Peritoneal Mesothelioma and Colorectal Peritoneal Metastases

Towards Improved Outcomes

Michelle V. Dietz

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Towards improved outcomes

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Chapter 1

General introduction and outline of this thesis

PERITONEAL SURFACE MALIGNANCIES

The term 'peritoneum' originates from the Greek word 'peritonaion'. The prefix 'peri' signifies 'around', while 'teino' means to 'to stretch'. The peritoneum is a membrane, consisting of only two layers of mesothelial cells, which 'stretches' over the abdominal cavity. The outer, parietal layer covers the abdominal wall, while the inner, the so-called visceral layer, covers the intra-abdominal organs. The peritoneum is a dynamic organ with an important lubricating function, which reduces friction between intra-abdominal organs and the abdominal wall, and it regulates intraperitoneal homeostasis by the secretion and the transport of various factors. Peritoneal surface malignancies (PSM) refer to a heterogeneous group of malignancies that are present in the peritoneum. PSM can either originate primarily from the peritoneum or arise from the dissemination of other tumors.

Peritoneal Mesothelioma

The primary onset of malignancies in the peritoneum is rare. Of the primary PSM, peritoneal mesothelioma (PeM) is the most common type, with an incidence of 0.15–0.25 cases per 100 000 person-years in the Netherlands, resulting in approximately 30 new patients each year.[1] PeM accounts for roughly 10% of all mesothelioma cases, whereas the majority of cases originate from the pleura (80-90%) and a very small proportion from the pericardium or tunica vaginalis testis (1%).[1-3] Exposure to asbestos is historically considered the main risk factor for the development of mesothelioma in general.[4,5] While this link is well established for the pleural variant, it is less evident for PeM, reflected by differences in incidence rates.[6-8] Whereas the incidence of PeM has remained relatively stable over time, pleural mesothelioma had a peak incidence in 2010, and its incidence is now declining.[1,9]

Colorectal Peritoneal Metastases

Unlike primary PSM, peritoneal metastases (PM) originating from other primary tumors are far more common. One of the most prevalent causes of PM is colorectal carcinoma (CRC).[10-12] In the Netherlands, about 12.000 patients present with CRC annually, of whom approximately 10% develop PM.[13-16] Of these patients, half present with PM at the time of diagnosis or treatment of the primary tumor (synchronous onset), while the other half is diagnosed with PM in the follow-up period after the initial treatment, known as metachronous onset. The majority of patients with colorectal PM also present with concurrent metastases at other sites, but 2-6% of the patients have isolated PM.[16,17]

A rare, but noteworthy underlying cause of PM is pseudomyxoma peritonei (PMP), which is characterized by peritoneal mucinous tumor deposits and mucinous ascites. The vast majority of PMP cases originate from appendiceal mucinous tumors, although other primary tumor locations have been described.[18]

TREATMENT STRATEGIES

Although PSM comprise a heterogeneous group of malignancies, they are generally associated with a poor prognosis. Most patients with PSM present with non-specific symptoms, like abdominal distension and pain. Combined with the limited ability of standard imaging techniques to visualize small peritoneal nodules, PSM are often diagnosed in an advanced stage, reflected by extensive local disease. Together with the aggressive character of most PSM, the prognosis and quality of life of these patients is generally poor. For both patients with PeM and colorectal PM that do not undergo anti-tumor treatment, the prognosis is often limited to less than three months.[14,19] Luckily, ongoing advances in systemic, as well as, local therapeutic options have resulted in the improvement of survival outcomes.

Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy

Both patients with PeM and colorectal PM have been limited to palliative interventions for a considerable time. The introduction of cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has resulted in a potentially curative treatment option for a select group of patients. The primary aim of CRS-HIPEC is to remove all macroscopic tumor tissue from the peritoneal cavity (CRS) and to eradicate any residual microscopic disease using HIPEC. This extensive surgical treatment was first described in 1995 by Sugarbaker and has become the cornerstone of treatment for patients with PSM.[20,21]

For patients with PeM, promising survival outcomes of patients undergoing CRS-HIPEC have been first reported early this century, with a median overall survival (mOS) of 33 months.[22] Although the benefit of CRS-HIPEC for these patients has never been investigated in a randomized controlled trial (RCT), results of retrospective studies indicate a considerable improvement in survival outcomes.[19,23-26] For patients with colorectal PM, an RCT by Verwaal et al. in 2003 reported improved survival in patients who underwent CRS-HIPEC (mOS 22 months) compared with systemic chemotherapy (mOS 13 months).[27,28] These survival outcomes have improved since, with reported mOS of 41 months for patients with colorectal PM in the most recent RCT (PRODIGE 7).[29] Despite these promising survival outcomes, this

trial also questioned the benefit of HIPEC, as the authors reported no difference in mOS between patients who underwent CRS alone and patients who underwent CRS with HIPEC. It should be noted that this trial included a very selected patient population but it illustrates that the addition of HIPEC might not be beneficial for all patients.

Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy – Patient selection

To avoid ineffective interventions that are accompanied by the risk of considerable morbidity, it is important to select those patients who are most likely to gain benefit from both CRS and HIPEC. For both patients and physicians, it is crucial to accurately assess the utility prior to surgery. An important element that reflects the efficacy of CRS-HIPEC is the completeness of the cytoreduction, assessed by the completeness of cytoreduction (CC) score.[24,30-32] Incomplete cytoreduction (CC-2/3) is associated with a worse prognosis, underlining the importance of the selection of patients in whom a complete cytoreduction is feasible.

This feasibility strongly depends on the extent of local disease. The extent of local disease can be assessed by the Peritoneal Cancer Index (PCI), calculated by dividing the abdomen into thirteen regions.[33] Each region gets a score from zero to three, resulting in a range from zero to 39. A higher PCI, reflecting more extensive disease, is associated with poor outcomes after CRS-HIPEC.[31,32,34] For patients with colorectal PM, the Dutch guideline considers a PCI of 20 or above an absolute contra-indication, as the survival benefit for these patients seems limited.[35,36] However, the optimal cut-off value for PCI remains under discussion.[37,38] For patients with PeM, current guidelines propose to combine the PCI score with the Ki-67 index.[39] A high Ki-67 index reflects a higher rate of proliferating cancer cells and consequently a more aggressive character.[40,41] Only patients with a Ki-67 of 9% or below, irrespective of the PCI, or a PCI of 17 or below, irrespective of Ki-67, are considered eligible for CRS-HIPEC.

For both PeM and colorectal PM, the histological subtype is also of prognostic relevance. PeM can be classified into three distinct subtypes: epithelial (90%), sarcomatoid (5%), and biphasic (or mixed; 5%). Due to its aggressive character, the sarcomatoid subtype is considered an absolute contra-indication for CRS-HIPEC.[7,42] For patients with colorectal PM, signet ring cell histology is associated with a poor prognosis, yet is only considered a relative contra-indication.[43]

Lastly, as CRS-HIPEC involves local treatment, patients with extra-peritoneal disease (systemic or retroperitoneal lymph node metastases) are generally considered ineligible. However, since patients with colorectal PM generally respond less to systemic therapy compared with patients with systemic metastases, CRS-HIPEC could confer a survival benefit.[17] Hence, CRS-HIPEC is considered in selected patients with colorectal PM and limited systemic metastases (i.e. ≤3 liver or lung metastases).[44-46]

Despite the current strict patient selection, not all patients seem to gain survival benefit from CRS-HIPEC. Even in patients in whom a complete cytoreduction is obtained, recurrence rates remain high.[14,23,34,47,48] One strategy to improve these outcomes is to optimize patient selection. The identification of preoperative defined prognostic factors, could aid in this optimization. Examples of factors that have been associated with outcomes after surgery in patients with colorectal PM are skeletal muscle mass and the time of onset of PM.[14,49,50] However, the value of these factors in patient selection remains unclear.

Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy – Perioperative treatment strategies

Another strategy to improve outcomes after CRS-HIPEC is the addition of perioperative systemic chemotherapy. In the aforementioned PRODIGE 7 trial, patients with colorectal PM were treated with both neo-adjuvant and adjuvant chemotherapy.[29] Although perioperative chemotherapy is not included in the current Dutch guideline, its potential value is under discussion.

For patients with PeM, perioperative systemic chemotherapy does not seem to result in any survival benefit.[23,51] As immunotherapy is emerging as a promising primary treatment option for various malignancies, there is also a growing interest in exploring its perioperative use to enhance surgical outcomes. Dendritic cell-based immunotherapy (DCBI) is a form of immunotherapy that has shown promising results in patients with pleural mesothelioma.[52,53] DCBI, in the form of MesoPher, uses autologous dendritic cells (DCs) pulsed with a human allogeneic tumor cell lysate, generated from five clinical-grade mesothelioma cell lines. These DCs present tumor-associated antigens (TAAs) to the immune system, aiming to generate a tumor-specific immune response. In PeM murine models, DCBI has proven to be more effective in mice with low tumor load.[54] This provided a rationale for combination with cytoreductive surgery.[55] Together with the limited toxicity, this could be a very promising strategy to reduce the risk of recurrence after CRS-HIPEC in patients with PeM.

Systemic therapy

Due to the aforementioned patient selection, most patients with PeM and colorectal PM are not eligible for CRS-HIPEC.[14,19,56] For these patients prognosis is often poor, though advances have also been made in the palliative treatment. Especially for patients with colorectal PM, the introduction of new systemic chemotherapeutic regimens resulted in better survival outcomes.[57,58] The promising results of immunotherapy are less emphasized for patients with CRC in comparison to other solid tumors. However, for patients with microsatellite instable (MSI) tumors, immunotherapy with checkpoint inhibitors (CPI) seems to be effective and is currently the first-line treatment option.[59,60] Some studies have, however, indicated that the efficacy for PM is limited compared with other metastatic sites.[61,62]

For patients with PeM who are ineligible for surgery, first-line treatment consists of systemic platinum-based chemotherapy combined with pemetrexed. This regimen is based on studies in patients with pleural mesothelioma but the evidence for its efficacy in patients with PeM is scarce. Small retrospective studies reported overall response rates limited to 20-25% and a two-year survival rate of only 20%, accompanied by high morbidity rates.[63,64] This is probably an important reason why most patients with PeM (63%) in the Netherlands do not receive anti-tumor treatment.[19] Based on studies in patients with pleural mesothelioma, CPI could also be a new treatment option for these patients.[65] However, morbidity rates are comparable to those of chemotherapy and its benefit has not yet been proven for PeM.[59,66]

Targeted therapy

Another rapidly evolving type of cancer treatment is targeted therapy. Targeted drugs possess major advantages compared with other systemic drugs due to their capacity to selectively target cancer cells. For patients with metastatic CRC, the addition of anti-VEGF (mainly bevacizumab) and anti-EGFR agents (cetuximab and panitumumab) to the chemotherapeutic regimen improves survival rates.[67,68] In addition, patients with BRAFV600-mutated CRC gain survival benefit from combination therapy with cetuximab and a BRAF inhibitor (encorafenib).[69]

For patients with PeM, the use of targeted therapies is limited. Due to its rarity, knowledge of the mutational landscape has long been limited. Recently, several studies have been published that reported on the molecular aberrations in PeM, providing more insights into potential targets for therapy.[70-73] However, another challenge in the implementation of targeted therapies is the conduct of clinical trials in a rare malignancy like PeM. Currently, several clinical trials are including patients based on tumor molecular characteristics, rather than cancer type or

location to facilitate the use of available drugs for the treatment of rare malignancies.[74-76] With these developments, it is important to gain more insights in the value of genomic characterization in PeM and to identify potentially effective targeted therapies.

Intraperitoneal chemotherapy

As both patients with colorectal PM and those with PeM seem to gain limited benefit from systemic therapy, local treatment could be a more effective strategy. This local treatment in the form of intraperitoneal (IP) chemotherapy can be delivered through an IP port-a-cath (PAC). A recent phase I dose-escalation trial, the INTERACT trial, treated patients with colorectal PM who were not eligible for surgery with intraperitoneal chemotherapy concomitant with standard systemic therapy.[77] This trial showed that this treatment was safe and well tolerated, with promising responses in some patients. Especially for PeM, which is less likely to disseminate systemically, local treatment might potentially have major advantages over systemic treatment and could provide a palliative treatment option.

AIM AND OUTLINE OF THIS THESIS

Although advances in the treatment of patients with PeM and colorectal PM have resulted in modest improvements in survival outcomes, the overall prognosis remains poor. Hence, there is an ongoing need for the improvement of the treatment of these patients. The aim of this thesis is to improve survival outcomes for both patients with PeM and colorectal PM by optimizing current treatment strategies and identifying possible new treatment strategies. In the first part of this thesis, we focus on the treatment of patients with PeM, whereas the second part addresses the optimization of patient selection for CRS-HIPEC in patients with colorectal PM.

Part I

As the recurrence rates for patients with PeM after CRS-HIPEC are high, there is a need for the improvement of perioperative treatment strategies. In **chapter 2**, we investigated the feasibility of a combination strategy of CRS-HIPEC and adjuvant immunotherapy in the form of DCBI to reduce the risk of recurrence. **Chapter 3** describes a case of a patient with pleural mesothelioma who received (neo-) adjuvant DCBI to surgery; in which we aimed to assess the intratumoral immune response to DCBI.

In the following chapters, we explored several new treatment strategies for patients with PeM who are not eligible for surgery, as their treatment options are limited. In **chapter 4,** we present the protocol of the INTERACT MESO trial, a dose escalation and safety study that investigates intraperitoneal chemotherapy in patients with PeM who are deemed ineligible for CRS-HIPEC. In **chapters 5** and **6,** we aimed to explore the genomic signature of PeM and its potential therapeutic targets, to identify possible targeted therapies.

Part II

An approach to improve outcomes for patients with colorectal PM undergoing CRS-HIPEC is the refinement of patient selection by optimizing the use of prognostic factors. In **chapters 7** and **8,** we explored prognostic factors that could be used in patient selection. In **chapter 9,** we present a retrospective cohort study of patients who were deemed ineligible for CRS-HIPEC, to provide insight into the patients' characteristics, reasons for ineligibility, and survival outcomes. Another approach to improve outcomes after CRS-HIPEC is perioperative treatment strategies. In **chapter 10,** we aimed to develop a prediction model to predict the risk of recurrence after CRS-HIPEC in patients with colorectal PM as these patients couldbenefit from perioperative systemic therapy.

In **chapter 11,** we provide a summary of the results of the aforementioned chapters. In **chapter 12,** we discuss these findings and provide future perspectives.

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Part I

Peritoneal Mesothelioma

Chapter 2

Adjuvant Dendritic Cell-Based Immunotherapy after Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy in Patients with Malignant Peritoneal Mesothelioma: a phase II clinical trial

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ABSTRACT

Background

Malignant peritoneal mesothelioma (MPM) is an aggressive malignancy with a poor prognosis. Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) improves survival outcomes, but recurrence rates remain high. Dendritic cell-based immunotherapy (DCBI) showed promising results in patients with pleural mesothelioma. The primary aim of this trial was to determine feasibility of adjuvant DCBI after CRS-HIPEC.

Methods

This open-label, single-center, phase II clinical trial, performed in the Erasmus MC Cancer Institute Rotterdam, the Netherlands, included patients with epithelioid MPM. Four to six weeks before CRS-HIPEC leukapheresis was performed. Eight to ten weeks after surgery, DCBI was administered three times biweekly. Feasibility was defined as administration of at least three adjuvant vaccinations in 75% of patients. Comprehensive immune cell profiling was performed on peripheral blood samples prior to and during treatment.

Results

All patients that received CRS-HIPEC (n=16) were successfully treated with adjuvant DCBI. No severe toxicity related to DCBI was observed. Median progression-free survival (PFS) was 12 months [IQR 5 – 23] and median overall survival was not reached. DCBI was associated with increased proliferation of circulating NK-cells and CD4+ T-helper (Th) cells. Co-stimulatory molecules, including ICOS, HLA-DR, and CD28 were upregulated predominantly on memory or proliferating Th-cells and minimally on CD8+ cytotoxic T-lymphocytes (CTLs) after treatment. However, an increase in CD8+ terminally differentiated effector memory (Temra) cells positively correlated with PFS, whereas co-expression of ICOS and Ki67 on CTLs trended towards a positive correlation.

Conclusion

Adjuvant DCBI after CRS-HIPEC in MPM patients was feasible and safe, and showed promising survival outcomes**.** DCBI had an immune modulatory effect on lymphoid cells and induced memory T-cell activation. Moreover, an increase of CD8+ Temra-cells was more pronounced in patients with longer PFS. These data provide rationale for future combination treatment strategies.

INTRODUCTION

Malignant Peritoneal Mesothelioma (MPM) is an aggressive malignancy. Due to its non-specific symptoms, such as abdominal pain, weight loss, and abdominal distension, it is often diagnosed at an advanced stage. The combination of late diagnosis and aggressive biology results in a poor prognosis. Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) can improve the prognosis for selected patients, resulting in a median overall survival ranging from 19 to 92 months.[1-4] Nonetheless, even after complete cytoreduction, recurrence rates are high. Perioperative systemic chemotherapy has shown no survival benefit for patients with MPM.[1, 5, 6] Therefore, there is a need for effective perioperative treatments, which can prevent or delay recurrence, and ultimately improve overall survival (OS) after CRS-HIPEC.

Dendritic cell-based immunotherapy (DCBI) in the form of 'MesoPher' has the potential to induce long-term specific anti-tumor immunity. MesoPher uses autologous monocyte derived dendritic cells, loaded with an allogeneic lysate obtained from mesothelioma cell lines (PheraLys).[7] In murine models with MPM and in clinical phase I-II studies for patients with pleural mesothelioma, MesoPher was well tolerated and induced durable responses with promising survival rates.[7-10]

Earlier murine models have shown that DCBI is more effective in mice with a small tumor load, providing a rationale for DCBI as an adjuvant treatment.[8] The aim of the current trial is to determine feasibility of administering adjuvant DCBI after CRS-HIPEC for MPM. Secondary objectives are to assess safety and systemic immune phenotyping over the course of DCBI.

METHODS

Study design

The MESOPEC trial was an open-label, single arm, single center phase II clinical trial, conducted in the Erasmus MC Cancer Institute, Rotterdam, the Netherlands. The MESOPEC study protocol, as well as a detailed description of MesoPher production, have been published earlier.[7, 11] This study was approved by the Central Committee on Research Involving Human Subjects (NL60856.000.17) and conducted in accordance with the Declaration of Helsinki.

Eligibility criteria

Patients diagnosed with epithelioid malignant peritoneal mesothelioma (MPM) and an indication for CRS-HIPEC, were screened to participate in the study. Patients undergoing palliative resections with HIPEC, in case of symptomatic tumor lesions and/or ascites, were also eligible to participate in the trial. Eligibility for CRS-HIPEC was based on multiple factors. Patients had to have a WHO-ECOG performance status of 0 or 1, the disease had to be confined to the abdominal cavity, and the expected survival had to be at least 6 months. Peritoneal Cancer Index (PCI) above 17 was considered a contra-indication for CRS-HIPEC, when the Ki67 index was higher than nine percent. When the Ki67 index was below ten percent, PCI was not considered in evaluating CRS-HIPEC eligibility. If feasible, PCI and feasibility of cytoreduction were determined up front by diagnostic laparoscopy (DLS).

Study procedures

A timeline of study procedures is given in **figure 1**. Four to six weeks prior to CRS-HIPEC, a leukapheresis procedure was performed to obtain autologous monocytes

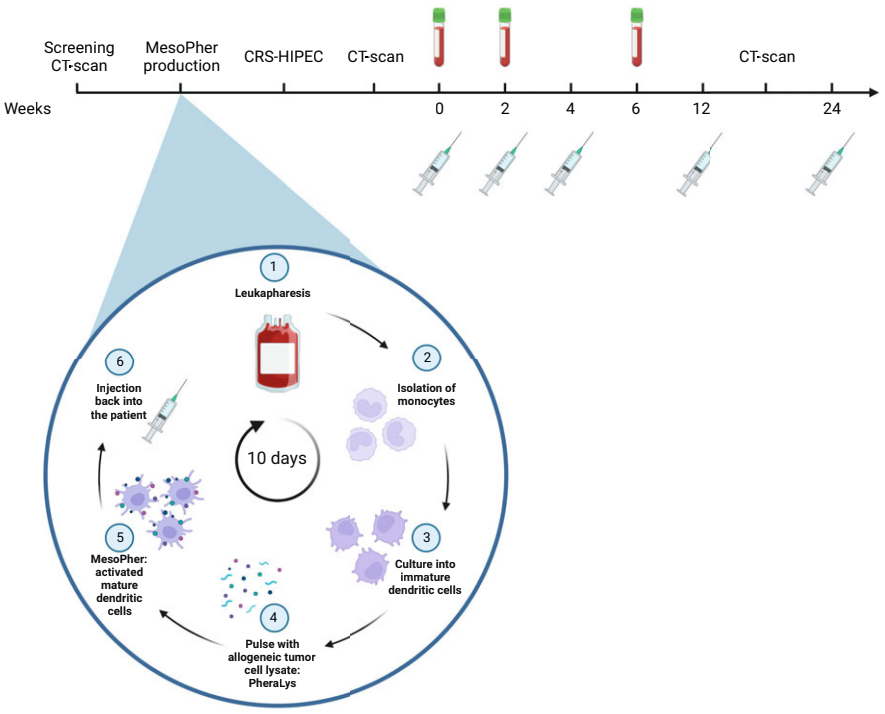

Figure 1. Illustration of the treatment regimen of malignant peritoneal mesothelioma patients treated with MesoPher dendritic cell vaccination (indicated by the syringes) after cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). Blood was drawn before every vaccination.

for DC vaccination production (a detailed description of the production of DC vaccination is provided in the **data supplement**). CRS-HIPEC was performed following standard of care. Eight to ten weeks after surgery, patients received DCBI in three biweekly vaccinations at the outpatient clinic, followed by a booster after 3 and 6 months. One-third of the MesoPher dose was injected intradermal (i.d.) and two-thirds were administered intravenous (i.v.). Prior to every vaccination, peripheral blood samples cells were obtained.

Safety evaluation

Safety and tolerability were assessed in terms of adverse events (AEs), physical examination (including vital signs), and laboratory testing (i.e., hematologic and biochemistry assessments), performed at each study visit. Toxicity related to DC vaccination was scored according to the CTCAE version 5.0 and reported for the first three vaccinations. All serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) related to the DC vaccination were monitored and reported.

Objectives

The primary objective was to determine feasibility of DCBI treatment after CRS-HIPEC in MPM patients. DCBI after CRS-HIPEC was deemed feasible when at least 75% of patients were able to receive the first three vaccinations after CRS-HIPEC. Secondary objectives were to assess safety of DCBI therapy after CRS-HIPEC and systemic immune phenotyping over the course of MesoPher treatment.

Surgical outcomes

PCI, intra operative blood loss, duration of surgery, completeness of cytoreduction, and characteristics regarding resections were registered. The completeness of cytoreduction score (CC-score) was used to characterize completeness of cytoreduction. A CC-score of 0 represents no macroscopic residual disease, CC-1 represents 0-2.5 mm of residual macroscopic disease, CC-2 represents 2.5-25 mm of residual macroscopic disease, and CC-3 represents more than 25 mm of residual macroscopic disease. Postoperative complications were defined by use of the Clavien Dindo (CD) classification: CD grade 1 denoting any deviation from the normal postoperative course without the need for an intervention; CD grade 2 denoting a complication requiring pharmacological treatment (including parenteral nutrition or blood transfusion); CD grade 3 denoting complications requiring surgical, endoscopic or radiological intervention not under general anesthesia (3a) or under general anesthesia (3b); CD grade 4 denoting life threatening complications requiring intermediate or intensive care unit management due to single

organ dysfunction (4a) or multi organ dysfunction (4b); and CD grade 5 denoting any complication resulting in the death of a patient.

Follow-up and clinical response evaluation

Further treatment and follow-up were performed according to standard protocol. Approximately six weeks after CRS-HIPEC, a CT-scan was performed to act as a baseline measurement. After this, CT-scans were made every six months during the first three years of follow-up. In postoperative years four and five, CT-scans were performed once a year. Recurrence of disease was defined as measurable disease on imaging or as recurrent disease found by laparotomy or laparoscopy. Progression free survival (PFS) was defined as the time interval between CRS-HIPEC and recurrence of disease for patients with a complete cytoreduction or progression of disease for patients with an incomplete cytoreduction. Patients who had no progression at the time of database lock were censored at the date of the last follow-up visit. OS was defined as the time interval between CRS-HIPEC and the date of death, or date of last follow-up visit in censored cases.

Immune cell profiling

Flow cytometry staining was performed on cryopreserved peripheral blood mononuclear cells (PBMCs) at baseline (before start of vaccination) and on treatment time points (two weeks after the first vaccination and two weeks after the third vaccination, **figure 1**). Three panels were designed to characterize the T-cells (co-stimulatory, co-inhibitory, cytokine) and one panel for the DC-myeloid fraction. An automated, computational pipeline, based on the one described by Quintelier et al. was developed to analyze the data (**figure 2A**).[12] First, margin events were removed, compensation, transformation, quality control, manual pre-gating and normalization were performed. Next, FlowSOM was used to cluster the data of the T-cell panels and the data of the DC-myeloid panel based on the cell type markers.[13] These clusterings were evaluated by mapping a manual gating of a subset of the data (**supplementary figure 1**) and as a result, cell subtype abundances were obtained. Additionally, thresholds were computed for all cell state markers to obtain the complete immune profiles. Detailed descriptions of the flow cytometry and computational analysis are provided in the **data supplement**.

Statistics

The sample size was calculated by: , assuming a sensitivity of diagnosing grade 3 toxicity of 99% and a prevalence of grade 3 toxicity in the study population of 2.5%. Total width of the confidence interval is 0.20 (0.10 below and 0.10 above). Confidence level of the interval is 95% and $\alpha = 0.05$. The sample size that was

necessary at least to obtain 95% confidence intervals with a width of 20% for a prevalence of 2.5% was 19 patients. Rounded, this is a total of 20 patients. Feasibility was defined as administration of at least three adjuvant vaccinations in 75% of patients (i.e., 15 patients). Kaplan-Meier survival analysis was used to estimate the median progression-free survival (PFS) and overall survival (OS). Wilcoxon signed rank tests (non-parametric, paired data) and Student's t test (parametric, paired data) were used to determine the statistical significance. Statistical analyses were executed using Graphpad Prism software (Graphpad Software Inc., San Diego, CA, USA). Continuous variables were shown as median with the range of values or interquartile range (IQR). Categorical variables were presented as counts with percentages. P-values of 0.05 and below were considered statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics are provided in **table 1** and an overview of the included patients is provided in **supplementary figure 2**. A total of 18 patients were included in the MESOPEC trial between March 2018 and September 2022. Most patients were male (78%) with a median age of 59 [range 30 – 75]. Two out of 18 patients had a known history of asbestos exposure, and two patients were carriers of a BAP1 germline mutation. Two patients dropped out of the study before CRS-HIPEC. One patient experienced progression of a secondary malignancy, thereby losing the indication to undergo CRS-HIPEC. Another patient had a rapid detoriation of performance status, making CRS-HIPEC no longer feasible. Three patients were included after CRS-HIPEC was performed (respectively 28, 5, and 7 weeks after CRS-HIPEC).

Perioperative characteristics

Table 1 provides the perioperative characteristics for patients that underwent CRS-HIPEC (n=16). In 14 patients, the HIPEC regimen consisted of cisplatin and doxorubicin. In two patients, Mitomycin-C (MMC) was used. For one patient this was because of induction therapy with carboplatin and possible resistance to cisplatin HIPEC. For the other patient MMC was used to minimize the risk of complications due to a perioperative expectancy of incomplete cytoreduction. In ten patients complete cytoreduction was performed, resulting in a completeness of cytoreduction (CC-) score of 0 (n=7) or 1 (n=3). In six patients, complete cytoreduction was not feasible and palliative resections (CC-score of 3) and HIPEC were performed. Most common complications after CRS-HIPEC were pneumonia (n=4,

25%) and chylous leakage (n=5, 44%). Severe complications (i.e., Clavien Dindo grade 3b) were reported in two patients (12.5%). One patient was diagnosed with an ileus and intra-abdominal hematoma, which was surgically evacuated. Exploratory laparotomy was performed in another patient, resulting in the diagnosis of jejunitis without additional resections. A detailed description of organ resections during cytoreduction and postoperative complications is provided in **supplementary table 1**.

Table 1. Baseline and perioperative characteristics.

	n=18
Age at inclusion in trial (range)	59 [30 – 75]
Gender	14 (77.8)
Male	4 (22.2)
Female	
History of asbestos exposure	2 (11.1)
Yes	11 (61.1)
No	5 (27.8)
Unknown [a]	
Epithelioid morphology	18 (100)
Ki67 index (range) [b]	8 [1 – 70]
Germline BAP1 mutation [c]	2 (11.1)
Prior therapy	5 (27.8)
Systemic chemotherapy [d]	1 (5.6)
Systemic immunotherapy	1 (5.6)
PIPAC	3 (16.7)
Prior surgery [e]	
CRS-HIPEC	16 (89)
PCI (range) [f]	39 [19 – 39]
Chemotherapy regimen [f]	14 (87.5)
Cisplatin/doxorubicin	2 (12.5)
Mitomycin-C	
CRS-HIPEC duration (minutes, range) [g]	494 [194 – 679]
Blood loss (liters, range) [h]	1.5 [0.2 – 5.4]
Perioperative bloodtransfusion [f i]	2 (12.5)
Organ resections (range) [f]	4 [0 – 9]
Completeness of cytoreduction [f]	7 (43.8)
CC-0	3 (18.8)
CC-1	0 (0)
CC-2	6 (37.5)
CC-3	

Table 1. Baseline and perioperative characteristics. (continued)

	n=18
In hospital length of stay [f]	16 [10 – 16]
Any postoperative complication [f]	13 (81.3)
Severe postoperative complications [f]	3 (18.8)
Ileus [j k]	1 (6.3)
Intra-abdominal hematoma [j k]	1 (6.3)
Other infection [j]	1 (6.3)
Malposition JJ-stent [l]	1 (6.3)
Reoperation [f m]	2 (12.5)

Continuous variables are shown as median [IQR] unless otherwise specified. Frequencies are shown as N (%)
IQR= interquartile range, CRS= cytoreductive surgery, HIPEC= hyperthermic intraperitoneal Chemotherapy, PIPAC= pressurized intraperitoneal aerosolized chemotherapy, PCI= peritoneal cancer index, CC= completeness of cytoreduction score, CD= Clavien-Dindo
[a] Reported as unknown by the patient
[b] Available for 17 out of 18 patients, for patients that received induction chemotherapy (n=3) Ki67 before chemotherapy was available for 2 patients
[c] Germline mutational analysis performed in three out of 8 patients with a BAP1 deficiency
[d] All patients received a combination of pemetrexed and a platinum based chemotherapeutic agent
[e] Surgery with resections for peritoneal disease
[f] Out of 16 patients that underwent CRS-HIPEC
[g] Data available for 12 patients
[h] Data available for 13 patients
[i] Both patients underwent leukapheresis before CRS-HIPEC
[j] Clavien Dindo grade 3b
[k] These complications were present in the same patient
[l] Clavien Dindo grade 3a
[m] Evacuation of an intra-abdominal hematoma (n=1) and exploratory laparotomy resulting in the diagnosis jejunitis (n=1)

Feasibility

Feasibility was determined based on the proportion of patients who were able to undergo leukapheresis with successful production of MesoPher and who received the first three adjuvant vaccinations. Four out of 16 patients underwent leukapheresis after CRS-HIPEC. For three patients this was because of inclusion in the trial after CRS-HIPEC was performed in another hospital. For one patient the leukapheresis was postponed due to pancytopenia after induction chemotherapy prior to CRS-HIPEC. The median time from leukapheresis to CRS-HIPEC was 4 weeks [2 – 5] for patients undergoing leukapheresis before CRS-HIPEC (**table 2**). In none of the patients serious adverse events (SAEs) or delay to CRS-HIPEC due to leukapheresis was reported. One patient underwent a second leukapheresis procedure after the administration of the first three vaccinations, as the yield of monocytes from the first procedure was not sufficient to produce all five DC vaccinations. All patients were sufficiently recovered to undergo DC-therapy within ten weeks after surgery and were able to undergo the first three DC treatments according to protocol. Five

Table 2. Leukapheresis and treatment with DC vaccination.

	n=16	Highest CTCAE
Interval leukapheresis to CRS-HIPEC [a] (weeks)	4 [2 – 5]	
Interval CRS-HIPEC to start DC vaccination [a] (weeks)	9 [8 – 11]	
Number of vaccinations (range)	5 [3 – 5]	
Any AE [b]	16 (100)	2
Injection site reaction	16 (100)	1
Cold chills	10 (63)	2
Fever	9 (56)	2
Fatigue	8 (50)	1
Malaise	6 (38)	1
Arthralgia	5 (31)	1
Myalgia	4 (25)	1
Headache	4 (25)	1
Nausea	3 (19)	1
Vomiting	2 (13)	1
Dizziness	1 (6)	1
Abdominal pain	1 (6)	1
SAE	0 (0)	n/a
SUSAR	0 (0)	n/a

Continuous variables are shown as median [IQR] unless otherwise specified. Frequencies are shown as N (%)
AE= adverse event, CRS= cytoreductive surgery, CTCAE= Common Terminology Criteria for Adverse Events, DC= dendritic cell, HIPEC= hyperthermic intraperitoneal chemotherapy, IQR= interquartile range, SAE= serious adverse event, SUSAR= suspected unexpected serious adverse reaction
[a] For patients (n=12) undergoing leukapheresis before CRS-HIPEC according to protocol
[b] Adverse events reported that were probably related to the first three DC vaccinations

patients (31.3%) showed progressive disease at first response evaluation and did therefore not receive all five DC vaccinations.

Safety of DC treatment

Safety was assessed in terms of adverse events (AEs) and suspected unexpected serious adverse reactions (SUSARs) based on the first three DC vaccinations. Sixteen patients received three vaccinations before the adverse events database lock (January 18th, 2023; **table 2**). None of the patients experienced a SAE or SUSAR that was related to the DC vaccination. Injection-site reactions (i.e., erythema, induration, itching, and pain) and infusion-related reactions (IRR) were reported at least once in all patients. The most reported IRRs were cold chills (63%), fever (56%), fatigue (50%), and malaise (38%). No AEs higher than CTCAE grade 2 related to the DC vaccination were reported.

Clinical outcomes

Clinical outcomes of all patients that underwent CRS-HIPEC and adjuvant DCBI (n=16) are shown in **figure 3** and a detailed description is provided by **supplementary table 2**. Median follow-up time after CRS-HIPEC was 26 months [IQR 16 – 35] for surviving patients (**figure 3**). Two patients did not complete the study treatment before the survival database lock (May 1st, 2023). Median PFS was 12 months [IQR 5 – 23] for all patients. Six out of 16 patients were deceased at time of the database lock, therefore median OS could not be determined. For patients with a complete cytoreduction (n=10), six patients had recurrence of disease with a median PFS of 20 months [IQR 8 – not reached], of whom two deceased. Five out of six patients with an incomplete cytoreduction had progression of disease with a median PFS of 4 months [IQR 4 – 16]. Four of these patients deceased, resulting in a median OS of 19 months [IQR 7 – 33]. Seven patients received palliative treatment after progression of whom five received treatment with a PD-1 checkpoint-inhibitor. One of these patients received the fourth and fifth DC vaccination during this treatment.

Vaccine-induced proliferation of (memory) T-helper cells and natural killer cells

Immune cell profiling was performed for 14 out of the 16 patients that were treated with DCBI. The FlowSOM clustering algorithm identified distinct lymphocyte subsets, i.e., natural killer (NK) cells, natural killer T (NKT) cells and T-cells, CD4+ (naive) regulatory T-cells and CD4+ T-helper (Th) cells and CD8+ T-lymphocytes (CTL), as well as naive and memory T-cell clusters (**figure 2A-C**). The treatment did not result in changes in the proportions of lymphocytes (data not shown). DCBI, however, did significantly increase the proportions of Ki67+ proliferating NK-cells and Th-cells (**figure 2D**). When investigating the abundance of different T-cell subsets, there was no difference upon treatment within the CD8+ T-cell compartment (**figure 2E**). Yet, the relative proportion of memory Th-cells (effector memory (Tem) and central memory (Tcm) cells) seemed to increase upon therapy, whereas naïve Th (Tn) cells were less abundant after three vaccinations (**figure 2E**). This was clarified by the increase in the percentage of Ki67+ memory Th-cells, which was not seen for Tn cells (**figure 2F**).

Phenotypic changes in CD4+ T-helper and CD8+ T-cells upon DC vaccination

In addition to lymphocyte proliferation, the expression of a variety of co-stimulatory and -inhibitory receptors on peripheral T-cells was assessed (**figure 4A**). Co-stimulatory molecules, including ICOS, HLA-DR and CD28 were significantly upregulated on Th-cells, specifically on memory or proliferating cells. This upregulation was most dominant after one vaccination in the total Th population but remained present two weeks after the third vaccination on the memory and proliferating cells.

