Novel therapeutic approaches in peritoneal metastasis from colorectal cancer

Ida Storhaug Frøysnes

Department of Tumor Biology
Institute of Cancer Research
The Norwegian Radium Hospital
Oslo University Hospital

Faculty of Medicine
University of Oslo
# Table of Contents

**Acknowledgements** ................................................................................................................ III  
**Abbreviations** .......................................................................................................................... IV  
**List of papers** ........................................................................................................................... V  
**Introduction** .............................................................................................................................. 1  
**Background** .............................................................................................................................. 2  
  - Colorectal cancer ..................................................................................................................... 2  
  - Classification .......................................................................................................................... 3  
  - Treatment of primary CRC ................................................................................................. 4  
  - Metastatic CRC ..................................................................................................................... 6  
**The peritoneum** ....................................................................................................................... 8  
  - Anatomy and physiology .................................................................................................... 8  
  - Immunological functions .................................................................................................... 8  
  - Transport across the peritoneum ....................................................................................... 12  
  - Peritoneal metastasis ......................................................................................................... 13  
  - Mechanisms for development of peritoneal metastasis .................................................... 14  
**CRS-HIPEC** ............................................................................................................................. 15  
  - Variations in intraperitoneal chemotherapy regimens ...................................................... 16  
  - Prognostic factors for outcome after CRS-HIPEC ........................................................... 16  
  - CRS-HIPEC in Norway .................................................................................................... 19  
**MOC31PE Immunotoxin** ......................................................................................................... 20  
  - MOC31PE- researcher driven drug development; “from bench to bedside“ ................. 21  
  - Rationale for intraperitoneal MOC31PE ........................................................................... 23  
**Aims of the study** ..................................................................................................................... 24  
**Summary of papers** ................................................................................................................ 25  
**Methodological considerations** .............................................................................................. 27  
  - Study design in medical research .................................................................................... 27  
  - Observational studies ....................................................................................................... 27  
  - Clinical trials ..................................................................................................................... 28
Acknowledgements

The work presented in this thesis was carried out at the Department of Tumor Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, between 2013 and 2018, including two periods of maternity leave. The financial support from The Norwegian Cancer Society is gratefully acknowledged.

First, I would like to thank my main supervisor Kjersti Flatmark, for giving me the opportunity to work on this exciting project. I am always motivated after our talks, and I feel lucky to have worked with you and to have learned from your broad knowledge of colorectal cancer. Thank you for letting me work independently, yet always helping me out and making time for me when needed.

I would also like to thank my great co-supervisors Yvonne Andersson and Stein Gunnar Larsen. Yvonne, you are a solid scientist, and your guidance, positive support and enthusiasm for our research project have been very important to me. Stein, your knowledge of and excitement for the treatment of peritoneal metastasis is impressive. Thank you both for always having an open door.

The contributions from all the co-authors are highly appreciated and I would like to thank all of you for your contribution to this work.

I would also like to thank the head of the Department of Tumor Biology, Gunhild Mari Mælandsmo and all my colleagues there for an exciting and including work environment. Especially I would like to thank all the great members (present and past) of Kjersti’s group, including the peritoneal metastasis group; Annette, Christin, Line, Torveig and Yvonne. I have also been so lucky to have the best of the best people in my office: first Birgit and Ingrid, later Christin and Bylgja. Thanks to Dawn for reading through my thesis, to Kjetil for input on different statistical problems and to Svein for discussions on metastatic colorectal cancer and for constructive feedback.

The main reason I applied for this PhD position was to work with the ImmunoPeCa trial. I have learned a lot through the planning and execution of the trial. A special thanks to Janne, for filling in for me when I was in maternity leave, and for introducing me to the do’s and dont’s of clinical trial administration. Big thanks to all the people involved in the ImmunoPeCa trial, including coordinators, nurses and surgeons. Most importantly, thanks to the 21 patients participating in the trial.

Finally, I would like to thank my mom and dad and the rest of my family, family in-law and friends for all their support. Erik, my love, your patience and super-daddy skills have made this possible. Thank you for help with illustrations and for always believing in me. Live and Ingrid, thank you for being exactly who you are- constant reminders of the most important things in life.

Ida Storhaug Froysnes
Oslo, August 2018
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>5-FU</td>
<td>Fluorouracil</td>
</tr>
<tr>
<td>CLS</td>
<td>Capillary leak syndrome</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>CRS</td>
<td>Cytoreductive surgery</td>
</tr>
<tr>
<td>CT</td>
<td>Computer tomography</td>
</tr>
<tr>
<td>CTCAE</td>
<td>Common toxicity criteria for adverse events</td>
</tr>
<tr>
<td>DFS</td>
<td>Disease-free survival</td>
</tr>
<tr>
<td>DLT</td>
<td>Dose-limiting toxicity</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>FLOX</td>
<td>5-FU, leucovorin, oxaliplatin</td>
</tr>
<tr>
<td>FLIRI</td>
<td>5-FU, leucovorin, irinotecan</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>5-FU, leucovorin, oxaliplatin</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>5-FU, leucovorin, irinotecan</td>
</tr>
<tr>
<td>HIPEC</td>
<td>Hyperthermic intraperitoneal chemotherapy</td>
</tr>
<tr>
<td>MMC</td>
<td>Mitomycin C</td>
</tr>
<tr>
<td>MTD</td>
<td>Maximum tolerated dose</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>PCI</td>
<td>Peritoneal cancer index</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression-free survival</td>
</tr>
<tr>
<td>PM</td>
<td>Peritoneal metastasis</td>
</tr>
<tr>
<td>PM-CRC</td>
<td>Colorectal peritoneal metastasis</td>
</tr>
<tr>
<td>UICC</td>
<td>Union for International Cancer Control</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, node, metastasis</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
List of papers

Paper I  Froysnes IS*, Larsen SG*, Spasojevic M, Dueland S, Flatmark K:
Complete Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Colorectal Peritoneal Metastasis in Norway: Prognostic Factors and Oncologic Outcome in a National Patient Cohort
*Shared first authorship

Paper II  Froysnes IS*, Andersson Y*, Larsen SG, Davidson B, Øien JMT, Olsen KH, Giercksky KE, Julsrud L, Fodstad Ø, Dueland S, Flatmark K:
Novel treatment with intraperitoneal MOC31PE immunotoxin in colorectal peritoneal metastasis - results from the ImmunoPeCa phase 1 trial
*Shared first authorship

Paper III  Froysnes IS, Andersson Y, Larsen SG, Davidson B, Øien JMT, Julsrud L, Fodstad Ø, Dueland S, Flatmark K:
ImmunoPeCa trial: Long-term outcome following intraperitoneal MOC31PE immunotoxin treatment in colorectal peritoneal metastasis
Manuscript
**Introduction**

The peritoneum is a common site of cancer spread from colorectal cancer (CRC), and outcome has generally been poor. Peritoneal metastasis (PM) is associated with worse prognosis than for example liver and lung metastasis from colorectal cancer [1, 2] (Figure 1), and most patients with PM-CRC are treated with palliative measures or best supportive care. Cytoreductive surgery (CRS), the removal of all visible intraperitoneal tumor, combined with hyperthermic intraperitoneal chemotherapy (HIPEC) performed at surgery, is a treatment option for selected patients with resectable disease, aiming to cure or give long-term survival. Unfortunately, the field has lacked representative randomized controlled trials investigating the benefit of CRS-HIPEC, and the role of CRS-HIPEC versus CRS alone. The great variability of CRS-HIPEC techniques also complicates interpretation of research within the field.

CRS-HIPEC involves major surgery and chemotherapy, and to avoid harmful effects of this extensive treatment in patients who will not benefit from it, correct patient selection is crucial. Most patients will also experience disease recurrence following CRS-HIPEC, showing the importance of developing treatment alternatives. Immunotoxins, hybrid proteins with antibody specificity and potent cytotoxicity, could be a potential targeted treatment alternative for PM-CRC [3]. The focus of this thesis was the current treatment for resectable PM-CRC and the investigation of a novel treatment approach involving intraperitoneal MOC31PE immunotoxin.

![Figure 1. Kaplan-Meier plot showing overall survival in colorectal cancer patients with metastases in a single organ (liver, lung or peritoneum). Modified and reprinted with permission from Elsevier [2].](image)
Background

Colorectal cancer

Cancer arising in the colon or rectum is commonly referred to as CRC. Worldwide, CRC is a common cancer with 1.36 million new cases in 2012, where the highest incidence rates are seen in developed countries [4]. In Europe, Norway has one of the highest incidence rates [5]. CRC is the second most common cancer in Norway, comprising 4363 new cases in 2016 [6, 7]. Colon cancer constitutes the majority of these cases (n=2935), with an equal distribution between men and women. Rectal cancer is about 50% more common in men. The incidence of colon cancer is slowly increasing, whereas the rectal cancer incidence has been stable for the past 30 years (Figure 2). The 5-year relative survival for colon cancer in Norway is 61% for men and 66% for women, and for rectal cancer it is 68% for both genders. Colon cancer incidence and mortality rates are high in Norway compared to other Nordic countries. In comparison to colon cancer, survival from rectal cancer has increased and mortality rates have declined by nearly 50% since the 1980s (Figure 2). The increase in rectal cancer survival is probably due to improved treatment, while the reason for the high colon cancer mortality is not known [6].

Figure 2. Incidence, mortality and survival in colorectal cancer for both genders in Norway 1965-2016. Modified and reprinted with permission [6].
Classification

Staging, a systematic examination of the cancer patient to determine disease extent, is crucial for therapeutic decision-making and determination of prognosis. The Tumor Node Metastasis (TNM) system categorizes cancers according to their anatomic extent; local invasion depth of primary tumor (T-stage), regional lymph node involvement (N-stage) and distant metastasis (M-stage) [8, 9] (Table 1). For CRC, substages have been introduced in the later TNM editions, but all subgroups can be collapsed back into the original 4 stages of previous TNM classifications covering a 30-year span and are compatible with the original Dukes classification [10]. An overall Union for International Cancer Control (UICC) stage is finally determined from the TNM-stages, guiding therapy decisions (Table 2). In addition to UICC-stage, the histological type and grade of the tumor are important for prognosis and treatment decisions. Colorectal cancers are adenocarcinomas originating from the glandular epithelium of the intestine, classified as low, intermediate or highly differentiated, referring to the similarity of the epithelium of origin.

<table>
<thead>
<tr>
<th>TABLE 1. TNM-staging of CRC, from the TNM classification of malignant tumors, 8th edn [9]</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
<tr>
<td>T4a</td>
</tr>
<tr>
<td>T4b</td>
</tr>
<tr>
<td>N0</td>
</tr>
<tr>
<td>N1</td>
</tr>
<tr>
<td>N1a</td>
</tr>
<tr>
<td>N1b</td>
</tr>
<tr>
<td>N1c</td>
</tr>
<tr>
<td>N2</td>
</tr>
<tr>
<td>N2a</td>
</tr>
<tr>
<td>N2b</td>
</tr>
<tr>
<td>M0</td>
</tr>
<tr>
<td>M1</td>
</tr>
<tr>
<td>M1a</td>
</tr>
<tr>
<td>M1b</td>
</tr>
<tr>
<td>M1c</td>
</tr>
</tbody>
</table>
### TABLE 2. UICC stage classification of colorectal cancer

<table>
<thead>
<tr>
<th>UICC-stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1, T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIA</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIB</td>
<td>T4a</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIC</td>
<td>T4b</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>IIIA</td>
<td>T1, T2</td>
<td>N1/N1c</td>
<td>M0</td>
</tr>
<tr>
<td>IIIB</td>
<td>T3, T4a</td>
<td>N1/N1c</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2-T3</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T4b</td>
<td>N1-N2</td>
<td>M0</td>
</tr>
<tr>
<td>IIIIC</td>
<td>T4a</td>
<td>N2a</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3-T4a</td>
<td>N2b</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T4b</td>
<td>N1-N2</td>
<td>M0</td>
</tr>
<tr>
<td>IVA</td>
<td>Any</td>
<td>Any</td>
<td>M1a</td>
</tr>
<tr>
<td>IVB</td>
<td>Any</td>
<td>Any</td>
<td>M1b</td>
</tr>
<tr>
<td>IVC</td>
<td>Any</td>
<td>Any</td>
<td>M1c</td>
</tr>
</tbody>
</table>

### Treatment of primary CRC

Surgery is the oldest cancer treatment approach, and also the therapeutic basis for most solid malignancies. Even though the major principles for treatment of CRC are common, there are local differences. In the description of treatment in this work, the focus is on Norwegian guidelines. For curative rectal cancer treatment, the standard surgical technique is total mesorectal excision (TME). TME involves the complete removal of the rectum, the surrounding mesorectum containing draining lymph vessels and the mesorectal fascia [11, 12]. This technique was developed in the 1980s [11] and established as gold standard in Europe in the 1990s [13]. The importance of a clear lateral or circumferential resection margin on disease recurrence and survival has been shown in several studies [14-17], and the implementation of TME as standard of care has improved rectal cancer survival [6, 18].

