall survival of about 18 months versus 10 months in those who were only treated with standard cytotoxic therapy. This suggests that there is possibly an effect of biological agents in PM of CRC. However this effect is still markedly less than the effect observed in patients with haematogenous metastases. This could mean that the biologic basis of PM is distinct, which in turn could translate into a relative resistance to treatment with systemic chemotherapy. Yet, the fact remains that very little is known about the effects of (modern) systemic chemotherapy treatment on isolated PM, in contrast to hepatic or pulmonary metastases of CRC.
Treatment - The advent and implementation of Cytoreductive Surgery and Hyperthermic IntraPeritoneal Chemotherapy

Surgical treatment of both hepatic and pulmonary metastases is the mainstay of current treatment for metastasized CRC, under the condition that these metastases are amenable to resection. Often the surgical resection is followed by treatment with systemic chemotherapy. Adjuvant systemic chemotherapy after radical resection of liver metastases, with or without the addition of so-called biologicals has been shown to give a disease-free survival rate of about 50%. However, for a considerable time this approach was not considered feasible or desirable for patients presenting with PM due to the observation that most of these patients succumb to the disease well within a year after diagnosis if either left untreated or if they are treated exclusively with (standard) palliative chemotherapy. It was however noted that there is a subgroup of patients that only present with PM, with no hepatic or pulmonary metastases. This led to the theory that PM are a form of localized spread, and thus should be amenable to a form of localized treatment, i.e. surgery. Risk factors described for tumour seeding on the peritoneal surface include advanced T-stage of the primary tumour (pT4) and bowel perforation. Subsequently, a novel treatment was developed in the late eighties-early nineties consisting of an aggressive surgical approach, CytoReductive Surgery (CRS), combined with Hyperthermic IntraPeritoneal Chemotherapy (HIPEC). The aim of this procedure is to surgically remove all macroscopically visible intra-abdominal tumour deposits. This treatment is administered only when the patient presents with isolated PM. Patients with other metastatic sites, such as liver and lung, are largely excluded.

During the initial laparotomy (performed from xyphoid to os pubis) the abdomen is systematically explored by dividing it into 13 different regions for inspection (fig.1). In this manner both the volume and location of the peritoneal implants are assessed. To record this in a standardized fashion, the Peritoneal Cancer Index (PCI) system is used. This tool aids in quantifying the distribution and implant size in both the abdomen and pelvis. The abdomen and pelvis are divided into nine regions and the remaining regions are composed of the jejunum and ileum (two regions each for upper and lower part). Subsequently the lesion size (LS) in each region is recorded. The size-score ranges from zero to three. LS-0 means there are no visible lesions in the inspected region. LS-1 is utilized for lesion up to 0.5cm in size. LS-2 is used to describe lesions that vary between 0.5 to five cm. And finally, lesions larger than 5cm are classified as LS-3. It should be noted that the scoring takes place after a complete adhesiolysis and complete inspection of the entire peritoneal surface. After completion of the scoring the score is presented as a numerical score that ranges from 1 up to 39. In the Netherlands, a simplified PCI (sPCI) is routinely used. This sPCI does not divide the abdomen into 13 regions, but into seven. These include: pelvis & sigmoid; right lower-abdomen; small bowel & mesentery; omentum & transverse colon;
The extent of peritoneal involvement is quantified by counting the number of affected regions (ranges from zero to seven). Generally patients with a PCI score of less than 20 or, if the sPCI is used, five or less regions affected, are considered most suitable for CRS and HIPEC. The extent of peritoneal involvement, measured as PCI or sPCI, is a well-documented prognostic indicator for patients undergoing CRS & HIPEC. It is believed that this is due to the fact that if the disease is “limited” there is a greater chance of successfully resecting all of the tumourous implants. Patients who are excluded from treatment based on the extent and resectability of their disease are commonly referred to the oncologist for palliative treatment.