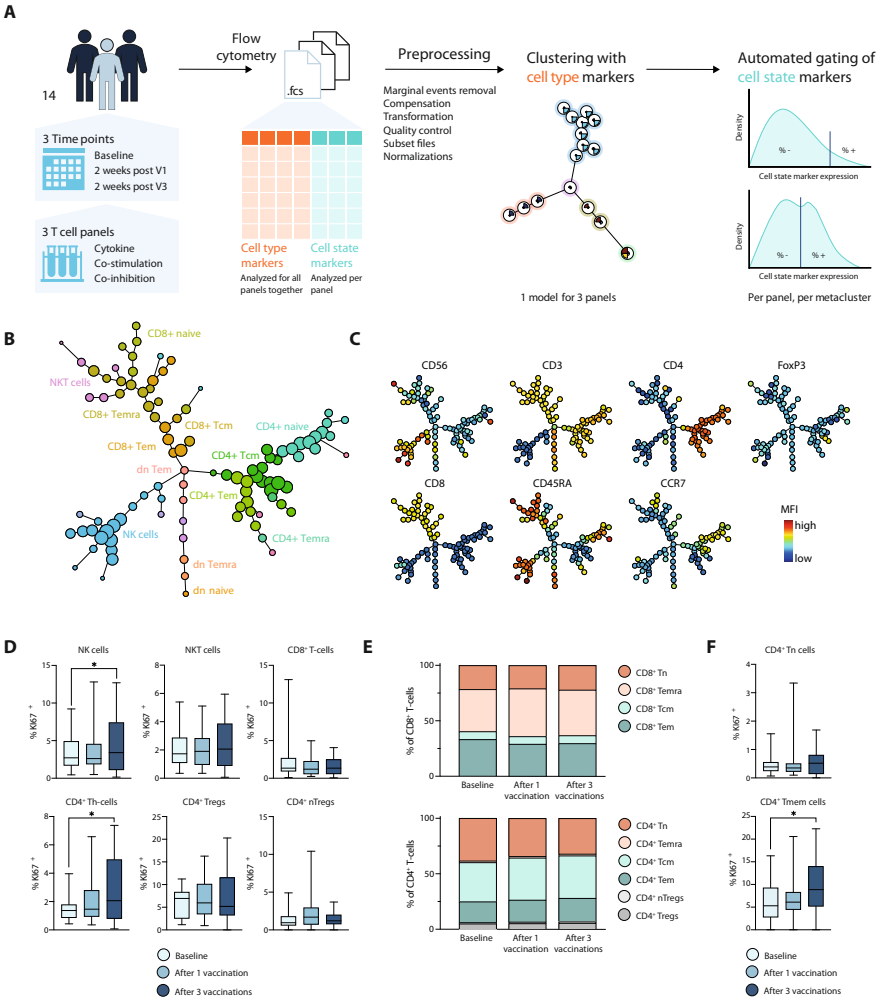

Figure 2. Treatment induced changes in the abundance and proliferation of CD4+ Th-cells. A: Pipeline for the FlowSOM unsupervised clustering analysis of the T-cell panels. B-C: Identification of different lymphocyte clusters (B) by cell type marker expression (C) from the FlowSOM algorithm. D: Percentage of Ki67+ NK-cells, NKT-cells, T-cells, CD8+ T-cells, CD4+ Th-cells and CD4+ (n)Tregs. E: Relative abundance of T-cell subsets within CD8+ T-cells (upper) or CD4+ T-cells (lower) before vaccination and after 1 or 3 vaccination(s). F: Percentage of Ki67+ CD4+ Tn-cells (upper) and CD4+ Tmem-cells (Tem + Tcm; lower). Th, T-helper cells; Tregs, regulatory T-cells; nTregs, naive regulatory T-cells; Tn, naive T-cells; Temra, terminally differentiated effector memory T-cells; Tcm, central memory T-cells; Tem, effector memory T-cells. *p<0.05.

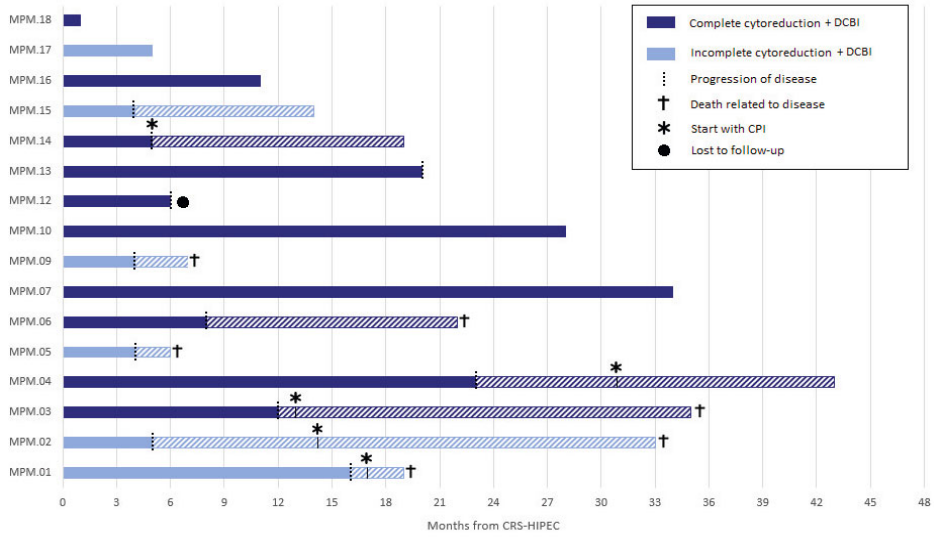

Figure 3. Progression-free (PFS) and overall survival (OS) for MPM patients treated with adjuvant dendritic cell-based immunotherapy (DCBI) after cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (CRS-HIPEC). The filled bars represent PFS and OS of patients since date of CRS-HIPEC. Time of progression is represented by the dotted vertical lines. Patients that deceased are depicted with a cross symbol. Patients treated with checkpoint inhibitors (CPI) are depicted with an asterisk symbol at time of start with treatment. Patients that were lost to follow-up are depicted with a black circle.

In addition, the expression of co-inhibitory molecules such as CD39 and LAG-3 changed with similar dynamics on Th-cells.

Expression of co-stimulatory and co-inhibitory markers appeared to a lesser extent on CTLs but were seen on Tem cells or proliferating CTLs (**figure 4A**). Along with limited CTL activation, treatment-induced changes in cytokine expression were lacking, except for TNF-α-producing Tem cells and IFN-γ+ terminally differentiated effector memory T (Temra) cells (**figure 4B**). However, when DCBI led to an increase in the proportion of Temra-cells after 1 vaccination (**figure 4C**), it positively correlated with PFS. Likewise, an increase in proliferating ICOS+ CTLs upon 3 vaccinations trended towards a positive correlation with PFS (**figure 4C**). Finally, DCBI led to few changes within the myeloid compartment. The relative abundance of classical monocytes slightly decreased upon vaccination (**supplementary figure 3A-C**). PD-L1 expression decreased during treatment on both classical and non-classical monocytes (**supplementary figure 3D**).

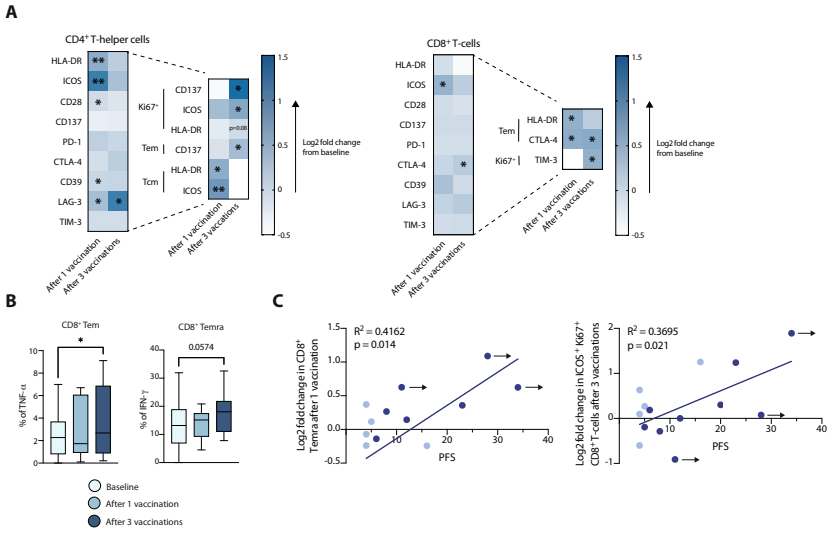

Figure 4. DC vaccination alters the phenotype of circulating CD4+ and CD8+ T-cells. A: Log2 fold change from baseline in stimulatory (HLA-DR, ICOS, CD28, CD137, PD-1) and inhibitory (PD-1, CTLA-4, CD39, LAG-3, TIM-3) by CD4+ Th-cells and CD4+ Th-cell subsets (left) and by CD8+ T-cells and CD8+ T-cell subsets (right) after 1 or 3 vaccination(s). For CD4+ Th-cell subsets and CD8+ T-cell subsets, only significant results are shown. B: Percentage of TNF-a+ cells of CD8+ Tem (left) and IFN-y+ of CD8+ Temra (right) cells. C: Linear regression analyses of the log2 fold change from baseline in percentage CD8+ Temra-cells after 1 vaccination (left) and percentage ICOS+ Ki67+ CD8+ T-cells after 3 vaccinations (right). Patients with complete cytoreduction are denoted in dark blue, incomplete cytoreduction in light blue. Patients with ongoing PFS are depicted with a horizontal arrow. No fold change in CD8+ Temra-cells could be determined for patient MPM.13 and MPM.14. This was due to no sample at baseline (MPM.13) or after 1 vaccination (MPM.14). PFS, progression-free survival; Tem, effector memory T-cells; Temra, terminally differentiated effector memory T-cells. *p<0.05; **p<0.01.

DISCUSSION

In the MESOPEC trial, patients with MPM were treated with adjuvant DCBI after CRS-HIPEC. This trial showed that this treatment is feasible and safe. In addition, DCBI demonstrated a diffuse immune modulatory effect on lymphoid cells, particularly on Th-cells. Activation of CTLs was limited, but when present, seemed to lead to better survival outcomes.

For patients with pleural mesothelioma and pancreatic adenocarcinoma, DCBI treatment has been proven feasible and safe.[10, 14] The current trial shows that DCBI is also feasible after major surgery for MPM. The cut-off value for feasibility

(i.e. 75% of patients treated with DCBI after CRS-HIPEC) was based on the rate of patients with colorectal carcinoma who are not able to undergo adjuvant systemic therapy due to severe complications after CRS-HIPEC (20 – 30%).[15, 16] The sample size was calculated at 20 patients, but inclusion was stopped when sixteen patients were successfully treated with adjuvant DCBI thereby meeting the feasibility endpoint. Severe complications after surgery were reported in 19% of patients. Despite these complications, all patients received their first three vaccinations within the protocol time frame (for patients treated according to protocol). For none of the patients leukapheresis resulted in delay of surgery. The two patients that dropped out of the trial before CRS-HIPEC was performed were not included in the feasibility determination, as the reason for exclusion was unrelated to DCBI treatment, but due to ineligibility for CRS-HIPEC.

To assure high quality analysis of blood immune monitoring, a computational preprocessing and analysis pipeline was used, and the semi-automated clustering made it possible to distinguish the different cell subsets in a comprehensive manner. Because evaluating co-expression of all different phenotypic marker combinations is much more feasible in a computational pipeline than when doing it manually, extensive immune monitoring could be performed. In line with previous studies, immune monitoring showed an increased proliferation of natural killer (NK) cells and Th-cells after DCBI.[14, 17, 18] In addition, an upregulation of co-stimulatory markers on Th-cells was detected. NK-cells have direct cytotoxic capacity, but also play a role in adaptive immunity by modulating DC responses.[19] The activation of Th-cells is also promising and recently there has been growing interest in the role of Th-cells in cancer immunology.[20] Although most cancer immunotherapies have been focusing on the CTL response, Th-cells play a pivotal role in developing and sustaining an effective anti-tumor response.[21, 22] Th-cells are key players in obtaining an optimal immune effect by providing help to CTLs, but also by the production of effector cytokines (i.e. IFN-γ and TNF-α) with direct anti-tumor activity. Th-cell signaling is also essential for the formation and survival of memory CTLs, contributing to a durable immune-mediated tumor response.[21, 23]

Next to an increased proliferation of Th-cells, an upregulation of co-stimulatory molecules (i.e., ICOS, HLA-DR and CD28) specifically on memory Th-cells was detected after DCBI treatment. In addition, a slight increase in TNF-α and IFN-γ production by memory CTLs was reported. Activation of memory T-cells is promising regarding clinical activity since memory T-cells are believed to show superior persistence and antitumor immunity compared to effector T-cells.[22]

Unlike the effect of DCBI treatment on Th-cell proliferation, the effect on CTLs was less profound. A slight upregulation of co-stimulatory molecules on CTLs after DCBI treatment was seen for all patients. For patients that had a complete cytoreduction (feasible in 63%) median PFS was 20 months, compared to four months for patients with an incomplete cytoreduction. CTL activation and proliferation seemed to be more pronounced in those patients that had a long PFS. This enhanced CTL response might be affected by the tumor load, which was lower in patients with a complete cytoreduction and is in line with earlier studies in mice.[8] The effect of the tumor load on the CTL response might be explained by the tumor microenvironment (TME).[20] Several studies have explored the TME of mesothelioma and reported that these tumors show variable degrees of T-cell infiltration.[24-26] The TME also consists of regulatory and inhibitory cells, among which regulatory T-cells, M2-like macrophages and myeloid-derived suppressor cells, that can hamper an effective anti-tumor response.[24, 27-31] This supports the rationale for the combination of DCBI treatment with cytoreduction.

The possible immunosuppressive role of the TME also provides a rationale for combination strategies to optimize the effectiveness of DCBI, especially for patients with incomplete cytoreduction. As PD-L1/PD-1 signaling plays a pivotal role in immune suppression, there is a rationale for combination therapy of DCBI with anti-PD-1 immunotherapy. A recent study by Gulijk et al. showed that DCBI and sequential anti-PD1 treatment in patients with pleural mesothelioma was safe and reported a synergistic effect of concurrent treatment in mice.[32] Future research should investigate the effect of this combination strategy in MPM patients.

As the current trial investigated DCBI as adjuvant treatment after CRS-HIPEC, no post-treatment tumor tissue was available. DCBI is known to induce a T-cell response in lymph nodes and the executive function of effector T-cells is located in the tumor.[33] The current trial showed that DCBI resulted in activation and proliferation of peripheral T-cells. It remains unknown whether systemic T-cell activation also resulted in activated tumor-specific T-cell infiltration in the tumor. The moderate immune activation after three vaccinations, as compared to after one vaccination, could suggest infiltration of activated T-cell in the tumor. Future studies also investigating the immune infiltration in the tumor might provide more insight into the effectiveness of DCBI in MPM.

This trial has some limitations, including small sample size and limited follow-up time. Therefore, robust statements about the effect of adjuvant DCBI after CRS-HIPEC on survival cannot be made. Another limitation were the protocol violations

regarding the time of inclusion for some patients. Inclusion of three patients was performed after CRS-HIPEC, as it was hypothesized that these patients might still benefit from adjuvant DCBI.

Regarding the computational analysis, a limitation was the data acquisition over multiple measurement days, introducting time-related batch effects. In a manual analysis, this can be accounted for by adjusting the gates on sample level, but clustering algorithms are more sensitive to numeric shifts. Therefore, a normalization step in the analysis pipeline was included. Future studies should include controls that are analyzed together with the patient data. Normalization algorithms can then employ these controls to characterize the batch effect more accurately.

CONCLUSIONS

The current trial shows that treatment with adjuvant DCBI after CRS-HIPEC in MPM patients is feasible and safe, and showed promising survival outcomes. DCBI has an immune modulatory effect on lymphoid cells, mainly Th-cells, and induces memory T-cell activation. Complete cytoreduction and an increase in CD8+ Temra-cells seemed to lead to better patient outcomes. Future research should be done to investigate the effect of DCBI on survival outcomes and identify possible combination treatment strategies to optimize the effect of DCBI.

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DATA SUPPLEMENT

Production of DC vaccination

The DCBI product was produced at the department of Pulmonary Medicine at the Erasmus MC Cancer Institute, following the protocol as published by Aerts et al. In short, dendritic cells (DCs) were derived from peripheral blood mononuclear cells, by differentiating monocytes towards immature DCs using specific culture conditions.[1] Immature DCs were exposed to tumor specific antigens in a co-culture with allogeneic mesothelioma cell lysate. This lysate (PheraLys) was derived from five well-specified mesothelioma cell lines. After exposure to PheraLys the immature dendritic cells were differentiated towards mature dendritic cells, which are ready to activate the immune system in vivo (MesoPher).

Data acquisition / Flow cytometry

Flow cytometry staining was performed on cryopreserved peripheral blood mononuclear cells (PBMCs) at baseline (before start of vaccination) and on treatment time points (two weeks after the first vaccination and two weeks after the third vaccination). For cytokine analysis, cells were stimulated for 4 hours in vitro with phorbol 12-myristate 13-acetate (PMA) and ionomycin (both from Sigma-Aldrich), supplemented with GolgiStop (BD Biosciences, Franklin Lakes, NJ, USA), prior to continuation of the cytokine staining. All stainings were performed at 4°C. Cells were first stained for membrane markers for 30 min. (**supplementary table 3**), followed by incubation with Fixable Viability Dye (eBioscience, ThermoFisher, Waltham, MA, USA) for 15 min. Subsequently, cells were fixed and permeabilized using the FoxP3 Transcription Factor Staining Buffer Set (eBioscience). Intracellular proteins were stained for 60 min. (**supplementary table 3**) and FACS acquisition was performed on a FACSymphony A5 (BD Biosciences) using BD FACSDiva software (BD Biosciences).

Computational analysis

The data were analyzed with a computational pipeline in R, based on the pipeline described in Quintelier et al.[2] Raw .fcs files were read into the R environment and margin events, i.e. events outside of the detection range, were removed with PeacoQC.[3] Next, the files were compensated with manually optimized compensation matrices, transformed with an arcsinh transformation with a cofactor of 150 and the FSC-A scatter channel was rescaled to the range of the transformed marker channels. Finally, good quality cells were selected with the PeacoQC quality control function and cells of interest (i.e. $CD56^{+}CD3^{-}$, $CD56^{+}CD3^{+}$ and $CD56^{-}CD3^{+}$ events for the T-cell panels and $LD^{-}FSC\text{-}A^{high}$ events for the DC-myeloid panel) were filtered out by applying a gate that was manually drawn in FlowJo.[4]

Next, for the T panels, two normalization steps were implemented with CytoNorm to solve the batch effects on two levels; the different panels and the two analysis days.[5] On the one hand, cell state markers were normalized by using *n panels x two days* batches (i.e. six for the T-cell panels) to prepare the data for the clustering. On the other hand, cell type markers were normalized per panel by using two batches to facilitate the downstream automated gating. The latter normalization was also applied in the DC-myeloid panel analysis pipeline to solve the batch effect on analysis day level.

FlowSOM, a two-level automated clustering algorithm, was used for a first, low-level SOM overclustering per cell type: a T-cell clustering (incl. cytokine, co-inhibition and co-stimulation panels) and a DC-myeloid cell clustering.[6] A subset of cells (T-cell: 5,446,594; DC-myeloid: 1,841,565) was sampled across all files to build each FlowSOM model with a ten by ten SOM grid with the cell type markers: seven and ten respectively (T-cell: CCR7, CD3, CD4, CD8, CD45RA, CD56, FoxP3; DC-myeloid: AXKL, HLA−DR, CD3 CD19 CD20, CD11c, CD14, CD16, CD56, CD123, IRF4, IRF8). Instead of the default consensus hierarchical clustering for a higher-level clustering, a manual approach was used. A manual threshold per cell type marker was defined, and every cluster was labeled as positive or negative depending on whether its median value was higher or lower than this threshold. These positive/negative patterns across all cell type markers were then used to combine the SOM clusters into meta-clusters, resulting in 28 meta-clusters for the T-cell model and 25 for the DC-myeloid FlowSOM model. **Supplementary table 4** shows how the meta-cluster definitions were translated to the actual cell types. In a next step, all preprocessed .fcs files were mapped on the associated model: 123 on the T-cell model (cytokine: 41; co-inhibition: 42; co-stimulation: 40) and 38 on the DC-myeloid model to obtain the (meta-)cluster abundances.

Finally, to obtain the complete immune profiles, gate values were computed for the cell state markers in an automated and unbiased way. For six files (three time points from two patients) the gates were manually drawn in FlowJo. To automate this, the deGate function of the flowDensity package was used to estimate the thresholds in the density distributions of the cell state markers.[7] 12 sets of parameters were composed to evaluate the deGate performance and the best set was selected based on the correspondence with the manually drawn gates. For each .fcs file, the median over the different time points of the patient was eventually chosen as final gate (**supplementary figure 4**). An iterative approach was implemented to obtain the percentages positive cells per file per marker and per combination of markers.

Supplementary table 1. Organ resections and all postoperative complications.

	n=16	
Organ resections	1 (6.3)	
Ovaries	2 (12.5)	
Uterus	14 (87.5)	
Omentum	5 (31.3)	
Rectum	6 (37.5)	
Colon/appendix	1 (6.3)	
Small bowel	3 (18.8)	
Galbladder	0 (0)	
Stomach	2 (12.5)	
Spleen	5 (31.5)	
Diaphragm	0 (0)	
Pancreas	0 (0)	
Bladder	1 (6.3)	
Urether	12 (75.0)	
Peritoneum	3 (18.8)	
Pelvis	2 (12.5)	
Other		
Postoperative complications	**N (%)**	**CD grade**
Pneumonia	4 (25.0)	2
Chylous leakage	5 (31.3)	2
Parenteral feeding	3 (18.8)	2
Acute on chronic kidney injury	2 (12.5)	2
Urine tract infection	2 (12.5)	2
Delirium	1 (6.3)	2
Ileus	1 (6.3)	3b
Intra-abdominal hematoma	1 (6.3)	3b
Other infection	1 (6.3)	3b
Malposition JJ-stent	1 (6.3)	3a

Frequencies are shown as N (%)
CD= Clavien-Dindo

Supplementary table 2. Individual patient outcomes.

Patient	CCR	Number of vaccinations	PFS [a]	OS [a]	Treatment after progression	Immunotherapy (number of cycles)	Chemotherapy (number of cycles)	Surgery
MPM.01	CCR 3	5	16	19	Yes	Nivolumab (4)	No	No
MPM.02	CCR 3	5	5	33	Yes	Nivolumab (24)	No	No
MPM.03	CCR 0	4	12	35	Yes	Nivolumab (8)	No	No
MPM.04	CCR 0	5	23	43+	Yes	Nivolumab + Ipilimumab (16) [b]	No	Yes
MPM.05	CCR 3	4	4	6	Yes	No	Pemetrexed + Platinum (1)	No
MPM.06	CCR 0	5	8	22	Yes	No	Pemetrexed + Platinum (4)	No
MPM.07	CCR 0	5	34+	34+	n/a	n/a	n/a	n/a
MPM.09	CCR 3	4	4	7	No	n/a	n/a	n/a
MPM.10	CCR 0	5	28+	28+	n/a	n/a	n/a	n/a
MPM.12 [c]	CCR 1	3	6	6+	Unknown	Unknown	Unknown	Unknown
MPM.13	CCR 1	5	20	20+	No	n/a	n/a	n/a
MPM.14	CCR 0	5 [d]	5	19+	Yes	Nivolumab + Ipilimumab (17) [b]	No	Yes
MPM.15	CCR 3	4	4	14+	No	n/a	n/a	n/a
MPM.16	CCR 1	5	11+	11+	n/a	n/a	n/a	n/a
MPM.17	CCR 3	4 [e]	5+	5+	n/a	n/a	n/a	n/a
MPM.18	CCR 0	3 [e]	1+	1+	n/a	n/a	n/a	n/a

CC= completeness of cytoreduction score, PFS= progression free survival, OS= overall survival

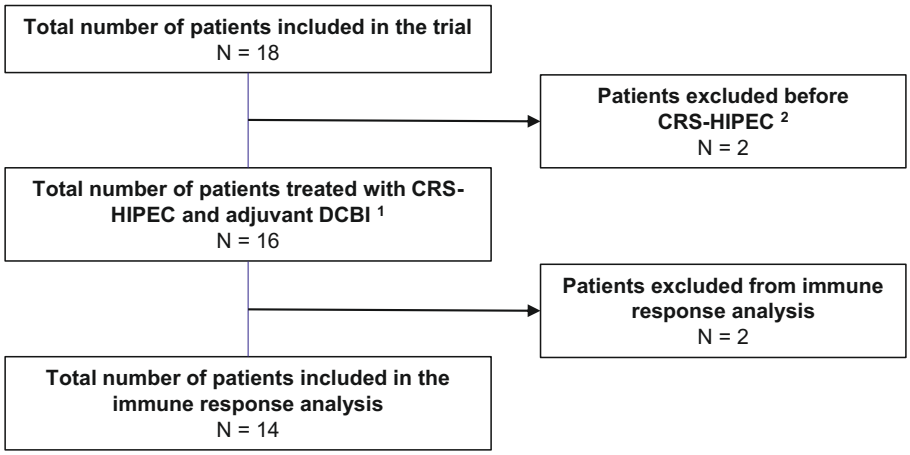
[a] Interval between CRS-HIPEC and recurrence or death in months

[b] Nivolumab was administered every three weeks and ipilimumab every six weeks according to national guidelines

[c] This patient was lost to follow-up after progression of disease

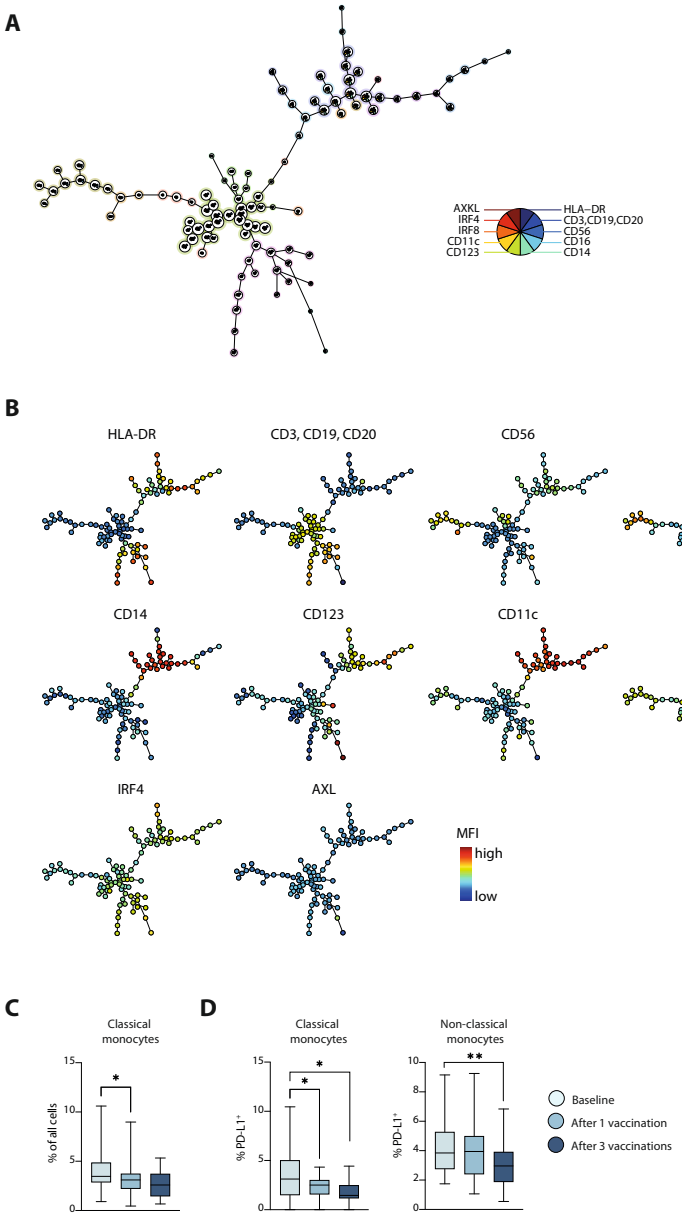
[d] DC vaccination 4 and 5 were given during treatment with nivolumab and ipilimumab

[e] These patients were still in the active treatment phase



Supplementary figure 2. Overview of included patients per analyses.
[1] These patients were included in the feasibility and safety analysis
[2] These patients were excluded from the study due to ineligibility for CRS-HIPEC

Supplementary tables 3-4, supplementary figure 1, and **supplementary figure 4** are available at https://doi.org/10.1136/jitc-2023-007070.



Supplementary figure 3. **Treatment induced changes in the abundance and phenotype of myeloid cells.** Identification of different myeloid clusters (A) by cell type marker expression (B) from the FlowSOM algorithm. Percentage of classical monocytes among all cells before vaccination and after 1 or 3 vaccination(s) (C). Expression of PD-L1+ cells within classical monocytes (left) and non-classical monocytes (right) before vaccination and after 1 or 3 vaccination(s) (D). *p<0.05, **p0.01.

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Chapter 4

Intraperitoneal Paclitaxel for Patients with Primary Malignant Peritoneal Mesothelioma – a Phase I/II Dose Escalation and Safety Study: INTERACT MESO

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ABSTRACT

Introduction

Malignant peritoneal mesothelioma (MPM) is a rare, aggressive tumor, arising primarily from the peritoneum. The only potentially curative treatment is cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC). However, the majority of patients are not eligible to undergo this treatment. The benefit of systemic treatment for these patients is limited, at the cost of considerable morbidity. Hence, there is need for appropriate palliative treatment options for MPM patients. As MPM rarely disseminates outside the abdominal cavity, these patients might benefit from local treatment. A higher, more effective dose of chemotherapy can directly be delivered at the site of disease. Systemic uptake will be limited, likely resulting in less toxicity. The aim of the INTERACT MESO trial is to determine the maximum tolerable dose (MTD) of intraperitoneal (IP) paclitaxel monotherapy in patients with MPM. Secondary endpoints are to assess safety and toxicity, feasibility, and the pharmacokinetic profile of this treatment.

Methods and analysis

The INTERACT MESO trial is a prospective, open-label, single-center, phase-1 study with a classic three-plus-three dose escalation design. The study population consists of adult patients with primary MPM, without extra-abdominal disease, that are not eligible to undergo CRS-HIPEC. According to standard of care work-up for CRS-HIPEC, patients will undergo diagnostic laparoscopy (DLS) to determine the feasibility of CRS-HIPEC. In case CRS-HIPEC is not considered feasible, a peritoneal port-a-cath (PAC) system will be placed. Through this PAC, 8-16 weekly cycles of intraperitoneal chemotherapy will be administered.

Ethics and dissemination

The Central Committee on Research Involving Human Subjects (CCMO, The Hague, The Netherlands) and the Research Ethics Committee (METC, Rotterdam, The Netherlands) have granted permission to carry out this study protocol. The results of this trial will be submitted for publication in a peer-reviewed scientific journal.

Trial registration number: Nederlands Trial Register: NL9718. EudraCT: 2021-003637-11.

INTRODUCTION

Malignant Peritoneal Mesothelioma (MPM) is a rare, but aggressive neoplasm with a poor prognosis, arising primarily from the serosal lining of the peritoneal cavity.[1] Currently, the only possibly curative treatment is cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC).[2, 3] In the Netherlands, only a minority of patients undergo this treatment.[1] For patients that are not eligible to undergo CRS-HIPEC, the treatment options are limited. Overall response rates to systemic chemotherapy are low (20-25%), though morbidity rates are high, with a grade 3-4 hematological toxicity rate up to 38%.[4-6] Moreover, the two-year survival rate for these patients is only 20%.[1] Combination checkpoint-inhibition-therapy with nivolumab and Ipilimumab has been proposed as a new treatment option for MPM patients. However, this treatment has comparable morbidity rates to that of systemic chemotherapy, and its benefit for MPM patients is not proven.[7, 8] Because of the high morbidity rate, and the limited effectiveness of systemic treatment it is debatable whether these therapies are suitable as palliative treatment for patients with MPM. Due to lack of appropriate palliative treatment options, the majority of MPM patients in the Netherlands (63%) currently receives no anti-tumor treatment.[1]

For peritoneal metastases from several types of cancer, local treatment with intraperitoneal (IP) chemotherapy has been proposed as a palliative treatment option. This therapy can be delivered through an IP port-a-cath (PAC), and potentially has major advantages over systemic treatment. A higher, more effective dose of chemotherapy can directly be delivered at the site of disease, while systemic uptake is limited, likely resulting in fewer toxicity. Paclitaxel is a chemotherapeutic agent that is considered extremely favorable for IP use.[9] Due to its large molecular weight and lipophilic properties, it is slowly cleared from the peritoneal cavity when administered locally. This results in an area under the curve (AUC) after IP- administration that is up to a 1000-fold (3-log) higher than that in plasma, while peritoneal concentrations persist up to 48 hours after administration.[10] This considerably increases drug activity.

Markman et al. presented the first phase-1 dose-escalation study of IP-paclitaxel in ovarian cancer patients, pre-treated with systemic chemotherapy.[10] They established the maximum-tolerable-dose (MTD) to be 175 mg/m^2 at a two-to-three week interval. Another dose-escalation study was performed by Francis et al., delivering a lower dose at a weekly frequency.[11] These patients were also pre-treated with systemic chemotherapy. Severe abdominal pain was uncommon and only low-

grade leukopenia, fatigue and stomatitis was observed. Grade 3-4 gastro-intestinal complications were observed in 15% of patients. Francis et al. recommended a dose of 60-65 mg/m^2 IP-paclitaxel in weekly cycles. Markman et al. performed a phase-2 trial in 80 ovarian cancer patients, using 60 mg/m^2 of IP-paclitaxel, in 16 weekly cycles after pre-treatment with systemic chemotherapy.[12] The majority of patients (70%) received all planned 16 courses. Grade-3 complications were rare, with abdominal pain, neuropathy, and neutropenia in one, two and one patients, respectively. Bowel perforation, a rare but potentially life-threatening complication, was observed once in the phase-1 trial (3%) but was not observed in the phase-2 trial. Five patients were removed from the study due to excessive toxicity, and three patients due to catheter malfunction. In total, 18 (24%) patients achieved a complete response.

As the effectiveness of systemic therapy is limit and MPM very rarely disseminates outside the abdominal cavity, the use of IP paclitaxel monotherapy seems a logical and promising step. The group of Paul Sugarbaker utilizes long-term IP-administration of paclitaxel as an adjuvant treatment to CRS-HIPEC for patients with MPM. They use doses of 20 mg/m^2 daily for five consecutive days monthly, starting 4-6 weeks postoperatively. Some of these patients showed remarkable survival, despite incompleteness of cytoreduction at CRS-HIPEC.[13-15] Another major advantage of the suggested treatment is that ascites, a common MPM-symptom that causes major morbidity, can be drained through the same PAC-system.

Currently, there are no studies that investigate IP paclitaxel as non-adjuvant monotherapy in MPM patients. The main objective of this clinical trial is to determine the maximum tolerable dose (MTD) of IP paclitaxel monotherapy in patients with MPM. Secondary endpoints are to assess safety and toxicity, feasibility, and the pharmacokinetic profile of this treatment. When the MTD is determined, further research is needed to determine the effect on survival.

METHODS AND ANALYSIS

This protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Statement (**supplementary appendix 1**).[16]

STUDY DESIGN

Trial setting

The INTERACT MESO trial is a prospective, open-label, single-center, phase-1 study with a classic three-plus-three dose escalation design (**figure 1**). The defined dose levels are 100 mg, 150 mg, and 200 mg paclitaxel. This study is conducted in the Erasmus MC Cancer Institute, a tertiary referral hospital, located in Rotterdam, the Netherlands. Trial registration details are described in **table 1**. The study started recruitment in February 2022, and as of 17 May 2022 one patient has been enrolled. The end of the study is planned in February 2026.

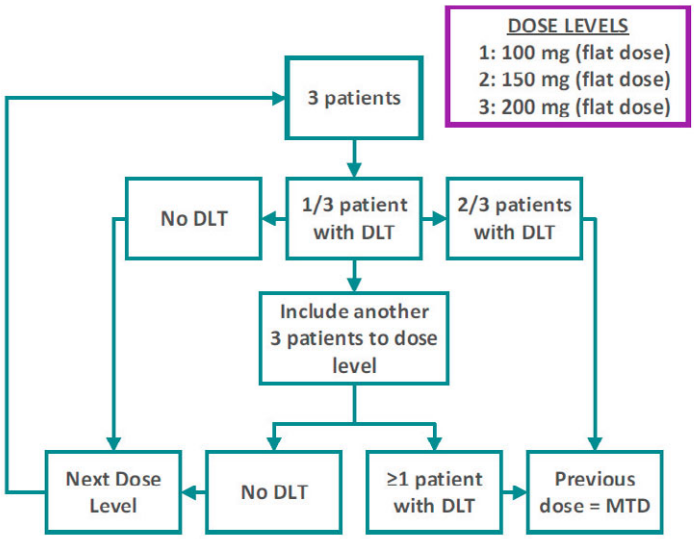

Figure 1. Three-plus-three dose escalation design. DLT, dose limiting toxicity; MTD, maximum tolerable dose.