The basis of curative colon cancer treatment is surgical resection of the tumor and the corresponding lymph nodes, where the localization and blood supply of the tumor determines the localization and extent of surgery. Ten centimeter (cm) free margins are recommended for colon cancer and five cm for cancers of the rectosigmoid colon. In addition, resection of regional lymph nodes should be performed. Like TME for rectal cancers, complete mesocolic excision with separation of the mesocolic plane from the parietal peritoneum has been proposed for colon cancer, and this technique is now recommended in Norway [19].
For locally resectable tumors without lymph node metastasis, surgery alone may be adequate for curative treatment. However, for locally advanced tumors with or without lymph node metastasis, surgery may be supplemented with radiotherapy, chemotherapy or both, given before (neoadjuvant) or after (adjuvant) surgery.

Radiotherapy kills cancer cells by causing DNA damage with radiation from X-rays, gamma-rays, neutrons, electrons or protons. It has been an effective method for treating cancer for more than a century. Curative radiotherapy can be classified as radical or adjuvant. Radical means that radiation is given as the definitive treatment, and adjuvant refers to radiotherapy given in addition to surgery. For locally advanced rectal cancer threatening or compromising the mesorectal fascia, neoadjuvant radiotherapy or chemoradiotherapy is given to reduce tumor size in order to facilitate complete surgical removal. The high risk of local recurrence and poor survival in locally advanced rectal cancer has been reduced from about 40% to 5% following the implementation of neoadjuvant chemoradiotherapy and TME [13, 20-22]. In cases where the mesorectal fascia is involved, surgery is performed as beyond TME surgery with resection of affected structures or organs [23].

Adjuvant chemotherapy, aiming to eliminate microscopic disease following curatively intended surgery is recommended for stage III colon cancer patients without contraindications and for high-risk stage II. Different chemotherapy regimens are recommended for patients according to age and comorbidity, but they usually contain 5-fluorouracil (5-FU) or capecitabine (oral prodrug of 5-FU). Evidence for adjuvant chemotherapy in stage II colon cancer is less clear than for stage III, and in this group only selected patients (T4 tumors, perforated tumors and <12 lymph nodes removed at surgery) are recommended adjuvant chemotherapy. There is extensive research activity in this field, aiming to identify high-risk patients in need of adjuvant therapy. For rectal cancer patients treated with neoadjuvant chemoradiotherapy, adjuvant chemotherapy is generally not recommended in Norway, but is considered in tumors with high-risk features (e.g. poor grade, blood or lymphatic vessel invasion, involved resection margins), where the patient did not receive preoperative treatment [24].
**Metastatic CRC**

A tumor’s ability to invade and metastasize is one of the well-known hallmarks of cancer [25, 26]. The process of tumor invasion and metastasis is known as the invasion-metastasis cascade [27], and has several steps. Cancer cells from the primary tumor (1) invade locally and intravasate into blood or lymphatic vessels, (2) survive transportation through the lymphatic- or hematogenous systems, (3) extravasate into the parenchyma of distant organs and (4) adapt in a foreign microenvironment, which may lead to (5) metastatic colonization and macroscopic metastases.

Approximately 20% of patients diagnosed with CRC will have metastasis (UICC stage IV) at the time of diagnosis [6, 28-32], while 18% of radically treated CRC patients will develop metastasis at a later time [33]. Across all CRC patients, almost 50% will have metastatic disease at some point [34].

Even though the most common site of metastasis in CRC is the liver, colon and rectal cancers have metastatic patterns that differ markedly. Rectal tumors metastasize more often to extraabdominal sites such as lung, bone and brain than colon cancers, but are less likely to spread to intraabdominal sites such as the peritoneum [35-37]. Blood from the colon and proximal rectum is drained through the portal vein to the liver, while the distal rectum drains into the inferior vena cava, and the first organ encountered are the lungs. Histologic subtype has also been shown to influence site of metastasis; regular adenocarcinomas predominantly metastasize to the liver, while mucinous adenocarcinomas and signet ring cell carcinomas more frequently show peritoneal metastases. Mucinous and signet ring cell carcinomas also commonly metastasize to multiple sites [37].

In the past, metastatic CRC was considered incurable, and for most patients it still is. For patients with stage IV disease at diagnosis, the cancer can only be cured through resection of both the primary tumor and the metastasis. The majority is not eligible for such treatment and is therefore placed into a palliative setting. Systemic chemotherapy is generally the best treatment choice for this group, except for patients with poor performance status who will often receive best supportive care only. The treatment goal is to improve survival and quality of life, and to reduce or delay symptoms. Chemotherapy is usually given at diagnosis of non-resectable metastasis and if possible before patients become symptomatic. For patients receiving best supportive care, the median overall survival (OS) is reported to be 5-6 months [38, 39], while for patients receiving combination chemotherapy the OS in clinical trials now exceeds 20-30 months [34, 40-43].
Surgical removal of the metastatic tumor is the only way patients can achieve cure once the disease has metastasized. In addition to peritoneal metastasis, which will be introduced later, the organs of interest for metastasis surgery are primarily the liver and lungs. Metastasis surgery is recommended for selected patients, but lack of randomized controlled trials is a problem within the field. For colorectal liver metastasis, 550 liver resections were performed in Norway in 2015, and out of the annual 150-200 lung metastasis resections performed in Norway each year, most are CRC patients. Common for both liver and lung surgery is that organ function following resection is important, and the patient must be able to tolerate the resection. This is generally more straightforward in the liver than in the lungs, mainly because liver tissue can regenerate. The 5-year OS for lung metastasis surgery ranges from 27-68% in different studies [44], while for liver metastasis surgery it ranges from 16-74% [7].

For decades, 5-FU was the only drug available for treatment of metastatic CRC. From the late 1990s, other drugs such as oxaliplatin, irinotecan and biological agents (bevacizumab, anti-VEGF and cetuximab and panitumumab, anti-EGFR) were introduced. Following this introduction, OS improved from 12 months for 5-FU monotherapy to 20-30 months in clinical trials with combination treatments [34, 40-43, 45]. Later also other agents such as regorafenib and TAS-102 have been approved and shown slightly superior progression-free survival (PFS) compared to placebo in late-stage metastatic CRC. They are not in regular use, due to toxicity and the short increase in OS of less than 2 months. Fluoropyrimidine (5-FU or oral capecitabine) combined with oxaliplatin or irinotecan comprise the first line chemotherapy backbone for most patients. In Norway, bolus administration of 5-FU in the combinatory FLOX (5-FU, leucovorin, oxaliplatin), or FLIRI (5-FU, leucovorin, irinotecan) regimens are usually preferred. Internationally, the infusional regimens FOLFOX (5-FU, leucovorin, oxaliplatin) or FOLFIRI (5-FU, leucovorin, irinotecan) are used. Survival is comparable between the bolus and infusional regimens, but the latter has a higher response rate [46, 47].

Second-line therapy describes treatment given after first-line therapy failure, and is normally offered to patients with good performance status. Choice of second-line therapy is dependent of the first-line therapy choice [34]. There is evidence that patients, if tolerated, should be offered all three available cytotoxic agents (fluoropyrimidines, oxaliplatin and irinotecan), as well as all targeted agents (anti-VEGF and if RAS-wildtype, anti-EGFR) during their course of treatment. The optimal therapy sequence is not known [34, 48, 49].
The peritoneum

Anatomy and physiology

The peritoneum, from Greek *peritonaion*, meaning “stretched around”, is a semi-permeable serous membrane lining most of the abdominal organs and the inner abdominal wall (Figure 3a). The structure of the peritoneum was extensively described by Baron in 1941 [50]. It consists of a monolayer of mesothelial cells, a basement membrane and submesothelial connective tissue (Figure 3b). The mesothelium is derived from the mesoderm that lines the body cavity during embryonic development. The surface area in adults is about 0.8-2 m², and it is highly capillarized with a relatively high blood flow (100-150 ml/min) [51-54]. This anatomically rather complex structure includes the organ-covering visceral peritoneum (serosa) and the parietal peritoneum lining the inner abdominal wall. The parietal and visceral peritoneum are continuous with each other, and the peritoneal cavity refers to the potential space located between the two layers. Normally, this space holds small amounts of fluid (approximately 50-100 ml), but has the capacity of large fluid accumulation (for example, in situations with ascites or hemorrhage). The visceral peritoneum is served by the same nerve supply, blood- and lymphatic vessels as the organs it covers, while the parietal peritoneum shares circulation and nerve supply with the abdominal wall. In men the peritoneal cavity is a closed sac, whereas in women the fimbriae of the fallopian tubes and ovaries are in connection with the intraperitoneal space. The peritoneum holds organs in place, and reduces friction between organs through the mesothelial production of lubricants, anticoagulants, and surface tension–lowering substances [55]. The mesenteries carry blood vessels, lymphatics and nerves, in addition to adipose tissue, which is also found in the peritoneal ligaments and especially in the greater omentum.

Immunological functions

The peritoneum is protected both through the innate and the adaptive immune system. The innate immune system works through mechanical clearance of the peritoneal cavity and antibacterial activity of the peritoneal fluid through complement and phagocytosis by polymorphonuclear leukocytes and macrophages. Diaphragmatic stomata are gaps in the mesothelium and basement membrane covering the diaphragm. These stomata allow peritoneal fluid carrying antigens and cells to be continuously drained from the peritoneal cavity, into the subdiaphragmatic lymphatic lacunae and further into lymphatic vessels. Normally, about 1/3 of the fluid drained from the peritoneal cavity passes through the
diaphragmatic lymphatics [56]. In preclinical models about half of the bacteria injected into
the peritoneal cavity were removed via the diaphragmatic lymphatics, appearing in the
thoracic duct after only 6 minutes [57-59].

Figure 3. (A) Gross anatomy of the peritoneum. The parietal peritoneum (blue) covers the
abdominal wall, and the visceral peritoneum (brown) covers intraabdominal organs. The peritoneal
cavity is located between the two layers of peritoneum, and is a potential space normally holding
small amounts of fluid. (B) Structure of the peritoneum. The peritoneum is composed of a
monolayer of mesothelial cells (mesothelium), supported by a basement membrane that rests on the
submesothelium. The glycocalyx produced by the mesothelial cells forms a slippery, non-adhesive
surface. The basement membrane is composed of a thin laminar network containing collagen,
glycoproteins and proteoglycans. The submesothelium includes extracellular matrix (collagen,
glycoproteins, glycosaminoglycans and proteoglycans), capillaries, lymphatics and various cell types.
The greater omentum, a “fatty apron" composed of 4 layers of visceral peritoneum hanging from the greater curvature of the stomach (Figures 3a and 4), is a well-vascularized visceral adipose tissue with unique immune functions. It is often referred to as “the policeman of the abdomen” because of its ability to isolate areas of contamination or inflammation and seal it off to prevent further spread.

The omentum contains “milky spots”, aggregates of perivascular leukocytes embedded in the adipose tissue, which in many ways resemble the follicles of secondary lymphoid tissues, but are also distinctively different [60, 61]. In the milky spots, B cells form a central cluster, while macrophages tend to accumulate around the periphery, and T-cells are intermixed with the B-cells or aggregate around blood vessels (Figure 5). This unusual leucocyte composition is possibly involved in adipocyte homeostasis and protection of the unique environment of the peritoneal cavity [61]. Milky spots contribute to peritoneal immunity by collecting pathogens and antigens from peritoneal fluid, and inducing different immune responses depending on the stimuli [61]. Other than the diaphragmatic stomata, the omental milky spots are the only sites in the peritoneum with the ability to absorb particles from the peritoneal cavity [58]. Cells and antigens are passively collected from the peritoneal cavity through openings in the mesothelial lining but can also be carried by macrophages. Cells can also enter the milky spots via blood and high endothelial venules. The omentum has unique angiogenic [62], fibrotic [63], stem cell [64, 65] and immune activities [60], which boost vascularization, improve wound healing and counteract infection. Unfortunately, these features are also involved in the rapid growth of tumor metastasis, which is often seen in the omentum [61, 66]. So even though the milky spots have been shown to exhibit cytotoxic activity against
tumor cells ex vivo [67], their ability to prevent intraperitoneal tumor growth is insufficient. 
Therefore omentectomy is recommended in surgical treatment for peritoneal metastases. In 
experimental models, live tumor cells injected into the peritoneal cavity were trapped by the 
milky spots, but also circulating ovarian tumor cells (SKOV3-OM3) in the blood have been 
shown to preferentially metastasize to the omentum [68]. After tumor cell entrapment, the 
milky spots increase in size and number, but this apparent immune activation does not prevent 
tumor growth [69]. Interestingly, intraperitoneal vaccination with lethally radiated tumor cells 
was, through an NK-mediated immune response, highly effective in preventing omental 
tumor growth in mice [70]. In summary, the omentum is a common site for peritoneal 
metastasis, and the host of important, but poorly understood immune responses.