The objective of the CRS is to remove all macroscopic disease from the abdomen or to leave only limited residual tumour (<2.5mm) if complete resection is not feasible. During the surgical procedure, often multiple resections are carried out to remove all intra-abdominal tumour, amongst which are resections of the intestines, parts of the stomach, the gall-bladder and even the spleen. In addition, peritonectomy procedures are frequently performed. The extent of resection is recorded using the Completeness of Cytoreduction Score (CC or CCS), of which there are several variants. Jacquet and Sugarbaker proposed a scoring system in which; a CC-0 indicates no peritoneal seeding was observed after complete exploration; a CC-1 score indicates the remaining tumour nodules to be smaller than <2.5mm; CC-2 is utilized to indicate residual disease that ranges between 2.5 and 25 mm in diameter and lastly, a CC-3 score indicates nodules greater than 25 mm in diameter are left in-situ or irresectable tumour deposits at any site within the abdominal cavity or pelvis. The French group scores complete cytoreduction as R0-R1 and incomplete as R2. The reason for grouping the R0 and R1 categories lies in the difficulty of confirming an R0 in peritoneal carcinomatosis while in addition the outcome for both groups has been described as being quite similar. The Dutch HIPEC group utilizes a similar scoring system, however the different categories are scored as R1 (no macroscopic residual tumour), R2a (largest residual nodule <2.5mm) and R2b (largest remaining nodule >2.5mm). Several studies have shown a direct relation between the completeness of CRS and the survival after treatment, making the completeness of the CRS one of the most important prognostic factors in this population.

After a macroscopically complete CRS has been performed, intraperitoneal chemotherapy is administered to eradicate the microscopic residual disease. Administration techniques differ slightly per region. Firstly, the intraperitoneal chemotherapy can be administered per-operatively, usually with hyperthermia (HIPEC) or it can be administered post-operatively, starting on the first day after the CRS through day five. The last technique is known as early post-operative intraperitoneal chemotherapy, EPIC for short. Additionally, the per-operative variant can be performed either in an open (coliseum technique) or closed...
fashion. Furthermore, the chemotherapeutic compounds and doses also can vary. Until now, in the Netherlands Mitomycin C (MMC) has mainly been the chemotherapeutic agent of choice for this procedure. The inflow temperature of the intra-abdominal perfusate lies between 41-42 degrees Celsius. At this point, the MMC is added at 35 mg/m$^2$ body surface in three fractions at 30 minute intervals. However, in recent years the use of Oxaliplatin for HIPEC has also gained popularity in the Netherlands, and was mostly used for repeat procedures. Reconstruction of the gastrointestinal continuity is classically performed after the intraperitoneal lavage in order to prevent entrapment of tumour cells in the suture lines that in theory could cause recurrence.

**Treatment Outcome – Morbidity, Mortality & Survival**

Since the introduction of the CRS & HIPEC technique several institutions have reported very promising results in the treatment of peritoneal carcinomatosis, showing 5-year survival rates of up to 35-45%. As with other major surgical procedures, the CRS & HIPEC procedure is accompanied by a relatively high major morbidity (grade III/IV) rate ranging from 12% to 52%. Common complications observed after CRS and HIPEC include: hematological complications, bleeding, intra-abdominal collections, infections, embolism and the formation of fistula.

The mortality rate reported for this approach ranges from one up to 10%. The complication rate accompanying CRS and HIPEC is considered acceptable, especially when compared to other major gastrointestinal surgical procedures carried out with curative intent. However, it must be noted that there is a learning-curve described for this procedure and that subsequently the morbidity and mortality figures for the more experienced centers are at the lower spectrum of the earlier stated range. High volume CRS and HIPEC centres show treatment associated morbidity rates ranging from 12-34% and mortality rates ranging from one to six percent. In addition, the type of resection performed during the CRS has been reported to have an impact on the perioperative outcome of CRS & HIPEC patients. Both left upper-quadrant peritonectomy and small bowel resection were described as having a negative effect on the perioperative outcome after CRS & HIPEC.