Study population

The study population consist of adult patients with primary MPM, without extra-abdominal disease, that are not eligible to undergo CRS-HIPEC. Potentially eligible patients will be referred by their local clinician or through self-referral to a medical specialist. A member of the study team will inform patients about the trial at the outpatient clinic, and an eligibility assessment will be performed. In order to be eligible to participate in the study, potential subjects must meet all of the following inclusion criteria:

Table 1. WHO trial registration data set

Primary registry and trial identifying number	EudraCT number: 2021-003637-11 Netherlands Trial Register: NL9718
Date of registration in primary registry	September 2021
Protocol version	Protocol version 4.0, date November 22nd, 2021
SPIRIT guidelines data set for clinical trials	See supplementary file
Secondary Identifying Numbers	Dutch competent authority (CCMO): NL78373.078.21 Local medical ethics committee (METC): MEC-2021-0697
Source(s) of monetary or material support	Erasmus MC Foundation, Rotterdam, the Netherlands
Primary sponsor	Erasmus University Medical Center, Rotterdam, the Netherlands
Secondary sponsors	Not applicable
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Public title	Treatment of abdominal mesothelioma with intra-abdominal chemotherapy: INTERACT MESO
Scientific title	Intraperitoneal Paclitaxel for Patients with Primary Malignant Peritoneal Mesothelioma – a Phase I/II Dose Escalation and Safety Study: INTERACT MESO
Countries of recruitment	The Netherlands
Health conditions or problems studied	Malignant peritoneal mesothelioma
Interventions	Patients undergo a diagnostic laparoscopy (DLS) according to standard work-up for CRS-HIPEC. If the disease is considered not resectable, a peritoneal port-a-cath (PAC) will be placed. Through this PAC, intraperitoneal paclitaxel will be administered in weekly cycles.
Key inclusion and exclusion criteria	Key inclusion criteria: Confirmed diagnosis of malignant peritoneal mesothelioma, WHO-ECOG performance status 0-1, age ≥ 18 years old, and adequate organ function and bone marrow reserves. Key exclusion criteria: Extra-abdominal disease/metastatic disease, serious concomitant disease or active infections, any medical or psychological impediment to probable compliance with the protocol, and pregnant or lactating women.
Study type	Open label single center phase I/II study
Date of first enrolment	Planned February 2022
Target sample size	11 – 21 according to dose escalation
Recruitment status	Pending
Primary outcome	Maximum tolerable dose (MTD) of intraperitoneal (IP) paclitaxel monotherapy in patients with MPM
Key secondary outcome(s)	Safety and toxicity, feasibility, and the pharmacokinetic profile of intraperitoneal paclitaxel monotherapy

CRS, cytoreductive surgery; DLS, diagnostic laparoscopy; ECOG, Eastern Cooperative Oncology Group; HIPEC, hyperthermic intraperitoneal chemotherapy; PAC, port-a-cath, SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials.

- Histological confirmed diagnosis of malignant peritoneal mesothelioma
- Patients that are not eligible (or willing) to undergo cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC)
- Age ≥ 18 years
- Written informed consent by the patient according to the ICH-GCP and national/ local regulations
- Patients must be ambulatory (WHO-ECOG performance status 0 or 1)
- Ability to return to the Erasmus MC for adequate follow-up as required by this protocol
- Patients must have normal organ function and adequate bone marrow reserve as assessed by the following laboratory requirements; absolute neutrophil count >1.5 * 10^9/l, platelet count >100*10^9/l and hemoglobin >6.0 mmol/l. Patients must have a bilirubin <1½ x upper limit of normal (ULN), serum AST and ALT <2.5 x ULN

A potential subject who meets any of the following exclusion criteria will be excluded from participation in the study:

- Incapacitated patients
- Extra-abdominal disease/metastatic disease established by preoperative CT-scan of thorax-abdomen and/or PET-scan. Imaging not older than two months at time of surgery
- Medical or psychological impediment to probable compliance with the protocol
- Serious concomitant disease or active infections
- History of auto-immune disease or organ allografts, or with active or chronic infection, including HIV and viral hepatitis
- Serious intercurrent chronic or acute illness such as pulmonary (COPD or asthma) or cardiac (NYHA class III or IV) or hepatic disease or other illness considered by the study coordinator to constitute an unwarranted high risk for participation in this study
- Pregnant or lactating women; for all women of child-bearing potential a negative urine pregnancy test will be required as well as the willingness to use adequate contraception during the study until 4 weeks after finishing treatment
- Absence of assurance of compliance with the protocol
- An organic brain syndrome or other significant psychiatric abnormality which would comprise the ability to give informed consent, and preclude participation in the full protocol and follow-up

PATIENT TIMELINE AND ADDITIONAL PROCEDURES

A flowchart of the study is shown in **figure 2**. A more detailed description of (additional) study procedures is presented in **table 2**.

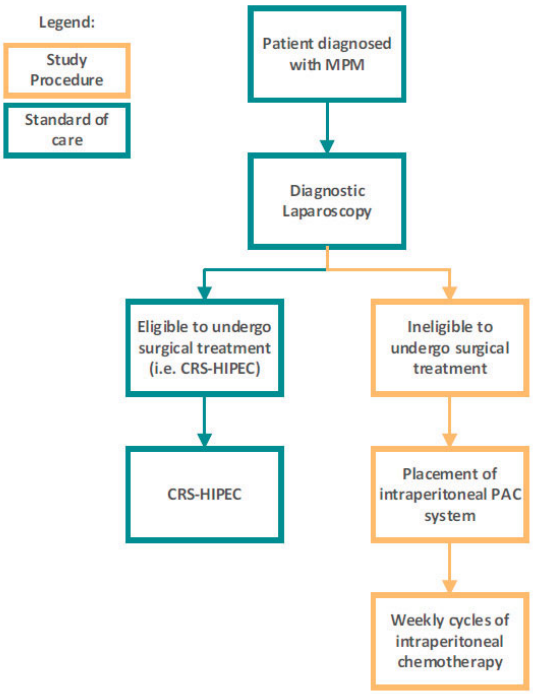

Figure 2. Study workflow. After patients are diagnosed with MPM, they will undergo a DLS, as a part of standard care. If the disease is deemed resectable, patients will undergo CRS-HIPEC as part of standard care. If the disease is considered not resectable during DLS, patients are eligible for inclusion in the currents study. A PAC system will be placed subcutaneously, while the catheter tip is placed inside the peritoneal cavity. After surgery, patients will receive weekly cycles of IP-chemotherapy. PAC, port-a-cath; CRS-HIPEC, cytoreductive surgery with hyperthermic intraperitoneal chemotherapy; DLS, diagnostic laparoscopy; IP, intraperitoneal.

Table 2. Study procedures.

	Before 1st visit	1st visit	2nd visit	DLS	1st post-op visit	IP-CTx								Response evaluation	9-16th cycle[6]	IP-CTx		
						1st cycle	2nd cycle	3rd cycle	4th cycle	5th cycle	6th cycle	7th cycle	8th cycle			Response evaluation	Response evaluation	Last study visit
MTB[1]	X															X		
Medical history	X	X																
In- / exclusion criteria		X																
Provide information about the study		X	X															
Written informed consent			X															
Vital signs			X		X	X	X	X	X	X	X	X	X		X			
Physical examination (Incl. weight)[2]			X		X	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]		X[2]			
Operability check (Anesthetist)			X															
Hematology and blood chemistry			X		X[2]	X	X	X	X	X	X	X	X		X			
Viral serology			X															
Pregnancy test[2]			X															
Placement of peritoneal PAC[3]				X														
Visit medical oncologist			X		X									X		X		
CT-scan chest/abdomen	X[4]					X[5]								X		X		
Intraperitoneal chemotherapy						X	X	X	X	X	X	X	X		X			
Performance status			X		X	X	X	X	X	X	X	X	X	X	X	X		
Chemotherapy toxicity evaluation (CTCAE 5.0)						X	X	X	X	X	X	X	X	X	X	X		
Collection of blood and peritoneal fluid for PK analysis						X			X									
Removal of peritoneal PAC																	X[8]	

[1)] Scans and reports of (referred) patients are first discussed in a multi-disciplinary tumor board. When patients are considered candidates for HIPEC-procedure, they are seen in the outpatient clinic.
[2)] If applicable.
[3)] In case complete cytoreduction is deemed impossible.
[4)] If not performed by referring center.
[5)] Maximum of four weeks before start of study treatment.
[6)] In case of no progression of disease (i.e., CR, PR, or SD) and if patient is willing.
[7)] At cycle 16 if applicable.
[8)] Optional, according to patient preference and life expectancy

Screening

The multidisciplinary tumor board will review all referred patients who are possibly eligible to participate in the study. Potential candidates for CRS-HIPEC will visit the surgical oncology outpatient clinic, where they will be informed about the treatment options, including the study, and undergo standard screening procedures. The standard of care CRS-HIPEC screening procedure includes a CT scan of the thorax and abdomen (not older than two months before surgery), lab testing (including kidney and liver panels, and blood cell count), anesthetic assessment, and a diagnostic laparoscopy (DLS). If the disease is considered not resectable during DLS, and if the patient meets the inclusion/exclusion criteria, the patient is eligible for inclusion. Patients who are considered ineligible for CRS-HIPEC, based on parameters that were obtained before DLS, but have no contra-indication for IP chemotherapy, can also participate in the study.

Surgical procedures

Patients will be operated under general anesthesia, according to local hospital procedures. During the diagnostic laparoscopy, the extent of disease is assessed according to the 'peritoneal carcinomatosis index' (PCI). Ascites fluid will be collected for storage in the local MPM biobank. The surgeon will determine feasibility of complete cytoreduction. If it is deemed impossible to achieve complete cytoreduction, a port-a-cath (PAC) system will be placed subcutaneously, while the catheter tip is placed inside the peritoneal cavity. After surgery, patients may leave the hospital that same day, with careful (including written) instructions for e.g., hygiene. Patients are seen in the outpatient clinic approximately a week after surgery by a medical oncologist. The start date of the first treatment cycle of chemotherapy will be determined.

Chemotherapy

Patients will receive intraperitoneal paclitaxel (dose according to current dose-level) dissolved in 1 liter of saline (0.9% NaCl), pre-warmed to 37°C through the PAC that was placed during laparoscopy. Patients will receive all necessary pre-medications prior to infusion, according to the local standard protocol for intravenous administration of paclitaxel. If present, prior to infusion, ascites will be drained through the PAC, and stored in the MPM biobank. Administration of IP-chemotherapy will take about 1.5-2 hours. After infusion, patients are instructed to switch position frequently to maximize distribution of chemotherapy in the peritoneal cavity. Patients will be observed for two hours after chemotherapy administration. If no adverse events occur during this period, patients will be discharged with careful instructions to contact the hospital if any alarming symptoms do develop. During

the first and the fourth cycle of IP-chemotherapy, additional blood samples and IP-fluid samples will be collected for pharmacokinetic (PK) analysis. The 24-hour AUC will be calculated for systemic and IP-paclitaxel. Other pharmacokinetic parameters such as the maximum concentration (Cmax) and the elimination half-life ($t_{1/2}$) will also be determined.

Patients will initially receive eight weekly cycles of IP-chemotherapy. After the start of the first cycles, following cycles can be delayed, at the discretion of the medical oncologist in case of a medical indication (e.g., neutropenia). If a cycle is delayed for more than two weeks, this is considered a dose limiting toxicity. After the first eight cycles, response evaluation will take place. Depending on this outcome, another eight cycles can be initiated. In case of ongoing therapy response, there is no limit to the number of cycles.

Follow-up

As the current proposal is a phase-1 trial, long-term follow-up is not applicable. However, (PET-)CT scans are performed at baseline, during response evaluation (if possible, according to RECIST-criteria), and every four months after the last treatment. By doing so, valuable preliminary data on the effectiveness of this treatment can be acquired. Also, in case of treatment response after 16 cycles, a second diagnostic laparoscopy can be performed to definitively assess response and possibly assess eligibility for surgical treatment.

Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Should a patient or the study coordinator decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Patients will receive treatment according to standard of care. Three patients within a dose level must be observed for 2 weeks (2 cycles of chemotherapy) before proceeding to the next higher dose level. If a patient is withdrawn from the study prior to completing 2 cycles of therapy and 1 week of follow-up without experiencing a DLT prior to withdrawal, an additional patient may be added to that dose level. The investigators also have the right to withdraw patients from the study if one of more of the following events occur:

- Significant protocol violation or noncompliance on the part of the patient or investigator
- Refusal of the patient to continue treatment or observations

- Any change in the condition of the patient that justifies discontinuation of treatment
- Decision by the study coordinator that termination is in the patient's best medical interest
- Unrelated medical illness or complication

Objectives and analysis

Primary objective

The primary objective is to determine the maximum tolerable dose (MTD) of intraperitoneal paclitaxel monotherapy for patients with MPM that are ineligible to undergo CRS-HIPEC. The MTD will be determined during the first eight cycles of IP-chemotherapy by a classic three-plus-three dose escalation design with three dose-levels (i.e., 100 mg, 150 mg, and 200 mg flat dose paclitaxel; see figure 1). To determine the MTD, dose limiting toxicities (DLTs) are predefined. DLTs are graded according to the CTCAE version 5.0. If less than 33% of subject in a dose-cohort experience DLT (i.e., one subject out of a maximum of six subjects in a dose-cohort), the next higher dose cohort will be assessed. Dose levels higher than 200 mg will not be assessed. If ≥ 33% of subjects experience DLT in the first dose-cohort (i.e., 100 mg), a dose-de-escalation to 80 mg will be assessed. There will be no dose-escalation within patients. The following events will be considered DLTs:

Hematologic:
- Absolute neutrophil count (ANC) $<0.5*10^9$/l (grade 4), lasting longer than 7 days
- Febrile neutropenia (ANC $<1.0*10^9$/l with fever ≥38.5°C) (grade 3-4)
- Platelet count $<25*10^9$/l (grade 4)

Non-hematologic
- Grade ≥3 non-hematological adverse events, except nausea/vomitus, diarrhea, or fatigue, for which the following DLT definition will apply:
    - Nausea grade ≥3, despite optimal anti-emetic use
    - Diarrhea grade ≥3, despite optimal loperamide use
    - Fatigue grade ≥3 lasting longer than 7 days
    - Delay of next cycle by >2 weeks due to any medical reason

Secondary objective(s):

Secondary objectives are to assess the safety, toxicity, and feasibility of this treatment, and to establish the pharmacokinetic profile of IP-paclitaxel. During the study, ascites and tumor material will be systematically collected, processed, and stored for translational research purposes.

Sample size calculation and statistical analysis

Because of the dose escalation design, the needed number of participants depends on data obtained during different dose levels (see figure 1). The minimum number of patients is four, if the first two patients in the first dose cohort immediately experience DLT, as well as the first two patients in the dose-de-escalation cohort. The minimum number of patients required to reach the primary endpoint (i.e., to find the MTD) is 11. If the first three patients experience no DLT, but the first two patients in the second dose-cohort both experience DLT. Then five patients were already included, after which an additional six patients have to be included at the first dose level, to come to nine patients treated at the MTD. The maximum number of patients that can possibly be required to reach the primary endpoint is 21. If there are six patients required in each dose cohort to reach the MTD, after which an additional three patients have to be included in the final dose cohort, to come to nine patients treated at the MTD. The statistical analyses/data summaries will be performed using R and Rstudio. Other tools may be used for exploratory summaries and graphical presentations. Descriptive statistics will be used to describe paclitaxel pharmacokinetics, dose linearity, and its relation to paclitaxel related side effects. Systemic bioavailability of peritoneal administration will be analyzed by comparing the AUC with the results of our many other pharmacological studies with paclitaxel. Relationship between toxicity and paclitaxel exposure will be explored graphically and with logistic regression (two sided and $P < 0.05$).

Harms and auditing

All adverse events (AE), serious adverse events (SAE) or suspected unexpected serious adverse reactions (SUSARs) will be recorded. All (S)AEs and SUSARs as a related to the administration of intraperitoneal paclitaxel will be reported through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening, followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States. The sponsor (Erasmus MC Cancer Institute, the Netherlands) is insured to provide cover for any patients who suffer harm from study participation.

Since this is a phase I dose escalation study, all (S)AEs and SUSARs will be evaluated by the study team before the decision will be made to continue with the next dose-level. Therefore, no data safety monitoring board will be installed.

Patient and public involvement

There was no patient or public involvement in the design, conduct, reporting, or dissemination plans of the INTERACT MESO trial. However, the design of this trial has been shared with the Asbestos Victims Association of the Netherlands (in Dutch 'Asbestslachtoffers Vereniging Nederland', AVN), and they support this research.

Ethics and dissemination

This study will be conducted in agreement with both the Declaration of Helsinki (latest amendment: 64th WMA General Assembly, Fortaleza, Brazil, October 2013), the Dutch laws and regulations with the WMO ("Wet Medisch-wetenschappelijk Onderzoek met mensen") in particular. In case of protocol modifications, the research medical ethics committee (METC) and the Dutch competent authority (CCMO) will be informed. The new protocol has to be approved by the METC, and CCMO, before it can be implemented. Data collection, data assessment and data analysis will be performed according to the local guidelines for data management of the Erasmus MC. The sponsor will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.The results of this clinical trial will be submitted for publication in a peer-reviewed scientific journal.

DISCUSSION

The main objective of the INTERACT MESO trial is to determine the maximum tolerable dose (MTD) of IP paclitaxel monotherapy in patients with MPM. Secondary endpoints are to assess safety and toxicity, feasibility, and the pharmacokinetic profile of this treatment. To our knowledge, the INTERACT MESO trial is the first clinical trial that investigates intraperitoneal (IP) paclitaxel as non-adjuvant monotherapy in MPM patients that are not eligible for CRS-HIPEC.

Currently, the majority of MPM patients in the Netherlands receive no anti-tumor treatment.[1] The morbidity of systemic treatment is high, and the effectiveness is limited.[4-8] Hence, there is a lack of appropriate palliative treatment for patients

with MPM. As MPM rarely disseminates outside the abdominal cavity, the use of intraperitoneal (IP) chemotherapy seems a logical and promising step. This has major advantages over systemic treatment, as a higher, more effective dose of chemotherapy can directly be delivered at the site of disease, while systemic uptake is limited. This will likely result in fewer systemic toxicity, and thus an increase in quality of life. In rare cases where metastases do develop, a switch can be made to systemic treatment. By first applying local treatment, most patients will be spared a toxic and often ineffective systemic therapy. The placement of the intraperitoneal PAC is performed during standard of care diagnostic laparoscopy, thus not associated with extra visits or procedures. The Erasmus MC Cancer Institute is experienced with the placements of intraperitoneal PACs and the administration of intraperitoneal chemotherapy. The INTERACT trial, a phase I, dose-escalation study with concomitant intraperitoneal irinotecan combined with FOLFOX in patients with peritoneal metastases from colorectal carcinoma, was conducted in the Erasmus MC Cancer Institute.[17] This trial recently finished and shows promising results. Another advantage of the peritoneal PAC is that ascites, a common symptom of MPM, causing major morbidity, can repeatedly be drained through the PAC system.

Paclitaxel is a well-known chemotherapeutic agent and is considered extremely favorable for IP use.[9] Due to its large molecular weight and lipophilic properties, it is slowly cleared from the peritoneal cavity when administered locally. This results in an area under the curve (AUC) after IP- administration that is up to a 1000-fold (3-log) higher than that in plasma, while peritoneal concentrations persist up to 48 hours after administration.[10] Based on earlier studies, intraperitoneal paclitaxel is expected to be a more effective treatment for patients with extensive peritoneal mesothelioma, compared to the current available systemic chemotherapy. Though systemic administration has not shown to result in survival benefit for MPM patients, the fact that up to a 1000-fold AUC can be achieved by peritoneal administration, provides the rationale for the hypothesis that intraperitoneal treatment can be effective.

The starting dose in this dose escalation study will be a 100 mg flat-dose. In earlier phase-1 and 2 studies that investigated the use of IP-paclitaxel in ovarian cancer patients in weekly cycles, the MTD was 60-65 mg/m2.[11, 12] This translates to a 120-130 mg flat-dose. The ovarian cancer patients in these studies were heavily pre-treated with systemic chemotherapy. As IP-paclitaxel will be used as first line monotherapy in the current study, a higher MTD is anticipated. Currently, the systemic effective dosage is 175-200 mg (flat-dose). As IP-administration can reach

up-to a 1000-fold higher AUC, there is no clinical rationale to pursue a dose escalation beyond a 200 mg flat-dose. Earlier studies have shown that intraperitoneal administration of paclitaxel causes mild toxicity. Common toxicities that occur from systemic administration, such as neuropathy, were not observed after intraperitoneal administration.[10-12] Bowel perforation is a rare, but potentially serious complication from intraperitoneal treatment. This was extremely rare in previous studies that investigated a similar treatment strategy.

During this study, ascites and tumor material will also be collected, processed, and stored for translational research purposes. As MPM is a rare disease, this could result in valuable information for all MPM patients.

If the MTD for IP-paclitaxel in the current study population is determined, and the treatment is found to be safe, a larger phase III clinical trial should be conducted to determine the effect on survival outcomes. Because the incidence of MPM in the Netherlands alone is low, a phase III clinical trial would have to be conducted internationally.

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Chapter 5

Molecular Alteration and Potential Actionable Mutations in Peritoneal Mesothelioma: A scoping review of high throughput sequencing studies

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ABSTRACT

Background

Peritoneal mesothelioma (PeM) is a rare malignancy with a poor prognosis. Currently there is a lack of effective systemic therapies. Due to the rarity of PeM, it is challenging to study new treatment options. Off-label use of targeted drugs could be an effective approach. This scoping review aims to explore the genomic landscape of PeM to identify potential therapeutic targets.

Methods

A systematic literature search of Embase, Medline, Web of Science, the Cochrane Library, and Google Scholar was performed up to November 1, 2022. Studies that reported on molecular alterations in PeM detected by high throughput sequencing techniques were included. Genes that were altered in ≥ 1% of PeM's were selected for the identification of potential targeted therapies.

Results

Thirteen articles were included, comprising 824 PeM patients. In total, 142 genes were altered in ≥ 1% of patients, of which seven genes were altered in ≥ 10%. *BAP1* was the most commonly altered gene (50%). Other commonly altered genes were *NF2* (25%), *CDKN2A* (23%), *CDKN2B* (17%), *PBRM1* (15%), *TP53* (14%), and *SETD2* (13%). In total, 17% of PeM patients were carriers of a germline mutation, mainly in *BAP1* (7%).

Conclusion

This scoping review provides an overview of the mutational landscape of PeM. Germline mutations might be a larger contributor to the incidence of PeM than previously thought. Currently available targeted therapy options are limited, but several targeted agents (like PARP, EZH2, and CDK4/6 inhibitors) were identified that might provide new targeted therapy options in the future.

INTRODUCTION

Peritoneal mesothelioma (PeM) is a rare and aggressive malignancy. The prognosis of patients with PeM is very poor due to its non-specific clinical presentation, aggressive nature, and limited treatment options.[1] Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) could potentially cure a selected group of patients.[2, 3] About one third of patients is eligible to undergo this extensive treatment and the recurrence rate is high.[1] Currently available systemic therapies have limited efficacy, in the first-line as well as in second line or adjuvant setting.[4-7] Hence, there is a pressing need for new treatment options.

As PeM is a rare malignancy, it is challenging and extremely time-consuming to study these new treatment options, and to gather randomized evidence for treatment effectiveness. An effective approach could therefore be the off-label use of readily available targeted drugs. Currently, several trials are investigating such an approach, for example the Dutch Drug Rediscovery Protocol (DRUP) trial.[8] In this trial, patients with (solid) malignancies are treated with approved targeted agents using a personalized strategy by molecular profiling. A tailored approach is only feasible, however, if the tumor harbors actionable mutations to begin with.

Several studies reported on the mutational landscape of pleural mesothelioma (PM), the more common variant of mesothelioma, but studies on genetic alterations in PeM are scarce.[9, 10] Due to the rarity of PeM, most currently available therapies are extrapolated from PM. However, as these malignancies harbor important differences, like sex distribution, age of onset, and asbestos exposure, it is likely that these diseases also present distinct molecular features.[10-12] This scoping review aims to explore the genomic signature of PeM, and its potential therapeutic targets.

METHODS

Selection of literature

This scoping review was performed (where possible) according to the 'Preferred Reporting Items for Systematic Reviews and Meta-analysis Extension for Scoping Reviews' (PRISMA-ScR) statement.[13] A systematic search for available literature was performed in the following databases: Embase (i.e. pubmed), Medline, Web of Science Core Collection, Cochrane Central Register of Controlled Trials, and Google Scholar (100-top ranked). The full search term per database is provided in the

supplementary table 1. Databases were searched for articles published between the date of initiation and 1st October 2022.

For every record, title and abstract were screened by two independent reviewers (JvK and MD). Studies that reported on molecular alterations (i.e., gene mutations, gene fusions and gene copy number variants) in mesothelioma, detected by high throughput sequencing techniques, were selected for full text review. Animal studies, studies with cell lines, case reports, conference abstracts, papers without an available full (English) text, and studies that only included pleural or pericardial mesothelioma were excluded. Studies that only used RNA-sequencing, comparative genomic hybridization, or targeted DNA-sequencing of one specific gene were also excluded. In case of disagreement over studies to be included in this report, the study was discussed with a third reviewer (JvdT).

Data extraction and quality assessment

Due to a wide variety in methods used by different groups, meta-analyses were not considered feasible. The risk of bias was not assessed, due to the descriptive nature of the included reports. Data regarding the following patient characteristics was extracted from the included studies: sex, histology, tumor mutational burden (TMB), and gene alterations. Somatic, as well as germline mutations were included. If data were not reported in the original article, it was reported as unknown. The included studies used various sequencing methods and different gene panels. The proportion of altered genes was based on the total number of patients included in articles that specifically tested for a particular gene. Only likely pathogenic genetic alterations were included, i.e., single nucleotide variants (SNVs) in oncogenes or tumor suppressor genes (TSG), amplifications of oncogenes, oncogenic gene fusions, and complete loss of TSGs. Single copy number variations were not included. Genes were reported if they were altered in ≥ 1% of all patients and were investigated in at least 10% of the PeM cases. In addition, an overview of gene alterations (i.e., all types of alterations) present in ≥ 10% of PeMs that were investigated by whole exome (WES), or genome sequencing (WGS) was provided.

Identification of targeted therapies

Genes that were altered in at least 1% of the patients were selected for identification of potential currently available targeted therapies. The selection of these therapies was based on the currently approved targeted therapies for solid malignancies by the European Medicines Agency (EMA) and targeted therapies that are available via the DRUP trial (NCT02925234).[14, 15] To gain insight into possible future perspectives, genes that were altered in ≥ 10% of the PeM samples were selected. Clinical trials

investigating potential targeted therapies for these altered genes were identified with mycancergenome.org. Trials were selected in case they specifically included patients with solid tumors and alterations in one of the genes. Vaccine trials were excluded. Additional clinical trials specifically investigating targeted therapies in patients with PM were identified using ClinicalTrials.gov. A search was done for "Malignant Mesothelioma", with the filter 'Interventional studies'.

RESULTS

Our search retrieved 631 records, of which 558 were excluded based on title/abstract screening (**figure 1**). Full text screening was performed for 73 records. A total of 13 articles were selected based on the in- and exclusion criteria (**table**

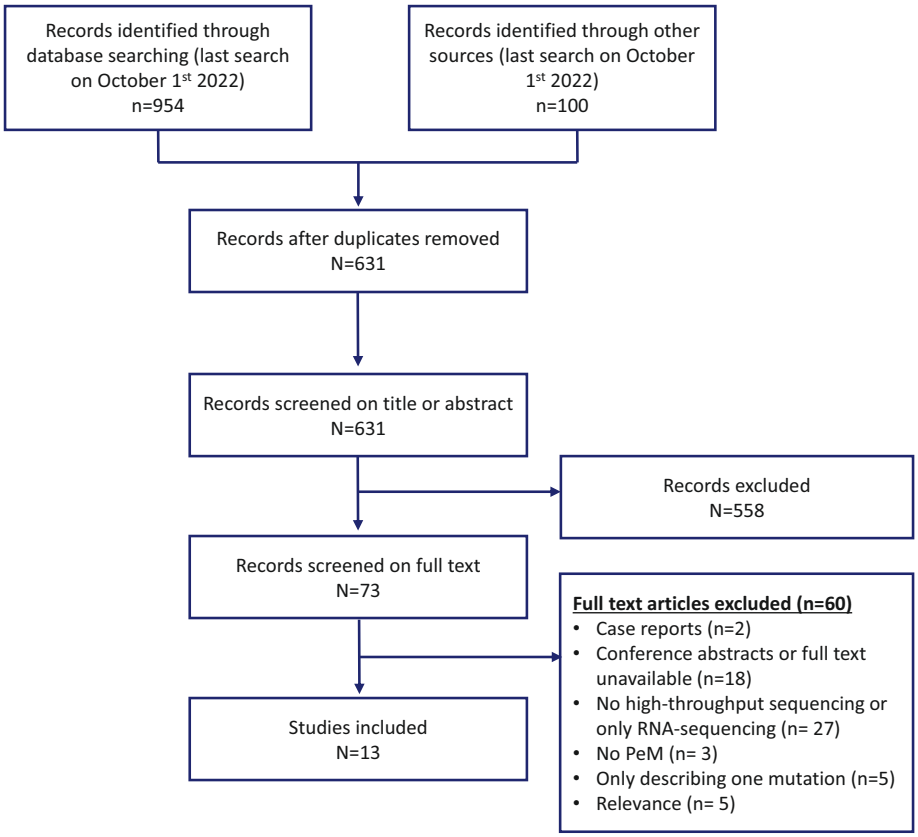

Figure 1. PRISMA flow diagram.

Table 1. Overview of the included articles.

	Reference	Year	N	Inclusion criteria	Sequencing method	Gene panel [a]	Type of samples	Origin of tumor material
1	**Sheffield et al.**	2015	2	NS	WGS	NA	FFPE tumor, blood	Diagnostic biopsy (n=2), resection (n=1)
2	**Alakus et al.**	2015	7	Epithelioid PeM patients undergoing CRS	WES	NA	FFPE tumor, blood	Resection
3	**Kato et al.**	2016	11	NS	NGS	Foundation Medicine	FFPE tumor	Unknown
4	**Ugurluer et al.**	2016	4	NS	NGS	Foundation Medicine	FFPE tumor	Unknown
5	**Joseph et al.**	2017	13	PeM limited to abdomen/ pelvis	NGS	UCSF500 Cancer	FFPE tumor, FFPE normal tissue	Unknown
6	**Panou et al.**	2018	10* 17	Unrelated mesothelioma patients with germline mutations NS	NGS NGS	Targeted [b] UCM-OncoPlus20,Foundation Medicine	FFPE tumor, blood, saliva FFPE tumor, blood, saliva	Unknown Unknown
7	**Kim et al.**	2018	4	PeM patients treated with first-line palliative chemotherapy	NGS	OncoPanel version 2	FFPE tumor	Unknown
8	**Shreshta et al.**	2019	18	Treatment-naïve PeM patients undergoing CRS	WES	NA	FFPE tumor, FFPE normal tissue or blood	Resection
9	**Hung et al.**	2020	26	NS	NGS	Targeted gene panel	FFPE tumor	Resection (n=21) or excisional biopsy (n=5)
10	**Taghizadeh et al.**	2020	3	Metastasized PeM refractory to standard treatment	NGS	Ion AmpliSeq Cancer Hotspot Panel v3	FFPE tumor	Unknown
11	**Offin et al.**	2021	50	NS	NGS	MSK-IMPACT platform	Tumor, blood	Unknown
12	**Dagogo-Jack et al.**	2022	314	Patients diagnosed with PeM	NGS	Foundation Medicine	FFPE tumor	Unknown
13	**Hiltbrunner et al.**	2022	355	NS	NGS	Foundation Medicine	FFPE tumor	Unknown

* Germline mutations

FFPE= Formalin-Fixed Paraffin-Embedded, PM= peritoneal mesothelioma, MSK-IMPACT= Memorial Sloan Kettering Cancer Center–IMPACT, NGS= next generation sequencing, NS= not specified, NA= not applicable,

WES= whole exome sequencing, WGS= whole genome sequencing.

1). Sequencing techniques that were used comprised (targeted-) next generation sequencing ((t-)NGS), whole exome sequencing (WES), and whole genome sequencing (WGS). Six out of 13 studies also analyzed blood, saliva, or normal tissue samples to identify germline mutations. The 13 included articles comprised 824 patients (**supplementary table 2**). Data regarding gender was available for 746 patients, of which 347 (47%) were male. For 268 patients the histology type was reported, which was epithelioid in 233 (87%) of the cases.

Gene alterations

A total of 52 genes (tested in at least 10% of the patients) harbored alterations in ≥ 1% of the patients (**figure 2**). Of these, the most commonly altered genes were *BAP1* (49%), *NF2* (25%), *CDKN2A* (23%), *CDKN2B* (17%), *PBRM1* (15%), *TP53* (14%), and *SETD2* (12%). These gene alterations were not mutually exclusive. Simultaneous gene alterations were common in *CDKN2A* and *CDKN2B,* as well as in *BAP1, PBRM1,* and *SETD2.* WES or WGS was performed in 27 patients. A total of 40 genes were mutated in ≥ 10% of these patients (i.e., ≥ 3 patients; **supplementary figure 1**). A complete overview of all genes that were altered in ≥ 1% of the patients is provided in **supplementary table 2**. Four studies reported on the tumor mutational burden (TMB). Shrestha et al. only reported the highest (0.04 mutations/Mb) and the lowest TMB (0.001 mutations/Mb).[23] Offin et al. and Dagogo et al. reported the median TMB for all patients with PeM, which were 1.8 mutations/Mb (range 0.0 – 14.9) and 1.25 mutations/Mb (IQR 0.00 – 1.25), respectively.[12, 26] Hiltbrunner et al. reported a high TMB (i.e. ≥10 mutations/Mb) in 5 patients (1.41%).[27] Seven out of 13 of the included articles also reported on pleural mesothelioma (PM). Table 3 provides an overview of the patient characteristics and the most common genomic alterations in PeM versus PM.

Germline mutations

Five out of 13 studies also reported on germline mutations specifically for PeM (**supplementary table 2**). Sheffield et al. (n=2) detected no germline mutations.[16] Alakus et al. (n=7) and Joseph et al. (n=13) both identified one patient with a germline *BAP1* mutation.[17, 20] Offin et al. performed germline testing for 30 out of 50 patients.[26] Three patients harbored a germline mutation: *POT1* (n=1), *MUTYH* (n=1), and *BAP1* (n=1). Panou et al. specifically screened unrelated mesothelioma patients for germline mutations.[21] Out of 44 patients with PeM, 11 (25%) harbored a germline mutation. BAP1 was the most frequently mutated gene in four patients (9%). Other mutated genes were *ATM, BRCA2, CDKN2A, CHEK2, MITF, SDHA*, and *WT1*, each reported in one patient. The patients with the *ATM* and *WT1* germline mutation also harbored a somatic *BAP1* mutation. One of the patients with a *BAP1* germline

mutation also harbored a *MITF* germline variant of unknown significance (VUS), and a somatic *BAP1* (possibly second hit) and *CSF1R* mutation. Lastly, a germline *BAP1* VUS was detected in one patient. In total, 96 patients underwent germline testing, of whom 16 (17%) harbored a germline mutation, with *BAP1* germline mutations in seven (7%) patients.

Targeted therapies

Currently there are no targeted therapies available for patients with PeM and one of the 52 identified genes. For 12 out of these genes (i.e., *ALK, BRCA2, CCND1, CDK4, CDK6, CDKN2A, CHEK2, GNAS, KRAS, MET, PIK3CA,* and *RAD50*) approved targeted therapies are available for other malignancies (**table 2**). For *BRAF* mutations (i.e., V600E) there are also targeted agents available, but the gene alterations reported in the current review consisted of copy number gains for which these targeted agents are not indicated. **Supplementary table 3** provides an overview of the targeted therapies and their approved indications or availability through the DRUP trial. Out of seven of the most commonly altered genes, for six genes, clinical trials were identified that investigate targeted agents for treatment of solid malignancies harboring alterations in these genes (**supplementary table 4**).

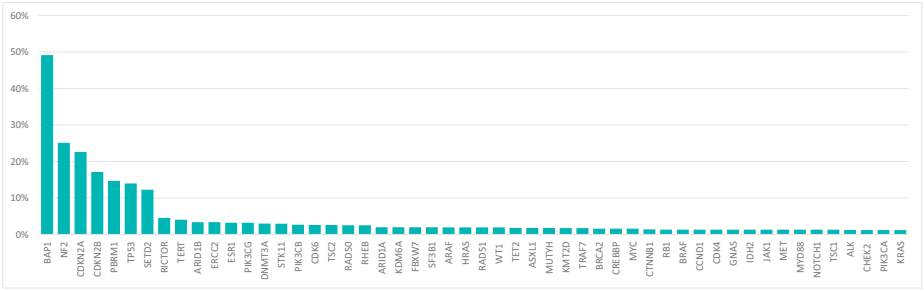

Figure 2. Gene aberrations present in ≥1% of the PeM patients

DISCUSSION

This scoping review aimed to provide an overview of the genomic landscape of PeM and its potential therapeutic targets, based on thirteen studies comprising 824 patients with PeM. This review identified multiple gene alterations, present in various proportions of patients with PeM, reflecting a heterogeneous mutational landscape. *BAP1* was the most commonly mutated gene (49%). Other commonly affected genes were *NF2* (25%), *CDKN2A* (23%), *CDKN2B* (17%), *PBRM1* (15%), *TP53* (14%), and *SETD2* (13%). Interestingly, out of 96 patients that underwent germline

Table 2. Genes with available targeted therapies for other malignancies.