Figure 5. Structure of omental milky spots. (A) Omentum in mice (B) Structure of milky spots. 
Milky spots are loose collections of leukocytes embedded between adipocytes and the 
mesothelial layer. B-cells form a central cluster, whereas macrophages and dendritic cells 
accumulate around the outside of the milky spots throughout the omentum. T cells and innate 
lymphoid cells (ILCs) can be intermixed with the B cells or cluster around blood vessels (not 
shown). Cells and antigens are passively collected from the peritoneal cavity through 
fenestrations in the mesothelium or actively carried by macrophages. Printed with permission 
from Elsevier [61].
**Transport across the peritoneum**

Because of the importance of the peritoneum in peritoneal dialysis and intraperitoneal treatment, transport across the peritoneum has been extensively studied. In intraperitoneal cancer treatment, the main goal is dose intensification within the peritoneal cavity with limited systemic drug uptake. In peritoneal dialysis the goal is efficient removal of waste metabolites and water from the blood. The peritoneal barrier is a complex structure made up of the mesothelium, the basement membrane and the underlying submesothelium with parenchymal cells, interstitial cells, pericytes and endothelial cells forming the peritoneal capillaries (Figure 3b) [71, 72]. Except for the normal cellular maintenance functions, there is no active transport of solutes across the mesothelium [71]. The mesothelium and basement membrane are highly permeable to water, small solutes and proteins. Transport of solutes and water between the blood and the peritoneal cavity occurs across the capillary wall, through the interstitium and across the mesothelium. Contrary to intuitive thinking, it is the endothelium that is the main barrier between the peritoneal cavity and the circulation, not the mesothelium [72]. This explains why peritonectomy, removal of the mesothelial lining and submesothelial connective tissue, does not affect the pharmacokinetics in patients treated with intraperitoneal chemotherapy [73, 74].

According to Rippes three-pore model [51, 75-78], the main blood-peritoneal transport route for water and small solutes is the “small pores” (radius 4-6 nm), which is situated between endothelial cells and accounts for about 99% of the total exchange (pore) area. Macromolecules can only pass through the few "large pores" (radius 20-30 nm), which constitute 0.01% of the total pore population, and the ultrasmall pores (radius <0.8 nm) are permeable only to water and not to solutes. Small solutes (<1000 Da) are transported mainly by diffusion while macromolecules such as immunoglobulin G (IgG) use the large pores for transport from serum to the peritoneal cavity. The exact mode of transfer for macromolecules is debated, but both size-selective diffusion and convection appear to contribute. For transport of macromolecules out of the peritoneal cavity, the route is the subdiaphragmatic lymphatics and to a lesser degree the peritoneal interstitium via interstitial trapping. This process is independent of molecular size [79]. It is under debate as to whether the lymphatic absorption of fluid and solutes from the peritoneal cavity should be calculated as the disappearance rate of intraperitoneally administered macromolecules or as the appearance rate in plasma [79, 80]. Disappearance rates are often much higher (5-10 times) than plasma appearance rates, which can be partly be explained by interstitial trapping.
**Peritoneal metastasis**

The peritoneum is a common site for metastasis from CRC and PM is also known as peritoneal carcinomatosis. At the time of diagnosis, about 4-8% will have peritoneal disease [81-84], and about 3-5% will develop PM after curative surgery [85, 86]. PM present at or around the time of diagnosis is known as synchronous PM, while PM that develops at a later time is called metachronous PM. However, the definition of synchronous and metachronous PM differs between studies (e.g. synchronous PM may be defined as PM present at primary surgery, or as PM occurring within 6 months of primary surgery). In total, about 8-13% of all CRC patients will be diagnosed with PM. Given the PM rate of up to 40% in autopsy studies, these percentages are probably an underestimation [87, 88]. PM-CRC is associated with poor prognosis. The TNM 8th edition has recognized this by including the presence or absence of PM as subgroups under “M-stage” (Table 1). A median OS of 5-16 months is reported for patients that are not eligible for CRS-HIPEC [2, 82, 89-91], and studies investigating combination chemotherapy for limited PM-CRC have shown median survival rates of 17-24 months [92, 93]. Patients with PM-CRC also have worse prognosis and respond less well to systemic chemotherapy than those with metastasis in other sites [1, 2].

Advanced T stage, lymph node metastases, free intraperitoneal cancer cells and poor tumor differentiation are associated with synchronous PM [84]. Risk factors for development of metachronous PM are advanced T and N stage, emergency surgery, primary tumor in the colon and free intraperitoneal cancer cells before and/or after primary tumor resection [83, 87, 94-96]. As well, mucinous adenocarcinomas and signet ring cell carcinomas carry a high risk of PM, metastasizing more often to the peritoneum [37]. Research on preventing metachronous PM in high-risk patients is ongoing, and in the Dutch randomized controlled COLOPEC trial (NCT02231086) patients with pT4 or perforated colon cancer who undergo curative resection receive adjuvant oxaliplatin-based HIPEC with adjuvant systemic chemotherapy or adjuvant systemic chemotherapy only to determine if adjuvant HIPEC will reduce the risk of peritoneal recurrence [87, 97]. The French PROPHYLOCHIP randomized phase 3 study (NCT01226394) aimed to evaluate the potential survival benefit of a systematic second-look surgery plus HIPEC in CRC patients at high risk of developing PM. After 6 months of adjuvant chemotherapy, patients without sign of recurrence were randomized into (1) surveillance and (2) systematic second-look surgery plus oxaliplatin-based HIPEC. The results were presented at the 2018 ASCO annual meeting, where the authors reported that second-look surgery plus HIPEC failed to improve survival, in comparison to surveillance only [98].
Mechanisms for development of peritoneal metastasis

In contrast to hematogenous or lymphatic cancer spread, which leads to metastasis to for instance liver, lungs and lymph nodes, PM develops by direct seeding of tumor cells into the peritoneal cavity. This typically occurs with cancers of the abdomen (colon and rectum, ovaries, stomach, pancreas) as a consequence of tumor growth reaching the surface of the organ, or if lymphatic vessels containing tumor cells are transected during surgery. Research on the biology of peritoneal spread from CRC is scarce compared to hematogenous and lymphatic spread, but certain well-defined steps are known to be involved. (1) Spontaneous or iatrogenic detachment of cancer cells from the primary tumor is the first step towards peritoneal dissemination of tumor cells. This spontaneous detachment is caused by high interstitial fluid-pressure within the tumor; a result of rapid cell proliferation and lack of effective lymphatic drainage. Fibrosis, contraction of interstitial matrix and leakage of plasma proteins also contribute [99]. Down-regulation of cell adhesion molecules has also been shown to play a role in the detachment of tumor cells [100]. Shedding is followed by tumor cell transport within the peritoneal cavity in predictable routes determined by the interaction between gravity, diaphragmatic- and bowel movements and peritoneal recesses. The flow is directed to and from the pelvis, via the right paracolic gutter to the subdiaphragmatic space [101]. (2) Step two is adhesion to the mesothelial layer. Adhesion is mediated by adhesion molecules expressed by mesothelial and endothelial cells. Tumor cells can adhere to mesothelial cells, the extracellular matrix or to specialized structures such as the omentum or the diaphragmatic peritoneum. The omentum, with its discontinuous mesothelial lining, is a preferred site for peritoneal cancer spread, but the mechanisms for tumor site specificity is not well understood [102]. Postoperative inflammation has been shown to enhance tumor growth and adhesion, and wounds on the peritoneal surface might promote peritoneal spread through fibrin clot entrapment of cancer cells [103]. (3) Step three in the formation of PM is invasion of the submesothelial layer. This happens when tumor cells enter discontinuous areas of the mesothelium, by tumor-induced mesothelial cell apoptosis [104] and by contraction of mesothelial cells and disruption of intercellular junctions in response to inflammatory mediators [102, 105]. Inhibition of proteases such as matrix metalloproteinases has been shown to suppress peritoneal invasion [102]. (4) Finally, in step four, tumor cells can access the lymphatic and systemic circulation via the submesothelial lymphatic lacunae. From the lymphatic lacunae peritoneal fluid, cells and proteins are drained into substernal, parasternal, mediastinal para-aortic and renal hilum lymph nodes [102, 106].
CRS-HIPEC

The theory of PM-CRC as a locoregional disease gave rise to a new surgical technique first described in 1980 and further developed in the early 1990s by Sugarbaker [107, 108]. The technique is now a highly specialized treatment combining CRS and HIPEC. The rationale behind HIPEC is to eradicate microscopic residual cancer cells not removed or shed during surgery. Due to the peritoneal-plasma barrier, relatively high intraperitoneal doses can be given with limited systemic toxicity, which is considered the pharmacokinetic advantage of HIPEC [109]. In line with this, the most used chemotherapeutic agents for HIPEC show a relatively high intraperitoneal to plasma area under the curve (AUC) ratio [110]. The addition of hyperthermia is hypothesized to enhance the effect of intraperitoneal chemotherapy [111], but evidence is contradictory [112].

### TABLE 3: Comparison of HIPEC techniques in PM-CRC, illustrating the great diversity between centers. Table modified and printed with permission from Springer Nature [113]

<table>
<thead>
<tr>
<th>Institution</th>
<th>Method</th>
<th>Drugs</th>
<th>Dosage</th>
<th>Timing</th>
<th>Outflow temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington Hospital Center</td>
<td>Open</td>
<td>IP MMC</td>
<td>15 mg/m²</td>
<td>All at time 0</td>
<td>41 °C</td>
<td>90 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP Dox</td>
<td>15 mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV SFU</td>
<td>400 mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV Leu</td>
<td>20 mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wake Forest University</td>
<td>Closed</td>
<td>MMC</td>
<td>40 mg</td>
<td>30 mg at time 0</td>
<td>40 °C</td>
<td>120 min</td>
</tr>
<tr>
<td>St Agnes Hospital</td>
<td>Closed</td>
<td>MMC</td>
<td>40 mg</td>
<td>30 mg at time 0</td>
<td>42 °C</td>
<td>90 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 mg at 45 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University California San Diego</td>
<td>Closed</td>
<td>MMC</td>
<td>10 mg/L perfusate up to 60 mg</td>
<td>2/3 at time 0</td>
<td>41-42 °C</td>
<td>60 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/3 at 45 minutes</td>
<td></td>
</tr>
<tr>
<td>Regensburg University</td>
<td>Closed</td>
<td>MMC</td>
<td>20 mg/m²</td>
<td>All at time 0</td>
<td>41-42 °C</td>
<td>60 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dox</td>
<td>15 mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxali</td>
<td>300 mg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>Open</td>
<td>Oxali</td>
<td>460 mg/m²</td>
<td>All at time 0</td>
<td>43 °C</td>
<td>30 min</td>
</tr>
<tr>
<td>Sweden</td>
<td>Open</td>
<td>IP Oxali</td>
<td>460 mg/m²</td>
<td>All at time 0</td>
<td>41 °C</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV 5-FU</td>
<td></td>
<td></td>
<td>1 hour before</td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Open</td>
<td>MMC</td>
<td>15 mg/m²</td>
<td>All at time 0</td>
<td>42 °C</td>
<td>60 min</td>
</tr>
<tr>
<td>Basingstoke</td>
<td>Open</td>
<td>MMC</td>
<td>25 mg/m²</td>
<td>1/3 every 30 min</td>
<td>42 °C</td>
<td>90 min</td>
</tr>
<tr>
<td>Switzerland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*MMC* mitomycin C, *Dox* doxorubicin, *Leu* leucovorin, *Oxali* oxaliplatin
Variations in intraperitoneal chemotherapy regimens

Several attempts have been made to standardize the delivery of HIPEC, but there is still great variability between the regimens in use [113-115]. There is variability in delivery method, temperature, carrier solution and volume, drug, drug dosage, drug concentration, timing of drug delivery, total perfusion time and more, illustrated by table 3. This variation is a huge obstacle to the interpretation of research within the field. Intraperitoneal chemotherapy can be administered as HIPEC, early postoperative intraperitoneal chemotherapy (EPIC) or sequential postoperative intraperitoneal chemotherapy (SPIC). The optimal delivery method has not been established, but HIPEC is now by far most common. In HIPEC, the drug is diluted in a carrier solution, often isotonic salt solutions or dextrose-based solutions, and the body surface area (BSA) is normally used to calculate drug doses. Volume of the carrier solution may also vary; some centers use a standard volume for all patients, some dilute the drug in volumes based on the BSA and others titrate volume in the operating room based on the capacity of the abdominal cavity to hold fluid [116]. The drug concentration will vary substantially as a result of the difference in carrier solution volume. Mitomycin C (MMC) or oxaliplatin are usually the drugs of choice for PM-CRC [117]. At our institution MMC (35 mg/m², maximum 70 mg) is given for 90 min at 41.5-42 °C, using a closed technique with open abdomen. In the HIPEC setting, MMC is usually administered for a longer time period compared to oxaliplatin. The relatively lower intraperitoneal to plasma AUC ratio for oxaliplatin compared to MMC, is thought to be compensated by rapid drug absorption of oxaliplatin into the tumor [118, 119]. Retrospective observational studies have compared the toxicity and effect of MMC- versus oxaliplatin-based HIPEC regimens with conflicting results. One study reported comparable survival results between the two drugs in a study of 95 patients [120], while another study of 539 patients reported survival benefit for patients receiving MMC-based HIPEC, if they had a low PCI and favorable histology [121]. A third study from Australia (n=201) reported improved OS in patients treated with oxaliplatin-based HIPEC (56 months OS) over MMC-based HIPEC (29 month OS) [122].