On average, CRC patients with stage IV disease show a five-year survival rate of approximately 12%. The only RCT conducted in 2003, comparing best systemic chemotherapy with CRS & HIPEC showed a gain in median survival from 12.6 to 22.4 months in patients with isolated PM. In this cohort, a subgroup of patients - in whom there was no residual macroscopic tumour – even showed a five-year survival equal to that of patients undergoing resection for CRLM’s (35-45%). Moreover, the median
overall survival reported in several studies on PM of CRC treated with CRS and HIPEC ranges from 19 to 62 months, showing the best outcome in patients in which a complete cytoreduction was achieved. The resection of hepatic metastases is the surgical procedure that is currently still the golden standard approach for the treatment a hepatic metastases. Seeing as both procedures carry comparable risks and clinical benefits, we propose that CRS and HIPEC has the potential of becoming the golden standard for the treatment of PM of CRC.

Systemic Chemotherapy versus CRS & HIPEC

Currently, there is no data available to compare patients that present with PM of CRC, treated with either curative resection with or without Heated IntraPeritoneal Chemotherapy (HIPEC) or best systemic chemotherapy. However, as stated previously, there is some indication that systemic chemotherapy is less beneficial in PM when compared to its effect in hepatic and lung metastases.

In general terms, the advent and further development of CRS and HIPEC has radically changed the clinical outcome of patients with isolated PM over the past two decades. Overall, the data presented support the belief that this treatment provides a very promising therapeutic option for this subgroup of patients with metastatic CRC.

There are still several clinical needs that remain to be addressed in order to achieve maximum benefit of this aggressive combined surgical approach in this particular population of metastatic CRC patients.

Figure 2. Schematic overview of the clinical course of the colorectal cancer patient presenting with peritoneal metastases
Clinical Needs

Currently, the treatment and prognosis of CRC patients is largely based on the tumour, node, metastasis staging classification. Patients with resectable metastatic colorectal cancer, either to the liver or the peritoneum, have benefitted from the aggressive surgical management. At present, the selection of patients with PM for CRS and HIPEC is made roughly on clinical parameters and intraoperative findings, such as the exclusion of hepatic and pulmonary metastases and the PCI score with the aim of successfully resecting all intra-abdominal tumour deposits. However, to date no strong pre-operative predictors of irresectability have been identified.

Thus, several clinical needs, or challenges if you will, still exist in this particular population of metastasized colorectal cancer patients. Notwithstanding the extensive work-up, sometimes even including laparoscopic PCI assessment, there are reports that approximately 25% of the patients deemed suitable for CRS and HIPEC are ultimately deemed irresectable and undergo a so-called open-close procedure. This is largely due to either a PCI score of more than 20 or extensive small-bowel involvement, which could not be identified pre-operatively. Extensive involvement of the small-bowel would mean that a significant portion of the small-bowel would need to be resected to achieve a macroscopically complete resection, which leads to short-bowel syndrome. Sadly, this constitutes a sizeable proportion (about 20%) of patients in specialized CRS and HIPEC centres. Secondly, it has been observed that patients with a “favourable profile”, based on both PCI and resection outcome after CRS and HIPEC still show a very different treatment outcome than was previously expected based on the aforementioned clinical parameters. Thus, additional stratification tools are essential for the optimization of the entire procedure.

In addition, the chemotherapy that is used in HIPEC is still based on the “one size fits all” principle, without any biological stratification. Lastly, when the patient has successfully undergone treatment and has been disease-free for an extended period of time also the prediction of prognosis and aftercare of these patients is of major clinical importance. For example; improving follow-up strategies, stoma-care and possible reversal procedures.

With the advent of newer surgical and medical treatments available, we believe the choice for certain strategies in patients with PM requires further personalization, i.e. the implementation of personalized medicine. Further insight into the baseline characteristics, natural course of this metastatic entity, pathobiology and treatment mechanisms is necessary in order to better understand and interpret treatment outcome after CRS and HIPEC. We
strongly believe an evident need exists for both clinical and molecular profiling to aid in addressing the aforementioned clinical needs.

**Aim & Outline of this thesis**

With the identification of several clinical needs, we aim to gain more insight into these clinical challenges that are encountered during the entire course of treatment of CRS and HIPEC patients. This thesis is divided into two main parts. Part one focuses on clinical decision making in order to address some of the challenges faced in this particular population of CRC patients. The second part focuses on how biology could aid clinical decision making in the future.