Gene	Aberration	Frequency in PeM	Targeted therapies Type	Targeted drugs Drug
CDKN2A	Loss/mutation	23%	CDK4/6 inhibitors	Palbociclib Ribociclib
CDK6	Amplification	3%	CDK4/6 inhibitors	Abemaciclib Palbociclib
BRCA2	Loss/mutation	2%	PARP inhibitors	Rucaparib
CCND1	Amplification	2%	CDK4/6 inhibitors	Abemaciclib Palbociclib
ALK	Fusion	1%	ALK inhibitors	Alectinib Crizotinib Lorlatinib
CDK4	Amplification	1%	CDK4/6 inhibitors	Abemaciclib Palbociclib Ribociclib
CHEK2	Mutation	1%	PARP inhibitors	Olaparib Talazoparib
GNAS	Mutation	1%	MEK inhibitors	Trametinib
MET	Amplification Fusion	1%	Multi-targeted receptor tyrosine kinase inhibitors	Crizotinib Cabozantinib
PIK3CA	Mutation	1%	PI3K inhibitors	Alpelisib
KRAS	G12C mutation	1%	KRAS inhibitors	Sotorasib

testing, 16 (17%) were carriers of a germline mutation, mainly in *BAP1* (7%). Another significant proportion of cases might be caused by rarely occurring germline mutations in other genes. Germline mutations seem to be a larger contributor to the incidence of PeM than previously thought.

Mutational landscape of PeM

The most common alterations in PeM were detected in TSGs. Inactivation of TSGs appears to play an important role in PeM development. Despite the heterogeneous mutational landscape of PeM, several pathways seem to be predominantly involved in PeM etiology.

DNA damage response (DDR)

The DDR pathway is essential for genomic stability and defects in this pathway have been associated with the development of cancer. The current review shows that the DDR pathway also seems to be involved in PeM, which is in line with literature on mesothelioma.[28] In almost half of the patients with PeM, *BAP1* was altered. *BAP1* is involved in multiple processes, including DDR, and acts as a TSG by binding to BRCA1, another well-known TSG.[29] *BAP1* is located on chromosome 3p21, which is often lost in various malignancies.[30] Other TSGs located on this locus are *SETD2* and

PBRM1. Alterations of these genes were also frequently observed (12% and 15%, respectively) in the current review. Germline mutations in *BAP1* are known to cause a tumor predisposition syndrome, which is accompanied by the risk of early-onset of several malignancies, like (uveal) melanoma, renal cancer, and PeM.[31] Other DDR associated genes that were altered in PeM were *BRCA2, ERCC2,* and *RAD50/51,* all present in less than 5% of the samples.

Chromatin remodeling/DNA methylation

Chromatin remodeling and DNA methylation play an essential role in gene expression and alterations can contribute to the development of cancer. Epigenetic mechanisms also have an important function in the DDR, as reorganization of the chromatin structure is essential for DNA repair. The aforementioned BAP1 gene performs its function in the DDR by binding to BRCA1, but also functions as a deubiquitinating enzyme, regulating chromatin remodeling. Another essential component of chromatin-remodeling is the SWI/SNF complex.[32] In the current review several SWI/SNF subunit genes were reported as altered in PeM, including *PBRM1* (15%), *ARID1B* (3%), and *ARID1A* (2%). Other genes involved in epigenetic gene regulation that were mutated in PeM are *DNMT3A, KDM6A, TET2, ASXL1, KMT2D,* and *IDH2,* all present in 3% or less of the PeM tumors.

Cell cycle regulation

Another pathway that seems to contribute to PeM development concerns cell cycle regulation. A key-player in this pathway is *TP53,* a well-known TSG that encodes p53, and was mutated in 14% of the PeM samples. Other reported genes involved in cell cycle regulation are CDKN2A/B, CDK4/6, CCND1, CHEK2, and Rb1. CDKN2A was inactivated in 23% of PeM's and encodes for two tumor suppressor proteins, p16 and p14, which are both involved in the cell cycle regulation through inhibition of CDK4/6 and stabilization of p53.[33-35] Adjacent to CDKN2A lies CDKN2B, altered in 17% of cases, which encodes a cyclin-dependent kinase inhibitor (p15) that functions as a cell growth regulator that controls cell cycle G1 progression.[36] Inactivation of *TP53,* as well as *CDKN2A/B* are associated with a variety of malignancies.[33, 37]

Kinase signaling pathways

Kinase signaling pathways are pivotal in cell growth and survival, and have been associated with the development of several malignancies.[38, 39] One of these pathways is the PI3K/AKT/mTOR signaling pathway. Genes that are involved in this pathway are *PIK3CA, PIK3CB, PIK3CG, RICTOR,* and *TSC1/2*, present in 5% or less of PeM cases. *NF2* is a TSG that encodes for the Merlin protein and is mainly involved in de Hippo pathway, but also impacts mTOR signaling.[40] Alterations in *NF2* are known for

causing the familial cancer predisposition syndrome neurofibromatosis type 2, but have also been associated with sporadic malignancies including mesothelioma, breast, colorectal, and renal cancers.[41] *NF2* was altered in 25% of the PeMs, but no germline *NF2* mutations were observed. Lastly, the MAP kinase signaling pathway has been associated with a variety of tumors, but apparently does not play a major role in the development of PeMs as mutations in this pathway were less common (*HRAS* 2% and *KRAS* 1%).[42]

PeM versus PM

Because PeM and pleural mesothelioma (PM) are known to harbor differences in clinical characteristics, like sex distribution, age of onset, and relation to asbestosis exposure, it was hypothesized that these differences might be reflected by the mutational landscape.[10-12] Of the 13 included articles, seven also reported on molecular alterations in PM (**table 3**). Clinical characteristics between PeM and PM also seemed to differ in the studies that were included in the current review. Conform the large cohorts of Dagogo-Jack et al. and Hiltbrunner et al, the mutational landscapes of PM and PeM seems to be similar.[12, 27] However, lower prevalence of *CDKN2A/B* alterations were detected in PeM compared to PM, whereas PBRM1 alterations were more common in PeM. The frequency of *BAP1* mutations in PM of 44% is conform other studies reporting on the genetic landscape of PM and is similar to the 49% reported in patients with PeM is.[9, 12, 43, 44] Other frequently altered genes in PeM like *NF2* and *TP53* are also common in PM.[9, 12, 43, 44] Although rare, *ALK* rearrangements were reported in ten patients (1%) in the current review. This alteration seems to be more common in PeM, as very few cases of patients with PM with *ALK* rearrangements have been described.[12, 27, 45-47]

The current review showed that 17% of all patients with PeM who underwent germline testing harbored a germline mutation. Panou et al. was the only included study that also reported on germline mutations in PM (7%), but the proportion of germline mutations is conform other studies reporting on mesothelioma in general, ranging from 0 to 8%.[21, 48-51] This indicates that genetic predisposition plays a larger role in the development of PeM compared to PM. Subsequently, this might explain why the association between PeM and asbestosis exposure is less evident for PeM compared to the pleural variant, and hence contributes to the difference in sex distribution and age of onset. Several studies have highlighted these differences between PeM and PM, but the role of germline mutations in the etiology of PeM has been relatively underexposed.[52, 53] Further investigation should be done to unravel the role of germline mutations in PeM etiology.

Table 3. Patient characteristics and genomic alterations in peritoneal versus pleural mesothelioma.

	Peritoneal mesothelioma [a]	Pleural mesothelioma [b]
Median age range [c]	48 – 64	56 – 71
Male gender [d]	347 (47)	1490 (70)
Epithelioid histology [e]	233 (87)	2825 (71)
Germline mutations [f]	16 (17)	11 (7)
Altered genes		
BAP1	405 (49)	966 (44)
NF2	207 (25)	706 (32)
CDKN2A	186 (23)	1040 (48) [g]
CDKN2B	141 (17)	895 (42) [h]
PBRM1	120 (15)	145 (7) [i]
TP53	115 (14)	389 (18)
SETD2	101 (12)	217 (10)

[a] total patients with PeM included, n=824
[b] total patients with PM included, n=2178
[c] reported for 420 patients with PeM, reported for 999 patients with PM
[d] reported for 739 patients with PeM, reported for 2118 patients with PM
[e] reported for 268 patients with PeM, reported for 395 patients with PM

Targeted therapies

In the Netherlands, there are currently no approved targeted drugs for patients with PeM and one of the reported gene alterations. The loss and/or inactivation of TSGs appear to play an important role in PeM development. Targeting TSGs is known to be challenging and most of the currently available targeted drugs target oncogenes. In the last decades, the development of drugs targeting TSGs is increasing, resulting in potential new therapies for patients with PeM. The availability of these targeted therapies might be hampered by the rareness of PeM and its heterogeneous mutational landscape. Therefore, a "tailored approach" with the off-label use of approved targeted drugs might be an effective strategy. This is not only relevant for PeM, but applies to other (rare) malignancies and provided the rationale for several multi-drug trials like the Dutch DRUP trial (NCT02925234), the MATCH Screening Trial (NCT02465060) in the United States, the CAPTUR trial (NCT03297606) in Canada, and the ProTarget trial (NCT04341181) in Denmark.[54]

The Dutch DRUP trial consist of multiple arms, including one in which mesothelioma patients with *CDKN2A* loss or mutation (present in 23% of patients with PeM) were treated with ribociclib, a CDK4/6 inhibitor. Another arm included mesothelioma patients with a *PDGFRA* mutation, which, according to our data, has not been observed in PeM. Four of the trial arms include patients independent of tumor type and one of the reported alterations in PeM: olaparib (PARP inhibitor)

for alterations in DDR related genes (*BRCA2, CHEK2,* and *RAD50),* trametinib (BRAF inhibitor) for *GNAS* mutations, and alectinib (ALK inhibitor) for *ALK* fusions. These alterations were rare in this review (present in 3% or less). A British trial with a similar approach specific for mesothelioma patients, is the MiST trial (NCT03654833). This trial includes five treatment arms, including rucaparib (a PARP inhibitor) for patients with BRCA1/BAP1 deficient tumors and abemaciclib (a CDK4/6 inhibitor) in patients with p16ink4A-negative, *CDKN2A*-mutated tumors, which are more common in PeM. The first results of this trial showed that rucaparib and abemaciclib were both well tolerated and showed promising activity.[55, 56]

Hopefully, the outcomes of these multi-drug trials will support the rationale for a tailored approach resulting in more treatment options for patients with PeM. In addition to these multi-drug trials, several agents are available or under investigation for the treatment of other solid malignancies that target common genetic alterations in PeM (i.e., present in ≥ 10%). Below, a brief overview of targeted therapies that might be beneficial for PeM based on its molecular signature is provided (an overview of clinical trials is provided in **supplementary table 4**).

PARP inhibitors

One of the targeted therapies that have been introduced in the last decade are PARP inhibitors, of which olaparib was the first approved inhibitor.[57] PARP is involved in the DDR and inhibition of PARP results in the inability to correct DNA single strand breaks, leading to cell death in DDR deficient cells. Assuming that DDR deficiencies are an important contributor to PeM development, PARP inhibitors might be a promising therapy for patients with PeM. PARP inhibitors are currently approved by the EMA for the treatment of several solid malignancies, including breast and ovarian cancer, and mutations in DDR related genes like *BRCA1/2, ATM* and *CHEK2.* Due to the role of *BAP1* in DDR, it has been hypothesized that PARP inhibition might also be effective in the treatment of *BAP1* altered tumors.[29, 58] In a recently published trial, 23 patients with mesothelioma (i.e. 16 with PM and 7 with PeM) were treated with olaparib, independent of mutational status.[59] Unfortunately, olaparib had limited activity in patients with mesothelioma, including in patients with BAP1 mutations. The MiST trial on the other hand showed that rucaparib showed promising activity in patients with *BAP1* deficient mesothelioma.[55] Currently, a phase 2 trial is investigating the effect of olaparib in patients with mesothelioma and a *BAP1* mutation (NCT04515836). Several other trials are currently investigating the efficacy of PARP inhibitors (i.e., niraparib, olaparib, talazoparib, and veliparib) in other solid tumors with *BAP1* mutations.

EZH2 inhibitors

Another targeted therapy of interest is EZH2 inhibition due to its function in transcriptional activation and suppression of important TSGs. In mesothelioma, high expression of *EZH2* has been reported, especially in patients with loss of BAP1.[60] Tazemetostat is the first EZH2 inhibitor that received an orphan designation by the EMA. In a recently finished multicenter phase 2 trial, mesothelioma patients with loss of *BAP1* were treated with Tazemetostat.[61] This trial showed that this therapy was safe and antitumor activity was observed in more than half of the patients. Currently, a phase 1/2 trial is investigating another EZ2H inhibitor, CPI-0209, in patients with various malignancies with the loss of *BAP1,* including mesothelioma (NCT04104776).

CDK4/6 inhibitors

Due to the involvement of the cell cycle regulation pathway in PeM, another promising targeted therapy is CDK4/6 inhibition. Currently, CDK4/6 inhibitors (i.e., abemaciclib, palbociclib, and ribociclib) are approved by the EMA for the treatment of HR-positive and HER2-negative breast cancer, independent of mutational profile. In the DRUP trial, patients with CDK4/6 amplified tumors are treated with CDK4/6 inhibitors.[14] Although, CDK4/6 amplifications were rare in PeM (1 and 3%, respectively), these inhibitors might also be beneficial for the treatment of tumors with loss of *CDKN2A/B* (23% and 17% of PeM, respectively), as these encode for proteins that inhibit CDK4/6. Currently, many trials are investigating the efficacy of CDK4/6 inhibitors in patients with CDKN2A/B-altered tumors, either as monotherapy or in combination with other targeted therapies. Lastly, in the DRUP trial, treatment with CDK4/6 inhibitors is also provided to patients CCND1 amplifications, due to the interplay of CCND1 with CDK4.

Other targeted therapies

Another popular target gene is *ALK,* as it is the driver oncogene in approximately 5% of patients with non-small cell lung cancer (NSCLC).[62] Several ALK inhibitors have been approved for treatment of patients with NSCLC and *ALK* alterations, and there is evidence that these agents are beneficial for other *ALK*-rearranged malignancies.[63-65] For patients with mutations in *PIK3CA* and HR-positive and HER2-negative breast cancer, alpelisib (a PI3K inhibitor) is approved by the EMA and the DRUP trial provides this treatment for several other *PIK3CA* mutated-tumors (not for PeM).[14] For patients with *NF2, SETD2,* or *TP53* alterations (all present in ≥10% of PeM cases) there are currently no approved targeted therapies, independent of type of tumor. However, several clinical trials are investigating various drugs targeting these genes, which might result in new treatment options in the future.

Current clinical implications

The heterogeneous mutational landscape of PeM together with the limited treatment options provide a rationale for mutational analysis. Although there are currently no approved targeted therapies for patients with PeM, several therapies are available in a clinical trial setting and might become available for patients with PeM in the future. Comprehensive screening for genetic alterations might be considered to simultaneously test for high- as well as low-frequency altered genes, with limited additional costs. Although most of the currently approved drugs target genes which are rarely altered in PeM (for example *PIK3CA* and *ALK,* altered in 1%), these patients could gain substantial benefit from these therapies. As the availability of targeted agents changes over time, the indication of mutational analysis (i.e., broad spectrum or selective mutational analysis) should be regularly reconsidered.

Another approach could be to identify predictive factors for specific mutations to select patients with PeM that are most likely to harbor these alterations. For example, Hiltbrunner et al. identified subgroups of patients with mesothelioma according to gene alterations as some mutations do not appear to be mutually exclusive and often occur simultaneously.[27] This subgroup identification might not only be relevant for treatment purposes, but might also have prognostic value. Hiltbrunner et al. suggested that patients with CDKN2A alteration only, or patients with simultaneous *CDKN2A* and *BAP1* alterations had poor survival outcomes. Lastly, mutational analysis can not only be used for selection of targeted therapies, but can also be used for prediction of sensitivity to other therapies.[66-68] Several genetic alterations have been associated with sensitivity to specific chemotherapeutic drugs or immunotherapy. For example, due to its role in DDR, loss of *BAP1* might enhance response to platinum and pemetrexed chemotherapy.[51, 69] In addition, TMB has been shown to be a predictive biomarker for the response to immunotherapy.[70] TMB was low in most PeM tumors, which may indicate limited benefit of immunotherapy.[12, 23, 26, 27] However, recent studies using different techniques for TMB assessment, unraveled higher rates of genomic alterations in mesothelioma.[71, 72] The value of both mutational analysis and TMB assessment as predictive biomarkers for chemo- and immunotherapy need to be further investigated before they can be implemented in daily practice.

Lastly, these data provide a rationale for referral of patients with PeM to a clinical geneticist for germline testing, as germline mutations were present in a large proportion of patients (17%). Panou et al. reported that patients with mesothelioma and germline mutations were younger at the onset of disease, more often had a second cancer diagnosis, and had minimal known asbestos exposure.[21] This is

conform other studies reporting on germline mutations in patients with mesothelioma in general and resulted in a recent addition of advice on germline testing in the Dutch mesothelioma guidelines.[73, 74] However, further research should be done to assess the involvement of germline mutations in the time of onset of PeM specifically.

Limitations

This scoping review has some limitations, mainly due to the heterogeneity of the included studies and lack of relevant data. It is important to take into account that the included studies comprised various populations of patient with PeM (i.e., treatment-naïve patients versus patients treated with palliative chemotherapy or surgery). Another contributing factor to the heterogeneity is the difference in DNA sequencing methods. Targeted NGS studies, exploring a specific set of genes based on recurrently altered genes, cannot be directly compared to WGS studies covering the whole genome. To process data from high-throughput sequencing analyses, a set of bioinformatics algorithms, referred to as a bioinformatics pipeline framework, is needed. These bioinformatics pipeline frameworks are needed to process and analyze sequencing data to identify clinically relevant genetic alterations and often vary between studies, resulting in varying sensitivity to detect genomic alterations. The same applies to the measurement of TMB, for which bioinformatic algorithms are also known to strongly influence the results.[70]

Not all of the included studies provided full mutational data, hampering good interpretation. Some studies did not report on the clinical significance of the detected alterations and some studies were very limited in clinical data. The current review only included likely pathogenic gene alterations (including homozygous losses and amplifications of oncogenes), therefore single copy number variants were excluded. However, some studies only reported on the whether a copy number variation concerned a loss or a gain but did not report any details on the depth of losses (homozygous versus allelic loss) or number of extra copies.

CONCLUSIONS

This scoping review provides an overview of the genetic landscape of PeM and aimed to identify targeted therapies. Alterations in *BAP1* were most common and present in almost half of the patients. Up to 17% of patients were carrier of a germline mutation, most frequently in *BAP1,* which adds to the understanding of PeM etiology and provides a rationale for further research. Based on the molecular

signature of PeM, currently available targeted therapy options are very limited. However, clinical trials, as well as currently available targeted therapies for other malignancies were identified that might provide benefit to patients with PeM, supporting the rationale for molecular diagnostics.

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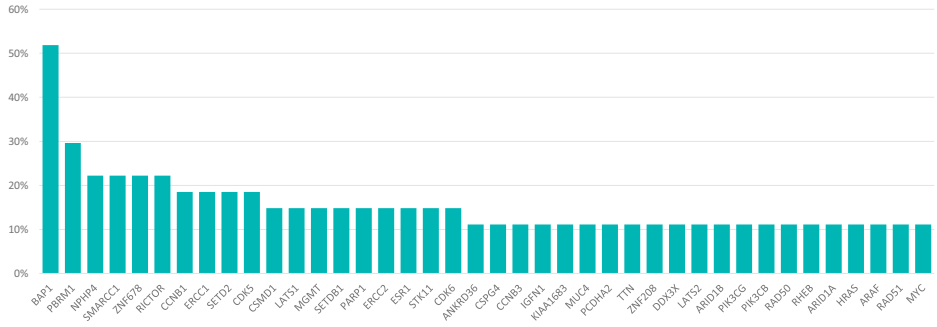
DATA SUPPLEMENT



Supplementary figure 1. Gene aberrations present in ≥10% of the PeM patients that underwent WGS/WES.

Supplementary tables 1-4 are available at https://doi.org/10.1016/j.esmoop.2023.101600.

Chapter 6

Genomic Characterization and Detection of Potential Therapeutic Targets for Peritoneal Mesothelioma

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ABSTRACT

Background

Peritoneal mesothelioma (PeM) is an aggressive tumor with limited treatment options. The current study aimed to evaluate the value of next generation sequencing (NGS) of PeM samples in current practice.

Methods

Foundation Medicine F1CDx NGS was performed on 20 tumor samples. This platform assesses 360 commonly somatically mutated genes in solid tumors and provides a genomic signature. Based on the detected mutations, potentially effective targeted therapies were identified.

Results

NGS was successful in 19 cases. Tumor mutational burden (TMB) was low in 10 cases and 11 cases were microsatellite stable. In the other cases, TMB and microsatellite status could not be determined. *BRCA1 associated protein 1* (*BAP1*) mutations were found in 32% of cases, *cyclin dependent kinase inhibitor 2A/B* (*CDKN2A/B*) and *neurofibromin 2* (*NF2*) mutations in 16%, and *ataxia-telangiectasia mutated serine/threonine kinase* (*ATM*) in 11%. Based on mutations in the latter two genes, potential targeted therapies are available for approximately a quarter of cases (i.e., protein kinase inhibitors for three *NF2* mutated tumors, and poly ADP-ribose polymerase (PARP) inhibitors for two *ATM* mutated tumors).

Conclusion

Extensive NGS analysis of PeM samples resulted in the identification of potentially effective targeted therapies for about one in four patients. Although these therapies are currently not available for patients with PeM, ongoing developments might result in new treatment options in the future.

INTRODUCTION

Peritoneal mesothelioma (PeM) is an aggressive tumor, arising from the peritoneum.[1] It comprises about ten to fifteen percent of all mesotheliomas, thereby being the second most common variant after pleural mesothelioma.[2] Due to its rarity and non-specific symptoms, it is often diagnosed at an advanced stage. Currently the best available treatment is a combination of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC).[3] Unfortunately, most patients experience disease recurrence, even after complete cytoreduction. Adding (neo)adjuvant systemic chemotherapy to the treatment does not result in improved disease-free, or overall, survival.[4], and only a small proportion of patients are eligible to undergo surgical treatment, while there is a lack of effective systemic treatment options.[5]

Because PeM is so rare, it is especially hard to gather (randomized) evidence on the effect of new therapeutics. The heterogeneity of the tumor further complicates this research. Personalized strategies, based on tumor molecular characteristics, could be promising.[6] One approach is to identify potentially targetable mutations, which can be treated with readily available therapies. However, data on the mutational landscape of PeM have long been lacking. Recently, several studies have been published that provide more insights in the mutational profile of PeM.[7-11] These data could aid to identify new treatment options for patients with PeM. Preferably, these treatments are already registered for the treatment of (other) cancers, but currently there are also clinical trials that include patients based on tumor molecular characteristics rather than cancer type or location.[12-14]

Foundation Medicine (FMI) offers a platform (Foundation One® CDx (F1CDx)) for next generation sequencing (NGS) of formalin fixed paraffin embedded (FFPE) tumor samples, which are often the only material available from diagnostic biopsies. The platform assesses a total of 360 genes that are known to be somatically mutated in solid tumors.[15] It also provides a genomic signature, by assessing tumor mutational burden (TMB) and microsatellite (in)stability (MSS/MSI). To evaluate the value of genomic characterization in patients with PeM in current daily practice, we performed broad targeted NGS on tumor biopsies from 20 patients who were referred to the Erasmus MC Cancer Institute from 2018 to 2021.

METHODS

Patient selection and data handling

From 2018 to 2021, 41 PeM patients were referred to the Erasmus MC Cancer Institute in Rotterdam, a Dutch mesothelioma expert center. From these 41 patients, we identified 23 patients for whom excess tumor tissue was available and who provided permission to use this tissue for research purposes. NGS by Foundation Medicine (FMI) F1CDx was available for 20 tumor samples. To maximize the chance of finding new significant mutations, we further selected the patients based on sex, age, and lack of asbestos exposure, thus enriching the cohort for females and younger patients.[16] All data were collected and managed according to the latest European privacy regulations (General Data Protection Regulation (GDPR), EU 2016/679). The study was approved by the EMC local ethics committee (MEC 2018-1286).

The Foundation One® CDx Assay

F1CDx uses DNA, acquired from FFPE tissue samples, for NGS of solid tumors. A comprehensive method description can be found in the technical information.[15] The assay is able to detect alterations in a total of 324 different genes, and another 36 introns of genes that are involved in rearrangements. Mutations in these genes and genetic rearrangements are known to occur in solid tumors and might be drive alterations for oncogenesis. Moreover, many of these mutations are susceptible to targeted therapies. A full list of included genes/rearrangements is rendered in the supplementary data (**supplementary table 1**). The assay also determines the genetic signature of the tumor, by providing microsatellite status (MSI), and tumor mutational burden (TMB). MSI status is determined by genome wide analysis of 95 microsatellite loci. The assay report that is provided by Foundation One® also includes suggested (targeted) therapies or clinical trials for individual patients, based on latest available clinical evidence and an up-to-date overview of current clinical trials that include patients based on certain mutations.

RESULTS

Patient and tumor characteristics

Broad targeted NGS on tumor biopsies from 20 individual patients was performed. Unfortunately, this resulted in one sample failure, leaving 19 samples to be fully analyzed. **Table 1** provides a comprehensive overview of patient and disease characteristics per patient. The patients included in the study had a median age of 54

years (IQR 48-63), and three (15%) were female. Epithelioid morphology was most common, observed in 18 patients (90%), while sarcomatoid and biphasic morphology were each present in one patient (5%), as determined by an experienced subspecialist pathologist (JT) by histological analysis of hematoxylin/eosin (H&E) stained sections of FFPE tissue. A minority of patients (40%) had been (occupationally) exposed to asbestos in the past. The median peritoneal cancer index (PCI), a measure used to determine the extent of peritoneal disease, was 39 (IQR 31 – 39).[17] Most patients (80%) presented with ascites at time of diagnosis and two patients (10%) had nodal dissemination. The Ki67 (or MIB) index reflects the percentage of proliferating cells and is a known prognostic indicator for PeM patients. Median Ki67 index was 8% (IQR 5-19%); while 11 tumors (58%) had a Ki67 index below 10% and eight tumors (42%) had a Ki67 index equal to or greater than 10%. Germline mutation analysis was performed in five out of 20 patients, of whom two patients were carrier of a BRCA associated protein 1 (BAP1) germline mutation.

Genomic signature

NGS data was available for 19 samples, as there was one sample failure (**table 1**). The TMB could not be determined in nine (47%) cases due to low tumor purity. In all of the remaining cases (n=10), TMB was low (defined as <10 mutations/Mb). Similar outcomes were observed for MSI, which could not be determined in eight (42%) cases and the remaining 11 tumors were microsatellite stable (MSS). In one patient, with a MSS tumor according to NGS, a frameshift mutation was detected in mutS homolog 6 (MSH6), encoding for the mismatch repair protein MSH6. Additional IHC for MMR proteins was performed on this sample, showing MLH-1, MSH-2, and PMS-2 proficiency and loss of MSH-6 (**supplementary figure 1**). No germline analysis was performed for this patient. The most commonly affected gene in this cohort was *BAP1*, with oncogenic mutations found in six out of 19 patients (32%). In two samples, a variant of unknown significance (VUS) was detected in *BAP1*. Both cyclin dependent kinase inhibitor 2A/B (*CDKN2A/B*) and neurofibromin 2 (*NF2*) harbored mutations in three (16%) tumors. Genes harboring oncogenic mutations in this cohort are depicted in **figure 1**. Besides *BAP1, CDKN2A/B*, and *NF2*: ataxia-telangiectasia mutated serine/threonine kinase (*ATM*), polybromo 1 (*PBRM1*), protein kinase C iota (*PRKCI*), telomerase reverse transcriptase (*TERT*), and tumor protein p53 (*TP53*) were aberrant in ≥10% of the sequenced tumors. **In table 2**, an overview of all affected genes is provided, including both significant mutations and VUS.

Table 1. Overview of patients and tumor characteristics.

Patient	Sex	Age at diagnosis	Histological subtype	Lymph node metastases	Ki-67 (%)	PD-L1 (%)	*BAP1* germline	BAP1 IHC	MTAP IHC	Tumor purity (%)
1	F	36	Epithelioid	No	3	UND	No	positive	positive	10,1
2	M	39	Epithelioid	No	4	UND	Yes	loss	positive	60,0
3	M	48	Epithelioid	No	10	UND	UND	loss	positive	48,7
4	M	51	Epithelioid	No	15	UND	No	positive	inconclusive	37,0
5	M	55	Epithelioid	No	7,5	5	UND	loss	UND	71,8
6	M	57	Epithelioid	No	2	UND	UND	positive	positive	57,7
7	M	61	Epithelioid	No	10	UND	UND	UND	UND	10,1
8	F	62	Epithelioid	No	4	UND	UND	loss	UND	28,5
9	F	63	Epithelioid	No	10	1	UND	UND	UND	62,6
10	M	41	Epithelioid	No	60	UND	No	positive	positive	13,8
11	M	40	Sarcomatoid	Yes	60	UND	UND	UND	UND	76,0
12	M	49	Epithelioid	No	5	2	Yes	UND	UND	46,0
13	M	52	Epithelioid	No	7,5	UND	UND	UND	UND	50,2

Gene alterations	VAF (%) [a]	TMB (muts/mb)	MS status	Mutations of unknown significance	Approved targeted therapies [b]	Targeted therapies investigated in clinical trials [c]
WT1 splice site 1340-1G>A	2,3	UND	UND	*AR, EP300, GRM3, LTK, NTRK1, PIK3C2B, SETD2*	None	None
BAP1 K61fs*11	77,5	4	MSS	*IRF2, NF1, NOTCH3, POLE, TBX3*	None	EZH2 inhibitors
BAP1 loss *PIK3CA* amplification *SOX2* amplification [#] *ATR* rearrangement exon 39 *EPHB1* amplification [#] *PBRM1* loss (exons 13-30) *PRKCI* amplification *TERT* promotor -124C>T	34,8	0	MSS	*CTNNA1, KMT2A, MAP3K13, PRKCI, RAR, TERC, TIPARP*	None	EZH2 inhibitors PARP inhibitors PI3K inhibitors mTOR inhibitors
NF2 Q212* *CDKN2A/B* loss	22,5	0	MSS	*ARID1A, ESR1, MDM4*	mTOR inhibitors	FAK inhibitors mTOR inhibitors CDK4/6 inhibitors Pan-ERBB inhibitors
FLT3 N841T [□] *PBRM1* rearrangement exon 26	1,5	0	MSS	*CXCR4, FANCA, HGF*	None	None
CDH1 R732Q [⊠] *MSH6* F1245fs*31 [d] *MUTYH* G382D *TP53* R175H [⊠] *TP53* R273C [⊠] *TP53* R158H [⊠] *TP53* R273H	2,6 62,8 47,9 1,1 1,1 1,4 26,6	1	MSS	*ALK, MSH3, ERRFI1, PPP2R2A, MDM4, ROS1, MEN1*	None	None
BAP1 splice site 554_580+12del39, Y33fs*1	5,1 5,6	UND	UND	*CSF1R, KDR, POLE*	None	EZH2 inhibitors
None		1	MSS	*BAP1, BRCA1, FANCA, KRAS, MAP3K1*	None	None
BAP1 loss *PRKC1* amplification [#] *TERC* amplification [#]		1	MSS	*IDH1, SDHA, ZNF703*	None	EZH2 inhibitors
TP53 R248W	2,9	UND	UND	*JAK2, KMT2A (MLL), MAP2K2 (MEK2), SETD2, TET2*	None	None
NF2 E463* *PTEN* loss (exons 4-9) *CDKN2A/B* loss *FAS* loss	69,3	UND	MSS	*ATM, SETD2, TSC2*	mTOR inhibitors	FAK inhibitors mTOR inhibitors CDK4/6 inhibitors Pan-ERBB inhibitors AKT inhibitors
BAP1 splice site *35_37*+2CAGGT>AGGG *TERT* promotor -124C>T	69,0 6,3	0	MSS	*CIC, KDM5A, MLL2, MYCI1, RICTOR, ZNF703*	None	EZH2 inhibitors
UND	UND	UND	UND	*UND*	UND	UND

Table 1. Overview of patients and tumor characteristics. (continued)

Patient	Sex	Age at diagnosis	Histological subtype	Lymph node metastases	Ki-67 (%)	PD-L1 (%)	*BAP1* germline	BAP1 IHC	MTAP IHC	Tumor purity (%)
14	M	51	Biphasic	No	20	UND	UND	UND	UND	10,0
15	M	58	Epithelioid	No	UND	UND	UND	UND	UND	20,0
16	M	64	Epithelioid	No	8	1	UND	UND	UND	20,0
17	M	76	Epithelioid	No	5	UND	UND	loss	positive	11,2
18	M	71	Epithelioid	No	7,5	UND	UND	UND	UND	26,2
19	M	53	Epithelioid	Yes	30	UND	UND	loss	positive	35,2
20	M	63	Biphasic	No	5	UND	UND	loss	positive	10,0

CPI= checkpoint inhibitor, IHC= immunohistochemistry, F= female, M= male, MSS= microsatellite stable, UND= undetermined, VAF= variant allele frequency,
[a] VAF is calculated as the number of variant reads divided by the number of reads covering the same location and the percentage is estimated based on tumor purity.
[b] Approved therapies in the European Union for other tumor types than mesothelioma
[c] Clinical trials that are investigating therapies that targeted genes that were found aberrant in the patient and in which patients with PeM could potentially participate
[d] Additional IHC for MMR proteins showed MLH-1, MSH-2, and PMS-2 proficiency and loss of MSH-6.
[#] Equivocal copy number alteration, i.e., sequencing data provide some, but not unambiguous, signal that the copy number exceeds the threshold for copy number events assigned to the relevant gene.
[¤] Subclonal copy number alteration, i.e. presence of the alteration in <10% of the assayed tumor DNA.
[¶] Sensitivity for the detection of copy number alterations was reduced due to low sample quality.

Variants of unknown significance

Besides known mutations involved in oncogenesis, the F1CDx analysis also provides a report of all VUSes. Variants in polymerase epsilon catalytic subunit (*POLE*), ROS proto-oncogene 1 receptor tyrosine kinase (*ROS1*), and zinc finger protein 703 (*ZNF703*) were determined to be a VUS in 15% of cases each. VUSes that were prevalent in ≥10% of cases were also included in **figure 1**. In two samples, a VUS in *BAP1* was detected, resulting in loss of BAP1 expression at IHC.

Gene alterations	VAF (%) [a]	TMB (muts/mb)	MS status	Mutations of unknown significance	Approved targeted therapies [b]	Targeted therapies investigated in clinical trials [c]
BARD1 L479fs*1 *CDK12* duplication exon 1	49,0	UND	UND	*ARID1A, FAM123B, HSD3B1, KDM5C, PBRM1, RAD51C, ROS1*	None	PARP inhibitors
NF2 L46fs*77	7,6	UND	UND	*BRCA2, FGFR3, INPP4B, MPL, PTCH1, ROS1*	mTOR inhibitors	FAK inhibitors mTOR inhibitors CDK4/6 inhibitors Pan-ERBB inhibitors
ATM E522fs*43	41,9	UND	UND	*BAP1, DNMT3A, ESR1, MYCN, NTRK1, POLE*	PARP inhibitors	ATR inhibitors PARP inhibitors
ATM V1729fs*20 *BAP1* rearrangement intron 10	10,1	UND	UND	*ABL1, MSH2, SMO*	PARP inhibitors	ATR inhibitors PARP inhibitors EZH2 inhibitors
SETD2 R2510fs*2 ¶	18,8	0	MSS	*ATM, DDR1, ERBB3, LTK, MUTYH, ZNF703*	None	None
CDKN2A loss *WHSC1* E1099K ¶	17,5	1	MSS	*CTNNB1, MLL2, PARP3, PIM1*	None	None
SF3B1 K700E	2,1	UND	UND	*ALOX12B, APC, CSF1R, mTOR, PDGFRA, SGK1, TEK*	None	None

Therapy recommendations

The analyses resulted in possible therapy recommendations for five patients (26%). All these recommendations were based on targeted therapies that were approved in the European Union for the treatment of other tumor types. None of these therapies is currently registered as a treatment for mesothelioma. For three (16%) patients with mutations in *NF2,* protein kinase inhibitor (PKI) therapy with either everolimus or temsirolimus could be of interest. For two (11%) other patients, therapy with poly ADP-ribose polymerase (PARP)-inhibitors might be effective, based on mutations of the *ATM* gene.