Prognostic factors for outcome after CRS-HIPEC

CRS-HIPEC involves major surgery and chemotherapy. To avoid potential harmful effects of this extensive treatment in patients who will not benefit from it, correct patient selection is crucial. Efforts have been made to identify parameters that are associated with outcome following CRS-HIPEC. The peritoneal cancer index (PCI) is a scoring tool used by surgeons to assess the extent of peritoneal disease during surgery, and probably the most important
prognostic factor for CRS-HIPEC survival outcome [87, 123] (Figure 6). The PCI ranges from 0 to 39, as the abdomen is divided into 9 regions and the small intestine into four, adding up to a total of 13 regions. Each region is given a score from 0-3 based on the size of the tumor deposits [124]. The PCI is a very reliable and reproducible score [125], and is now recognized as the standard for quantifying the distribution of peritoneal metastasis [126, 127].

Residual tumor size after CRS is of great importance. It has been shown that small tumors respond better to treatment than larger ones and that there is limited penetration of drug into peritoneal tumor tissue [129-131]. The completeness of cytoreduction (CC) score assesses tumor burden left in the peritoneal cavity after CRS, and is like the PCI, essential when considering the indication for HIPEC: CC-0: no residual tumor, CC-1: residual tumors < 2.5 mm, CC-2: tumors 2.5-25 mm and CC-3: tumors >25 mm. It is widely accepted that HIPEC is probably not beneficial in cases of incomplete cytoreduction, but the definition of “incomplete cytoreduction” is unclear. CC-0/1 is often defined as complete cytoreduction in

Figure 6. The Peritoneal cancer index (PCI) is a scoring tool used by surgeons to assess the extent of peritoneal disease during surgery. The abdomen is divided into 9 regions and the small intestine into four, adding up to a total of 13 regions. Each region is given a score from 0-3 based on the size of the tumor deposits, and a final PCI score ranging from 0-39 is then calculated. Reprinted from [128] with permission from Elsevier.
pseudomyxoma peritonei (PMP), a rare malignant condition characterized by intraperitoneal accumulation of mucin, usually originating from tumors in the appendix. For PM-CRC, a consensus statement from 2014 defined CC-0/1 as “complete cytoreduction” also for PM-CRC [132]. In contrast, other studies have defined only CC-0 as complete cytoreduction [133], and this is the practice in our institution today. In a study of 523 patients, CC-0 was associated with longer OS than CC-1, which again had a better outcome than CC-2 (Figure 7).

It has been debated whether patients with resectable liver metastasis should be considered eligible for CRS-HIPEC. A recent review investigated outcome in patients treated with CRS and intraperitoneal chemotherapy for PM-CRC coupled with curative treatment for colorectal liver metastasis. Median OS was longer in PM-CRC patients with isolated PM than in those who also had liver metastasis [135]. A meta-analysis found a hazard ratio of 1.30 for death for liver metastases and PM versus isolated PM, when treated with curative intent [133, 136]. It is necessary to approach patients with both resectable liver metastasis and PM in an individualized way, as they may achieve long-term survival, although OS is somewhat shorter than for isolated PM-CRC.
CRS-HIPEC in Norway

As the only center in Norway, CRS and intraperitoneal chemotherapy have been performed since 1994 at the Norwegian Radium Hospital, part of Oslo University Hospital Comprehensive Cancer Center. Initially, only PMP patients were treated, but from 2004 PM-CRC patients were also included. The Norwegian National Unit for Hyperthermic Intraperitoneal Chemotherapy in Colorectal Cancer, Pseudomyxoma Peritonei and Abdominal Mesothelioma was established in 2009 (Figure 8). Between 2001 and 2009, intraperitoneal chemotherapy (EPIC to 2003, HIPEC from 2004) was part of the national unit for perfusion chemotherapy. In 2017, 282 patients were referred to the unit, 123 patients underwent laparotomy and 80 were treated with CRS-HIPEC.

Figure 8. Timeline showing key events in peritoneal cancer surgery at the Norwegian Radium Hospital. The number of complete CRS-HIPEC procedures/year are indicated. In addition, a number of explorative and palliative surgical procedures are performed every year. PM-CRC peritoneal metastasis from colorectal cancer, PMP pseudomyxoma peritonei
MOC31PE Immunotoxin

In the work presented in this thesis (paper II and III), MMC-based CRS-HIPEC was combined with intraperitoneal MOC31PE immunotoxin therapy. Immunotoxins are proteins composed of an antibody or antibody fragment linked to a toxin. The antibody binds to antigens expressed on cancer cells, and the toxin kills the cell upon internalization [137, 138]. The most potent immunotoxins are made from bacterial and plant toxins, which are among the most cytotoxic agents found in nature [3]. One molecule of diphtheria toxin can kill a cell [139]. The bacterial toxins diphtheria toxin and *Pseudomonas* exotoxin A (PE) are the most commonly used toxins in immunotoxins, and both trigger cell death by irreversibly inhibiting protein synthesis and by inducing apoptosis [137, 140-142]. Protein synthesis inactivation is a unique mechanism of action because it is toxic to both dividing and non-dividing cells. Theoretically, such immunotoxins will therefore induce cell death also in multi-drug resistant and dormant tumor cells [143].

The first successful use of immunotoxin in vivo was described in Nature in 1981 [144], followed by several clinical trials in solid tumors [145]. So far, clinical responses to immunotoxins have mainly been seen in hematological malignancies, and not in solid tumors: Tumors in blood and bone marrow are easily accessible to the immunotoxin, and the impaired immune system in hematological malignancies will hinder the formation of neutralizing anti-immunotoxin antibodies [146, 147]. In line with this, known obstacles to successful treatment of solid tumors include poor tumor penetration and the formation of anti-immunotoxin antibodies, which prevents repeat dose regimens. Much effort has been put into development of immunotoxins that are more active and less immunogenic [148]. Yet, only one immunotoxin has to date been approved for clinical use. Denileukin diftitox (Ontak), with a diphtheria toxin payload, was approved for cutaneous T-cell lymphoma in 1999 [149, 150], but was discontinued in 2014 due to a number of serious side effects [151]. An ongoing phase 3 trial (NCT01871727) is investigating the efficacy and safety of E7777, a purified version of denileukin diftitox. Another phase 3 trial (NCT01829711) investigating the effect of the CD22-targeting moxetumomab pasudotox on refractory or relapsed hairy cell leukemia has completed inclusion with promising results [152], and the U.S. Food and Drug Administration (FDA) has accepted the Biologics license application. Several other phase 1 and 2 trials investigating different immunotoxins are also ongoing [145]. Immunotoxin-associated toxicity can be nonspecific or the result of target interaction on non-target cells. Capillary leak syndrome and hepatocyte injury causing elevated serum
transaminases are examples of toxicity often caused by nonspecific uptake of immunotoxins by normal cells. The normal cells mediating these injuries may be hepatocytes, endothelial cells or macrophages that secondarily release cytokines, producing liver and endothelium damage [153]. Capillary leak syndrome, characterized by increased capillary permeability and extravasation of fluid, can give generalized edema, hypoalbuminemia and hypotension. The incidence and severity of capillary leak syndrome varies with the specific agents, and for PE-based immunotoxins it has only rarely been dose limiting. Targeted toxicity is a result of the immunotoxin binding to normal cells expressing the target antigen, and the symptoms will vary depending on which tissues are affected [154]. Targeted toxicity was for example seen in the OVB3-PE phase 1 trial, where OVB3-PE was given intraperitoneally in patients with PM from ovarian cancer. The dose-limiting toxicity (DLT) was neurotoxicity, fatal in one patient, due to cross-reactivity of OVB3 to normal brain tissue. [155]. Other common side effects in patients treated with immunotoxins are infusion reactions [154].

MOC31PE- researcher driven drug development; “from bench to bedside“
MOC31PE is an immunotoxin composed of the monoclonal mouse IgG antibody MOC31, covalently linked to PE, a toxin secreted by Pseudomonas aeruginosa. PE is composed of 613 amino acids and is synthesized as a proenzyme. MOC31PE targets EpCAM and when internalized into the cell the toxin triggers cell death by irreversibly inhibiting protein synthesis through catalytic inactivation of elongation factor 2 [156] and by direct induction of apoptosis (Figure 9) [137].

Epithelial cell adhesion molecule (EpCAM/CD326) was identified in the 1970s as one of the first tumor-associated antigens, and the first monoclonal antibody ever applied for human cancer therapy was a murine antibody (edrecolomab) targeting EpCAM in the early 1980s [157, 158]. EpCAM is now an established epithelial cell marker used by pathologists, and one of the most frequently and highly expressed tumor-antigens known. This 37 kDa type I transmembrane glycoprotein of 314 amino acids is highly expressed in epithelial cancers, and could therefore be an ideal target for diagnosis and therapy [159, 160]. Importantly, while EpCAM is uniformly overexpressed in the cell membrane of cancer cells, it is only expressed basolaterally in normal epithelial tissue [161], meaning that the EpCAM target is relatively inaccessible. This explains why therapeutics targeting EpCAM are believed to not harm normal epithelial tissue [159, 162].
Figure 9. Mechanism of action of MOC31PE. MOC31PE consists of the MOC31 antibody targeting the EpCAM surface antigen, linked to *Pseudomonas* exotoxin A (PE). The antibody directs the immunotoxin to tumor cells and when internalized through endocytosis, the catalytic part of PE is cleaved off and transported from the acidic vesicle to the cytosol via the Golgi and endoplasmatic reticulum (ER). In the cytosol PE triggers cell death through irreversible inhibition of protein synthesis through adenosine diphosphate (ADP)-ribosylation of elongation factor (EF) 2. PE also induces apoptosis, involving depolarization of the mitochondrial membrane, activation of caspase-3, and inactivation of poly (ADP-ribose) polymerase (PARP). Figure based on the work of Y. Andersson.

The development of MOC31PE is a story of researcher driven drug development at our department, evolving over decades. Initially, research was focused on the plant toxins ricin and abrin [163-168], and an early interest in immunotoxins developed in the 1980s after the discovery of monoclonal antibody technology and its potential as Ehrlich's “magic bullets” [169] when conjugated to a toxin or drug [144]. Several monoclonal antibodies and immunotoxins were investigated in different cancer types, and the antibody MOC31 was found to be interesting as it targeted EpCAM-expressing cancer cells, while sparing normal cells [170]. When conjugated to PE, MOC31 was highly effective in killing cancer cells in preclinical studies [171-176]. In 1998, MOC31PE was produced for clinical use and toxicity was investigated in a 21-day intravenous injection study in cynomolgus monkeys in addition to rodents. In 2003 the first phase 1 intravenous study was initiated (#NCT01061645) [177]. In this study, MOC31PE was investigated in 63 patients with EpCAM positive carcinomas, alone and in combination with Cyclosporin A (given to reduce the formation of neutralizing
antibodies). The drug was very well tolerated, with transient liver enzyme elevation as the only observed severe drug-related toxicity.

**Rationale for intraperitoneal MOC31PE**

PM-CRC accounts for a significant proportion of CRC recurrences, and even though CRS-HIPEC can give long-term survival in many patients, treatment outcome is strikingly variable. The peritoneal cavity can be viewed as a unique compartment where the peritoneum acts as a relative barrier against metastatic tumor spread [178], with the possibility of dose intensification due to the relative transport barrier created by the peritoneum [109]. In animal models of PM-CRC using human tumor tissue and cell lines, MOC31PE was given intraperitoneally, alone and together with MMC, and the combination treatment had at least additive effect on intraperitoneal tumor growth (Figure 10) [175, 176]. Intraperitoneal administration of MOC31PE following CRS-HIPEC was considered ideal for exploring clinical efficacy for several reasons: Tumor burden following surgery is low and restricted to the peritoneal cavity, which would allow optimal interaction between MOC31PE and remaining tumor cells [175, 176], and neutralizing antibodies would not be of concern due to the single dose regimen. EpCAM is highly expressed in gastrointestinal malignancies [179], and previous clinical experience with MOC31PE indicated excellent tolerability [177]. Finally, because mesothelial cells do not express EpCAM, targeted toxicity caused by interaction with normal intraperitoneal tissue was considered unlikely. The ImmunoPeCa trial was initiated on this basis, and the overriding hypothesis was that intraperitoneal MOC31PE would safely improve survival in patients with EpCAM positive PM-CRC.