**Clinical Decision Making / Treatment Allocation**

Firstly, adequate patient selection for CRS and HIPEC is paramount in effectively treating the individual patient for his or her disease and/or effective aftercare. For this purpose, the first part of this thesis focuses on clinical profiling for treatment allocation and aftercare. *Chapter 2* focuses on whether or not patients with both hepatic and peritoneal metastases should be treated with a combination of CRS of the liver and peritoneal surfaces and HIPEC. In *Chapter 3* we analyzed the outcome of colostomy reversal procedures in CRS and HIPEC patents in order to enable us to improve prediction of success or failure based on several clinical parameters. This knowledge will ultimately facilitate the counseling of these patients during aftercare.

**Molecular Approach – Possible Aids in Future Clinical Decision Making**

Based on the hypothesis that the phenotype, and thus clinical behaviour, of PM in CRC is driven by underlying biological mechanisms, read outs of disease biology, i.e. biomarkers, will be of help for more refined identification of suitable patients.

*Chapter 4 and 5* provide two reviews on biomarkers in PM of CRC with the aim to provide an overview of promising molecular changes reported to be involved in peritoneal dissemination of CRC and to evaluate the potential for clinical application of these biomarkers.

Adhesion to the peritoneal surface is considered one of the key steps in peritoneal dissemination of malignant disease. In addition to attachment, angiogenesis, i.e. the formation of new blood vessels, is crucial for the progression of micrometastases to clinically relevant macrometastases. This process depends on interactions between several cell types and extracellular matrix components. Even though this process is not
fully understood, there are indications of involvement of the adhesion molecule Versican (VCAN), by possible interaction with Vascular Endothelial Growth Factor (VEGF), a well-known and potent angiogenic factor. In Chapter 6 we subsequently assess the possible role of VCAN, an adhesion molecule, in combination with VEGF as a prognostic biomarkers in CRC patients with PM after treatment with CRS and HIPEC.

Multiple studies have shown the relevance of angiogenesis, measured by the formation of microvessels in colorectal cancer. These studies were predominantly carried out in the setting of either the primary tumour or haematogenous metastases. To date the expression of the angiogenesis related markers HIF1α, SDF1, CXCR4 and VEGF in peritoneally disseminated CRC is largely unknown and remains to be elucidated. In addition, it is not known if these markers are of prognostic relevance. In Chapter 7 we describe the expression of the aforementioned angiogenesis related markers and microvessel density and whether or not these have prognostic implications in patients with PM of CRC undergoing CRS and HIPEC. Additionally, in Chapter 8 we retrospectively investigated the individual and combined prognostic value of HIF1α, GLUT1 and VEGF expression in patients with colorectal liver metastases and assessed the added value of these molecular biomarkers to established clinicopathological prognostic factors.

Patients with PM originating from CRC are curatively treated by CRS and HIPEC with MMC. In order to better stratify patients and ultimately improve upon the treatment itself, in Chapter 9 we tackle the patient selection and personalization of treatment for CRS and HIPEC patients by attempting to predict MMC sensitivity in-vitro and assess its possible clinical relevance in patient derived tissue from a cohort of CRS and HIPEC patients.

Lastly, it has been described that tumours with the microsatellite instability (MSI) genotype tend to metastasize less to the liver. In contrast, tumours that have the tendency to metastasize to the peritoneum often show mucinous histology and MSI genotype. Hypothetically this could imply that these tumours preferentially metastasize to either the peritoneum or lymph nodes. In Chapter 10 we sought to further characterize the clinical features and oncologic outcomes of BRAF and KRAS mutant early-stage MSI CRCs. However, we only focus on the effect of these changes on cancer specific and overall survival, not directly on pattern of metastatic spread. Because in general PM has a worse outcome, we believe this to be a first step in the future development of molecular profiling of early stage tumours in order to be able to predict which of these tumours will develop PM in the future.
References


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