Clinical trials

For patients with mutations in genes for which currently no targeted therapy is available, participation in clinical trials might be beneficial. Based on the NGS data, ten (53%) cases were possibly eligible to participate in clinical trials, based on thirteen observed mutations. Tumors with inactivating mutations, or loss of *BAP1,* are possibly susceptible to treatment with enhancer of zeste homolog 2 (EZH2) inhibitors. This resulted in a clinical trial recommendation for six (30%) cases with such a mutation. Two (11%) patients with mutations in *ATM* were possibly eligible to participate in various phase 1 and 2 clinical trials investigating ATR serine/

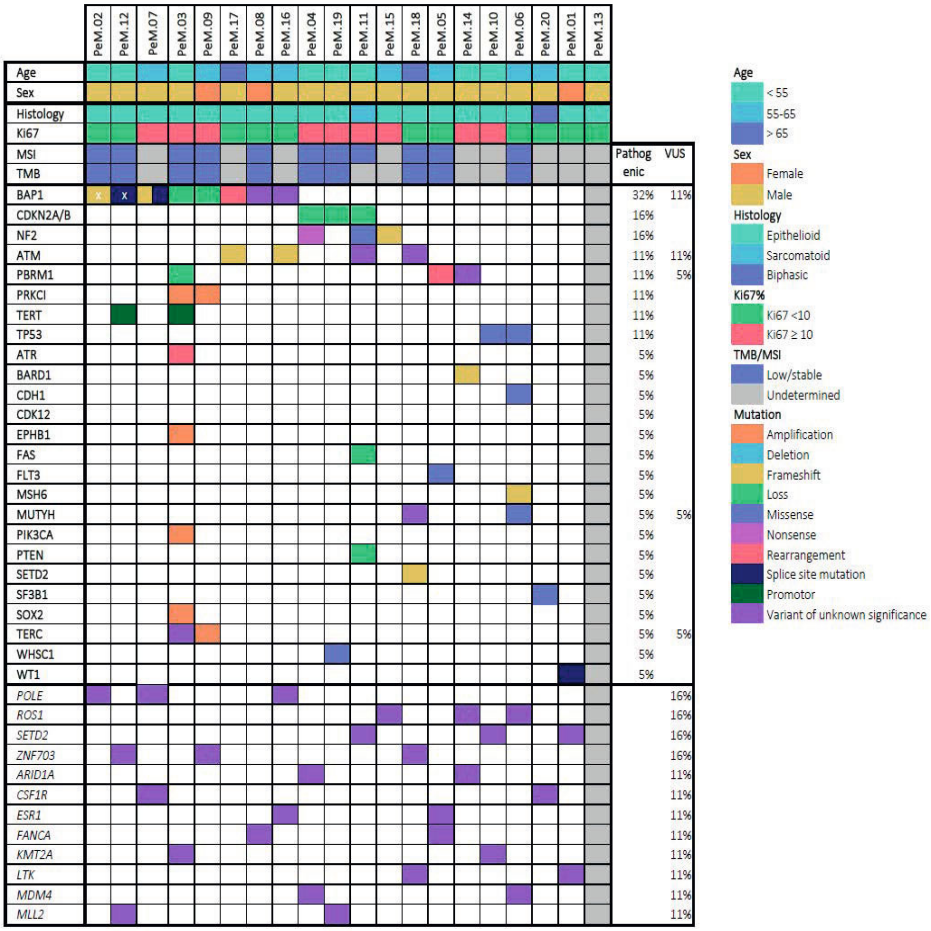

Figure 1. Mutational landscape of 20 peritoneal mesothelioma (PeM) cases.
x = *BAP1* germline mutation

threonine kinase (ATR) inhibitors, PARP inhibitors and/or DNA-dependent protein kinase catalytic subunit (DNA-PKcs) inhibitors. Another two (11%) patients were possibly eligible for participation in various clinical trials targeting focal adhesion kinase (FAK), programmed cell death 1 (PD1) and mammalian target of rapamycin complex 1/2 (mTORC1/C2) based on mutations in *NF2.* Mutations in *phosphatase and tensin homolog* (*PTEN)* and BRCA1 *associated ring domain 1* (*BARD1*) resulted in similar recommendations, involving among others PARP and immune checkpoint inhibition. It should be noted that none of the patients in the current cohort participated in any of these trials, as these trials were not conducted in The Netherlands.

Table 2. Overview of mutated genes, number and percentage of affected cases, percentage of VUS.

Gene	N[a]	%	VUS	%	Gene	N[a]	%	VUS	%	Gene	N[a]	%	VUS	%	Gene	N[a]	%	VUS	%	Gene	N[a]	%	VUS	%
BAP1	6	32%	2	11%	*SF3B1*	1	5%	2	11%	*AR*	0	0%	1	5%	*JAK2*	0	0%	1	5%	*PIK3C2B*	0	0%	1	5%
CDKN2A/B	3	16%	0	0%	*SOX2*	1	5%	0	0%	*BRCA1*	0	0%	1	5%	*KDM5A*	0	0%	1	5%	*PIM1*	0	0%	1	5%
NF2	3	16%	0	0%	*TERC*	1	5%	0	0%	*BRCA2*	0	0%	1	5%	*KDM5C*	0	0%	1	5%	*PPP2R2A*	0	0%	1	5%
ATM	2	11%	2	11%	*WHSC1*	1	5%	1	5%	*CIC*	0	0%	1	5%	*KDR*	0	0%	1	5%	*PTCH1*	0	0%	1	5%
PBRM1	2	11%	1	5%	*WT1*	1	5%	1	5%	*CTNNA1*	0	0%	1	5%	*KRAS*	0	0%	1	5%	*RAD51C*	0	0%	1	5%
PRKCI	2	11%	1	5%	*POLE*	0	0%	3	16%	*CTNNB1*	0	0%	1	5%	*MAP2K2 (MEK2)*	0	0%	1	5%	*RAR*	0	0%	1	5%
TERT[b]	2	11%	2	11%	*ROS1*	0	0%	3	16%	*CXCR4*	0	0%	1	5%	*MAP3K1*	0	0%	1	5%	*RICTOR*	0	0%	1	5%
TP53	2	11%	0	0%	*ZNF703*	0	0%	3	16%	*DDR1*	0	0%	1	5%	*MAP3K13*	0	0%	1	5%	*SDHa*	0	0%	1	5%
ATR	1	5%	0	0%	*ARID1A*	0	0%	2	11%	*DNMT3A*	0	0%	1	5%	*MEN1*	0	0%	1	5%	*SGK1*	0	0%	1	5%
BARD1	1	5%	0	0%	*CSF1R*	0	0%	2	11%	*EP300*	0	0%	1	5%	*MPL*	0	0%	1	5%	*SMO*	0	0%	1	5%
CDH1	1	5%	0	0%	*ESR1*	0	0%	2	11%	*ERBB3*	0	0%	1	5%	*MSH2*	0	0%	1	5%	*TBX3*	0	0%	1	5%
CDK12	1	5%	0	0%	*FANCA*	0	0%	2	11%	*ERRFI1*	0	0%	1	5%	*MSH3*	0	0%	1	5%	*TEK*	0	0%	1	5%
EPHB1	1	5%	0	0%	*KMT2A*	0	0%	2	11%	*FAM123B*	0	0%	1	5%	*mTOR*	0	0%	1	5%	*TERC*	0	0%	1	5%
FAS	1	5%	0	0%	*LTK*	0	0%	2	11%	*FGFR3*	0	0%	1	5%	*MYCI1*	0	0%	1	5%	*TET2*	0	0%	1	5%
FLT3	1	5%	0	0%	*MDM4*	0	0%	2	11%	*GRM3*	0	0%	1	5%	*MYCN*	0	0%	1	5%	*TIPARP*	0	0%	1	5%
MSH6	1	5%	0	0%	*MLL2*	0	0%	2	11%	*HGF*	0	0%	1	5%	*NF1*	0	0%	1	5%	*TSC2*	1	5%	2	11%
MUTYH	1	5%	1	5%	*ABL1*	0	0%	1	5%	*HSD3B1*	0	0%	1	5%	*NOTCH3*	0	0%	1	5%					
PIK3CA	1	5%	0	0%	*ALK*	0	0%	1	5%	*IDH1*	0	0%	1	5%	*NTRK1*	0	0%	1	5%					
PTEN	1	5%	0	0%	*ALOX12B*	0	0%	1	5%	*INPP4B*	0	0%	1	5%	*PARP3*	0	0%	1	5%					
SETD2	1	5%	3	16%	*APC*	0	0%	1	5%	*IRF2*	0	0%	1	5%	*PDGFRA*	0	0%	1	5%					

[a] Known oncogenic mutations
[b] Promotor mutation

DISCUSSION

The lack of effective treatments for peritoneal mesothelioma (PeM) makes it interesting to explore the use of targeted therapies that might benefit these patients. Although also rare, pleural mesothelioma is relatively more common and treatment strategies for PeM are commonly derived from the pleural variant. Recently, large cohorts of both pleural and PeM have provided more insights in their mutational profiles and provided possible targets or therapies.[7-11, 18] The mutational profile of the current study cohort is comparable to the TCGA pleural mesothelioma cohort, which is in line with the large cohorts of Hiltbrunner et al. and Dagogo-Jack et al.[10, 11, 19]

To evaluate the value of broad NGS in patients with PeM in current practice, we performed broad targeted NGS on tumor biopsies from 20 individual PeM patients. Based on the molecular signature of these tumors, for about one in four patients, potentially effective targeted therapies are available. It should be noted that these targeted treatments have so far not been proven effective against mesothelioma (pleural or peritoneal). Therefore, the value of NGS in the current practice for these patients seems limited.

We did identify some clinical trials in which patients with PeM could potentially participate. There are also numerous ongoing trials in other tumor types that are investigating targeted therapies that might be beneficial for patients in our cohort based on the detected aberrations. As new targeted treatments, as well as combination therapies, are being continuously investigated, molecular characterization of individual patient tumors will be increasingly relevant in the future. Below, we reviewed biomarkers generated by NGS that could predict response to certain treatments and the most frequently mutated genes (i.e., oncogenic mutations in ≥10% of cases) in the current cohort, for which targeted therapies are currently available.

TMB and MSI status

TMB was low and tumors were MSS in all cases for which this could be determined. For one patient in our cohort a MSH6 deficiency was reported. As MSI is a result of a deficient DNA MMR system, MSH6 deficient tumors are per definition MSI. Nonetheless, this tumor was reported as MSS by molecular MSI analysis. Several studies have indicated that molecular MSI analysis has lower sensitivity for MMR deficiency (dMMR) detection compared to IHC, which might be dependent on the origin of the primary tumor; hence, the value of molecular MSI analysis to detect

dMMR tumors remains a subject of debate.[20, 21] Likewise, molecular MSI, but also TMB analysis, requires samples with sufficient tumor purity. Low tumor purity is an important challenge to these analyses in daily practice. Panel-based TMB estimation by targeted NGS has been proposed to result in a better estimate of the TMB, compared to the general method of measuring the TMB with the whole exome.[22]. Moreover, increasing tumor purity by microdissection is valuable, but unfortunately not possible for send-out FMI tests.

Though MSI and TMB status could not be determined for eight and nine cases, respectively, it is likely that TMB and MSI are mostly low or absent in PeM. Arulananda and colleagues could not identify a single patient with MSI in a cohort of 335 patients with pleural mesothelioma, performed by IHC.[23] There are some studies that reported MSI in patients with mesothelioma, but these cases are rare.[10, 24] With regard to TMB, several studies reported low TMB in the majority of mesothelioma cases (both pleural and peritoneal).[10, 11, 25] As both MSI and high-TMB tumors are associated with a good response to immune checkpoint inhibition (CPI) therapy, one might expect that these therapies are ineffective against mesothelioma.[26] Indeed, the recent checkmate 743 study by Baas et. al showed only modest responses to combination CPI therapy with nivolumab (anti-PD1) and ipilimumab (anti-CTLA4) as a first line treatment for pleural mesothelioma, although long term responders were established.[27] Hence, it is questionable whether MSI and TMB are optimal biomarkers to predict response to CPI.

BAP1

BAP1 is the most frequently mutated gene found in mesothelioma (pleural and peritoneal), with about 30-50% of cases harboring somatic mutations.(AACR GENIE and COSMIC, February 2022)[28, 29] Also, a significant proportion of PeM patients might be affected by the so-called 'BAP1 tumor predisposition syndrome' (BAP1-TPDS), as they are carriers of a germline *BAP1* mutation.[30] Besides a predisposition for mesothelioma, these patients are also commonly affected by BAP1-inactivated melanocytic tumors, uveal melanoma, cutaneous melanoma and renal cell carcinoma.[31] In line with other studies, we found oncogenic *BAP1* mutations in 32% of tumors in the current cohort, of which two patients were known carriers of a *BAP1* germline mutation.[7, 10, 11] *BAP1* encodes for the tumor suppressor protein 'ubiquitin carboxyl-terminal hydrolase', which plays a role in several cellular processes involved in oncogenesis.[32] Though there are currently no treatments directly targeting BAP1, there are therapies targeting molecular pathways in which BAP1 is involved. BAP1 is associated with BRCA1 activation, thereby playing a key role in homologous recombination repair (HRR).[32-34] Similar to *ATM* deficient tumors, *BAP1*

and *BRCA1* deficient tumors might be susceptible to PARP inhibition and promising results have been reported in a phase 2 clinical trial.[35] However, in vitro results of sensitivity to PARP inhibition and its relationship to *BAP1* status are inconsistent.[36-38] Another potential target is EZH2, which is upregulated in BAP1 deficient tumors. A preclinical showed increased sensitivity to EZH2 inhibition in BAP1 deficient mice.[39] A phase 2 trial including 74 patients with BAP1 deficient mesothelioma treated patients with PeM with the EZH2 inhibitor tazemetostat as a monotherapy.[40] A disease control rate of 51% at twelve weeks and 25% at 24 weeks was reported, but no complete and only two partial responses were observed. Due to its involvement in HRR, *BAP1* has also been studied as a biomarker for response to chemotherapy. Wildtype *BAP1* has been associated with sensitivity to gemcitabine treatment in mesothelioma cell lines, but this has not been confirmed in patients with PeM.[41, 42]

NF2

Based on several mutations in *NF2*, protein kinase inhibitors everolimus and temsirolimus could be a potential treatment option for 16% of patients in our cohort. *NF2* is a tumor suppressor gene that plays an important role in cell proliferation and survival.[43, 44] *NF2* is involved in the mammalian target of rapamycin (mTOR) signaling pathway. Inactivating mutations of *NF2* lead to cell cycle progression and cell proliferation.[45, 46] *NF2* mutations are reported by previous studies in around 25% of cases of PeM.[10, 11] Some clinical studies and some preclinical evidence suggests that *NF2* inactivation might be associated with response to mTOR inhibitors.[47, 48] Everolimus and temsirolimus are both mTOR inhibitors and have been approved by the FDA for the treatment of neuroendocrine tumors of the gastro-intestinal tract or lung, HER2/neu-negative breast cancer and renal cell carcinoma, among others. A phase 2 study in pleural mesothelioma only showed a 2% response rate to everolimus.[49] This study, however, did not stratify patients based on mutational status. Considering that only about 15% of mesothelioma cases show mutations in *NF2*, the response rate might be higher when only these patients are included. However, some studies suggest that combination treatment might be indicated.[50, 51]

ATM

Mutations in *ATM* were present in two patients in our cohort (11%), but were reported in only 2% of the patients in the large cohort of Hiltbrunner et al.[9] Although rare, patients with PeM and mutations in *ATM* could benefit from treatment with PARP inhibitors. *ATM* is located on chromosome 11, and codes for the ATM serine/threonine kinase protein. This protein plays a role in the HRR pathway, among others by p53 activation, which has an important role in cell cycle arrest and apoptosis.[41] Mateo et al. found that deleterious *ATM* mutations in metastatic prostate cancer

were associated with good response to olaparib, a PARP inhibitor that is approved for the treatment of several solid tumors in the European Union.[32, 42] However, the same group found no survival benefit for castration resistant prostate cancer patients, but these findings were the result of an underpowered interim analysis.[43] For other malignancies, such as gastric-cancer and renal cell carcinoma, similar relations between *ATM* mutations and response to PARP inhibition have been reported.[44, 45] Fennell et al. performed a phase 2 trial, treating 26 mesothelioma patients (*25 pleural, 1 peritoneal*) with the PARP inhibitor rucaparib after at least one cycle of systemic chemotherapy. They found a disease control rate of 58% at twelve weeks and 23% at 24 weeks, while toxicity was limited.[46] They selected patients with BAP1 and/or BRCA1 deficient tumors, other key proteins in HRR. HRR deficient tumors, such as ATM inactivated tumors, might have similar responses to PARP inhibition.

Strengths and limitations

The main strength of this study is the in-depth analysis of PeM molecular characteristics and the evaluation of its value in current daily practice. The current study provides more comprehensive data compared with recently published studies reporting on larger cohorts, which can be valuable for the guidance of future treatment strategies.[8, 9] Though our cohort only included 20 patients, with successful NGS in 19, PeM is such a rare tumor that data of its molecular characteristics remains valuable.

There are some limitations to the current study. As NGS was available for only 20 samples, we selected those patients that were most likely to harbor relevant mutations, resulting in selection bias. In addition, NGS requires sufficient amount of high-quality DNA. For NGS, FMI does not perform any tumor purification, requiring high-quality samples and resulting in a lower sensitivity for the detection of mutations. Selection of high-quality samples might also have resulted in selection bias. Despite this selection, there was one sample failure and TMB/MSI could not be determined in approximately half of the patients due to low tumor purity. This underlines the challenges of NGS in current daily practice, as the success of NGS highly depends on the sample quality and quantity. Despite low tumor purity, we were able to detect relevant mutations in the majority of patients. As the value of TMB/MSI in the treatment of patients with PeM seems limited, low tumor purity might not pose a serious problem in this patient population. Though not a limitation of the current study, another important factor to take into consideration with the interpretation of NGS data is tumor heterogeneity. Tumor heterogeneity results in the possibility of an unrepresentative tumor biopsy, which can be especially

relevant in guiding possible treatment choices. Likewise, NGS often identifies variants of unknown significance (VUS), which have no clear clinical implications (yet). For example, one patient in our cohort [8] had a VUS in BAP1, but also showed loss of BAP1 on IHC, making it likely that this is actually a pathogenic mutation. Ongoing research will probably identify the nature of these mutations in the future.

CONCLUSIONS

The value of genomic characterization of PeM tumor samples in daily practice in the Netherlands is currently limited. NGS poses several practical challenges and effective targeted therapies are limited. For about one in four patients in our cohort, NGS resulted in the identification of potentially effective targeted therapies that are currently available for other tumor types than PeM. Ongoing developments in targeted therapies will result in new treatment options, making genomic characterization increasingly relevant in the future.

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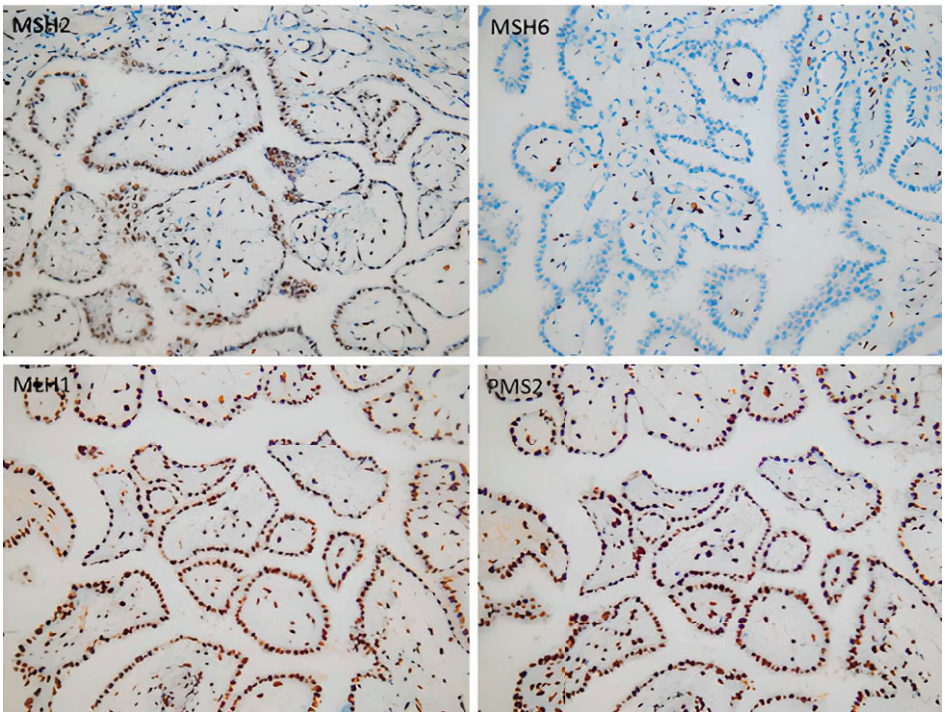
Supplementary table 1. Genes included in FoundationOne CDx panel.

ABL1	*BRAF*	*CDKN1A*	*EPHA3*	*FGFR4*	*IKZF1*	*MCL1*	*NKX2-1*	*PMS2*	*RNF43*	*TET2*
ACVR1B	*BRCA1*	*CDKN1B*	*EPHB1*	*FH*	*INPP4B*	*MDM2*	*NOTCH1*	*POLD1*	*ROS1*	*TGFBR2*
AKT1	*BRCA2*	*CDKN2A*	*EPHB4*	*FLCN*	*IRF2*	*MDM4*	*NOTCH2*	*POLE*	*RPTOR*	*TIPARP*
AKT2	*BRD4*	*CDKN2B*	*ERBB2*	*FLT1*	*IRF4*	*MED12*	*NOTCH3*	*PPARG*	*SDHA*	*TNFAIP3*
AKT3	*BRIP1*	*CDKN2C*	*ERBB3*	*FLT3*	*IRS2*	*MEF2B*	*NPM1*	*PPP2R1A*	*SDHB*	*TNFRSF14*
ALK	*BTG1*	*CEBPA*	*ERBB4*	*FOXL2*	*JAK1*	*MEN1*	*NRAS*	*PPP2R2A*	*SDHC*	*TP53*
ALOX12B	*BTG2*	*CHEK1*	*ERCC4*	*FUBP1*	*JAK2*	*MERTK*	*NT5C2*	*PRDM1*	*SDHD*	*TSC1*
AMER1	*BTK*	*CHEK2*	*ERG*	*GABRA6*	*JAK3*	*MET*	*NTRK1*	*PRKAR1A*	*SETD2*	*TSC2*
APC	*C11orf30*	*CIC*	*ERRFI1*	*GATA3*	*JUN*	*MITF*	*NTRK2*	*PRKCI*	*SF3B1*	*TYRO3*
AR	*CALR*	*CREBBP*	*ESR1*	*GATA4*	*KDM5A*	*MKNK1*	*NTRK3*	*PTCH1*	*SGK1*	*U2AF1*
ARAF	*CARD11*	*CRKL*	*EZH2*	*GATA6*	*KDM5C*	*MLH1*	*P2RY8*	*PTEN*	*SMAD2*	*VEGFA*
ARFRP1	*CASP8*	*CSF1R*	*FAM46C*	*GID4 (C17orf39)*	*KDM6A*	*MPL*	*PALB2*	*PTPN11*	*SMAD4*	*VHL*
ARID1A	*CBFB*	*CSF3R*	*FANCA*	*GNA11*	*KDR*	*MRE11A*	*PARK2*	*PTPRO*	*SMARCA4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARCB1*	*WHSC1L1*
ATM	*CCND1*	*CTNNA1*	*FANCG*	*GNAQ*	*KEL*	*MSH3*	*PARP2*	*RAC1*	*SMO*	*WT1*
ATR	*CCND2*	*CTNNB1*	*FANCL*	*GNAS*	*KIT*	*MSH6*	*PARP3*	*RAD21*	*SNCAIP*	*XPO1*
ATRX	*CCND3*	*CUL3*	*FAS*	*GRM3*	*KLHL6*	*MST1R*	*PAX5*	*RAD51*	*SOCS1*	*XRCC2*
AURKA	*CCNE1*	*CUL4A*	*FBXW7*	*GSK3B*	*KMT2A (MLL)*	*MTAP*	*PBRM1*	*RAD51B*	*SOX2*	*ZNF217*
AURKB	*CD22*	*CXCR4*	*FGF10*	*H3F3A*	*KMT2D (MLL2)*	*MTOR*	*PDCD1*	*RAD51C*	*SOX9*	*ZNF703*
AXIN1	*CD274*	*CYP17A1*	*FGF12*	*HDAC1*	*KRAS*	*MUTYH*	*PDCD1LG2*	*RAD51D*	*SPEN*	
AXL	*CD70*	*DAXX*	*FGF14*	*HGF*	*LTK*	*MYC*	*PDGFRA*	*RAD52*	*SPOP*	
BAP1	*CD79A*	*DDR1*	*FGF19*	*HNF1A*	*LYN*	*MYCL*	*PDGFRB*	*RAD54L*	*SRC*	
BARD1	*CD79B*	*DDR2*	*FGF23*	*HRAS*	*MAF*	*MYCN*	*PDK1*	*RAF1*	*STAG2*	
BCL2	*CDC73*	*DIS3*	*FGF3*	*HSD3B1*	*MAP2K1*	*MYD88*	*PIK3C2B*	*RARA*	*STAT3*	
BCL2L1	*CDH1*	*DNMT3A*	*FGF4*	*ID3*	*MAP2K2*	*NBN*	*PIK3C2G*	*RB1*	*STK11*	
BCL2L2	*CDK2*	*DOT1L*	*FGF6*	*IDH1*	*MAP2K4*	*NF1*	*PIK3CA*	*RBM10*	*SUFU*	
BCL6	*CDK4*	*EED*	*FGFR1*	*IDH2*	*MAP3K1*	*NF2*	*PIK3CB*	*REL*	*SYK*	
BCOR	*CDK6*	*EGFR*	*FGFR2*	*IGF1R*	*MAP3K13*	*NFE2L2*	*PIK3R1*	*RET*	*TBX3*	
BCORL1	*CDK8*	*EP300*	*FGFR3*	*IKBKE*	*MAPK1*	*NFKBIA*	*PIM1*	*RICTOR*	*TEK*	



Supplementary figure 1. Immunohistochemical staining for MSH-2, MSH-6, MLH-1, and PMS-2.

Part II

Colorectal Peritoneal Metastases

Chapter 7

The Impact of Low Skeletal Muscle Mass on Short and Long Term Outcomes after Cytoreductive Surgery and Hyperthermic Intrapertioneal Chemotherapy

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ABSTRACT

Background

Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) is a potentially curative treatment for peritoneal metastases (PM) from colorectal cancer (CRC) or pseudomyxoma peritonei (PMP). Because of the considerable morbidity of this treatment, optimal patient selection is key. This study aimed to assess the impact of low skeletal muscle mass (SMM) on outcomes after CRS-HIPEC.

Methods

Patients who underwent CRS-HIPEC between 2014 and 2020 at a tertiary center, were included. SMM was measured on computed tomography by means of the L3 muscle index. Postoperative complications and survival outcomes were compared between groups by use of logistic regression and Kaplan-Meier survival analyses.

Results

Of 284 included patients, 149 had low SMM. Occurrence of severe postoperative complications did not differ between groups (28.9% for patients with low versus 34.1% for patients with normal SMM). Low SMM was not associated with postoperative complications (p=0.344). For CRC patients, no significant differences were observed in disease-free (DFS) or overall survival (OS) between patients with low (median DFS 7 months [IQR 4 – 14], median OS 33 months [IQR 14 – NR]) and patients with normal SMM (median DFS 8 months [IQR 5 – 20], median OS 35 months [IQR 18 – NR]). Regarding PMP, survival outcomes did not significantly differ between groups (3-year DFS 47.3% for patients with low SMM versus 54.5% for patients with normal SMM, p=0.676; 3-year OS 70.8% versus 90.9% respectively, p=0.172).

Conclusion

Low SMM could not be identified as a predictor of severe complications or survival outcomes after CRS-HIPEC.

INTRODUCTION

Cytoreductive surgery (CRS) combined with intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC) is considered to be a potentially curative treatment for selected patients with peritoneal metastases (PM) from colorectal carcinoma (CRC) or pseudomyxoma peritonei (PMP). This extensive surgical treatment significantly improves the survival of CRC patients compared to systemic chemotherapy, resulting in 5-year survival rates of up to 40%.[1-5] For PMP patients CRS-HIPEC is considered the golden standard with 5-year survival rates of 74%.[6] Despite these improvements in survival, CRS-HIPEC is associated with considerable postoperative morbidity. Severe postoperative complications are reported in approximately 30% of the patients.[4, 7, 8] Previous studies have shown an association between the occurrence of postoperative complications and impaired survival outcomes.[6, 9, 10] Hence, for this select patient population, it is of great importance to identify risk factors for postoperative outcomes that could aid in preoperative patient selection.

With the increasing emphasis on prehabilitation in cancer surgery, potential risk factors associated with the nutritional status are widely investigated. As the number of obese cancer patients is rising, the utility of factors like BMI and weight loss is under debate.[11] A potential risk factor of interest is sarcopenia, which is mainly determined by the loss of skeletal muscle mass (SMM) and can easily be remained unnoticed in obese patients. Several studies showed that SMM was an independent predictor of outcomes after colorectal cancer surgery, and was better in predicting outcomes than other factors representing the patients' nutritional status, like BMI and albumin.[11-16] A few studies report on the impact of SMM in patients undergoing CRS-HIPEC for PM from CRC or PMP and conflicting results have been published.[17-20]

The aim of this retrospective study is to identify the impact of low SMM on postoperative outcomes after CRS-HIPEC for these patients. The hypothesis is that low SMM can be a valid predictor of severe postoperative complications and impaired survival outcomes. Hence, preoperative SMM measurement can potentially aid in preoperative patient selection.

METHODS

Study population

All patients who underwent CRS-HIPEC for PM from CRC or PMP at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands, between March 2014 and June 2020 were included in this study. Erasmus MC is a university hospital and tertiary referral center for patients with extensive (metastasized) colorectal cancer. Patients were excluded if a suitable preoperative computed tomography (CT) image or patient body height, both essential of SMM measurement, were not available. Relevant patient and disease-related characteristics, operation details, and postoperative outcomes were extracted from a prospectively maintained database. This study was approved by the local Medical Ethics Review Committee of Erasmus MC (MEC-2018-1286).

Surgical procedure

CRS-HIPEC procedures were performed by a specialized surgical team, in accordance with the Dutch CRS and HIPEC protocol.[21, 22] After abdominal access via laparotomy, the peritoneal cancer index (PCI) according to Jacquet and Sugarbaker was used to estimate the tumor load.[23] For patients with PM from CRC, cytoreductive surgery was performed if the PCI score was under 20 and/or the specialized surgeons presumed the PM to be resectable. For patients with PM from PMP, the PCI score was not considered for determining CRS-HIPEC feasibility.

Postoperative monitoring

Patients were postoperatively treated following standard local protocol for CRS-HIPEC. Postoperative complications were categorized according to the Clavien-Dindo classification.[24] Severe postoperative complications were defined as Clavien-Dindo grade 3 or higher (i.e. re-intervention, extended ICU stay/readmission to ICU, or treatment-related death). In case of multiple complications, the highest grade complication was registered. The postoperative period was defined as 30 days after CRS-HIPEC, or the duration of the entire hospital stay, when exceeding 30 days.

Follow-up

Follow-up was performed at the outpatient clinic by use of CT imaging and monitoring of the Carcino-Embryonal Antigen (CEA). CEA was determined every 3 months, and a CT examination was performed every six months during the first two postoperative years. After two years, the follow-up interval for CEA was 6 months and a CT scan was made every 12 months. A CT scan was also performed in case of

increasing CEA levels or complaints, suspicious for recurrent disease. Follow-up was completed after a disease-free interval of 5 years following CRS-HIPEC.

Skeletal muscle mass (SMM) measurement

Abdominal CT was routinely performed during preoperative assessment. For patients treated with neo-adjuvant chemotherapy, SMM measurements were performed on the CT scan that was made after neo-adjuvant treatment. SMM was determined on using FatSeg software [developed by the Biomedical Imaging Group Rotterdam of Erasmus MC, Rotterdam, the Netherlands, based on MeVisLab (Mevis Medical Solutions, Bremen, Germany)].[25] In summary, SMM was measured twice at the level of the third lumbar vertebra (L3) on two different slices showing both transversal processes. The psoas, paraspinal, transverse abdominal, external oblique, internal oblique, and rectus abdominis muscles were manually traced (**figure 1**). The SMM area was computed automatically using the preset Hounsfield unit (HU) intensity thresholds (between – 30 and + 150), and was expressed in square centimeters. The L3 muscle index was calculated by dividing the average of the two L3 muscle area measurements by the squared patient height (cm^2/m^2). Low SMM was defined using the cut-off values of 43 cm^2/m^2 for men with BMI<25 cm^2/m^2, 53 cm^2/m^2 for men with BMI≥25 and 41 cm^2/m^2 for women, independent of BMI. These were developed in an oncological population to predict survival.[11] The SMM measurements were performed by a member of the research group (MD). A second investigator (JK) performed a random control on 10% of the examinations.

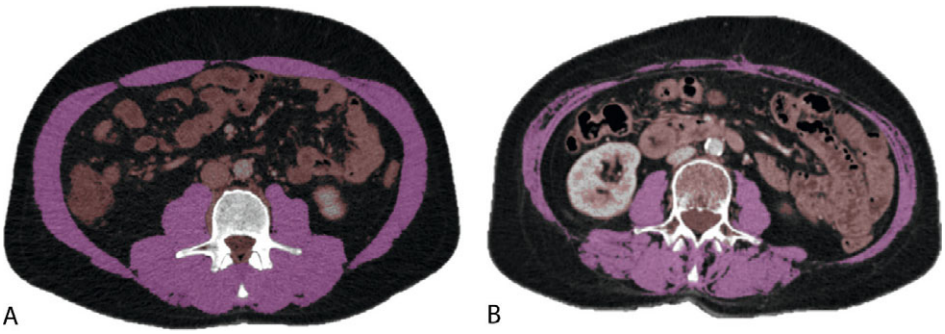

Figure 1. Axial CT slice at the level of the third lumbar vertebra of a male patient with normal SMM (A) and of a female patient with low SMM (B) with peritoneal metastasis from colorectal carcinoma. The psoas, paraspinal, transverse abdominal, external oblique, internal oblique, and rectus abdominis muscles are outlined in purple (threshold of – 30 to + 150 HU).

Primary and secondary outcomes

The primary outcome of this study was defined as the occurrence of severe postoperative complications (i.e. Clavien-Dindo grade 3 or higher). Secondary outcomes were disease-free survival (DFS) and overall survival (OS). DFS was defined as the time interval in months between CRS-HIPEC and date of recurrence or death. OS was defined as the time interval in months between CRS-HIPEC and date of death. Information on survival status was obtained from the national civil registry. When no event occurred, patients were censored at the date of the last follow-up visit for DFS or the date of last request of survival status for OS.

Statistical analysis

Continuous variables were expressed as median with interquartile range (IQR). Categorical variables were presented as counts with percentages. Continuous variables were compared between patients with low and normal SMM using the Mann-Whitney U test. Categorical variables were compared using the Chi-square test or Fisher's exact test if less than 5 events occurred. Intra-class correlation coefficient (ICC) based on a mean-rating (k =3), absolute-agreement, 2-way mixed-effects model, was estimated to investigate the reliability of the SMM measurements of the two investigators. To determine the effect of low SMM on the occurrence of severe postoperative complications, corrected for other possible risk factors, multivariable linear regression with backward selection was used. Gender, age, BMI, ASA (American Society of Anesthesiologists) classification and primary tumor type were entered in the multivariable model. Smoking history, PCI, presence of anastomosis and intraoperative blood loss are known predictive variables and were also entered in the model.[26-28] The Log-Rank was used to compare OS and DFS between patients with low and normal SMM. Survival analysis was performed separately for CRC and PMP patients because of different prognosis. All tests were performed two-sided, and differences were considered statistically significant when $p < 0.05$. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corporation, Armonk, NY, USA). Kaplan-Meier survival curves were created using R version 4.0.2 (https://www.r-project.org/).

RESULTS

During the study period, 244 patients (83.6%) underwent CRS-HIPEC for PM from CRC and 48 patients (16.4%) from PMP. Five CRC patients and three PMP patients were excluded from analyses because of the absence of a suitable preoperative CT for SMM measurement. The degree of reliability between the SMM measurements

of the two investigators was high. The single measure ICC was 0.924 (95% CI 0.824 – 0.964, p<0.001).

Baseline and intra-operative characteristics

A total of 149 patients (52.5%) had low SMM. Baseline and intra-operative characteristics are displayed in **table 1** and **2**, respectively. Patients with low SMM were significantly more often woman than patients with normal SMM (55.0% versus 42.2%, p=0.031). Body mass index (BMI) was significantly lower for patients with low SMM (median 25.0 versus 27.5, p<0.001). Other baseline characteristics and intra-operative characteristics did not significantly differ between the groups. Median interval from preoperative CT to surgery was 5 weeks for both patients with low (IQR 3 – 8) and normal SMM (IQR 2 – 8).

Postoperative outcomes

There were no differences in the occurrence of severe postoperative complications in general (i.e., Clavien-Dindo 3 or higher) between the groups (**table 3**). Patients with normal SMM significantly more often had a perforation (i.e., bowel perforation n=6, gallbladder perforation n=1) than patients with low SMM (bowel perforation n=1, p=0.022). Other postoperative outcomes did not significantly differ between the groups. Low SMM was not associated with severe postoperative complications in univariate logistic regression analysis (OR 0.79, 95% CI 0.48 – 1.30, p=0.344; **table 4**). Significant risk factors in univariate analysis were male gender (OR 2.67, 95% CI 1.58 – 4.53, p<0.001), smoking (OR 1.76, 95% CI 1.06 – 2.94, p=0.030) and more intraoperative blood loss (OR 1.34, 95% CI 1.04 – 1.73, p=0.021). In a multivariable analysis, male gender (OR 2.67, 95% CI 1.58 – 4.53, p<0.001), smoking (OR 1.76, 95% CI 1.06 – 2.94, p=0.030) and more intraoperative blood loss (OR 1.34, 95% CI 1.04 – 1.73, p=0.021) remained significantly associated with the occurrence of severe postoperative complications.