**Figure 10. Efficacy of single intraperitoneal injections of vehicle, mitomycin C (MMC), MOC31PE and drug combinations in an animal model of peritoneal metastasis.** Error bars indicate standard deviation, and all treatment groups were significantly different from vehicle ($p = 0.005$ for MMC, $p < 0.001$ for MOC31PE and the combination). The combination treatment was more efficacious than either of the drugs alone ($p < 0.001$). Modified from [176], with permission from John Wiley and sons.
Aims of the study

CRS and intraperitoneal chemotherapy have increasingly been included in the treatment of peritoneal malignancies for about 3 decades. Despite the improved survival following implementation of CRS-HIPEC in PM-CRC, most patients will experience disease relapse, illustrating the need for improved treatment. Correct patient selection is also important, as the procedure is associated with morbidity. Because PM-CRC does not respond well to systemic chemotherapy and because it can be viewed as a locoregional disease, intraperitoneal treatment is theoretically the optimal treatment combined with surgery. Based on this, the overall aims of this project were to investigate outcome and associated prognostic factors following CRS-HIPEC for PM-CRC and to explore a novel treatment approach involving intraperitoneal MOC31PE immunotoxin in the same patient group. More specifically, this work aimed to:

I. Investigate short- and long-term outcome following CRS-HIPEC for PM-CRC in Norway and to examine associated prognostic factors

II. Evaluate safety and toxicity, pharmacokinetics, neutralizing anti-immunotoxin antibody response and long-term outcome upon intraperitoneal MOC31PE immunotoxin treatment in the ImmunoPeCa trial
Summary of papers

PAPER I: Complete Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Colorectal Peritoneal Metastasis in Norway: Prognostic Factors and Oncologic Outcome in a National Patient Cohort

The aim of paper I was to investigate short- and long-term outcome in patients treated with CRS-HIPEC with MMC for PM-CRC in Norway from 2004 through 2013, and to identify factors associated with disease outcome. Patients were retrospectively identified from a prospective database, and all patients with histologically verified non-appendiceal PM-CRC in the time period were included (n=119). Outcome and potential prognostic factors were examined using uni- and multivariable survival- and ROC-curve analysis. Median PCI was 9 (0-28), 113 (95%) patients had a CC-0 resection, 18 patients (15%) experienced one or more severe postoperative complication (Clavien-Dindo ≥ 3), and there was no 100-day mortality. Median OS was 47 months and median DFS was 10 months. 5-year OS and DFS were 36% and 14%, respectively, with a 45-month median follow-up for OS and 42 months for DFS. Peritoneal relapse was associated with worse prognosis than distant metastasis (p=0.002). The only factor associated with OS in multivariable analysis was the PCI with a HR of 1.05 (p=0.015), and ROC-curve analysis identified PCI > 12 as a marker with 100% specificity for prediction of disease relapse. Based on the possibility of long-term survival combined with no postoperative mortality and acceptable postoperative morbidity in this study, we concluded that CRS-HIPEC remains an important treatment option for patients with resectable PM-CRC.

PAPER II: Novel Treatment with Intraperitoneal MOC31PE Immunotoxin in Colorectal Peritoneal Metastasis: Results From the ImmunoPeCa Phase 1 Trial

Although CRS-HIPEC may offer long-term survival in PM-CRC, most patients will experience disease recurrence and novel therapeutic options are needed. MOC31PE immunotoxin was developed to rapidly kill cells expressing EpCAM, which is highly expressed in CRC. Based on promising preclinical studies it was hypothesized that this drug would improve outcome in PM-CRC patients when administered intraperitoneally. The ImmunoPeCa trial was a dose-finding phase 1 trial aiming to evaluate the safety and toxicity (primary endpoint) upon intraperitoneal administration of MOC31PE in patients with PM-CRC undergoing CRS-HIPEC with MMC. Pharmacokinetics, neutralizing antibody response and long-term outcome were secondary endpoints. The study was recruiting between
September 2014 and September 2016. In this paper we reported the results from the first 15 patients, who received the study drug at four dose levels ((2.5 (n=3), 5.0 (n=3), 7.5 (n=3) and 10.0 μg/kg (n=6)), administered intraperitoneally as a single dose the day after CRS-HIPEC. No DLT was observed, and the maximum tolerated dose (MTD) was not reached. There was only very low systemic absorption of the study drug in 6 patients at the two upper dose levels. Drug concentrations in peritoneal fluid samples were in the cytotoxic range and increased in a dose-dependent manner. MOC31PE recovered from peritoneal cavity retained its cytotoxic activity in cell-based assays, indicating that it is stable under physiological conditions. All 15 patients developed neutralizing antibodies. Intraperitoneal administration of MOC31PE was deemed safe and well tolerated, and combined with low systemic uptake we concluded that the drug seemed ideal for intraperitoneal treatment.

PAPER III: ImmunoPeCa trial: Long-term outcome following intraperitoneal MOC31PE immunotoxin treatment in colorectal peritoneal metastasis

The ImmunoPeCa trial investigated the use of intraperitoneal MOC31PE immunotoxin as a novel therapeutic principle to target PM-CRC, aiming to establish a safe dose level and ultimately improve treatment for PM-CRC. This dose-finding trial explored safety and tolerability upon intraperitoneal administration of MOC31PE in patients with PM-CRC undergoing MMC-based CRS-HIPEC, and OS and DFS. Patients were treated at four dose levels (2.5, 5.0, 7.5 and 10.0 μg/kg), and study drug was administered intraperitoneally as a single dose the day after CRS-HIPEC. There was no severe drug-related toxicity in the expansion cohort of 6 patients. With a 34-month follow-up, the median OS was not reached and the estimated 3-year OS was 78% for the entire study population. Median DFS was 21 months and the 3-year DFS 33%, with a median follow-up of 31 months. High levels of MOC31PE in peritoneal fluid samples following study treatment showed a non-significant trend towards improved DFS after adjusting for PCI in multivariable analysis (HR 0.37, p=0.065). Although encouraging, the results may reflect the study patient selection, and investigation in a larger cohort would be necessary to study efficacy. We concluded that intraperitoneal, postoperative administration of MOC31PE was safe and well tolerated also when including additional 6 patients. The promising long-term outcome combined with the previously shown low systemic absorbance, high drug concentration and cytotoxic activity in peritoneal fluid, support further studies on efficacy.
Methodological considerations

Study design in medical research

Clinical research within the field of medicine can be divided into epidemiological observational studies and clinical interventional studies. In the observational study, the investigator observes the relationship between different factors and outcome, while in the interventional trial patients are subjected to an intervention, e.g. a new drug or surgical technique. Patients in an observational study may also receive interventions, but as part of routine medical care and not because they are assigned to it by an investigator as in an interventional trial. This thesis includes one observational cohort study (paper I) and one interventional clinical trial (papers II and III).

Observational studies

In an observational cohort study, a group of individuals who have specific features in common are followed over time and disease and/or treatment related data are recorded [180]. Such studies can be labeled prospective or retrospective. In a prospective design, the study cohort is defined at baseline, exposures assessed and patients are followed over time to study the development of for example disease or mortality. In a retrospective design, the patient cohort is established at a later time, and exposures and outcome are studied looking back. In short, the major strength of a prospective cohort study is the accuracy of data collection, but at the expense of cost and time consumption. Retrospective studies are in contrast more time and cost efficient, but one has to rely on data collected in the past, often for another purpose, such as patient care. Importantly, there has been some confusion regarding terminology, as some epidemiologists have considered all follow-up studies “prospective” because follow-up always goes forward in time [181]. In addition, prospective cohort studies are typically ranked higher in the study hierarchy than retrospective studies [182], and therefore studies are sometimes named “prospective” in an attempt to improve impact. Considering this, instead of only using the terms “prospective” or “retrospective” when describing a study, researchers should give a thorough explanation of what has been done in the methods section [183]. Observational studies are well suited to assess associations between multiple exposures and multiple outcomes, and are particularly well suited for exploration of rare exposures or situations where a randomized controlled trial is not an option for practical or ethical reasons [183]. Furthermore, because observational studies are usually not subjected to the same strict
inclusion and exclusion criteria as interventional studies, the results are more generalizable, giving a better picture of the “real world”. However, for the same reasons, observational studies are more prone to various types of bias.

Clinical trials
The U.S. National Institutes of Health defines a clinical trial as “a research study in which one or more human subjects are prospectively assigned to one or more interventions (which may include placebo or other control) to evaluate the effects of those interventions on health-related biomedical or behavioral outcomes.” The interventions are typically a new drug, drug combination or surgical technique, but could also be a new diet or a behavioral intervention. The new medical approach under investigation may be compared to the standard-of-care, to a placebo or to no intervention, and it is not known beforehand whether the intervention will be harmful, beneficial or indifferent. Clinical trials used in drug development are often divided into development phases. These phases are defined by the FDA, and follow consecutive steps from initial, small-scale phase 1 trials to large-scale phase 3 and 4 trials (Figure 11). Drug development is time consuming and costly. Only one in ten of all drugs entering clinical development in phase 1 will later be approved, and for oncology drugs the number is even lower with a 1 in 15 likelihood of approval [184].

Figure 11: The drug development process. Prior to clinical testing, the study drug has gone through discovery, development and preclinical (in vivo and in vitro) toxicity testing. In phase 1, the aim is to study safety and determine a tolerated dose. In phase 2, the aim is to analyze efficacy and side effects, while in phase 3 the purpose is to compare the efficacy and safety of the candidate drug to conventional treatment. Because phase 3 studies are longer in duration and require more participants, less common side effects and long-term effects will usually be exposed. Phase 4 trials are executed after the drug is approved by further monitoring safety and effectiveness in the general population.
The cohort study

Patient selection

In paper I we reported on the oncologic outcome and postoperative complications following CRS-HIPEC for PM-CRC in Norway between 2004 and 2013, through an observational cohort study. The cohort was retrospectively identified from the institutional peritoneal surface malignancy database, where all patients surgically treated for PM at The Norwegian Radium Hospital have been prospectively registered (Figure 12). Medinsight, a tool for storage and collation of patient data, was used for this purpose. From this database we identified all patients with CRC that had undergone surgery for suspected or verified PM. Patients not treated with CRS-HIPEC, patients with appendiceal cancers, and those without histologically verified PM were excluded from the study. Data not registered in the database was retrospectively obtained from patient records. Because all CRS-HIPEC patients with PM-CRC within the selected time interval were included in the study, the risk of selection bias was reduced.

Figure 12. Flow diagram of the patient selection process for paper I. Patients with surgery for suspected or verified PM from CRC or appendiceal cancer between 2004-2013 (n=229) were retrospectively identified from the prospectively maintained institutional peritoneal surface malignancy database, where all patients surgically treated for PM at The Norwegian Radium Hospital are registered. Patients treated with CRS-HIPEC for non-appendiceal, biopsy verified PM-CRC between 2004-2013 were included in the study.
Endpoints

In all research studies, it is important to critically evaluate which endpoints to use and the associated potential biases. Follow-up data including patient records and radiologic work-up from referring hospitals were collected and thoroughly examined for information on disease recurrence. The date of CRS-HIPEC was chosen as baseline for survival calculations, and time from CRS-HIPEC to death or the censoring date was calculated for OS. For DFS, time from CRS-HIPEC to the time of recurrence, new primary cancer or death (whichever came first) or to last follow-up was calculated. It is common to choose the date for CRS-HIPEC as baseline for survival analysis. Some patients will thus already have been treated with one or more lines of chemotherapy at the time of CRS-HIPEC. If the dates of PM diagnosis- or primary tumor diagnosis were chosen as the baseline for survival calculations, the reported survival would be longer. Survival data was obtained from the Norwegian National Registry and was censored on June 8, 2015. Since all deaths in Norway are registered here without exception, postoperative mortality and OS are reliable endpoints. More uncertainty is associated with the exact time of cancer recurrence, and the DFS could be influenced by several factors, such as the thoroughness and experience of the radiologist and the investigator, and the follow-up intervals. The follow-up modalities, which may differ substantially between centers, and interval between follow-up could affect the shape of the survival curve, especially in PM-CRC where many patients experience early recurrence. If a recurrence is diagnosed early due to a follow-up regimen with short intervals, the survival curve would be steeper and the DFS shorter than in a less tight regimen, which should be considered when comparing results from different studies. The median DFS would in this setting be more influenced by the different follow-up regimens than the 3- and 5- year DFS. Only the first site(s) of recurrence was reported in our study; either as PM only, PM and distant metastasis or distant metastasis only. Distant metastasis was defined as metastasis in any extraperitoneal location. Ovarian metastases were defined as PM, because of the direct contact with the peritoneal cavity. If the recurrences were detected within a 3-month interval, they were considered simultaneous. Metastasis in other locations following the first recurrence was not reported to avoid bias as most patients are not followed with CT (computed tomography) -scans of thorax, abdomen and the pelvis regularly while in a palliative setting.
The ImmunoPeCa trial

Study design

In paper I and II we reported the results from the ImmunoPeCa trial. Careful design of study protocol and dedicated study management is crucial for trial execution, and for producing relevant data and results in order to make correct conclusions. In the ImmunoPeCa trial (Immunotoxin in Peritoneal Carcinomatosis (NCT00769405)) MOC31PE was investigated for intraperitoneal use for the first time. 21 patients were treated with MMC-based CRS-HIPEC and intraperitoneal MOC31PE on the following day (figure 13), with the intention to kill remaining tumor cells [185].