Survival outcomes

Median follow-up time for surviving CRC patients was 24 months (IQR 12 – 37). A total of 179 CRC patients (73.4%) had recurrence of disease during follow-up and the median DFS was 8 months (IQR 4 – 16). There was no significant difference in median DFS between patients with low and patients with normal SMM (7 [IQR 4 – 14] versus 8 months [IQR 5 – 20], p=0.078; **figure 2A**). Median OS for CRC patients was 33 months (IQR 17 – NR [not reached]). The median OS (33 months, IQR 14 – NR) for CRC patients with low SMM did not significantly differ from CRC patients with normal SMM (35 months, IQR 18 – NR, p=0.195, **figure 2B**).

Table 1. Baseline characteristics.

	Total n = 284 (100)	**Low SMM** n = 149 (52.5)	**Normal SMM** n = 135 (47.5)	**p-value**
Gender				
Male	145 (51.1)	67 (45.0)	78 (57.8)	**0.031***
Female	139 (48.9)	82 (55.0)	57 (42.2)	
Age (years)	62 [53 – 70]	63 [53 – 70]	61 [53 – 69]	0.593
BMI (kg/m^2)	25.9 [23.1 – 29.2]	25.0 [22.0 – 27.4]	27.5 [24.3 – 30.5]	**<0.001***
Smoking (past or current)				
Yes	126 (46.2)	69 (49.6)	57 (42.5)	0.239
No	147 (53.8)	70 (50.4)	77 (57.5)	
Missing	11 (3.9)	10 (6.7)	1 (0.7)	
Diabetes				
Yes	31 (11.0)	19 (12.8)	12 (8.9)	0.288
No	252 (89.0)	129 (87.2)	123 (91.1)	
Missing	1 (0.4)	1 (0.7)	0 (0)	
Hypertension				
Yes	77 (27.4)	37 (25.2)	40 (29.9)	0.380
No	204 (72.6)	110 (74.8)	94 (70.1)	
Missing	3 (1.1)	2 (1.3)	1 (0.7)	
ASA-classification				
1	45 (16.1)	23 (15.8)	22 (16.5)	0.975
2	174 (62.4)	91 (62.3)	83 (62.4)	
≥ 3	60 (21.5)	32 (21.9)	28 (21.1)	
Missing	5 (1.8)	3 (2.0)	2 (1.5)	
Primary tumor				
PMP	45 (15.8)	24 (16.1)	21 (15.6)	0.467
Appendix	16 (5.6)	6 (4.0)	10 (7.4)	
CRC	223 (78.5)	119 (79.9)	104 (77.0)	
Primary location CRC				
Ascending colon	82 (36.8)	48 (40.3)	34 (32.7)	0.613
Transverse colon	19 (8.5)	9 (7.6)	10 (9.6)	
Descending colon	23 (10.3)	14 (11.8)	9 (8.7)	
Sigmoid	69 (30.9)	34 (28.6)	35 (33.7)	
Rectum	30 (13.5)	14 (11.8)	16 (15.4)	
T stage primary tumor [a]				
T1	6 (2.6)	2 (1.6)	4 (3.6)	0.651
T2	10 (4.3)	4 (3.3)	6 (5.4)	
T3	105 (44.9)	57 (46.7)	48 (42.9)	
T4	113 (48.3)	59 (48.4)	54 (48.2)	
Missing	5 (2.1)	3 (2.4)	2 (1.8)	
N stage primary tumor [a]				
N0	64 (28.4)	39 (32.8)	25 (23.6)	0.311
N1	88 (39.1)	44 (37.0)	44 (41.5)	
N2	73 (32.4)	36 (30.3)	37 (34.9)	
Missing	14 (5.9)	6 (4.8)	8 (7.0)	
M stage primary tumor [a]				
M0	100 (48.5)	51 (46.4)	49 (51.0)	0.503
M1	106 (51.5)	59 (53.6)	47 (49.0)	
Missing	33 (13.8)	15 (12.0)	18 (15.8)	

Table 1. Baseline characteristics. (continued)

	Total n = 284 (100)	Low SMM n = 149 (52.5)	Normal SMM n = 135 (47.5)	p-value
Liver metastases [a b]				
Yes	23 (9.6)	16 (12.8)	7 (6.1)	0.081
Differentiation [a]				
Good	31 (16.0)	18 (17.0)	13 (14.8)	0.930
Moderate	129 (66.5)	71 (67.0)	58 (65.9)	
Poor	18 (9.3)	9 (8.5)	9 (10.2)	
Signet	16 (8.2)	8 (7.5)	8 (9.1)	
Missing	45 (18.8)	19 (15.2)	26 (22.8)	
Mucinous [a]				
Yes	50 (23.1)	21 (18.3)	29 (28.7)	0.069
No	166 (76.9)	94 (81.7)	72 (71.3)	
Missing	23 (9.6)	10 (8.0)	13 (11.4)	
Histopathology PMP [d]				
DPAM	39 (86.7)	21 (87.5)	18 (85.7)	0.860
PMCA	6 (13.3)	3 (12.5)	3 (14.3)	
PMCA-I	0 (0)	0 (0)	0 (0)	
PM onset [a]				
Synchronous	113(47.3)	65 (52.0)	48 (42.1)	0.126
Metachronous	126 (52.7)	60 (48.0)	66 (57.9)	
PSS				
0	25 (8.8)	12 (8.1)	13 (9.6)	0.925
1	47 (16.5)	25 (16.8)	22 (16.3)	
2	201 (70.8)	107 (71.8)	94 (69.6)	
3	11 (3.9)	5 (3.4)	6 (4.4)	
Neo-adjuvant chemotherapy [e]				
Yes	33 (11.6)	22 (14.8)	11 (8.1)	0.082
CT-to-surgery interval (weeks)	5 [2 – 8]	5 [3 – 8]	5 [2 – 8]	0.326

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%), excluding 'missing'
BMI= body mass index, ASA= American Association for Anesthesiology, PMP= pseudomyxoma peritonei, CRC= colorectal carcinoma, PM= peritoneal metastasis, DPAM= disseminated peritoneal adenomucinosis, PMCA= peritoneal mucinous carcinomatosis, PMCA-I= peritoneal mucinous carcinomatosis with intermediate features, PSS= prior surgical score
[a] Proportion of CRC patients (n=239)
[b] Presence liver metastasis at CRS-HIPEC
[c] Synchronous liver metastasis to primary colorectal tumor
[d] Proportion of PMP patients (n=45)
[e] Neo-adjuvant chemotherapy to CRS-HIPEC
* $\alpha < 0.05$

Table 2. Intra-operative characteristics.

	Total n = 284 (100)	**Low SMM** n = 149 (52.5)	**Normal SMM** n = 135 (47.5)	**p-value**
PCI	11 [6 – 16]	10 [6 – 16]	12 [7 – 17]	0.156
CCR-score				
R1	277 (97.5)	147 (98.7)	130 (96.3)	0.340
R2a	5 (1.8)	1 (0.7)	4 (3.0)	
R2b	2 (0.7)	1 (0.7)	1 (0.7)	
Procedure time (min)	373 [304 – 438]	380 [306 – 435]	365 [303 – 451]	0.880
Blood loss (L)	1.0 [0.6 – 1.6]	0.9 [0.6 – 1.5]	1.0 [0.6 – 1.8]	0.265
HIPEC Regimen				
MMC	261 (91.9)	140 (94.0)	121 (89.6)	0.182
Oxaliplatin	23 (8.1)	9 (6.0)	14 (10.4)	
Resections				
Omentum	267 (94.0)	142 (95.3)	125 (92.6)	0.336
Peritoneum	214 (75.4)	116 (77.9)	98 (72.6)	0.304
Diaphragm	60 (21.1)	28 (18.8)	32 (23.7)	0.311
Stomach	4 (1.4)	3 (2.0)	1 (0.7)	0.363
Small bowel	75 (26.4)	41 (27.5)	34 (25.2)	0.656
Colon	169 (59.5)	82 (55.0)	87 (64.4)	0.107
Rectum	60 (21.1)	31 (20.8)	29 (21.5)	0.889
Gallbladder	18 (6.3)	10 (7.4)	8 (5.4)	0.481
Pancreas	12 (4.2)	6 (4.4)	6 (4.0)	0.861
Spleen	22 (7.7)	8 (5.9)	14 (9.4)	0.275
Pelvic organs [a]	160 (56.3)	89 (59.7)	71 (52.6)	0.226
Synchronous liver treatment b	33 (11.6)	22 (14.8)	11 (8.1)	0.082
Anastomosis				
Yes	163 (57.4)	81 (54.4)	82 (60.7)	0.278
Median number/patient	1 [0 – 1]	1 [0 – 1]	1 [0 – 1]	0.723
Stoma				
Ileostomy	15 (5.3)	7 (4.7)	8 (5.9)	0.106
Colostomy	85 (29.9)	37 (24.8)	48 (35.6)	

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%), excluding 'missing'
PCI= Peritoneal Cancer Index, CCR= completeness of cytoreduction, MMC= Mitomycin-C
[a] Pelvic organs including urinary bladder, ovaries, uterus, ureters, and pelvis
[b] Liver treatment during CRS-HIPEC procedure: hepatic resection (n=24) or radiofrequency ablation (RFA, n=9)

Table 3. Postoperative outcomes.

	Total n = 284 (100)	**Low SMM** n = 149 (52.5)	**Normal SMM** n = 135 (47.5)	**p-value**
Length of stay (days)	16 [12 – 20]	16 [12 – 19]	16 [12 – 22]	0.594
Complications (any grade)				
Any complication	181 (63.7)	98 (65.8)	83 (61.5)	0.453
Anastomotic leakage [a]	26 (9.2)	13 (8.7)	13 (9.6)	0.792
Perforation [b]	8 (2.8)	1 (0.7)	7 (5.2)	**0.022***
Postoperative hemorrhage	11 (3.9)	8 (5.4)	3 (2.2)	0.170
Intra-abdominal abscess	33 (11.6)	16 (10.7)	17 (12.6)	0.626
Ileus/gastroparesis [c]	48 (16.9)	28 (18.8)	20 (14.8)	0.372
Wound infection	20 (7.0)	7 (4.7)	13 (9.6)	0.105
Wound dehiscence	8 (2.8)	2 (1.3)	6 (4.4)	0.115
Chylous leakage	10 (3.5)	5 (3.4)	5 (3.7)	0.874
Pneumonia	15 (5.3)	10 (6.7)	5 (3.7)	0.258
Pulmonary embolism	9 (3.2)	3 (2.0)	6 (4.4)	0.243
Cardiac complications	13 (4.6)	5 (3.4)	8 (5.9)	0.301
UTI	20 (7.0)	13 (8.7)	7 (5.2)	0.244
Severe complication [d]	89 (31.3)	43 (28.9)	46 (34.1)	0.344
Reoperations	43 (15.1)	20 (13.4)	23 (17.0)	0.396
Clavien-Dindo grade				
I	19 (6.7)	11 (7.4)	8 (5.9)	0.946
II	73 (25.7)	42 (28.2)	31 (23.0)	
IIIa	39 (13.7)	19 (12.8)	20 (14.8)	
IIIb	32 (11.3)	16 (10.7)	16 (11.9)	
IVa	10 (3.5)	5 (3.4)	5 (3.7)	
IVb	2 (0.7)	1 (0.7)	1 (0.7)	
V	6 (2.1)	2 (1.3)	4 (3.0)	
Adjuvant chemotherapy				
Yes	38 (13.4)	20 (13.4)	18 (13.3)	0.982

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%), excluding 'missing'
UTI= Urinary Tract Infection
[a] Proportion of patients with a bowel anastomosis after CRS-HIPEC (n=163)
[b] Perforation: bowel perforation (n=7), gallbladder perforation (n=1)
[c] Ileus (n=11), gastroparesis (n=42)
[d] Clavien-Dindo classification ≥ III (i.e., re-intervention, extended ICU stay/readmission to ICU, or treatment-related death)
* $\alpha < 0.05$

Table 4. Logistic regression for predictors of severe postoperative complications (i.e., Clavien-Dindo ≥3).

	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Low SMM	0.79 (0.48 – 1.30)	0.344		
Gender				
Female	1	**<0.001***	1	**0.001***
Male	2.67 (1.58 – 4.53)		2.54 (1.44 – 4.48)	
Age (years)	1.02 (0.99 – 1.04)	0.199		
BMI (kg/m^2)	1.01 (0.96 – 1.07)	0.648		
Smoking (past or current)	1.76 (1.06 – 2.94)	**0.030***	2.19 (1.24 – 3.84)	**0.007***
ASA-classification				
1	1	0.725		
2	1.14 (0.55 – 2.34)	0.628		
≥ 3	1.23 (0.53 – 2.85)			
Primary tumor				
CRC	1	0.508		
Appendix[1]	1.43 (0.50 – 4.09)	0.281		
PMP	1.44 (0.74 – 2.82)			
PCI	1.03 (0.99 – 1.06)	0.061		
Blood loss (L)	1.34 (1.04 – 1.73)	**0.021***	1.46 (1.11 – 1.94)	**0.008***
Anastomosis				
Yes	1.40 (0.83 – 2.33)	0.204		

BMI= body mass index, ASA= American Association of Anesthesiology, PMP = pseudomyxoma peritonei, CRC = colorectal carcinoma, PCI= Peritoneal Cancer Index

* $\alpha < 0.05$

[1] Appendiceal adenocarcinoma

Median follow-up time for surviving PMP patients was 18 months (IQR 9 – 50). A total of 13 PMP patients (27.1%) had recurrence of disease during follow-up. For PMP patients the median DFS and OS were not yet reached. The 1- and 3-year DFS rates were respectively 84.2 and 51.8% for all PMP patients. For patients with low SMM, the 1- and 3-year DFS rates were 81.3 and 47.3% respectively, compared to 87.8 and 54.5% for patients with normal SMM (p=0.676, **figure 2C**). The 1- and 3- year OS rates were respectively 97.7 and 80.4% for all PMP patients. The 1-, and 3-year OS rates were respectively 95.8 and 70.8% patients with low versus 100 and 90.9% for patients with normal SMM (p=0.172, **figure 2D**).

Additional analyses of patients that did not receive neo-adjuvant chemotherapy to CRS-HIPEC

There was no significant difference in the occurrence of severe postoperative complications in general between groups for patients that did not receive neo-adjuvant chemotherapy to CRS-HIPEC (32.2% for patients with normal SMM versus 29.9%

for patients with low SMM, p=0.689). In a multivariable analysis, male gender (OR 2.75, 95% CI 1.49 – 5.06, p=0.001), smoking (OR 2.19, 95% CI 1.23 – 4.13, p=0.008), and more intraoperative blood loss (OR 1.45, 95% CI 1.08 – 1.93, p=0.013) were significantly associated with the occurrence of severe postoperative complications. For CRC patients that did not receive neo-adjuvant chemotherapy to CRS-HIPEC, median DFS was significantly shorter for patients with low SMM (7 [IQR 4 – 13] versus 8 months [IQR 5 – 20], p=0.019; **supplementary figure 1A**). Median OS was 35 months for the normal SMM group [IQR 19 – NR] versus 33 months in the low SMM group [IQR 15 – 56] (p=0.124, **supplementary figure 1B**). None of the PMP patients received neo-adjuvant chemotherapy to CRS-HIPEC.

DISCUSSION

The aim of this study was to identify the impact of low skeletal muscle mass (SMM) on postoperative outcomes in patients undergoing CRS-HIPEC for peritoneal metastases (PM) from colorectal carcinoma (CRC) or pseudomyxoma peritonei (PMP). The current study found no association between low SMM and the occurrence of severe postoperative complications or survival outcomes.

Previous studies, reporting on the impact of sarcopenia in general colorectal cancer surgery, showed that low SMM was associated with higher rates of postoperative complications and impaired survival.[12, 16, 29, 30] Therefore, low SMM has been proposed to aid in preoperative patient selection. This could be especially helpful for patients suffering from PM from CRC, because of their limited prognosis and considerable postoperative morbidity after CRS-HIPEC. CT scans are routinely performed as part of the preoperative assessment for CRS-HIPEC. Measurement of SMM on these CT scans could be used in preoperative patient selection. However, the current study could not reproduce an association between low SMM and postoperative outcomes in patients undergoing CRS-HIPEC. This is in line with other studies that investigated the impact of SMM in this specific patient population.[18-20] An explanation for the discrepancy between general colorectal surgery and CRS-HIPEC might be the strict preoperative patient selection for CRS-HIPEC, mainly based on fitness for major surgery, leading to (strong) selection bias. Indeed, the vast majority of patients (around 80%) had an ASA classification of 1 or 2. The impact of low SMM on postoperative outcomes might be smaller in this strictly selected population, in contrast to the less selected patient population undergoing general colorectal cancer surgery.

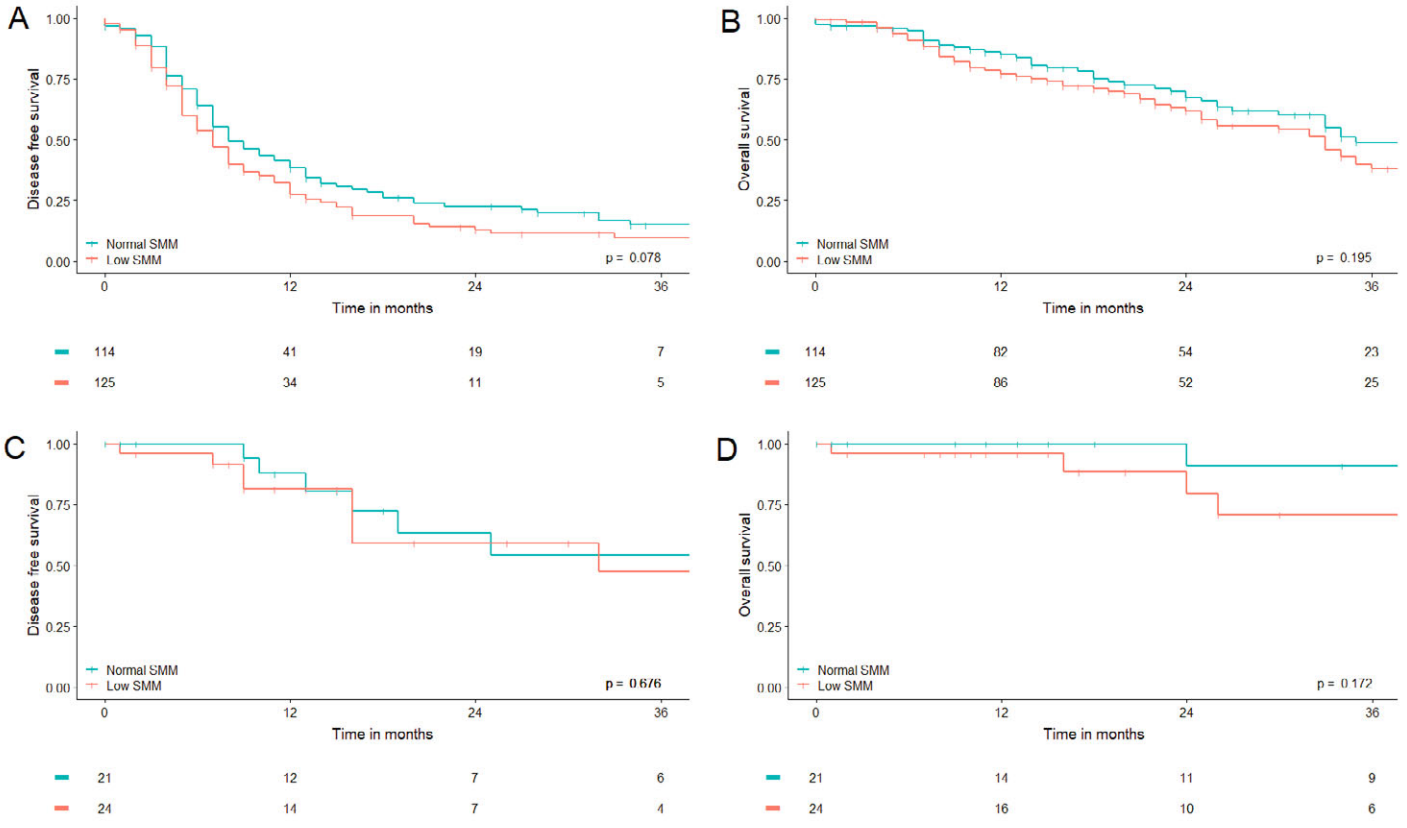

Figure 2. Kaplan-Meier survival curves for disease-free survival and overall survival for CRC (A, B) and PMP (C, D) patients with low versus normal SMM. The numbers at risk are displayed in the table below the graphs. The Log rank p-values are displayed in the bottom right corner.

One study regarding postoperative complications in patients undergoing CRS-HIPEC for PM from CRC, by Van Vugt et al., reported that patients with skeletal muscle depletion had significantly more reoperations than patients with normal SMM. They also found that lower SMM as a continuous measure, was independently associated with a higher rate of severe postoperative complications.[17] However, they did not find this association when using SMM as a dichotomous variable (i.e. low versus normal SMM). To increase the potential for clinical use, the current authors decided to use the SMM as a dichotomous variable. However, there is no consensus in the field which cut-off values are to be used to define low SMM. Previous studies, including the study by Van Vugt et al., used cut-off values defined by Prado et al.[31] These values have been acknowledged in an international consensus statement on cancer cachexia and have been validated for mortality prediction in obese patients with pulmonary and gastrointestinal cancer.[32] It is questionable whether these values are applicable in the current, mostly non-obese, cohort. Martin et al. more recently proposed cut-off values based on a general patient population with pulmonary and gastrointestinal malignancies, which were stratified for sex and BMI.[11] These cut-offs were applied because they were considered more appropriate for the current study. Nevertheless, we could not demonstrate a relation between low SMM and severe postoperative complications or reoperations after CRS-HIPEC. Van Vugt et al. included patients that underwent CRS-HIPEC between 2005 and 2013, whereas the current cohort consists of patients that underwent CRS-HIPEC from 2014. Preoperative patient selection regarding CRS-HIPEC has most likely improved during the last decade, resulting in stricter patient selection.

Another explanation could be that patients with low SMM were significantly more often women. This is in line with the study of Martin et al., in which these cut-off values were proposed.[11] In the current cohort male gender was independently associated with severe postoperative complications. However, in multivariate analysis including sex, low SMM remained unassociated with severe postoperative complications. Besides one previous study that reported on a proportion of the current study population, the association between sex and postoperative complications is not previously described for CRS-HIPEC.[33] Previous studies on colorectal surgery reported an association between male sex and increased risk of anastomotic leakage.[34, 35] One of these studies reported that the leak rate was especially high in men with low cancers.[35] This study proposed that this might be explained by anatomical differences in the narrower male pelvis and hormonal differences that might influence the intestinal microcirculation. In the current cohort other factors that were associated with severe postoperative complications were smoking

and intraoperative blood loss. This is conform previous studies on complications after CRS-HIPEC.[26, 27]

The cut-off values that were proposed by Martin et al., to classify SMM as low or normal, were based on SMM measurements on CT scans that were made before receiving any treatment for the measurement of SMM.[11] In the current study, SMM measurements were performed on the most recent CT scan that was made for CRS-HIPEC. For patients that were treated with neo-adjuvant chemotherapy for CRS-HIPEC, this was the CT scan that was made after neo-adjuvant treatment. Previous studies have shown that neo-adjuvant therapy was associated with the loss of SMM.[36-38] The post-therapy CT scan provides a more reliable view of the patients' physical status at time of surgery. However, concerning the utility of SMM measurements in patient selection for CRS-HIPEC, the possible effect of neo-adjuvant chemotherapy on the SMM should be taken into account. In the current cohort, only a minority of patients (12%) received neo-adjuvant chemotherapy, and there was no significant difference between groups. An additional analysis, excluding the patients that were treated with neo-adjuvant chemotherapy to CRS-HIPEC, showed similar outcomes regarding the occurrence of severe postoperative complications and OS. CRC patients with low SMM that did not receive neo-adjuvant chemotherapy had a significantly shorter DFS (7 months) than patients with normal SMM (8 months). Although not statistically significant, slightly more patients in the low SMM group received neo-adjuvant chemotherapy (14.8%) than in the normal SMM group (8.1%). This supports the hypothesis that neo-adjuvant chemotherapy is associated with the loss of SMM. Besides the significant difference in DFS in this subgroup analysis, there is a trend towards a slightly better DFS and OS for patients with normal SMM in the general study population. Low SMM might affect survival outcomes, but larger numbers of patients may be needed to support this hypothesis due to the highly selected population of patients undergoing CRS-HIPEC.

Regarding PMP, a study by Galan et al. showed that OS was significantly higher in patients with normal SMM.[20] This difference was found in the first months after CRS-HIPEC, without a significant difference in the occurrence of severe postoperative complications. Galan et al. stated that patients with low SMM might have a higher risk of death when major complications occur. In the current cohort the postoperative mortality for PMP patients was very low (i.e., n=1), which explains why this difference in OS was not found.

The current study had some limitations. Due to the retrospective nature of the current study, additional data on muscle function or nutritional status could not be

obtained. The EWGSOP recently updated the European consensus on the definition and diagnosis of sarcopenia.[39, 40] Whereas the EWGSOP previously recommended using the presence of both low muscle mass and low muscle function for the diagnosis of sarcopenia, the new consensus uses low muscle strength as the primary element of sarcopenia. Several studies proposed that muscle function (defined by factors like hand grip strength or cardiopulmonary exercise testing) might be better in reflecting a patients' physical function or nutritional status than skeletal muscle mass.[41-44] As skeletal muscle mass can be measured on routinely performed CT-scans, it is an easily available measure of the patients' physical status. A more comprehensive picture of the patients' physical and nutritional status might contribute to the prediction of postoperative outcomes in cancer patients. However, as aforementioned, the patients undergoing CRS-HIPEC consist of a selected patient population. It is questionable whether a more comprehensive picture of the patients' physical function provides significant additional value to patient selection for this specific patient population. Another limitation of this study was the limited follow-up period for surviving patients. Therefore, we presented 3-year survival data. For PMP patients, the sample size was small and median DFS and OS were not reached. Therefore, statements on the impact of low SMM on survival in these patients could not be made. Lastly, this study only investigated SMM as a predictor of postoperative outcomes. Sarcopenia, also defined by muscle weakness, might be a better predictor of postoperative outcomes. Previous studies reporting on the impact of sarcopenia on postoperative outcomes after general colorectal cancer surgery and CRS-HIPEC are inconsistent in the definition of sarcopenia. Future studies should investigate whether sarcopenia, defined by low SMM and muscle weakness, is a valid predictor of postoperative outcomes after CRS-HIPEC. In addition, other factors, such as weight loss and nutritional depletion, might also be relevant for the prediction of frailty and consequently of postoperative outcomes.

CONCLUSIONS

This study showed that low SMM was not a predictor of postoperative outcomes after CRS-HIPEC. This is probably explained by strict patient selection, based on factors like fitness for major surgery. Morbidity after CRS-HIPEC is considerable nonetheless. This morbidity might be more acceptable in patients with long term disease-free and overall survival. Hence, future research should focus on the identification of prognostic factors, useful in preoperative patient selection.

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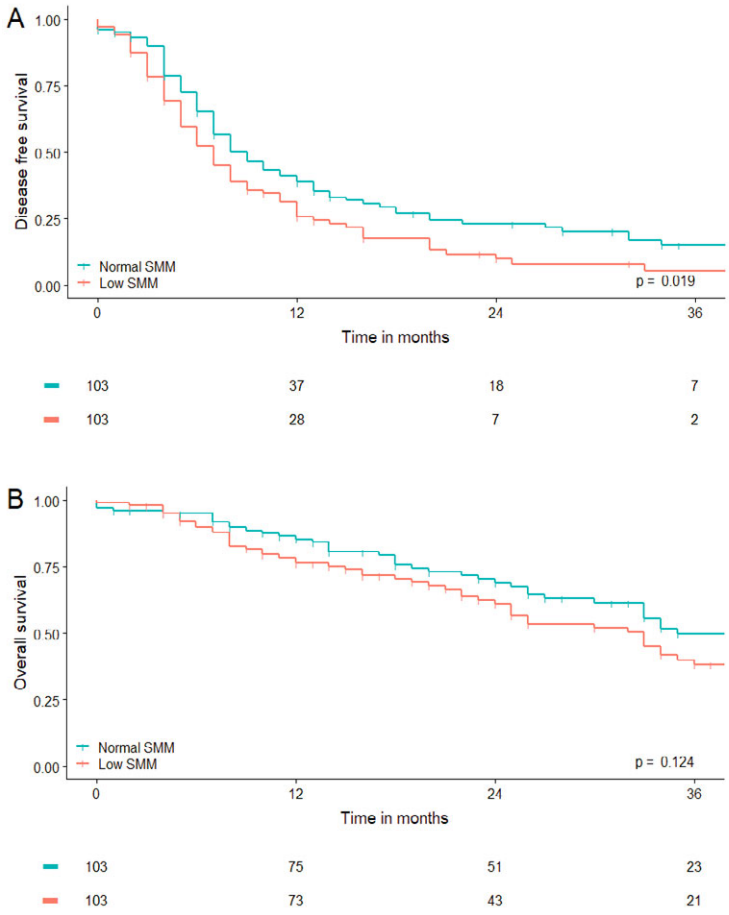
DATA SUPPLEMENT



Supplementary figure 1. Kaplan-Meier survival curves for disease-free survival (A) and overall survival (B) for CRC patients with low versus normal SMM. The numbers at risk are displayed in the table below the graphs. The Log rank p-values are displayed in the bottom right corner.

Chapter 8

Survival outcomes after Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy in Patients with Synchronous versus Metachronous onset of Peritoneal Metastases of Colorectal Carcinoma

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ABSTRACT

Background

Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) is a treatment option for peritoneal metastases (PM) from colorectal carcinoma (CRC). Because of considerable morbidity, optimal patient selection is essential. This study aimed to determine the impact of the onset of PM (synchronous versus metachronous) on survival outcomes after CRS-HIPEC.

Methods

Patients undergoing CRS-HIPEC for colorectal PM in two academic centers in the Netherlands between 2010 and 2020 were eligible for inclusion. Patients were classified as synchronous (s-PM, i.e., diagnosis at time of presentation, staging, or primary surgery) or metachronous onset (m-PM, i.e., diagnosis during follow-up) of colorectal PM. Survival outcomes were compared between groups by Kaplan-Meier survival and Cox regression analyses.

Results

Of 390 included patients, 179 (45.9%) had synchronous onset of colorectal PM. These patients more often presented with higher TN-stage and poor differentiation/signet ring cell histology. Treatment with perioperative chemotherapy was more common in s-PM patients. m-PM patients experienced more serious postoperative complications (Clavien Dindo ≥ III). There was no significant difference in disease-free survival (DFS) between s-PM (median 9 months, IQR 5 – 15) and m-PM patients (median 8 months, IQR 5-17). Overall survival (OS) was significantly shorter for s-PM (median 28 months, IQR 11-48) versus m-PM patients (median 33 months, IQR 18-66, p=0.049). Synchronous onset of PM was not independently associated with OS in a multivariable analysis.

Conclusion

Synchronous onset of colorectal PM was associated with poor tumor characteristics and more advanced disease but was not an independent predictor of survival outcomes after CRS-HIPEC.

INTRODUCTION

Approximately 4-6% of the patients with colorectal carcinoma (CRC) present with peritoneal metastases (PM) at the time of primary diagnosis (synchronous onset; s-PM).[1-3] Another 4-6% of the CRC patients will develop PM during follow-up (metachronous onset; m-PM). Patients with colorectal PM have a poor prognosis with a median survival of about 16 months in patients treated with systemic chemotherapy.[4] Selected patients might gain survival benefit from cytoreductive surgery (CRS) combined with intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC). This treatment results in median disease-free survival (DFS) and overall survival (OS) up to 20 and 41 months, respectively.[5-10]

Due to the extent of this treatment, CRS-HIPEC is associated with severe postoperative morbidity. It is essential to select patients who will most likely benefit from this treatment. Previous studies have identified multiple factors as predictors of survival after CRS-HIPEC. Important prognostic factors are the peritoneal cancer index (PCI) and completeness of cytoreduction (CCR).[7,11,12] However, CCR is determined intraoperatively and thus has no value in preoperative patient selection. The PCI can be estimated preoperatively by radiological assessment and/or diagnostic laparoscopy but is commonly underestimated.[13] Hence, there is a need to identify prognostic factors that could be used in preoperative patient selection.

For colorectal liver metastases (CRLM), several studies proposed the timing of onset as a predictor of survival. [14-16] A recent study in metastatic CRC patients reported impaired survival in patients with synchronous onset of these metastases.[17] A few groups have published conflicting data on the impact of s-PM versus m-PM on survival outcomes in patients undergoing CRS-HIPEC (**table 1**).[1,12,18-20] The aim of this retrospective multicenter study was to determine the prognostic value of time of onset of colorectal PM in patients undergoing CRS-HIPEC. We hypothesize that the synchronous onset of colorectal PM is a negative prognostic factor. More information contributes to a better estimation of the prognosis and could aid in shared decision making.

Table 1. Previous studies on the impact of the onset of PM on survival outcomes after CRS-HIPEC for PM from CRC.

Study	Number of patients	s-PM (%)	DFS s-PM [a] (months)	DFS m-PM [a] (months)	p-value	OS s-PM [a] (months)	OS m-PM [a] (months)	p-value
Hentzen et al. 2019 [17]	433	53.3%	15.0	11.0	**<0.001**	34.0	33.0	0.819
Wong et al. 2020 [18]	102	19.6%	13.1	9.5	0.917	26.9	45.2	**0.025**
Bakkers et al. 2021 [19]	88	38.6%	14.1	21.5	0.094	35.8	37.8	0.553

[a] Median

METHODS

Study population and definitions

Patients who underwent a complete CRS-HIPEC procedure for colorectal PM in the Erasmus Medical Center in Rotterdam between March 2014 and June 2020 and the Radboud University Medical Center in Nijmegen between March 2010 and October 2020 were eligible for inclusion. Patients with appendiceal carcinomas or without histologically proven PM were excluded. Relevant patient and disease-related characteristics, operation details, postoperative and survival outcomes were obtained from a prospectively maintained database.

Synchronous onset of PM (s-PM) was defined as a diagnosis of colorectal PM at the time of presentation, during routine staging, or at primary surgery. If colorectal PM were diagnosed in the follow-up period, the patients were stratified in the metachronous onset (m-PM) group. The disease-free interval (DFI) was defined as the time between diagnosis of the primary tumor and the diagnosis of the PM. A cut-off value of 12 months was used to stratify the DFI as short or long. The primary outcomes of this study were disease-free survival (DFS) and overall survival (OS). DFS was defined as the time interval in months between CRS-HIPEC and date of recurrence or date of last follow-up visit in censored cases. OS was defined as the time interval in months between CRS-HIPEC and date of death or date of the last update of survival status in censored cases. Information on survival status was obtained from the national civil registry, when not available in the electronic patient file.

Preoperative course

After referral for CRS-HIPEC, all patients were preoperatively screened. Dedicated radiologists reviewed preoperative CT scans to determine the extent of the dis-

ease. If possible, patients underwent diagnostic laparoscopy (DLS). The peritoneal cancer index (PCI) was recorded according to Jacquet and Sugarbaker.[21] Patients were eligible for CRS-HIPEC if they were fit for major surgery and had an estimated PCI below 20 without extra-abdominal metastasis. The presence of liver metastases was no definite contra-indication for CRS-HIPEC.

Perioperative course

CRS-HIPEC procedures were performed by a specialized surgical team, in accordance with the Dutch CRS-HIPEC protocol.[22,23] After median laparotomy, PCI was determined. CRS was performed when the PCI score was below 20 points and/or the surgeons presumed the PM resectable. Patients were postoperatively treated following standard of care for CRS-HIPEC procedures. The Clavien-Dindo classification of surgical complications was used to classify postoperative complications.[24] Severe postoperative complications were defined as Clavien-Dindo grade 3 or higher (i.e. re-intervention, prolonged ICU stay/readmission to ICU, or treatment-related death). If a patient had multiple complications, the highest Clavien-Dindo grade was registered. The postoperative period was defined as the 30 days after CRS-HIPEC, or the duration of the entire hospital stay when exceeding 30 days.

Follow-up

Follow-up was performed in the outpatient clinic. In the Erasmus Medical Center Carcino-Embryonal Antigen (CEA) was determined every three months and a CT scan was made every six months, or in case of rising CEA levels, during the first two years of follow-up. When patients were disease-free after two years, CEA was determined every six months and a CT scan was performed every 12 months, or in case of rising CEA levels. Follow-up was completed after a disease-free interval of 5 years following CRS-HIPEC. In the Radboud University Medical Center, CEA measurements and CT scans were performed every six months during the five years of follow-up. In both centers, an additional CT scan was performed in case of suspicion of recurrent disease.

Statistical analysis

Continuous variables were presented as median with interquartile range (IQR). Categorical variables were presented as counts with percentages. Continuous variables were compared between s-PM and m-PM patients using a Mann-Whitney U test. Categorical variables were compared using the Chi-square test, or Fisher's exact test if less than five events occurred. The Kaplan-Meier method was used to estimate the median OS and DFS. To compare OS and DFS between the groups, the Log-Rank test was used. To determine predictive factors for OS and DFS mul-

tivariable cox regression analyses with backward selection were performed. The variables age, ASA (American Society of Anesthesiologists) score, primary tumor differentiation, lymph node status, PCI, CCR score, and postoperative complications were entered in the model, as these have shown prognostic value in earlier studies.[7,11,18,25] All tests were performed two-sided, and differences were considered statistically significant when $p < 0.05$. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corporation, Armonk, NY, USA). Kaplan Meyer survival curves were created using R version 4.0.2 (http://www.r-project.org).

Ethical considerations

The local Medical Ethics Review Committees approved the collection of data for this study of the Erasmus Medical Center and the Radboud University Medical Center.

RESULTS

Between March 2010 and October 2020, 394 patients underwent a first CRS-HIPEC procedure for colorectal PM in the Erasmus Medical Center in Rotterdam and the Radboud University Medical Center in Nijmegen. Four patients were excluded because colorectal PM were not histologically proven in the preoperative workup or at CRS-HIPEC. The median follow-up was 26 months for all survivors (IQR 13 – 44).

Baseline and intra-operative characteristics, and postoperative outcomes

Of 390 patients included in this study, 179 (45.9%) patients had s-PM, whereas 211 (54.1%) patients were stratified as m-PM. Baseline characteristics are displayed in **table 2**. s-PM patients presented with higher TN-stages ($p<0.001$) and more often had a poorly differentiated primary tumor (29.8% versus 13.7%, $p=0.001$). Mucinous adenocarcinomas (27.5% versus 21.1%) and signet ring cell carcinomas (13.7% versus 3.3%) were more common in the s-PM group ($p=0.001$). Of the s-PM patients, 78.2% underwent prior colorectal cancer surgery and the primary tumor was resected in 45.8% prior to CRS-HIPEC. The median time between primary surgery and CRS-HIPEC procedure was 60 days [IQR 28 – 105] for s-PM patients that underwent prior surgery. These patients were stratified in two groups based on the median time interval of 60 days. For patients with a time interval of 60 days or more, prior surgery was more often performed in an acute setting (29.9% versus 15.1%, $p=0.035$), and the primary tumor was more often resected before CRS-HIPEC (68.7% versus 49.3%, $p=0.020$). s-PM patients more often received perioperative chemotherapy to CRS-HIPEC (44.5% versus 27.3%, $p=0.001$). Intra-operative char-

Table 2. Baseline characteristics.

	Total n = 390	**Synchronous** n = 179 (45.9%)	**Metachronous** n = 211 (54.1%)	**p-value**
Gender				
Male	191 (49.0)	85 (47.5)	106 (50.2)	0.588
Female	199 (51.0)	94 (52.5)	105 (49.8)	
Age (years)	64 [55 – 71]	64 [54 – 71]	64 [55 – 71]	0.480
BMI (kg/m^2)	25.7 [23.1 – 29]	25.6 [23.0 – 28.9]	25.8 [23.5 – 29.1]	0.488
Preoperative CEA	7.9 [3.7 – 14.0]	7.8 [2.8 – 19.7]	7.9 [4.4 – 14.0]	0.665
Smoking (past or current)				
Yes	125 (34.6)	61 (36.3)	64 (33.2)	0.531
No	236 (65.4)	107 (63.7)	129 (66.8)	
missing	*29 (7.4)*	*11 (2.8)*	*18 (4.6)*	
ASA-classification				
1	53 (13.8)	31 (17.6)	22 (10.6)	0.122
2	249 (64.8)	111 (63.1)	138 (66.3)	
≥ 3	82 (21.4)	34 (19.3)	48 (23.1)	
missing	*6 (1.5)*	*3 (1.7)*	*3 (1.4)*	
Primary tumor location				
Ascending colon	154 (39.5)	74 (41.3)	80 (37.9)	0.059
Transverse colon	30 (7.7)	19 (10.6)	11 (5.2)	
Descending colon	33 (8.5)	9 (5.0)	24 (11.4)	
Sigmoid	121 (31.0)	52 (31.0)	69 (32.7)	
Rectum	52 (13.3)	25 (14.0)	27 (12.8)	
T stage primary tumor				
T1	6 (1.6)	0 (0)	6 (2.9)	**<0.001**
T2	12 (3.1)	2 (1.2)	10 (4.8)	
T3	173 (45.3)	59 (34.3)	114 (54.3)	
T4	191 (50.0)	111 (64.5)	80 (38.1)	
missing	*8 (2.1)*	*7 (3.9)*	*1 (0.5)*	
N stage primary tumor				
N0	90 (23.9)	21 (12.4)	69 (33.5)	**<0.001**
N1-N2	286 (76.1)	149 (87.6)	137 (66.5)	
missing	*14 (3.6)*	*9 (5)*	*5 (2.3)*	
Synchronous liver metastases [a]				
Yes	42 (10.8)	21 (11.7)	21 (10.0)	0.572
Differentiation				
Good/moderate	245 (78.5)	106 (70.2)	139 (86.3)	**0.001**
Poor	67 (21.5)	45 (29.8)	22 (13.7)	
missing	*78 (20.0)*	*28 (15.6)*	*50 (23.7)*	
Histology				
Adenocarcinoma	205 (67.2)	90 (58.8)	115 (75.7)	**0.001**
Mucinous adenocarcinoma	74 (24.3)	42 (27.5)	32 (21.1)	
Signet ring cell carcinoma	26 ((8.5)	21 (13.7)	5 (3.3)	
missing	*85 (21.8)*	*26 (14.5)*	*59 (28.0)*	

Table 2. Baseline characteristics. (continued)

	Total n = 390	**Synchronous** n = 179 (45.9%)	**Metachronous** n = 211 (54.1%)	**p-value**
Prior colorectal cancer surgery				
Yes	351 (90.0)	140 (78.2)	211 (100)	**<0.001**
No	39 (10.0)	39 (21.8)	0 (0)	
Prior surgery type				
Acute	67 (19.6)	31 (22.1)	36 (17.9)	0.333
Elective	274 (80.4)	109 (77.9)	165 (82.1)	
missing	*10 (2.6)*	*0 (0)*	*10 (4.7)*	
Primary tumor status at HIPEC				
In situ	117 (30.0)	97 (54.2)	1 (0.5)	**<0.001**
Resected	273 (70.0)	82 (45.8)	210 (99.5)	
Prior chemotherapy				
Yes	104 (26.7)	0 (0)	104 (49.5)	**<0.001**
Perioperative chemotherapy [b]				
Yes	131 (35.3)	77 (44.5)	54 (27.3)	**0.001**

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%). BMI= body mass index, ASA= American association for anesthesiology, PM= peritoneal metastasis

[a] Synchronous liver metastases to primary tumor

[b] Neo-adjuvant and/or adjuvant chemotherapy around CRS-HIPEC

acteristics and postoperative outcomes are shown in **table 3**. Severe complications (Clavien-Dindo grade ≥ III) and reoperations after CRS-HIPEC occurred more often in the m-PM group (29.9% versus 18.4%, p=0.009; 14.7% versus 7.3%, p=0.021; respectively). m-PM patients with severe complications were less often treated with adjuvant chemotherapy to CRS-HIPEC (9.7%) than patients who did not experience a severe complication (27.2%, p=0.006). In the s-PM group, the rate of severe complications did not significantly differ between patients who underwent prior colorectal surgery (19.3%) and patients who did not (15.4%, p=0.578).

Disease-free survival

A total of 287 patients (77.2%) had a recurrence of disease during follow-up. Of these patients, 108 (37.6%) had peritoneal recurrence, 78 patients (27.2%) had systemic recurrence, and 101 patients (35.2%) had local as well as systemic recurrence of disease. The location of recurrence of disease did not significantly differ between the s-PM and m-PM groups (p=0.627). The median DFS for all patients was 8 months. For the s-PM patients, the median DFS was 9 months, compared with 8 months for the m-PM patients (p = 0.962; **figure 1a**). Multivariable analysis showed that age (HR 0.99, 95% CI 0.97 – 1.00, p=0.035) and PCI (1.05, 95% CI 1.02 – 1.07, p=0.001) were independently associated with DFS (**table 4**).

Table 3. Intra-operative characteristics and postoperative outcomes.

	Total n = 390	**Synchronous** n = 179 (45.9%)	**Metachronous** n = 211 (54.1%)	**p-value**
PCI	10 [5 – 15]	11 [5 – 15]	9 [5 – 14]	0.389
CCR-score				
R1	380 (97.4)	174 (97.2)	206 (97.6)	0.417
R2a	4 (1.0)	1 (0.6)	3 (1.4)	
R2b	6 (1.5)	4 (2.2)	2 (0.9)	
Procedure time (min) [a]	375 [304 – 449]	379 [316 – 452]	373 [300 – 449]	0.358
Blood loss (L) [b]	1.0 [0.5 – 1.6]	1.0 [0.5 – 1.9]	0.9 [0.6 – 1.5]	0.434
HIPEC Regimen				
MMC	274 (70.3)	118 (65.9)	156 (73.9)	0.085
Oxaliplatin	116 (29.7)	61 (34.1)	55 (26.1)	
Anastomosis [c]				
Yes	231 (60.6)	117 (66.5)	114 (55.6)	**0.030**
Median number/patient	1 [0 – 1]	1 [1 – 1]	1 [0 – 1]	0.196
Stoma				
Total	144 (36.9)	78 (43.6)	66 (31.3)	**0.012**
Ileostomy	29 (7.6)	19 (10.6)	10 (4.7)	0.170
Colostomy	115 (30.2)	59 (33.0)	56 (26.5)	
Length of stay (days)	14 [11 – 18]	14 [11 – 19]	14 [11 – 18]	0.798
Complications (any grade)				
Any complication	203 (52.1)	94 (52.5)	109 (51.7)	0.866
Anastomotic leakage	24 (6.2)	7 (3.9)	17 (8.2)	0.081
Postoperative hemorrhage	17 (4.4)	5 (2.8)	12 (5.8)	0.154
Intra-abdominal abscess	30 (7.8)	10 (5.6)	20 (9.7)	0.136
Ileus/gastroparesis [d]	57 (14.6)	30 (16.8)	27 (12.8)	0.270
Wound complications	35 (9.0)	17 (9.5)	18 (8.5)	0.739
Pneumonia	24 (6.2)	13 (7.3)	11 (5.3)	0.429
Pulmonary embolism	5 (1.3)	3 (1.7)	2 (1.0)	0.539
Cardiac complications	24 (6.2)	10 (5.6)	14 (6.8)	0.633
UTI	28 (7.3)	14 (7.8)	14 (6.8)	0.689
Complications Clavien-Dindo ≥ III [e]	96 (24.6)	33 (18.4)	63 (29.9)	**0.009**
Reoperations	44 (11.3)	13 (7.3)	31 (14.7)	**0.021**
Clavien-Dindo grade				
I	36 (9.2)	19 (10.6)	17 (8.1)	0.148
II	85 (21.8)	47 (26.3)	38 (18.0)	
IIIa	47 (12.1)	18 (10.1)	29 (13.7)	
IIIb	31 (7.9)	11 (6.1)	20 (9.5)	
IVa	11 (2.8)	2 (1.1)	9 (4.3)	
IVb	1 (0.3)	0 (0)	1 (0.5)	
V	6 (1.5)	2 (1.1)	4 (1.9)	

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%). PCI= Peritoneal Cancer Index. CCR= completeness of cytoreduction. MMC= mitomycin-C.

[a] Procedure time was available for 371 patients

[b] Blood loss data was available for 370 patients

[c] Anastomosis data was available for 381 patients

[d] Ileus (n=16), gastroparesis (n=45)

[e] Clavien-Dindo classification ≥ III (i.e., re-intervention, extended ICU stay/readmission to ICU, or treatment-related death)

Overall survival

Median OS for all patients was 32 months, and during follow-up 215 patients deceased. Median OS was significantly shorter for s-PM (28 months) compared to m-PM patients (33 months, p=0.045; **figure 1b**). In multivariable analysis, the onset of PM was not associated with OS (p= 0.193). Factors that were independently associated with OS in multivariable analysis were N stage (HR 1.76, 95% CI 1.9 – 2.84, p=0.020) and poor differentiation of the primary tumor (HR 1.95, 95% CI 1.32 – 2.90), as well as PCI (HR 1.07, 95% CI 1.03 – 1.10, p<0.001) (**table 5**).

Disease-free interval

The median disease-free interval (DFI) between the diagnosis of the primary tumor and PM was 19 months (IQR 11 – 30) for m-PM patients. DFI was not associated with DFS (HR 1.00, 95% CI 0.99 – 1.01, p=0.375), or OS (HR 1.00, 95% CI 0.99 – 1.01, p=0.974) in these patients. Median DFS was 8 months [IQR 5 – 17] for m-PM patients with a short DFI, compared to 9 months [IQR 5 – 17] for patients with a long DFI (p=0.660). Regarding OS, the median was 40 months [IQR 15 – NR] for the patients with a short DFI, versus 33 months [IQR 20 – 52] for the patients with a long DFI (p=0.747).

DISCUSSION

This study showed that patients with synchronous colorectal peritoneal metastasis (s-PM) had impaired overall survival compared to patients with metachronous PM (m-PM). However, this is probably explained by other factors, as synchronous onset of PM was not identified as an independent predictor of OS in multivariable analysis. Disease-free survival (DFS) did not differ between s-PM and m-PM patients.

Because of considerable morbidity after CRS-HIPEC for colorectal PM (24.6% in the current cohort), the identification of prognostic factors for optimal patient selection is needed. Some previous studies proposed that the timing of onset of metastases from CRC could be of prognostic value.[14-17] Few studies investigated the impact of synchronous onset in patients with colorectal PM undergoing CRS-HIPEC, and conflicting results were published (**table 1**).[1,12,18-20] A study by Hentzen et al. in the Netherlands reported a decreased DFS, but not OS, in m-PM patients after CRS-HIPEC.[19] This is the opposite of the hypothesis that synchronous onset would predict poor survival. This might partially be explained by the use of perioperative chemotherapy. In the study by Hentzen et al., s-PM patients were treated

Table 4. Cox proportional regression analysis for predictors of DFS.

	Univariate analysis HR (95% CI)	p-value	Multivariate analysis HR (95% CI)	p-value
Onset of PM				
Synchronous	1	0.929		
Metachronous	1.01 (0.80 – 1.27)			
Gender				
Male	1	0.860		
Female	1.02 (0.81 – 1.29)			
Age (years)	0.99 (0.98 – 1.00)	0.238	0.99 (0.97 – 1.00)	**0.035**
ASA-classification				
1	1	0.099	1	0.118
2	1.33 (0.95 – 1.87)	0.406	1.44 (0.91 – 2.29)	0.066
≥ 3	1.19 (0.79 – 1.79)		1.64 (0.97 – 2.77)	
Primary tumor location				
Ascending colon	1	0.291		
Transverse colon	1.27 (0.82 – 1.97)	0.444		
Descending colon	1.19 (0.77 – 1.83)	0.227		
Sigmoid	1.19 (0.90 – 1.58)	0.193		
Rectum	1.27 (0.89 – 1.82)			
N stage primary tumor				
N0	1	0.142	1	0.092
N1-2	1.24 (0.93 – 1.65)		1.37 (0.95 – 1.99)	
Differentiation				
Good/Moderate	1	0.051		
Poor	1.36 (1.00 – 1.85)			
Histology				
Adenocarcinoma	1	0.790		
Mucinous adenocarcinoma	0.96 (0.71 – 1.30)	0.519		
Signet ring cell carcinoma	1.18 (0.72 – 1.92)			
PCI	1.05 (1.03 – 1.07)	**<0.001**	1.05 (1.02 – 1.07)	**0.001**
CCR-score	1	0.300		
R1				
≥ R2a	1.60 (0.66 – 3.89)			
Complications				
Clavien-Dindo ≥ III	1.26 (0.96 – 1.65)	0.099		
Perioperative chemotherapy				
Yes	0.76 (0.60 – 0.97)	**0.030**	0.80 (0.58 – 1.09)	0.158

PM = peritoneal metastasis, ASA= American association of anesthesiology, PCI= peritoneal cancer index, CCR= completeness of cytoreduction.

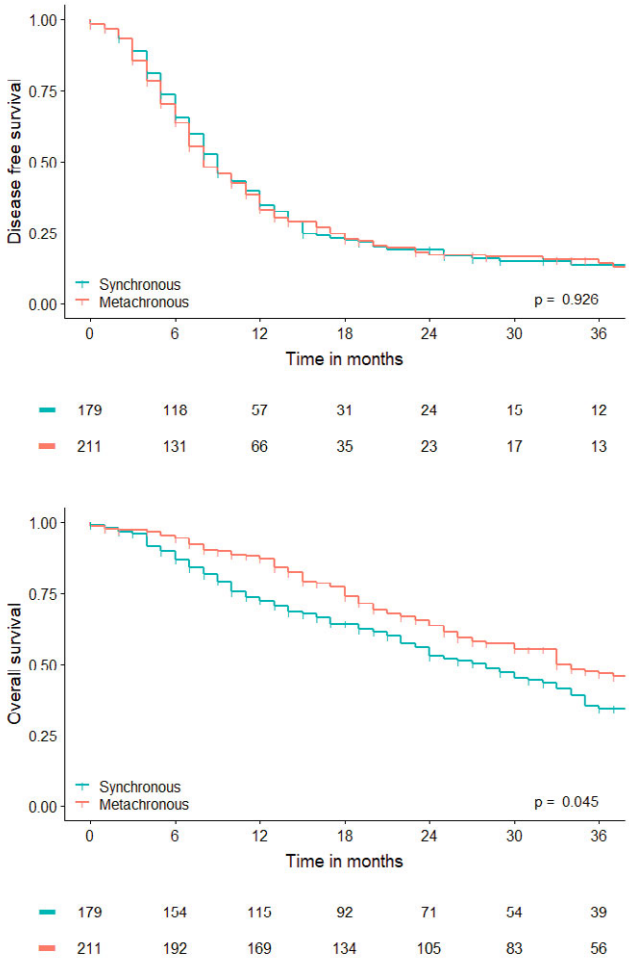

Figure 1. Kaplan-Meier survival curves for disease-free survival (A) and overall survival (B) for patients with synchronous onset versus metachronous onset of peritoneal metastasis. The Log rank p-values are displayed in the bottom right corner.

Table 5. Cox proportional regression analysis for predictors of OS.

	Univariate analysis HR (95% CI)	**p-value**	**Multivariate analysis** HR (95% CI)	**p-value**
Onset of PM				
Synchronous	1	**0.048**		
Metachronous	0.76 (0.58 – 1.00)			
Gender				
Male	1	0.976		
Female	1.00 (0.76 – 1.30)			
Age (years)	1.01 (1.00 – 1.02)	0.167	1.02 (1.00 – 1.03)	0.099
ASA-classification				
1	1	0.322		
2	1.22 (0.82 – 1.80)	0.526		
≥ 3	1.17 (0.73 – 1.87)			
Primary tumor location				
Ascending colon	1	0.583		
Transverse colon	1.16 (0.69 – 1.94)	0.858		
Descending colon	0.95 (0.57 – 1.60)	0.388		
Sigmoid	0.86 (0.62 – 1.20)	0.429		
Rectum	1.17 (0.79 – 1.73)			
N stage primary tumor				
N0	1	**0.034**	1	**0.020**
N1-2	1.44 (1.03 – 2.02)		1.76 (1.9 – 2.84)	
Differentiation				
Good/Moderate	1	**<0.001**	1	**0.001**
Poor	2.09 (1.50 – 2.92)		1.95 (1.32 – 2.90)	
Histology				
Adenocarcinoma	1	0.648		
Mucinous adenocarcinoma	1.09 (0.76 – 1.56)	**<0.001**		
Signet ring cell carcinoma	2.79 (1.74 – 4.48)			
PCI	1.07 (1.05 – 1.01)	**<0.001**	1.07 (1.03 – 1.10)	**<0.001**
CCR-score				
R1	1	**0.001**		
≥ R2a	3.26 (1.66 – 6.37)			
Complications				
Clavien-Dindo ≥ III	1.45 (1.08 – 1.96)	**0.015**	1.40 (0.94 – 2.09)	0.097
Perioperative chemotherapy				
Yes	0.81 (0.61 – 1.08)	0.151		

PM = peritoneal metastasis, ASA= American association of anesthesiology, PCI= peritoneal cancer index, CCR= completeness of cytoreduction.

more often with perioperative systemic chemotherapy around CRS-HIPEC than m-PM patients. They also reported that perioperative chemotherapy was associated with longer DFS, but not OS. In the current cohort, the use of perioperative chemotherapy was associated with longer DFS in univariable, but not in multivariable analysis. Because currently there is no consensus in the field regarding the use of perioperative systemic chemotherapy around CRS-HIPEC, the CAIRO-6 trial was initiated.[26,27] In this ongoing randomized controlled trial in the Netherlands, perioperative systemic therapy and CRS-HIPEC is compared to CRS-HIPEC alone. Hopefully, this trial will give clarity about the role of perioperative chemotherapy.

In the current cohort, s-PM patients were also more commonly treated with perioperative chemotherapy, but this difference was smaller than for the cohort of Hentzen et al. The difference in treatment regimen between s-PM and m-PM patients is partially explained by the (intended) treatment of the primary tumor. For some s-PM patients, PM was diagnosed at the (intended) resection of the primary tumor. Some of these patients had received neo-adjuvant systemic therapy. This treatment was classified as neo-adjuvant therapy to the completion surgery consisting of CRS-HIPEC. Another explanation is the difference in adjuvant treatment to CRS-HIPEC. The rate of severe complications after CRS-HIPEC was higher in the m-PM group, and these patients were less likely to receive adjuvant chemotherapy.

Hentzen et al. reported a remarkably longer median DFS (15 months for s-PM versus 11 months for m-PM patients) than the current study (9 months for s-PM versus 8 months for m-PM patients), while median OS was comparable. A recent population-based study by Bakkers et al. in the Netherlands reported an even longer DFS (14.1 months for s-PM versus 21.5 months for m-PM patients).[1] An explanation for the difference in DFS might be that there is no nationwide protocol for follow-up after CRS-HIPEC. In the cohort of Hentzen et al., CT scans were only performed when recurrence was suspected (e.g., clinical symptoms or increasing CEA levels). CT scans were performed every six months during the first two years of follow-up in the current study. This might have led to earlier detection of recurrence, resulting in a difference in DFS, but not OS. It is debatable whether earlier detection of recurrence after CRS-HIPEC is preferable because the curative options for recurrence after CRS-HIPEC are limited.

A study by Wong et al. described the same follow-up protocol as the current study and reported a similar median DFS (9.5 months) in 102 patients who underwent CRS-HIPEC from 2003 to 2018.[20] Corresponding to the findings of the current study, Wong et al. reported an impaired OS, but not DFS, in s-PM patients. In addition,

synchronous onset of PM could not be identified as an independent predictor of OS. This is in line with two previous meta-analyses reporting on prognostic factors after CRS-HIPEC and the study by Bakkers et al.[1,12,18] This suggests that s-PM is probably not a predictor of early recurrence, but that it illustrates poor tumor characteristics and a more advanced disease. At baseline, s-PM patients had higher TN-stages, poor differentiation, and signet ring cell histology of the primary tumor. These factors, which are associated with poor tumor characteristics and more advanced disease, independently predict survival.[7,11,18] This probably explains why s-PM patients had impaired OS, but synchronous onset was not significantly associated with OS in multivariable analysis. For patients with metachronous onset of PM, the time interval between diagnosis of the primary tumor and the diagnosis of PM (DFI) was not associated with DFS, nor with OS. This supports the hypothesis that the time of onset of PM is not an independent prognostic factor.

In the current cohort, lymph node metastasis, poor primary tumor differentiation, and PCI were independently associated with poorer OS. Signet ring cell histology was also associated with OS in univariable, but not in multivariable analysis. As CRS-HIPEC was more often the primary treatment for s-PM patients, bowel resections, and the creation of an anastomosis and/or stoma were more often performed in this group. However, significantly more severe complications (i.e., Clavien Dindo 3 and higher) and reoperations after CRS-HIPEC were reported in m-PM patients. m-PM patients more often underwent prior colorectal cancer surgery, with a longer time interval between primary surgery and CRS-HIPEC. Several previous studies showed that (extensive) prior surgery is a risk factor for the occurrence of complications after CRS-HIPEC.[28,29] These studies did not report on the time interval between prior surgery and CRS-HIPEC. In the current cohort, a substantial number of s-PM patients also underwent prior colorectal cancer surgery (78%). The primary tumor was resected prior to CRS-HIPEC for almost half of the s-PM patients, reflecting extensive surgery. Prior surgery is often performed in the referring center and in an acute setting, resulting in a considerable time interval (median 60 days) between primary surgery and completion surgery consisting of CRS-HIPEC. Contrary to the aforementioned hypothesis, the rate of severe postoperative complications was not higher for s-PM patients that underwent prior colorectal cancer surgery. Hence, the time interval between prior surgery and CRS-HIPEC seems to play a role in the risk of postoperative complications. Previous studies showed that postoperative complications were associated with impaired survival after CRS-HIPEC.[25,30] In the current study, severe postoperative complications (CD≥3) were associated with poorer OS in univariate, but not in multivariate analysis. This is

probably explained by the association of postoperative complications with higher PCI, reflecting extensive surgery.

PCI was the only variable that was independently associated with both DFS and OS. PCI is preferably determined by laparoscopy, or if not possible, by radiological imaging. However, preoperative underestimation of PCI is not uncommon.[13] To improve patient selection, future research should focus on improving preoperative prediction of PCI. A currently ongoing study in the Netherlands, the DISCO-trial, was initiated to determine the role of MRI in detecting colorectal PM in patients who are considered for CRS-HIPEC. In this multicenter randomized study, a diagnostic workup with MRI is compared to the standard workup with surgical staging. The results of this study will hopefully contribute to improved preoperative PCI estimation.

Limitations

This study was mainly limited by its retrospective nature, which could have resulted in selection bias. Patients with a high PCI (i.e., 20 or higher) were not eligible for CRS-HIPEC and were thus not included in this study. Patients with aggressive synchronous PM probably present with higher PCI and could therefore have been excluded. The study by Bakkers et al. showed that s-PM patients were less often treated with CRS-HIPEC than m-PM patients.[1] s-PM patients might have worse survival outcomes in the general population of patients with colorectal PM, but not in this selected cohort of patients undergoing CRS-HIPEC. Another limitation of the current study was the relatively short follow-up for surviving patients. Therefore, we presented the 3-year survival data.

CONCLUSIONS

In conclusion, the current study showed that synchronous onset of colorectal PM was associated with impaired overall survival, but probably due to confounding factors associated with poor tumor characteristics and advanced disease. Tumor differentiation, lymph node status, and PCI are more valuable predictors for survival after CRS-HIPEC and are important factors that could aid in shared decision making.

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Chapter 9

Treatment and Survival Outcomes of Patients with Colorectal Peritoneal Metastases deemed Ineligible for Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC): Results of a Retrospective Study

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ABSTRACT

Background

Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) is a treatment option for selected patients with colorectal peritoneal metastases (PM). This study provides an overview of treatment and survival outcomes of patients deemed ineligible for CRS-HIPEC.

Methods

Colorectal PM patients referred to a tertiary center from 2014 to 2020 that were ineligible for CRS-HIPEC were included. Patient, tumor, and treatment characteristics were provided. Survival analyses were performed using the Kaplan-Meier method.

Results

Of 476 patients referred for CRS-HIPEC, 227 (48%) were deemed ineligible. Median follow-up was 15 months [IQR 10 – 22]. Data on follow-up treatment was available for 198 patients, of which 73% received systemic therapy. These patients had a median overall survival (OS) of 17 months [IQR 9 – 25]. For patients receiving best supportive care (BSC) median OS was 4 months [IQR 2 – 9]. The main reason for ineligibility was extensive PM (42%), with a median OS of 11 months [IQR 5 – 18]. Patients deemed ineligible due to (extensive) liver (9%) or lung metastases (8%) showed longer OS (median 22 months, IQR 8 – 27, and 24 months, IQR 12 – 29, respectively) than patients with extensive PM (median 11 months, IQR 5 – 18) or distant lymph node metastases (median 14 months, IQR 4 – 25).

Conclusion

The main reason for CRS-HIPEC ineligibility was extensive PM. The majority of patients received systemic therapy. Patients deemed ineligible due to extra-peritoneal metastases had better survival outcomes than patients deemed ineligible due to extensive PM.

INTRODUCTION

Approximately 10% of patients with colorectal carcinoma (CRC) develop peritoneal metastases (PM) at some point.[1] Patients with colorectal PM (CRC-PM) have a worse prognosis compared to patients with other isolated sites of dissemination.[2-4] The introduction of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) resulted in a potential curative treatment option for selected CRC-PM patients, with a 5-year survival up to 39.4%.[5-8]

The recently published PRODIGE 7 trial has questioned the role of HIPEC in this survival benefit.[8] Although this trial led to discussion and changes in CRS-HIPEC regimens, this procedure is still broadly performed worldwide.[9] However, not all CRC-PM patients are eligible for this extensive treatment. In the Netherlands, 12 to 23% of CRC-PM patients undergo CRS-HIPEC.[1, 10] Irresectable or extensive PM (i.e. peritoneal cancer index (PCI) of 20 or higher), extra-abdominal or irresectable liver metastases, and poor performance status are considered contraindications for this treatment.[11, 12]

Despite a comprehensive preoperative workup, approximately 25% of the intended CRS-HIPEC procedures result in open-close (OC) procedures due to findings during laparotomy.[13] For patients deemed ineligible for CRS-HIPEC, or patients that are not willing to undergo extensive surgery, systemic therapy only is an alternative treatment option. Studies reported on outcomes of CRC-PM patients undergoing CRS with or without HIPEC, but the literature is scarce about the outcomes of patients deemed ineligible for this procedure.

This retrospective study aims to give an overview of the reasons for CRS-HIPEC ineligibility for patients referred to a tertiary center and the treatment, and survival outcomes for this specific patient population.

MATERIAL AND METHODS

Study population and data collection

The Erasmus Medical Center Cancer Institute is a tertiary referral hospital for patients with stage IV colorectal cancer. Patients with PM from colorectal adenocarcinoma, referred between April 2014 and July 2020, who were rejected for CRS-HIPEC, were eligible for inclusion. Patients were excluded in the current analysis if CRC-PM was not histologically confirmed. Data were obtained retrospectively from

electronic health records and the Dutch Personal Records Database. In case data regarding therapy and disease progression were unknown, additional data were requested from referring hospitals. This study was approved by the local medical ethics committee (registration number MEC-2018-1286). Data were handled according to the European privacy regulations (General Data Protection Regulation (GDPR), EU 2016/679).

Preoperative assessment

All patients were discussed by a multidisciplinary tumor board (MTD). Preoperative workup to assess the feasibility of CRS-HIPEC included staging by radiographic imaging, generally with an abdominal and thoracic CT-scan. Preferably, diagnostic laparoscopy (DLS) was performed to determine the surgical PCI as described by Jacquet and Sugarbaker.[14] When imaging revealed clear contraindications for CRS-HIPEC or if adequate oversight of the abdominal cavity was not expected (e.g. due to extensive prior abdominal surgery), DLS was not performed.

Outcomes and definitions

The primary outcomes of this study were reasons for CRS-HIPEC ineligibility, treatment regimens, and overall survival (OS). OS was defined as the time interval between diagnosis of peritoneal metastases and death due to any cause. Synchronous onset of PM was defined as diagnosis of PM at time of presentation of the primary tumor, during staging, or at primary surgery. Patients with PM diagnosed during follow-up were classified as metachronous onset of PM. The Clavien-Dindo classification was used to score postoperative complications in patients undergoing an open-close (OC) procedure.[15] Severe complications were defined by Clavien-Dindo grade 3 or higher (i.e. re-intervention, life-threatening organ dysfunction, or treatment-related death) occurring within 30 days after surgery, or the duration of the entire hospital stay, when exceeding 30 days. Postoperative mortality was defined as death, of any cause, within 30 days after surgery. The main reason for CRS-HIPEC ineligibility was determined based on the report of the multidisciplinary tumor board.

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS) version 25.0.0.1 (IBM Corporation, Armonk, NY, USA) and R version 4.0.2 (http://www.r-project.org). Continuous variables were presented as medians with interquartile range [IQR]. Categorical variables were compared between groups with the Mann-Whitney U test. Categorical variables were shown as absolute numbers with percentages and compared using the Chi-squared test. The Kaplan-Meier method

was used to create survival curves and estimate median OS and PFS. Two-sided p-values smaller than 0.05 were considered statistically significant.

RESULTS

Patient and tumor characteristics

A total of 476 patients with colorectal PM were referred to the Erasmus Medical Center Cancer Institute for CRS-HIPEC between April 2014 and July 2020. A total of 227 patients (48%) were deemed ineligible for this treatment at some point during the work-up or at intended CRS-HIPEC procedure (OC-procedure; **supplementary figure 1**). Baseline characteristics are displayed in **table 1**. Surgically determined PCI (i.e., during DLS or laparotomy) was available for 100 (44%) patients, with a median PCI of 17 [IQR 8 – 25]. The median follow-up was 11 months for the total cohort [IQR 5 – 19] and 15 months for event-free patients [IQR 10 – 22].

Table 1. Baseline characteristics.

	Total n = 227
Gender	
Male	127 (55.9)
Female	100 (44.1)
Age (years)	65 [57 – 71]
Primary tumor location	
Ascending colon	97 (42.7)
Transverse colon	19 (8.4)
Descending colon	17 (7.5)
Sigmoid colon	61 (26.9)
Rectum	33 (14.5)
T stage [a b]	
T1	2 (0.9)
T2	6 (2.6)
T3	96 (42.3)
T4	91 (38.8)
Missing	*35 (15.4)*
N stage [a b]	
N0	49 (21.6)
N1	53 (23.3)
N2	91 (40.1)
Missing	*34 (15.0)*
Systemic metastases [c]	
Liver	45 (19.8)
Lung	11 (4.8)
Liver and lung	3 (1.3)

Table 1. Baseline characteristics. (continued)

	Total n = 227
Differentiation [a]	
Well	85 (37.4)
Moderately	38 (16.7)
Poorly	48 (21.1)
Missing	*56 (24.7)*
Histology [a]	106 (46.7)
Adenocarcinoma	
Mucinous	42 (18.5)
Signet ring cell	20 (8.8)
Missing	*59 (26.0)*
Primary tumor resection	140 (61.7)
Prior chemotherapy [d]	68 (30.0)
PM onset	
Synchronous	118 (52.0)
Metachronous	109 (48.0)
Surgically determined PCI [e]	100 (44.1)
PCI at surgical assessment	17 [8 – 25]
Synchronous systemic metastases [f]	
Liver	56 (24.7)
Lung	17 (7.5)
Liver and lung	6 (2.6)

Continuous variables are shown as: median [IQR]. Frequencies are shown as: N (%). CRS-HIPEC = cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, PM = peritoneal metastasis, PCI = peritoneal cancer index

[a] Tumor characteristics at primary diagnosis

[b] Staging according to the TNM staging system of the American Joint Committee on Cancer (AJCC); pathological stage was available for 138 patients; clinical stage was available for 55 patients

[c] Synchronous to primary tumor

[d] Treatment with chemotherapy prior to PM diagnosis

[e] During workup for CRS-HIPEC

[f] Systemic metastases synchronous to diagnosis of PM

Reasons for CRS-HIPEC ineligibility

An overview of the reasons for CRS-HIPEC ineligibility is provided by **table 2**. Of 227 patients, 96 (42%) were deemed ineligible for CRS-HIPEC due to extensive PM. Surgical PCI was available for 79 out of 96 patients, with a median PCI of 25 [IQR 23 – 28]. Of these patients, 45 were deemed ineligible during diagnostic explorative surgery (i.e., 41 patients at DLS and four patients at laparotomy). For 17 patients extensive PM was observed at radiographic imaging or during explorative surgery at the referring hospital without reporting the PCI.

Table 2. Main reasons of ineligibility for CRS-HIPEC with corresponding overall survival.

Main reason	Total n = 227	Median PCI	Median OS (months)
Extensive PM [a]	96 (42.3)	25 [23 – 28] [f]	11 [5 – 18]
Distant lymph node metastases [b]	26 (11.5)	4 [3 – 16] [g]	14 [4 – 25]
(Rapid) progression [c]	25 (11.0)	12 [8 – 15] [h]	7 [5 – 24]
Extensive liver metastases [d]	20 (8.8)	10 [8 – 15] [i]	22 [8 – 27]
Patients' preference	19 (8.4)	6 [5 – 14] [j]	13 [9 – 37]
Performance status	17 (7.5)	18 [2 – NA] [k]	10 [3 – 14]
Lung metastases	17 (7.5)	6 [3 – 14] [l]	24 [12 – 29]
Irresectable PM [e]	7 (3.1)	7 [4 – NA] [m]	23 [12 – 48]

Continuous variables are shown as: median [IQR]. Frequencies are shown as: N (%)
[a] Peritoneal cancer index (PCI) of 20 or higher
[b] Retroperitoneal, mediastinal, or inguinal lymph node metastases
[c] I.e., rapid progression during work-up for CRS-HIPEC or during treatment with chemotherapy, based on radiological or surgical assessment
[d] Presence of more than 3 liver metastases
[e] Radical resection of PM deemed impossible
Median PCI available for [f] 79, [g] 5, [h] 10, [i] 5, [j] 8, [k] 3, [l] 4 and [m] 2 patients

Treatment regimens

Data regarding treatment regimen was available for 198 patients (87%). For 29 patients, data on treatment was not provided by the treatment center. Systemic therapy was given to 145 out of 198 patients (73%), of whom 112 (77%) started this treatment after rejection for CRS-HIPEC. The remaining 33 patients (23%) started before CRS-HIPEC work-up, either in an intended neo-adjuvant setting, or before referral for CRS-HIPEC. **Table 3** displays an overview of the administered first-line systemic treatment regimens. CAPOX (43%) and capecitabine monotherapy (24%) were most frequently administered as first-line regimen. Data on biological therapy was available for 143 out of 145 patients. Bevacizumab, an anti-angiogenic agent, was added to systemic chemotherapy in 51% of the cases. The majority of the patients that were treated with palliative systemic therapy received one (49%) or two lines (28%) of therapy. Of the 198 patients, 53 (27%) received best supportive care (BSC). Patients deemed ineligible for CRS-HIPEC due to poor performance status or patients' preference more often received BSC (47% and 56% respectively) compared to patients deemed ineligible due to other reasons (22%).