This was a dose-finding, single center, single arm, open-label clinical trial carried out to evaluate the safety and tolerability (primary endpoint) upon intraperitoneal MOC31PE treatment. Pharmacokinetic profile, neutralizing anti-immunotoxin antibody response at 4 and 8 weeks, OS and DFS were secondary endpoints. Oslo University Hospital was sponsor, and the study was carried out at the Norwegian Radium Hospital (part of Oslo University Hospital). Adult patients with suspected PM from histologically verified EpCAM positive CRC could be included in the study if they met all inclusion and exclusion criteria (Table 4). Decisions regarding which patients to invite to participate in the trial were made by the investigators during the weekly multidisciplinary team meeting. The inclusion and exclusion criteria were based on commonly applied criteria used in early phase clinical trials. Since DLT in the previous MOC31PE intravenous trial was transient elevation of liver enzymes, patients with a history of liver disease and patients using drugs that could influence hepatic
function were excluded. Information on OS was obtained from the Norwegian National Registry and information regarding the DFS was obtained from follow-up data, which included CT-scans (thorax, abdomen, pelvis), laboratory work-up and physical exams. Information on recurrence, complications and symptoms was prospectively registered, reducing the risk of bias. Patients are followed at the Norwegian Radium Hospital for 5 years or until disease relapse.

**TABLE 4. Inclusion and exclusion criteria for the ImmunoPeCa trial**

**Inclusion criteria**

- Histologically verified EpCAM positive CRC
- Ambulatory with ECOG performance status 0-1 at the time of surgery
- At least 18 years of age
- Suspected isolated PM upon radiological work-up
- Complete cytoreduction at surgery (CC-0) and mitomycin C given as standard HIPEC
- PCI ≤20
- Laboratory values: ANC ≥ 1.5 x 10^9/L, Platelets ≥ 100 x 10^9/L, Hb ≥ 9g/dL, Creatinine ≤ 2x ULN, Bilirubin < 2.0x ULN, AST and ALT ≤ 2. 5x ULN, Albumin levels > 30 g/L, INR<1.3
- Signed informed consent and expected cooperation with respect to treatment and follow-up must be obtained and documented according to ICH GCP, and national/local regulations

**Exclusion criteria**

- Other synchronous metastatic lesions. Patients may be included if they have had curative resection of metastatic CRC disease more than 2 years prior to inclusion and have no relapse at this location is detected
- History of prior other malignant disease the last 3 years, except for adequately treated carcinoma of the cervix or basal or squamous cell skin cancer
- History of CNS or bone metastases
- History of any liver disease including Hepatitis B or C infection
- Significant cardiac or other medical illness that would limit activity or survival, such as severe congestive heart failure, unstable angina, or serious cardiac arrhythmia
- Chemotherapy/radiation therapy within the last 4 weeks before start of treatment
- BMI > 35
- Pregnant or breast-feeding patients
- Alcohol or drug abuse
- Use of drugs that can influence hepatic function (e.g. phenytoin or phenobarbital)
- Use of anticoagulants
- Any reason why, in the opinion of the investigator, the patient should not participate in the study protocol

*ALT alanine aminotransferase, ANC absolute neutrophil count, AST aspartate aminotransferase, BMI body mass index, CNS central nervous system, CRC colorectal cancer, ECOG eastern cooperative oncology group, EpCAM epithelial cell adhesion molecule, Hb hemoglobin, HIPEC hyperthermic intraperitoneal chemotherapy, ICH GCP International Conference on Harmonization Good Clinical Practice guidelines, INR International normalized ratio, PCI peritoneal cancer index, PM peritoneal metastasis, ULN upper limit of normal*
The MOC31PE solution was administered as single rapid intraperitoneal instillations through each of two abdominal drainage catheters on the morning after CRS-HIPEC (figure 14). The catheters were clamped for 6h following drug instillation, and then reopened. This was a compromise between wanting to keep the drug in the peritoneal cavity for as long as possible, and the need for open catheters to remove postoperative excess fluid. A 3+3 dose escalation design was applied, with four dose levels (2.5, 5.0, 7.5 and 10.0 μg/kg, Table 5). Safety and toxicity were evaluated on days 3, 4, 5 and 9 after CRS-HIPEC, and at follow-up out-patient visits, with special attention on hepatobiliary laboratory tests as transient elevation of liver enzymes were DLT in the MOC31PE intravenous trial. DLT was thus defined as any drug related toxicity ≥ grade 3, except ASAT/ALAT and ALP/GT elevation, which was a DLT if the lab values reached grade 4. If a DLT was observed in one patient within one dose level, up to five additional patients could be included at this dose level. If 2 patients on the same dose level experienced a DLT, the dose level below would be considered MTD.

**Table 5. Algorithm for preparation of MOC31PE for intraperitoneal administration**

<table>
<thead>
<tr>
<th>Dose level</th>
<th>MOC31PE dose (μg/kg)</th>
<th>MOC31PE concentration (μg/ml)</th>
<th>Body weight (kg)</th>
<th>Instillation volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>0.5</td>
<td>60</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>1.0</td>
<td>70</td>
<td>350</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>1.5</td>
<td>80</td>
<td>400</td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>2.0</td>
<td>90</td>
<td>450</td>
</tr>
</tbody>
</table>

**Figure 14** (A) Illustration of the MOC31PE instillation through indwelling abdominal catheters and (B) image of an ImmunoPeCa patient receiving MOC31PE. The patient gave written consent for publication of photographs.
Dose rationale and dose finding

After the discovery and development of a potential new drug through preclinical efficacy studies, the toxicity has to be evaluated before it can be tested in patients. Such preclinical research is performed through in vitro and in vivo experiments and should give detailed information on dosage and toxicity levels. It is then decided whether the new drug should be tested in patients and, based on algorithms, which dose to start with. A too high dose will give an increased risk of toxicity, while a too low dose will not be efficacious. In the MOC31PE intravenous trial, the starting dose of 0.5 μg/kg was based on calculations from animal data to the equivalent human dose using common conversion factors described by Freireich et al [186]. DLT for systemic MOC31PE was observed at 8.0 μg/kg, and recommended dose for further intravenous studies was set to 6.5 μg/kg. As MOC31PE in the ImmunoPeCa trial was given intraperitoneally, a lower plasma concentration was expected, and a starting dose of 2.5 μg/kg was considered safe. A too low starting dose is however also considered unethical in cancer patient trials, and should be avoided. Based on results from animal experiments, the starting dose was considered acceptable also from an efficacy perspective. When a DLT was not seen at dose level 4, a decision was made by the investigators not to increase the dose further, as the intraperitoneal drug concentrations were considered more than high enough for biological effect.

Severity grading of adverse events

National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE version 4.0) is a system for grading deviation from expected laboratory tests, subjective complaints or abnormal physical findings. Adverse events (AEs) are defined as all untoward signs or symptoms observed in study patients regardless of a causal relationship to the study drug. Adverse drug reactions (ADRs) are defined as AEs considered causally related to the drug under investigation. The CTCAE grades are: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening) and Grade 5 (death related to AE). The primary endpoints for the ImmunoPeCa trial were safety and toxicity, and the patients were closely monitored in regards to laboratory tests, vital signs and symptoms, using the CTCAE system. The investigators assessed the causal relationship of the event and study medication as unrelated, unlikely related, possibly related, probably related and definitely related. In addition to the CTCAE version 4.0, events were registered using the Accordion classifications for postoperative complications [187].
**Statistical methods**

Survival analysis is based on time-to-event outcomes and censored observations (when the event did not occur during follow-up). Survival analysis give more information than solely whether an event has occurred in a group or not, and so they are widely used in medical research. In the Kaplan-Meier method, the probability of survival at a given time is provided by the survival function and can be drawn as a step-wise curve where a vertical drop illustrates an event. Comparing survival curves of different groups is often of particular interest, and the log-rank test is the most used method. In this thesis, univariable analysis was performed using the Kaplan-Meier method, and the log-rank test was used to compare differences in survival. Median follow-up time was estimated using the reverse Kaplan-Meier method.

The Cox proportional hazards model is a regression model for survival data, which investigates the relationship of predictor variables and time-to-event through the hazard function. The hazard function is an indicator of the risk of experiencing the event per unit time, and the Cox model is interpreted using hazard ratios (HR), which is defined as the ratio of the predicted hazard functions under two different values of a variable. An HR greater than 1 means that an event is more likely to occur, while an HR of less than 1 means that the event is less likely to occur [188]. Where the Kaplan Meier method can only analyze one variable at a time and the log-rank test can only compare categorical data, Cox regression models can operate with multiple variables and continuous data.

A ROC curve is used in medicine to evaluate the diagnostic accuracy of a continuous variable for prediction of a binary outcome such as disease or no disease and is a plot of sensitivity (true positives) versus 1-specificity (false positives) for all possible cutoff values of the continuous variable. The performance of the variable is evaluated by the area under the ROC curve (AUC), where a higher AUC indicates a better performance [189]. However, if the binary outcome is time-dependent, such as death (everyone will die eventually) or cancer recurrence, time-dependent ROC curves are more appropriate than conventional ones, especially in studies including individuals with short follow-up time [190]. In paper I, conventional ROC curve analysis for PCI versus death and recurrence were applied, and time-dependent ROC curve analysis for censored survival data using the Nearest Neighbor Estimation (NNE) method [190] was performed for quality assurance.
Results and discussion

OS in CRS-HIPEC for PM-CRC

Reported OS varies greatly between studies in this field, but the study criteria also vary substantially. Short- and long-term outcome and associated prognostic factors for non-appendiceal PM-CRC undergoing CRS-HIPEC in Norway between 2004 and 2013 were examined in paper I. Median OS was 47 months and the 3- and 5-year OS was 65% and 36%, respectively, with a median follow-up of 45 months. In the literature, median OS usually ranges from 32-47 months [116, 191], with one case-control study standing out with a 62.7-month OS [93]. In comparison, median OS for patients with limited PM treated with systemic chemotherapy was 17-24 months [92, 93].

CRS-HIPEC was implemented without validation from randomized controlled trials, and the available evidence has to date been mostly observational and retrospective in nature, with some exceptions. There is a paucity of basic research in the field, and the procedure moved quickly from bench to bedside [192-194]. Numerous cohort studies report on outcome, in addition to several case-cohort studies [92, 93, 195], meta-analysis [196-198] and systematic reviews [116, 199-202]. Many studies conclude that there is superior survival in CRS-HIPEC compared to systemic chemotherapy. Two randomized controlled trials have investigated the effect of CRS and intraperitoneal chemotherapy versus systemic chemotherapy alone in PM-CRC [90, 203, 204].

In the first, an often-referenced study by Verwaal et al, MMC-based CRS-HIPEC and adjuvant systemic chemotherapy (5-FU and leucovorin) were compared to systemic chemotherapy alone (5-FU and leucovorin). A longer OS was reported in the CRS-HIPEC arm (22.3 months) than in the control arm (12.6 months) [90, 203], but a shortcoming of the study is the outdated chemotherapy regimen. In the Swedish peritoneal study, patients who were deemed resectable preoperatively were randomized to surgery and intraperitoneal 5-FU for 6 days with repeated courses every month or to a systemic oxaliplatin- and 5-FU regimen every second week. Both treatments continued for 6 months. Higher OS was reported in the intraperitoneal chemotherapy arm (25 vs 18 months OS, p=0.04) [204]. The study was unfortunately terminated early because of problems with recruitment. As the Swedish study illustrates, it has been difficult to perform randomized controlled clinical trials comparing systemic chemotherapy to CRS and intraperitoneal chemotherapy, because the latter treatment has been implemented as the standard-of-care in many countries.
To date, most of the research in the field has focused on describing and investigating the effect of CRS and HIPEC combined, and in the literature, these two entities are almost inseparable. Until now, the additional effect of HIPEC beyond surgery has been undetermined. The main much-awaited results from the French prospective randomized phase 3 multicenter trial PRODIGE 7 (NCT00769405) were recently presented at the 2018 ASCO annual meeting [205], and the paper was not yet published when this thesis was submitted. The trial was designed to evaluate the effect of adding HIPEC to CRS. Patients were randomized to oxaliplatin-based HIPEC for 30 min or to no HIPEC in the operating room following CRS with residual disease < 1mm. The study finished accrual in 2014. With a 63.8-month follow-up, no difference in OS was seen between patients undergoing CRS-HIPEC compared to CRS only (41.7 vs 41.2 months, respectively). There was no difference in postoperative mortality and 30-day morbidity, but there was a higher grade 3-5 morbidity rate in the HIPEC group at 60 days (24.1% vs 13.6%, p= 0.030). Interestingly, a retrospective study from 2015 on PM-CRC treated with CRS only combined with systemic chemotherapy reported similar results: a 44% 5-year OS and median 47.5 months OS [206]. Several questions remain unanswered following PRODIGE 7, where one of the most obvious ones is whether the drug used for CRS-HIPEC is important, and in particular whether addition of MMC-based HIPEC would influence outcome differently. With these questions unanswered, continued treatment of patients should ideally be conducted in trials designed to answer this and other research questions. It will be interesting to see how PRODIGE 7 will impact clinical practice. The timing is good for exploring treatment alternatives in both basic and clinical research.

**Cancer recurrence after CRS-HIPEC**

We reported a 10-month median DFS, and the estimated 3- and 5-year DFS were 21% and 14%, respectively. In comparison, a meta-analysis including 76 papers recently reported a median DFS of 12.6 months [198], and median PFS in the HIPEC arm of PRODIGE 7 was 13.1 months. Patients with relapse following CRS-HIPEC were equally distributed between PM only, PM and distant metastasis and distant metastasis only. Similar results have been shown elsewhere [207]. OS was less favorable in patients with PM only or PM with distant metastasis, compared to patients with distant metastasis only (19% vs 53% 5-year OS, p=0.002). Some of this effect could be attributed to metastasis surgery in the distant metastasis only group, where approximately half of the patients received such treatment. However, after excluding patients undergoing curative interventions for their metastases, the
5-year OS was 18% for patients with PM with or without distant metastasis and 32% for patients with distant metastasis only treated with palliative chemotherapy or best supportive care (p=0.09), in line with previous studies reporting worse outcome in PM-CRC than in other metastatic locations [1, 2].

The long OS of 47 months observed for PM-CRC patients undergoing CRS-HIPEC is remarkable, considering the much shorter DFS of 10 months. The time from recurrence until death may reflect disease aggressiveness and treatment response, and can be explored by looking at the ratio between OS and PFS [208] or DFS. Patients treated with first line chemotherapy in the NORDIC VII phase 3 trial had a PFS of 8 months and an OS of 20 months OS, yielding a PFS/OS ratio of 2.7 [209]. In our study the OS/DFS-ratio was 4.8, suggesting that CRS-HIPEC could be beneficial to patients by improving OS despite a relatively short DFS. In other large studies on CRS-HIPEC in PM-CRC the OS/DFS-ratios were lower, ranging from 2.0-3.2 [134, 205, 210]. Differences in follow-up regimens could influence the timing of discovery of recurrences, and the use of systemic chemotherapy before, during or after CRS-HIPEC could influence DFS, OS or both. There have been no randomized controlled trials investigating addition of neoadjuvant, adjuvant and concurrent systemic chemotherapy to CRS-HIPEC, and the available evidence is inconclusive despite widespread use [211]. In Norway, adjuvant systemic chemotherapy is not routinely given after CRS-HIPEC unless there are other factors indicating such therapy. This might contribute to a shorter DFS as a more liberal chemotherapy policy could postpone disease recurrence in some patients. Comparing with the literature, our restrictive approach does not appear to influence OS negatively. However, only randomized controlled studies could answer this important question.

**Can CRS-HIPEC cure PM-CRC?**

With a noticeable number of patients in the 5-year DFS group, the question of whether PM-CRC can be cured arises. Although it was once believed that this was impossible, studies have shown the opposite [212, 213]. Upon reexamination of our dataset following an update of survival- and recurrence data 2 years after publishing, the following outcome was seen: 47 months median OS and 38% 5-year OS, with a 78-month follow-up time. 10-year OS was estimated to be 27%. The estimated 5-year DFS was 16%, with a 71-month follow-up time. Fourteen (12%) of our 119 study patients had been followed for more than 5 years after CRS-HIPEC without recurrence and were thus likely to be cured of their cancer (Table 6). The median PCI was 6 (1-10) among these patients. Three additional patients, who experienced
metastasis development following CRS-HIPEC, underwent surgical resection and have lived for 5 years without recurrence since the last cancer surgery. When including these patients, an actual cure rate of 14% was seen. Similar results were shown in other PM-CRC cohorts [212, 213], and after resection of CRC liver metastasis [214-216].

**TABLE 6. Characteristics of the 14 patients with no recurrence**

<table>
<thead>
<tr>
<th>ID</th>
<th>Follow-up (months)</th>
<th>Gender</th>
<th>Timing of PM</th>
<th>Location of primary tumor</th>
<th>PCI</th>
<th>CC-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>Female</td>
<td>Metachronous</td>
<td>Ascending colon</td>
<td>1</td>
<td>CC-0</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>Female</td>
<td>Metachronous</td>
<td>Sigmoid colon</td>
<td>6</td>
<td>CC-0</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>Female</td>
<td>Metachronous</td>
<td>Ascending colon</td>
<td>9</td>
<td>CC-0</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>Female</td>
<td>Metachronous</td>
<td>Ascending colon</td>
<td>6</td>
<td>CC-0</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>Female</td>
<td>Metachronous</td>
<td>Rectum</td>
<td>6</td>
<td>CC-0</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>Female</td>
<td>Metachronous</td>
<td>Transverse colon</td>
<td>8</td>
<td>CC-0</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>Female</td>
<td>Synchronous</td>
<td>Sigmoid colon</td>
<td>5</td>
<td>CC-0</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>Female</td>
<td>Metachronous</td>
<td>Sigmoid colon</td>
<td>10</td>
<td>CC-0</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>Female</td>
<td>Metachronous</td>
<td>Sigmoid colon</td>
<td>2</td>
<td>CC-0</td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>Female</td>
<td>Synchronous</td>
<td>Ascending colon</td>
<td>3</td>
<td>CC-0</td>
</tr>
<tr>
<td>11</td>
<td>64</td>
<td>Male</td>
<td>Synchronous</td>
<td>Ascending colon</td>
<td>8</td>
<td>CC-0</td>
</tr>
<tr>
<td>12</td>
<td>63</td>
<td>Female</td>
<td>Synchronous</td>
<td>Ascending colon</td>
<td>6</td>
<td>CC-0</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>Female</td>
<td>Synchronous</td>
<td>Transverse colon</td>
<td>6</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>63</td>
<td>Female</td>
<td>Synchronous</td>
<td>Rectum</td>
<td>7</td>
<td>CC-0</td>
</tr>
</tbody>
</table>

*CC completeness of cytoreduction, PCI peritoneal cancer index, PM peritoneal metastasis*

**Factors associated with survival**

Since patient selection for CRS-HIPEC is one of the major challenges within the field, research on prognostic factors that could help the selection is important. The PCI, representing the extent of PM, was the only variable associated with OS and DFS in multivariable analysis in this study, with a HR of 1.05 for death for every increase in PCI (p=0.015), in line with several other studies [134, 217, 218]. Different PCI cut-off values have been proposed to improve patient selection for CRS-HIPEC. PCI greater than 20-25 has in many centers, ours included, been regarded as a relative or absolute contraindication to CRS-HIPEC for PM-CRC [134, 218, 219]. However, a definitive PCI cut-off value might not exist [220, 221]. Studies have reported a linear correlation between PCI and survival, suggesting that an absolute or “true” cut-off value cannot be established [222]. We performed ROC-curve analysis to further investigate the ability of PCI to predict disease relapse, and this gave an AUC of 0.82, meaning that the likelihood is 0.82 for a patient with relapse to have higher PCI than a patient without relapse. The AUC for PCI and OS was 0.74. Furthermore, PCI >12
yielded a 100% specificity for predicting relapse, meaning that all patients with a PCI>12 experienced recurrence. However, the PCI alone is not enough to predict outcome, as reports have shown long-term survival in patients with high volume-disease [213], and we did not find differences in OS in patients with PCI 11-20 and >20. CRS-HIPEC may therefore be considered in some patients with extensive disease, if the cancer is completely resectable. Different scoring tools, such as the peritoneal surface disease severity score, (PSDSS) [223], the colorectal peritoneal metastases prognostic surgical score (COMPASS) [217] and colorectal peritoneal score (COREP) [224] have been proposed to help patient selection, but they have pros and cons and the ultimate scoring tool has yet to be developed.

In contrast to some studies [225], we did not find negative associations between the presence of signet ring cells and outcome. All pathology reports were reexamined before statistical analysis, and in some patients, the presence of signet ring cells was not given as a percentage in the pathology report. Therefore the term “signet ring cell carcinoma” was not used in the paper, as these cases might not fulfill the definition of > 50% signet ring cells. This could be a contributing factor as to why the presence of signet ring cells did not have a negative impact on outcome in this study, in addition to the limited number of patients with signet ring cell tumors (n=14, 12 %). Others reports show similar results [226]. On the other hand, it has been shown that also tumors with < 50% signet ring cells present hold a poor prognosis [227].

Postoperative complications
Short-term outcome after major surgery can be a reflection of how well a treatment has been implemented in the surgical center. In this cohort, there was no 100-day mortality, 15% experienced severe postoperative complications and 8% underwent re-operations within 30 days. Others have reported postoperative mortality rates between 0.7 -7.7% [228-234], and re-operation rates between 4 - 20.8% [116]. Our center had 10 years of experience with CRS and intraperitoneal chemotherapy prior to treating PM-CRC from treatment of PMP patients. This means that the presented results do not include the institution’s learning curve, a period characterized by higher frequency of per- and postoperative events [235]. A multidisciplinary team evaluates all referred patients, and possible cost in terms of morbidity and mortality is carefully weighed against benefit for the patient before acceptance for surgery. The retrospective collection of patient information is a possible source of bias. In contrast, postoperative mortality data was obtained from the Norwegian national registry where all deaths in Norway are registered, meaning that these results are unbiased.
**ImmunoPeCa: Toxicity and drug development in a complex surgical setting**

The primary endpoint of the ImmunoPeCa trial was safety and toxicity upon intraperitoneal treatment with MOC31PE and additional endpoints were pharmacokinetics, anti-immunotoxin antibody response and long-term survival. In paper II we reported toxicity, pharmacokinetics and neutralizing antibody response for the first 15 study patients and in paper III long-term outcome for the entire study population and toxicity for the expansion cohort of 6 patients. No DLT was observed at any dose level, and an MTD was not reached. CTCAE Grade 3 hepatobiliary laboratory abnormalities were observed in 5 patients, with no dose-response pattern, and the same changes were seen in similar patients undergoing CRS-HIPEC without immunotoxin treatment. Because of the lack of dose-response relationship, in addition to the low or absent levels of MOC31PE in serum, we concluded that the hepatobiliary laboratory abnormalities were not likely to be caused by the study drug. The frequency of postoperative complications was similar in the ImmunoPeCa trial and in the cohort study (15% vs. 14% severe complications).

The ImmunoPeCa trial faced the challenge of being an early-phase study in a complex surgical setting where the study patients had received major surgery and MMC-based HIPEC, both associated with toxicity. Postoperative pain, nausea, anorexia etc. are common and expected following this treatment, and more severe AEs are also relatively common [116, 228-234]. To investigate side effects from a new drug in this setting was challenging. Nevertheless, all AEs were recorded, and an assessment of whether they were likely to be related to the study drug was made. There is a possibility that less severe AEs could have been camouflaged by symptoms associated with the postoperative setting and attributed to surgery or HIPEC instead of MOC31PE, and the low number of patients will also prevent absolute conclusions regarding drug toxicity. However, the benign toxicity profile previously observed in the intravenous MOC31PE phase 1 trial including 63 patients [177], support our understanding of MOC31PE as a safe and relatively non-toxic drug.

The favorable toxicity profile seen in both intraperitoneally- and intravenously administered MOC31PE is encouraging, and in contrast to reports from investigations of other immunotoxins. Only one immunotoxin has to date been approved by the FDA. Denileukin diftitox (Ontak) was discontinued in its current formulation in 2014 due to a number of serious side effects such as capillary leak syndrome, infusion reactions, and loss of visual acuity [151]. Another immunotoxin that may receive approval in 2018 is the CD22-targeting moxetumomab pasudotox. In a study including 80 patients, 2 patients had increased
blood creatinine, 7 patients had reversible haemolytic uraemic syndrome and 7 patients had reversible capillary leak syndrome, including four patients who had both [152].

OV3B-PE immunotoxin was the first PE-based immunotoxin that was investigated in a clinical trial [155]. It was composed of a murine antibody targeting an unknown antigen on human ovarian cancers, coupled to PE, and in many regards similar to MOC31PE. In a dose-escalating study published in 1991, refractory ovarian cancer patients (n=23) were treated with escalating doses of intraperitoneal OV3B-PE (1-, 2-, 5- and 10 μg/kg), administered at least twice. Dose-limiting neurotoxicity was reported in three patients. Two patients treated at 10 μg/kg experienced confusion, apraxia and dysarthria, but they recovered after several months. Fatal neurotoxicity was seen in one patient treated at 5 μg/kg, after the third dose, and the study was immediately terminated. The neurotoxicity was likely caused by crossover reactivity of the antibody OV3B with brain cells. This was not detected in preclinical primate studies, as monkey brain tissue does not show reactivity to OV3B-PE. Except for the specific neurotoxicity, the authors report no other grade 3 toxicity, and the drug was well tolerated in patients not experiencing neurotoxicity, in line with a low uptake of drug in serum.

Catumaxomab (Removab), although not an immunotoxin, bears some similarities to MOC31PE. It is a trifunctional rat/murine hybrid antibody that has the ability to bind to three different types of cells: tumor cells expressing EpCAM, T-lymphocytes expressing CD3 and accessory cells with FcY receptor. The European Medicines Agency approved Catumaxomab in 2008 for intraperitoneal treatment of malignant ascites. The overall safety profile of this drug is characterized by mild to moderate cytokine-release related symptoms and gastrointestinal reactions, all fully reversible. A randomized phase 2 trial comparing CRS followed by two different dosing regimens of intraperitoneal catumaxomab in gastric cancer was completed last year (NCT01504256). The results are not yet published. Due to insolvency of the manufacturer, the marketing authorization in the European Union was withdrawn in 2017.

**Pharmacokinetics, drug stability and neutralizing antibody response**

MOC3PE was not detectable in serum using the quantitative sandwich ELISA [177]. Using the more sensitive PE-assay, low concentrations of MOC3PE (median 6.5 (1.5-10) ng/ml) was detected in 6 out of the first 15 patients at dose level 3 (n=1) and 4 (n=5) at 6, 12 and 24 h. This suggests that the systemic uptake was low, in line with what other clinical studies on intraperitoneal antibody or immunotoxin treatment have shown [155, 236]. It is also in line with the favorable toxicity profile seen in the ImmunoPeCa trial. Furthermore, analysis of
peritoneal fluid samples showed that adequate MOC31PE concentrations were obtained, also at the lower dose levels. In cell based assays, peritoneal fluid samples retained cytotoxic activity at 6, 24 and 48h, indicating that the drug was active throughout the period of peritoneal exposure. This underlines the suitability of MOC31PE for intraperitoneal treatment. Finally, neutralizing anti-immunotoxin antibodies were present in all patients tested (n=15). Such antibodies are known obstacles to treatment response in multiple dose regimens for solid tumors. In the ImmunoPeCa trial, MOC31PE was given as a single dose, and neutralizing antibodies were therefore not of concern.

Promising long-term outcome

Safety was the primary endpoint for the ImmunoPeCa trial, but improved outcome is the overriding goal for this novel treatment approach for PM-CRC. Median OS was not reached and the 3-year OS was estimated to be 78% with a 34-month follow-up. Median DFS was estimated to be 21 months and the 3-year DFS was 33%, with a median follow-up of 31 months.

Comparing with results obtained in the observational cohort of 119 similar patients (paper I), the median DFS was twice as long in the ImmunoPeCa trial (21 vs 10 months) and the 3-year DFS was 33% vs 21%. Three-year OS was 78% vs 65% in favor of the ImmunoPeCa trial. The results are interesting, although not statistically significant due to the low number of patients in the clinical trial. The small study population of n=21 could prevent the detection of small or modest treatment effects, and more patients should ideally have been included to improve the power of the study for long-term outcome estimation. Unfortunately, since MOC31PE has not yet been commercialized, our stock of clinical grade drug was depleted, limiting the size of the expansion cohort.

The median PCI was relatively similar in the two studies, with 9 in the cohort study and 7 in the ImmunoPeCa trial. The ImmunoPeCa trial included four patients that theoretically could be expected to have a more favorable prognosis: three patients with appendiceal cancer (signet-ring cell carcinoma (n=1), mucinous- (n=1), and highly differentiated adenocarcinoma (n=1)), which as a group hold a better prognosis than CRC. Two of the patients with appendiceal cancer had a PCI of 0, and were treated with HIPEC due to tumor perforation. Additionally, one more patient was included, that did not have biopsy-verified PM, despite a PCI clinically estimated at surgery of 6. When excluding the four patients with a theoretically more favorable prognosis from the survival analysis, the median OS was still not reached and the 3-year OS was a considerable 72%, and the median DFS was
13 months. Although the observed survival in the ImmunoPeCa study is encouraging, the results could be influenced by favorable patient selection, and investigations in a larger cohort should be performed.

Factors that may influence intraperitoneal MOC31PE efficacy
The aim of intraperitoneal anticancer therapy is to achieve high drug concentrations in the peritoneal cavity for a prolonged period of time, to again achieve high drug concentrations in the peritoneal tumor cells or nodules, without significant systemic toxicity. Several factors could influence the efficacy of intraperitoneally administered therapies, such as peritoneal contact area, exposure time, timing of drug administration in relation to surgery, drug characteristics and concentration [110]. In HIPEC, it is common to calculate dose by BSA, and since the volume of the carrier solution often varies, drug concentrations will also vary between patients. Some argue that since the drug in intraperitoneal therapy is administered directly into the peritoneal cavity, therefore does not need to be distributed before it reaches the target organ as in systemic therapy, fixed drug concentrations should be applied instead of dosing by BSA [237]. The algorithm for dosing in the ImmunoPeCa trial was based on body weight (as in the intravenous MOC31PE trial), and the drug concentration in each dose level was constant (0.5, 1.0, 1.5 and 2.0 $\mu$g/ml for dose level 1-4, respectively).

The contact area between the therapeutic solution instilled into the peritoneal cavity and the peritoneum is important for treatment or dialysis effect and a general assumption has been that a large volume (2-3 L) is necessary to make contact with the entire peritoneum. Using CT imaging and stereologic principles, the contact area in six dialysis patients who received 2 L of intraperitoneal dialysate containing a contrast agent was shown to be 0.55 m$^2$, which is only a fraction of measurements of the peritoneum from autopsy studies (0.8 m$^2$ -2 m$^2$) [54]. Animal studies have also shown that the contact area of dialysate is considerably less that the peritoneum [238], and that maneuvers such as agitation, use of surfactant and increase in exposure time increased the fraction of the contact area [239]. A major challenge for intraperitoneal treatment is ensuring that the drug reaches all potential sites for metastasis in the peritoneum in high enough concentrations. In the ImmunoPeCa trial, agitation of the patient, use of surfactant and large instillation volumes were not an option. A considerable exposure time of 6h was chosen, based on in vitro studies, and patients were encouraged to rest on both sides to increase the contact area. In practice exposure time was more than 6h, as the drug was not instantly removed when the abdominal catheters were reopened. Treatment with MOC31PE was given on the morning of the first postoperative day, before wound
healing and adhesion formation, hoping to circumvent these potential challenges. However, the actual exposure of the peritoneal surfaces remain a challenge in intraperitoneal drug administration.

**Associations between DFS and intraperitoneal MOC31PE concentrations**

In paper II we showed that MOC31PE in peritoneal fluid increased with increasing dose levels, and that it retained cytotoxic capacity in peritoneal fluid samples, suggesting that the drug is very stable under physiological conditions. There were large inter-individual differences in the concentration of MOC31PE in peritoneal fluid samples, partly explained by the large differences in peritoneal fluid production following CRS-HIPEC. In paper III we analyzed associations between MOC31PE concentrations in peritoneal fluid samples 6 h after administration and DFS. High levels of MOC31PE in peritoneal fluid showed a non-significant trend towards improved DFS compared with low levels. After adjusting for PCI in multivariable cox regression analysis, this survival benefit was borderline significant with a HR of 0.37 (p=0.065), meaning that patients with high levels had a 63% reduction in the risk of recurrence. These results have to be interpreted cautiously, as the sample size was low in both groups, and the actual dose and dose level did not correlate with survival. However, it is in line with our hypothesis of a treatment effect of MOC31PE and we are hoping to investigate this further in future trials.
Conclusions and future perspectives

The work in this thesis focused on the current treatment for resectable PM-CRC and on the development of a potential treatment alternative for the same patient group. In paper I we reported a median OS of 47 months in patients undergoing CRS-HIPEC for PM-CRC. The median DFS was 10 months, and there was acceptable morbidity and no postoperative mortality. The OS was considerably longer than the DFS, warranting increased focus on clarifying the role of treatment regimens following disease relapse, but also on the use of perioperative systemic chemotherapy with CRS-HIPEC. The extent of peritoneal disease, expressed by the PCI, was the only factor associated with OS and DFS in multivariable analyses, where one point increase in the PCI was associated with 5% increase in the risk of death. ROC-curve analysis suggested a PCI-cutoff of 12 to identify patients with high risk of recurrence. However, even patients with a high tumor burden (PCI>20), had long OS in this study, with a median OS of 43 months, suggesting that CRS-HIPEC could also be beneficial in selected patients with a high tumor burden. The possibility of long-term survival combined with acceptable postoperative morbidity and mortality leave CRS-HIPEC as an important treatment option for eligible patients with PM-CRC.

The PRODIGE 7 trial recently questioned the benefit of adding HIPEC to CRS. Previously, CRS and HIPEC have been almost inseparable in the literature, even though many have called for randomized controlled trials investigating the contribution of the two entities separately. It is still not known whether the drug used for HIPEC is of importance and if MMC-based HIPEC would contribute differently to outcome than oxaliplatin-based HIPEC. There might be subgroups of patients that would benefit from CRS-HIPEC and others that would not. The optimal regimen for HIPEC delivery, if any, is also unknown, as is the optimal regimen for perioperative systemic chemotherapy. Hopefully, we will see an increased focus on preclinical and clinical research in the time to come, as several important questions still need answers. Ideally, all patients treated with CRS-HIPEC should be included in studies aiming to answer these or other research questions.

The ImmunoPeCa trial investigated intraperitoneal administration of MOC31PE immunotoxin for the first time. Paper II reported on the safety and toxicity, pharmacokinetics and neutralizing anti-immunotoxin antibody response in the first 15 patients. Intraperitoneal MOC31PE was safe and well tolerated without DLTs, and the high intraperitoneal drug concentrations combined with absent or low systemic drug exposure suggested that the drug was ideal for local treatment of EpCAM-expressing cancer in the peritoneal cavity. In paper
III, we reported on the long-term outcome for the ImmunoPeCa trial and safety and toxicity for the 6 patients constituting the expansion cohort. Only four study patients had died at the time of censoring, and with a 34-month median follow up, an estimated 78% 3-year OS was observed. We reported a trend towards reduction in the risk of recurrence in patients with high levels of MOC31PE in peritoneal fluid samples, compared to patients with low levels. This could suggest a relationship between drug concentration and effect, but the low number of patients included makes the study vulnerable to patient selection and results should be interpreted cautiously.

Most patients with resectable PM-CRC will not be cured, whether the treatment is CRS or CRS-HIPEC, and developing new therapy options for PM-CRC is therefore still important. In light of new insight arising from the PRODIGE 7 trial, timing is right for a careful evaluation of contemporary treatment regimens, but also for exploring novel therapeutic approaches. Targeted therapy with intraperitoneal MOC31PE immunotoxin is in our opinion an excellent candidate in this regard, exploiting direct and rapid cytotoxicity and tumor-cell selectivity. The low intraperitoneal tumor burden after CRS and the single-dose regimen can help overcome previously described obstacles to successful immunotoxin therapy. The encouraging long-term outcome and very favorable toxicity profile reported in this work support further clinical research on intraperitoneal MOC31PE.
References


98. Goere, D., et al., *Results of a randomized phase 3 study evaluating the potential benefit of a second-look surgery plus HIPEC in patients at high risk of developing colorectal peritoneal metastases (PROPHYLOCHIP- NTC01226394)*. J Clin Oncol, 2018(36 (suppl; abstr 3531)).


152. Kuruvilla, D., et al., *Efficacy and safety of moxetumomab pasudotox (moxe) in adult patients (pts) with relapsed/refractory hairy cell leukemia (HCL) in relation to drug exposure, baseline disease burden, and immunogenicity*. J Clin Oncol 2018(36 (suppl; abstr 7060)).


203. Verwaal, V.J., et al., *8-year follow-up of randomized trial: cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy in...*


205. Quenet, F., et al., A UNICANCER phase III trial of hyperthermic intra-peritoneal chemotherapy (HIPEC) for colorectal peritoneal carcinomatosis (PC): PRODIGE 7. J Clin Oncol, 2018(36 (suppl; abstract LBA 3503)).