Survival outcomes

Median overall survival for the total study cohort was 13 months [IQR 5 – 23]. For patients treated with systemic therapy, median OS was 17 months [IQR 9 – 25] (**figure 1A**). For patients receiving BSC, median OS was 4 months [IQR 2 – 9]. Patients receiving bevacizumab as part of systemic treatment had a median OS of

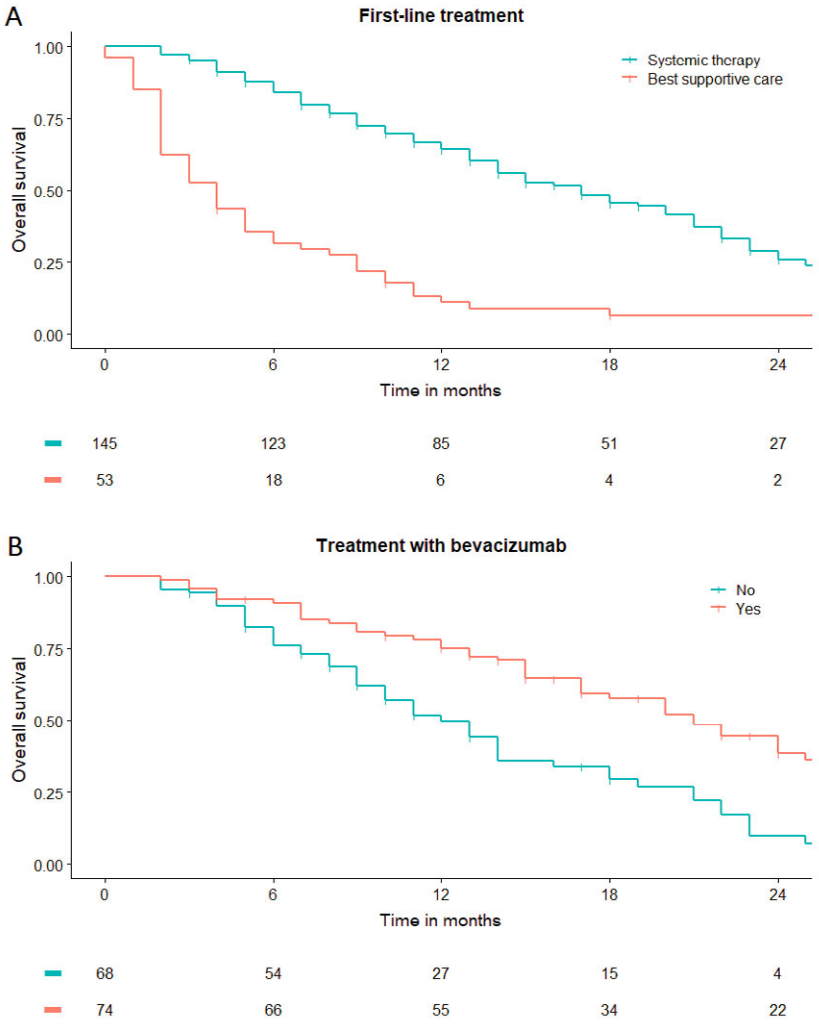

Figure 1. Kaplan-Meier survival curves for overall survival of patients receiving systemic therapy versus BSC (A) and of patients receiving systemic therapy with versus without bevacizumab as first-line treatment (B). The numbers at risk are displayed in the table below the graphs. Data on treatment regimen was available for 198 out of 227 patients. For patients receiving systemic therapy, data on biological therapy was available for 142 patients.

21 months [IQR 13 – 29], compared to 12 months [IQR 7 – 21] in patients treated without bevacizumab (**figure 1B**). Patients deemed ineligible for CRS-HIPEC due to extensive PM had a median OS of 11 months [IQR 5 – 18] (**table 2**). Patients deemed ineligible due to extensive liver metastases (9%), lung metastases (8%), or irresectable peritoneal metastases (3%) showed the longest overall survival rates,

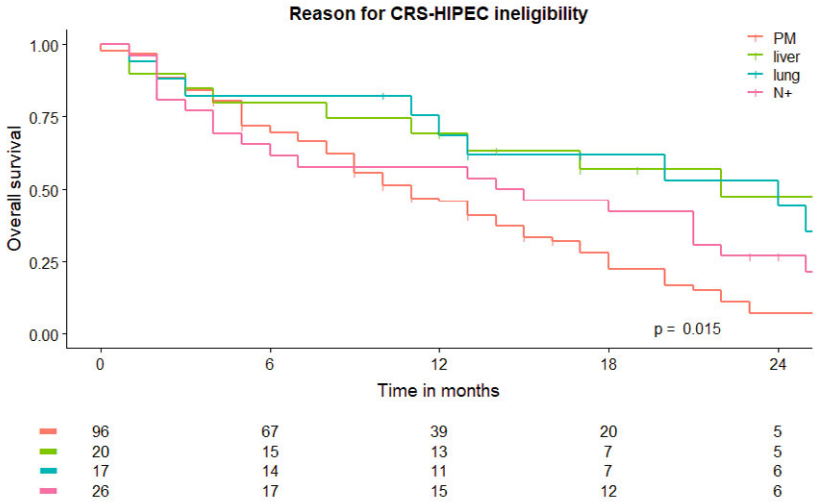

Figure 2. Kaplan-Meier survival curves for overall survival of patients deemed ineligible for CRS-HIPEC due to extensive PM, extensive liver metastases, lung metastases, or distant lymph node involvement. The numbers at risk are displayed in the table below the graph.

Table 3. First-line systemic therapy regimens.

	Total n = 145	**With bevacizumab** n = 74 (51.0)	**Median OS** (months)	**No bevacizumab** [a] n = 68 (46.9)	**Median OS** (months)
Capecitabine	35 (24.1)	23 (15.9)	21 [12 – 27]	12 (8.3)	11 [6 – 14]
CAPOX [b]	62 (42.8)	34 (23.4)	24 [12 – 33]	27 (18.6)	18 [8 – 22]
FOLFOX [c]	15 (10.3)	7 (4.8)	20 [17 – 22]	8 (5.5)	7 [4 – 13]
FOLFIRI [d]	11 (7.6)	5 (3.4)	15 [7 -21]	6 (4.1)	9 [9 – 21]
Irinotecan [e]	12 (8.3)	0 (0)	NA	12 (8.3)	10 [5 – 18]
Other	8 (5.5)	5 (3.4)	13 [9 – 15]	3 (2.1)	9 [2 – NA]
Unknown	2 (1.4)	0 (0)	NA	0 (0)	NA

Continuous variables are shown as: median [IQR]. Frequencies are shown as: N (%)
[a] One patient (0.7%) was treated with cetuximab, and one patient (0.7%) with panitumumab. For three patients (2.1%) data on biological therapy were not availablepunt weg
[b] CAPOX = combination of capecitabine and oxaliplatin
[c] FOLFOX = combination of folinic acid, 5-FU and oxaliplatin
[d] FOLFIRI = combination of folinic acid, 5-FU, and irinotecan
[e] One combination of irinotecan and cetuximab was reported

with 22 [IQR 8 – 27], 24 [IQR 12 – 29], and 23 months [IQR 12 – 48] median OS, respectively. Patients deemed ineligible for CRS-HIPEC because of extensive liver or lung metastases showed longer OS than patients rejected for extensive PM or distant lymph node metastases (**figure 2**). The proportions of patients receiving systemic therapy were 69%, 89%, 78%, and 67%, respectively.

Table 4. Characteristics open-close.

	Total n = 42
PM onset	
Synchronous	23 (54.8)
Metachronous	19 (45.2)
Procedure type	
Explorative only	32 (76.2)
Palliative surgery	9 (21.4)
Palliative surgery + HIPEC	1 (2.4)
Reason open-close	
Extensive PM [a]	34 (81.0)
Distant lymph node metastases [b]	3 (7.1)
Extensive liver metastases	2 (4.8)
(Rapid) progression	2 (4.8)
Irresectable [c]	1 (2.4)
PCI at previous surgical assessment [d]	13 [7 – 15]
PCI at open-close	25 [22 – 27]
Delta PCI [d e]	11 [9 – 15]
Interval surgical assessment PCI – intended CRS-HIPEC (weeks) [d]	5 [2 – 6]
Interval radiological imaging – intended CRS-HIPEC (weeks)	3 [2 – 5]
Hospital stay (days)	7 [5 – 10]
Severe postoperative complications [f]	2 (4.8)
30-days postoperative mortality	3 (7.1)

Continuous variables are shown as: median [IQR]. Frequencies are shown as: N (%).
[a] Peritoneal cancer index (PCI) above 20
[b] Retroperitoneal lymph node metastases
[c] Radical resection impossible due to tumor ingrowth
[d] PCI score at previous surgical assessment was available for 26 patients (61.9%)
[e] Median difference in PCI between previous surgical assessment and OC procedure
[f] Clavien-Dindo grade 3 or higher

Open close procedures

A total of 42 out of 227 patients (19%) underwent an OC procedure (**table 4**). The main reason not to proceed with CRS-HIPEC was extensive PM (81%). The median PCI at OC procedure was 25 [IQR 22 – 27]. For 26 out of 42 patients (62%) PCI was determined at prior surgical assessment, with a median PCI of 13 [IQR 7 – 15]. These patients had a median PCI of 25 [IQR 22 – 27] at OC procedure, with a median time interval between PCI assessment of 5 weeks [IQR 2 – 6]. In 24% of the OC procedures palliative surgery (i.e., resections and/or bowel diversion surgery) was performed. Severe complications (i.e., Clavien Dino ≥ 3) were observed in two patients (5%). There was no in-hospital mortality. Three patients (7%) died within 30 days after OC-procedure. These patients had no severe complications after OC

procedure. The proportion of patients treated with systemic therapy after an OC procedure (71%) did not differ from patients that did not undergo an OC procedure (74%, p=0.736). The time to start of systemic treatment was 6 weeks for OC patients [IQR 3 – 9], compared to 3 weeks [IQR 1 – 4] for patients that did not undergo an OC procedure (p<0.001). Median OS for OC patients was 10 months [IQR 5 – 18], compared to 13 months for patients without an OC procedure [IQR 5 – 24].

DISCUSSION

The current retrospective study showed that approximately half of the patients with colorectal peritoneal metastases (CRC-PM) who were referred for cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) were deemed ineligible for this procedure. Median overall survival (OS) for these patients was 13 months. The majority of patients received systemic treatment (73%), with a median overall survival (OS) of 17 months. Median OS was 4 months for patients receiving best supportive care (BSC). The main reason for CRS-HIPEC ineligibility (42%) was extensive PM (i.e., PCI of 20 or higher). These patients had a poor prognosis, with a median OS of 11 months.

The primary aim of this study was to provide a descriptive overview of the reasons for CRS-HIPEC ineligibility and the treatment and survival outcomes of this specific patient population. No statistical tests were performed to demonstrate significant differences between groups regarding survival outcomes, as this was not the aim of this study. Most of the patients in this study underwent a comprehensive work-up for CRS-HIPEC, resulting in a more complete picture of both patient and tumor characteristics. For example, surgical PCI was obtained for almost half of the patients during CRS-HIPEC work-up. This resulted in an accurate estimate of the extent of the PM in these patients. Most studies reporting on CRC-PM patients who do not undergo CRS-HIPEC reported only an estimation of the extent of PM by radiological imaging.

The proportion of patients treated with systemic therapy in the current cohort corresponds with other studies reporting on CRC-PM patients.[1, 10, 13, 16] In line with previous studies, patients treated with systemic therapy had longer OS (median 17 months) compared to patients receiving BSC (median 4 months).[1, 10, 13, 16, 17] This is probably explained by selection bias, as patients receiving systemic therapy probably have better performance status and less extensive disease. The current study showed that patients deemed ineligible for CRS-HIPEC due to poor performance

status more often received BSC compared to patients deemed ineligible due to other reasons. The median OS for patients receiving BSC in the current cohort was longer than reported by a recent population-based study in the Netherlands by Bakkers et al. (1.8 months).[1] This difference is probably also explained by patient selection. The cohort of Bakkers et al. included all CRC-PM patients, whereas the current cohort consists of patients that were referred for CRS-HIPEC, who were assumed to be fit for major surgery by the referring physician. Median OS for CRC-PM patients treated with systemic therapy in the present study was also longer than previously reported (ranging from 12 – 16.3 months).[1, 2, 17, 18] This might partially be explained by patient selection, but another important explanation is the continuing improvement of systemic therapy. The introduction of novel cytostatic agents and targeted therapies (e.g. bevacizumab) improved survival of patients with metastatic CRC.[19-22] In the current cohort, patients treated with bevacizumab showed longer OS. In the Netherlands, bevacizumab is currently part of standard care for patients with metastatic CRC. Bevacizumab targets vascular endothelial growth factor (VEGF), which is a key regulator of angiogenesis and plays an important role in the formation of CRC metastases.[23, 24] Several studies have also shown involvement of VEGF in the formation of peritoneal metastases.[25-27] In line with the current study, previous studies reported survival benefit of the addition of bevacizumab to the systemic therapy for patients with PM from CRC.[28, 29]

Frequently reported reasons for ineligibility were systemic metastases, distant lymph node metastases, patients' preference, and irresectable disease. Interestingly, patients deemed ineligible for CRS-HIPEC due to systemic metastases showed longer overall survival (median OS 22 months for liver and 24 months for lung metastases) compared to patients deemed ineligible for CRS-HIPEC due to extensive PM or distant lymph node metastases (median OS 11 and 14 months respectively). This suggests that prognosis is mainly determined by the extent of PM and not by the presence of lung or liver metastases. In line with this hypothesis, previous studies reported impaired survival outcomes in patients with PM compared to patients with other metastatic sites of colorectal cancer.[3, 4, 30] A study by Franko et al. reported similar survival outcomes for patients with isolated versus non-isolated colorectal PM receiving first-line systemic chemotherapy.[2] This supports the hypothesis that the presence of PM is the main predictor of survival in this patient population. Controlling the extent of PM is therefore an important factor in the improvement of the prognosis of both patients with isolated and non-isolated colorectal PM. As the effectiveness of systemic therapy alone on colorectal PM seems to be limited, there is a rationale for the addition of local treatment. Local treatment with CRS-HIPEC could be beneficial for selected patients but is

also associated with considerable morbidity. More importantly, only a minority of patients is eligible for this extensive treatment.

Other local treatment strategies are currently investigated. In a recently finished phase I, dose-escalation study: the INTERACT trial, concomitant intraperitoneal irinotecan combined with FOLFOX are administered in cycles of two weeks.[31] Another treatment strategy is pressurized intraperitoneal aerosol chemotherapy (PIPAC).[32, 33] During recurrent laparoscopic procedures, chemotherapy is nebulized into the peritoneal cavity. PIPAC is expected to enforce the cytotoxic effect of the chemotherapeutic agent by increasing the local drug penetration with less systemic toxicity. Although this is a treatment with palliative intent, some patients with good response were subsequently treated with CRS-HIPEC.[34] If the extent of PM can be managed with these new treatment strategies, this will hopefully lead to an improved prognosis for CRC-PM patients.

The current study also included patients that were deemed ineligible for CRS-HIPEC at open close (OC) procedure. An OC procedure resulted in hospitalization for approximately one week and severe complications occurred in two out of 42 patients. Although the time to start of systemic therapy was significantly prolonged (i.e., a median delay of 3 weeks) in patients with an OC procedure, the proportion and survival of patients that were treated with systemic therapy did not differ from patients that did not undergo an OC procedure. Despite being statistically significant, a delay of three weeks is probably not clinically relevant in most cases. Another explanation could be the performance of simultaneous palliative surgery in 24% of OC patients. Like CRS-HIPEC, palliative surgery is accompanied by the risk of complications and substantial hospitalization.[35, 36] Hence, its indication should be considered carefully.

Limitations

Due to the retrospective nature of this study, the main limitation was selection bias. As patients were referred for CRS-HIPEC, these were mostly patients with limited metastatic disease, deemed fit for major surgery by the referring center. Hence, these results are not applicable for the general population of CRC-PM patients. Selection bias also resulted in differences in performance status between patients receiving BSC and systemic therapy, most likely affecting survival outcomes. After rejection for CRS-HIPEC, most patients were referred back to the initial referring center for systemic treatment or follow-up. Follow-up data had to be requested retrospectively, resulting in missing data. Data on performance status was missing for the majority of patients receiving BSC. Likewise, for most patients deemed ineli-

gible for CRS-HIPEC due to other reasons than the extent of PM, surgical PCI score was not available. This should be taken into account when making statements on factors affecting the prognosis of these patients. Another important limitation was the limited sample size. Most patients were rejected for CRS-HIPEC due to extensive PM. Groups of patients rejected due to other reasons were small, making it impossible to make firm statements about the outcomes in these groups.

CONCLUSIONS

In the present study, the majority of patients received systemic therapy and had a median overall survival of 17 months. The main reason for CRS-HIPEC ineligibility in this cohort was extensive PM. Patients deemed ineligible due to lung or extensive liver metastases had better survival outcomes compared to patients rejected for CRS-HIPEC due to extensive PM. The occurrence of an OC procedure did not affect the proportion of patients treated with systemic therapy, nor resulted in impaired survival.

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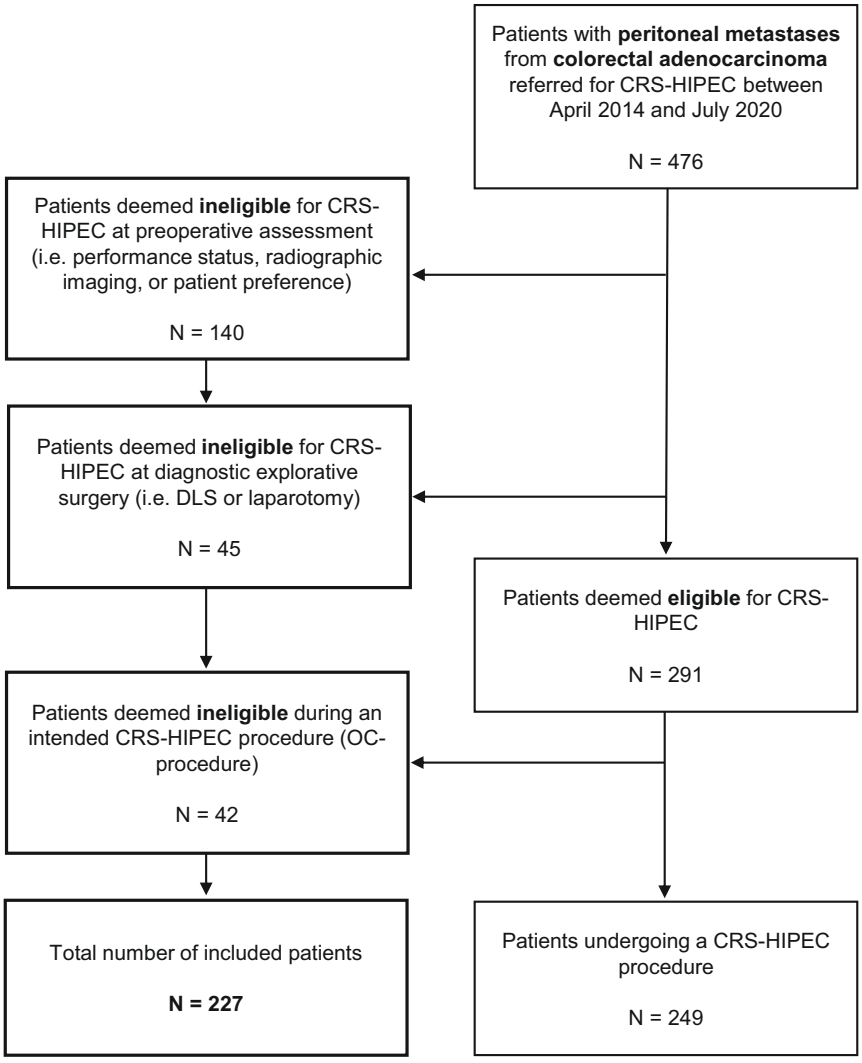
DATA SUPPLEMENT



Supplementary figure 1. Flow diagram of patient selection for the current study cohort.

Chapter 10

Development of a Prediction Model for Recurrence in Patients with Colorectal Peritoneal Metastases undergoing Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy

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ABSTRACT

Introduction

Cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) improves survival outcomes for selected patients with colorectal peritoneal metastases (PM), but recurrence rates are high. The aim of this study was to develop a tool to predict recurrence in patients with colorectal PM who undergo CRS-HIPEC.

Methods

For this retrospective cohort study, data of patients who underwent CRS-HIPEC for colorectal PM from four Dutch HIPEC centers were used. Exclusion criteria were perioperative systemic therapy and peritoneal cancer index (PCI) ≥20. Nine previously identified factors were considered as predictors: gender, age, primary tumor characteristics (location, nodal stage, differentiation, and mutation status), synchronous liver metastases, preoperative Carcino-Embryonal Antigen (CEA), and peritoneal cancer index (PCI). The prediction model was developed using multivariable Cox regression and validated internally using bootstrapping. The performance of the model was evaluated by discrimination and calibration.

Results

In total, 408 patients were included. During the follow-up, recurrence of disease occurred in 318 patients (78%). Significant predictors of recurrence were PCI (HR 1.075, 95% CI 1.044 – 1.108) and primary tumor location (left sided HR 0.719, 95% CI 0.550 – 0.939). The prediction model for recurrence showed fair discrimination with a C-index of 0.64 (95% CI 0.62, 0.66) after internal validation. The model was well-calibrated with good agreement between the predicted and observed probabilities.

Conclusion

We developed a prediction tool that could aid in the prediction of recurrence in patients with colorectal PM who undergo CRS-HIPEC.

INTRODUCTION

Colorectal carcinoma (CRC) is the third most common malignancy worldwide and its burden is expected to increase in the upcoming years.[1] Approximately 10% of these patients develop peritoneal metastases (PM) at some point in the disease course.[2, 3] Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) improves the survival of selected patients with colorectal PM. This extensive treatment results in a median overall survival (mOS) up to four years, which is limited to about a year in patients treated with systemic chemotherapy.[4-6]

To achieve these favorable outcomes, patient selection is of utmost importance. Despite careful patient selection, this extensive local treatment does not result in cure for all patients, which is reflected by high recurrence rates.[5, 7-9] About half of the patients with disease recurrence are diagnosed with systemic metastases which are commonly diagnosed within a year after surgery.[2, 5, 8, 10] For these patents, it is questionable whether CRS-HIPEC alone is the appropriate treatment. Systemic therapy, possibly (neo-)adjuvant around CRS-HIPEC, might be a better treatment option for these patients. Preoperative prediction of recurrence could therefore aid in the guidance of treatment choices. Identifying patients with an elevated risk of recurrence could help select patients who would benefit from additional systemic treatment.

Previous studies aimed to identify predictive factors for recurrence after CRS-HIPEC.[5, 6, 11, 12] These studies included cohorts of patients in which the majority received perioperative systemic chemotherapy. We argue that a cohort of patients who did not receive any perioperative systemic therapy would be more suitable for the prediction of recurrence in a preoperative setting. The current study aimed to develop a tool to predict recurrence after CRS-HIPEC in a cohort of patients who were not treated with perioperative systemic therapy, which could help identify patients who would benefit from additional therapy.

METHODS

Study design and data collection

This retrospective cohort study included patients who underwent a first CRS-HIPEC procedure for colorectal PM in the Erasmus MC Cancer Institute (EMC) in Rotterdam between 2014 and 2021, the Radboud University Medical Center in Nijmegen

between 2010 and 2020, the University Medical Center Groningen between 2006 and 2019, and the Catharina Hospital Eindhoven (CHE) between 2013 and 2017. Exclusion criteria were treatment with perioperative systemic therapy to CRS-HIPEC, appendiceal carcinomas, a peritoneal cancer index (PCI) of 20 or higher, and no histologically proven PM. Patients who were included in the CAIRO-6 trial (NCT02758951) were also excluded from this study.[13]

Relevant patient, disease, and perioperative characteristics, as well as postoperative outcomes were obtained from prospectively maintained databases from the aforementioned centers. Information on survival status was obtained from the national civil registry, when not available in the electronic patient file. Approval for the collection of these data was approved by the local Medical Ethics Review Committees of the Erasmus Medical Center (MEC-2018-1286). This study was conducted in compliance with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement (checklist is provided in the data supplement.[14]

Variable definitions and outcomes

Onset of colorectal PM was defined synchronous if PM was diagnosed at the time of presentation, during routine staging, or at surgery of the primary tumor. Metachronous onset was defined as PM diagnosed in the follow-up period after primary treatment. Nodal stage was determined by clinical staging for patients with synchronous PM and pathological staging for patients with metachronous PM. Disease-free survival (DFS) was defined as the time between CRS-HIPEC and the diagnosis of recurrence, death, or last follow-up in censored cases. Overall survival (OS) was defined as the time between CRS-HIPEC and death or last follow-up in censored cases.

The primary aim of this study was to develop a prediction model for recurrence, irrespective of location. Variables of interest for the development of the prediction model were selected based on previous studies reporting on risk factors for the development of metastases from CRC or recurrence after CRS-HIPEC (irrespective of location).[5, 6, 8, 11, 12, 15-18] Nine predictors were selected for the development of the model. Patient-related characteristics: sex (dichotomous) and age (continuous). Disease-related characteristics: location (right or left-sided), differentiation (good/moderate or poor), and nodal stage (positive or negative) of the primary tumor (all dichotomous), synchronous liver metastases (dichotomous), PCI (continuous), preoperative Carcino-Embryonal Antigen (CEA, continuous), mutational status (categorical, BRAF or KRAS or no BRAF/KRAS mutation).

Perioperative course

The perioperative course of these patients has been described earlier.[8] In summary, all patients were screened by preoperative imaging and if possible a diagnostic laparoscopy (DLS) to determine the extent of the disease, assessed by the PCI.[19] Patients were eligible for CRS-HIPEC if they were fit for major surgery, had an estimated PCI below 20, and no or limited systemic metastases (maximum of three liver metastases). CRS-HIPEC procedures were performed by a specialized surgical team, in accordance with the Dutch CRS-HIPEC protocol.[20, 21] Patients were postoperatively treated following standard of care for CRS-HIPEC procedures.

Follow-up

Follow-up was performed in the outpatient clinic according to a local protocol. In general, during the first two years of follow-up, CT scans were performed every six months, or in case of rising CEA levels or clinical suspicion of recurrent disease. Follow-up was completed after a disease-free interval of five years following CRS-HIPEC. For patients treated in the EMC, CHE, and UMCG, CEA was determined every three months in the first two postoperative years and every six months thereafter. The interval between CT scans increased to 12 months if no recurrence was detected after two years in the EMC and UMCG or three years in the CHE. In the Radboud University Medical Center, CEA measurements and CT scans were performed every six months during the complete five years of follow-up.

Statistical analysis

Descriptive statistics were used to summarize patient, disease, and treatment-related characteristics. Categorical variables were presented as counts with percentages and continuous variables as median with interquartile range (IQR). The reversed Kaplan–Meier method was used to calculate median follow-up and median time to recurrence for patients who were diagnosed with recurrent disease within follow-up. The Kaplan–Meier method was used to estimate the median DFS and OS. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corporation, Armonk, NY) and R (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria).

Model development

The sample size was calculated according to the methods described by Riley et al.[22, 23] Input for the sample size calculation was obtained using data from the development cohort. We used estimated event ratios of .45 and .22, for recurrence, respectively, and a median follow-up of 12.5 months. We selected our time-point of interest for prediction at 12 months. An r-squared value of .15 was used as

suggested.[22] With 10 parameters to be estimated (nine predictors) this resulted in a sample size of ~500 patients. To reduce bias in results due to missing data, multiple imputation by chained equations was used. Additional information about handling of missing data can be found in Data Supplement 1. Predictors were entered into a Cox proportional hazards regression model. Nonlinear associations between continuous predictors (e.g., PCI) and the outcomes were assessed using restricted cubic splines. Plausibility of nonlinear associations was evaluated graphically and benefit for model fit was assessed using likelihood-ratio testing. In the final models no nonlinear associations were modeled. Statistical analysis was performed in each imputed dataset and the resulting estimates were subsequently pooled using Rubin's rule.

Internal validation

To address potential overfitting, internal validation using bootstrapping was performed. For bootstrapping, 500 random samples were drawn from the development dataset (with replacement). A shrinkage factor was then calculated and used to adjust the regression coefficients of the prediction model. Calibration was assessed with a calibration plot for predictions at six months, one year, and two years. Harrell's C was used to determine the discriminatory performance of the model.[24] A Harrell's C of 1.0 indicates perfect discrimination, whereas 0.5 suggests poor discriminative ability (≤0.6 poor, 0.6–0.7 fair, 0.7–0.8 good, 0.8–0.9 very good, 0.9 excellent).

RESULTS

In total, 465 patients underwent a first CRS-HIPEC procedure for colorectal PM in one of the four HIPEC centers and did not receive neo-adjuvant and/or adjuvant systemic therapy. A total of 47 patients were excluded because the location of recurrence was not reported, or they had a PCI of 20 or higher. In total, 408 patients were included in the development cohort. Baseline characteristics are provided in **table 1**.

The median follow-up time for all patients was 14 months (7 – 37). Median DFS was 8 months (5 – 16) for the complete cohort. A total of 318 patients (78%) had recurrence of disease with a median time to recurrence of 7 months (4 – 12). Out of these patients, 182 (57%) were diagnosed with extra-peritoneal metastases and 79 patients (25%) were diagnosed with extra-peritoneal metastases only (without local recurrence, **table 2**). Extra-peritoneal metastases most commonly involved

Table 1. Baseline characteristics.

	Total n = 408
Gender	
Male	193 (47.3)
Age (years)	65 (56 – 71)
ASA-classification	
1-2	322 (80.3)
3-4	79 (19.7)
Missing	*7 (1.7)*
Primary tumor location	
Right-sided	189 (46.3)
Left-sided	219 (53.7)
T stage primary tumor	
T1-2	17 (4.2)
T3-4	385 (95.8)
Missing	*6 (1.5)*
N stage primary tumor [a]	
N-	111 (29.9)
N+	260 (70.1)
Missing	*37 (9.1)*
M stage primary tumor	
M0	164 (46.6)
M1	188 (53.4)
Missing	*78 (18.1)*
Location metastases [b]	
Local/PM	140 (83.3)
Systemic	14 (7.4)
Local/systemic	14 (7.4)
Missing	*20 (10.6)*
Differentiation primary tumor	
Good/moderate	267 (65.4)
Poor	64 (15.7)
Missing	77 (18.9)
Histology primary tumor	
Adenocarcinoma	204 (72.1)
Mucinous	50 (17.7)
Signet ring cell	29 (10.2)
Missing	*125 (30.6)*
Mutation status [c]	
BRAF [d]	13 (3.2)
KRAS [e]	31 (7.6)
Prior chemotherapy [f]	
Yes	117 (28.7)

Table 1. Baseline characteristics. (continued)

	Total n = 408
Time of onset of PM	
Synchronous	154 (37.7)
Synchronous liver metastases	
Yes	36 (8.8)
PCI at DLS [g]	4 (3 – 8)
Preoperative CEA [h]	7.2 (3.6 – 16.5)
HIPEC chemotherapy	
MMC	342 (84.0)
Oxaliplatin	65 (16.0)
Missing	1 (0.2)
PCI at HIPEC [i]	9 (5 – 13)
R score	
R1	405 (99.3)
R2a	2 (0.5)
R2b	0 (0.0)
Missing	1 (0.2)
Severe postoperative complications [j]	
Yes	111 (27.2)
Reoperation [k]	
Yes	47 (12.1)

Continuous variables are shown as median [IQR]. Frequencies are shown as N (%). ASA, American association for anesthesiology; BRAF, B-Raf proto-oncogene; CEA, carcinoembryonic antigen; CCR, completeness of cytoreduction; HIPEC, hyperthermic intraperitoneal chemotherapy; KRAS, kirsten rat sarcoma viral; MMC mitomycin-C; PCI, peritoneal cancer index; PM, peritoneal metastasis.

[a] Nodal stage was determined by clinical staging for patients with synchronous PM and pathological staging for patients with metachronous PM [b] Location of metastases synchronous to primary tumor [c] Data available for 73 patients (17.9%) [d] Most common type of BRAF mutation p.V600E in 12 patients (92.3%) [e] Most common type of KRAS mutation pG12D in 12 patients (38.7%) [f] Data available for 378 patients (92.6%) [g] Data available for 170 patients (41.7%) [h] Data available for 155 patients (62.0%) [i] Data available for 390 patients (95.6%) [j] According to Clavien–Dindo classification ≥ III (i.e., reintervention, extended ICU stay/readmission to ICU, or treatment-related death); available for 397 patients (97.3%) [k] Data available for 388 patients (95.1%)

the liver in 62 patients (40%), lungs in 41 patients (27%), or both in 20 patients (13%). A total of 218 patients deceased during follow-up, resulting in a median OS of 34 months (IQR 18 – 56).

Prediction of disease recurrence

Table 3 displays regression coefficients and hazard ratio's (HR) of the predictors for recurrence. There was no evidence for multicollinearity between the predictors. Through bootstrapping by internal validation a shrinkage factor of 0.90 was estimated. Significant predictors for recurrence of disease in multivariable analysis

Table 2. Location of recurrence.

	Total n = 318	**Median time to recurrence** (months)
Recurrence location		
Local	132 (41.5)	8 (5 – 12)
Systemic	79 (24.8)	6 (4 – 11)
Local and systemic	103 (32.4)	7 (4 – 9)
Systemic location [a]		
Liver	62 (40.0)	6 (3 – 9)
Lung	41 (26.5)	7 (4 – 8)
Liver and lung	20 (12.9)	5 (4 – 8)
Other	32 (20.6)	6 (4 – 12)
Missing	*27 (14.8)*	

[a] For all patients (n=182) with systemic recurrence.

Table 3. Predictors for recurrence after CRS-HIPEC.

	Univariable HR (95% CI)	**Regression coefficient** (β) [a]	**Multivariable HR** (95% CI) [a]
Gender			
Male	Ref	Ref	Ref
Female	1.052 (0.845 – 1.309)	0.139	1.149 (0.801 – 1.647)
Age (years)	0.995 (0.988 – 1.002)	-0.009	0.991 (0.978 – 1.005)
Primary tumor location			
Right sided	Ref	Ref	Ref
Left sided	0.845 (0.678 – 1.054)	-0.330	0.719 (0.550 – 0.939)
N stage primary tumor [b]			
N-	Ref	Ref	Ref
N+	1.158 (0.899 – 1.489)	0.117	1.124 (0.866 – 1.459)
Synchronous liver metastases [c]			
Yes	0.988 (0.653 – 1.497)	-0.123	0.884 (0.543 – 1.440)
Differentiation primary tumor			
Good/moderate	Ref	Ref	Ref
Poor	1.211 (0.890 – 1.647)	0.130	1.139 (0.773 – 1.678)
PCI at HIPEC	1.088 (1.062 – 1.116)	0.072	1.075 (1.044 – 1.108)
CEA	1.005 (1.001 – 1.008)	0.003	1.0035 (1.000 – 1.007)
Mutational status			
No	Ref	Ref	Ref
BRAF	1.837 (0.650 – 5.194)	0.654	1.923 (0.611 – 6.048)
KRAS	1.596 (0.643 – 3.961)	0.502	1.653 (0.686 – 3.980)

CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; KRAS, kirsten rat sarcoma viral; MMC mitomycin-C; PCI, peritoneal cancer index

[a] After internal validation, adjustment with shrinkage factor 0.901. [b] Nodal stage was determined by clinical staging for patients with synchronous PM and pathological staging for patients with metachronous PM. [c] Synchronous to colorectal peritoneal metastases.

were PCI (HR 1.075, 95% CI 1.044 – 1.108) and left-sided primary tumor location (HR 0.719, 95% CI 0.550 – 0.939). After shrinkage, the model demonstrated a C-index of 0.64 (95% CI 0.62, 0.66) for the development cohort, which is defined as fair discriminative capacity. Calibration of this model was satisfactory, with a tendency towards an underestimation of probabilities in low-risk patients at six months after surgery and in high-risk patients at two years after surgery (**figure 1**). **Supplementary figure 1** shows the risk-prediction nomogram.

Additional prediction of extra-peritoneal recurrence

We hypothesized that specifically patients with extra-peritoneal recurrence (with or without peritoneal recurrence) could gain benefit from systemic perioperative therapy. For this reason, we developed a second prediction model to predict extra-peritoneal recurrence as shown in **supplementary table 1**. The only significant predictor for extra-peritoneal recurrence in multivariable analysis was PCI (internally validated HR 1.698 (1.254 – 2.298). Internal validation resulted in a shrinkage factor of 0.84 with a C-index of 0.64 (95% CI 0.62, 0.66) also defined as fair discriminative capacity. The calibration plots showed good agreement between the predicted and observed probabilities of systemic recurrence at six months, one year, and two years after surgery (**supplementary figure 2**). **Supplementary figure 3** displays the risk-prediction nomogram.

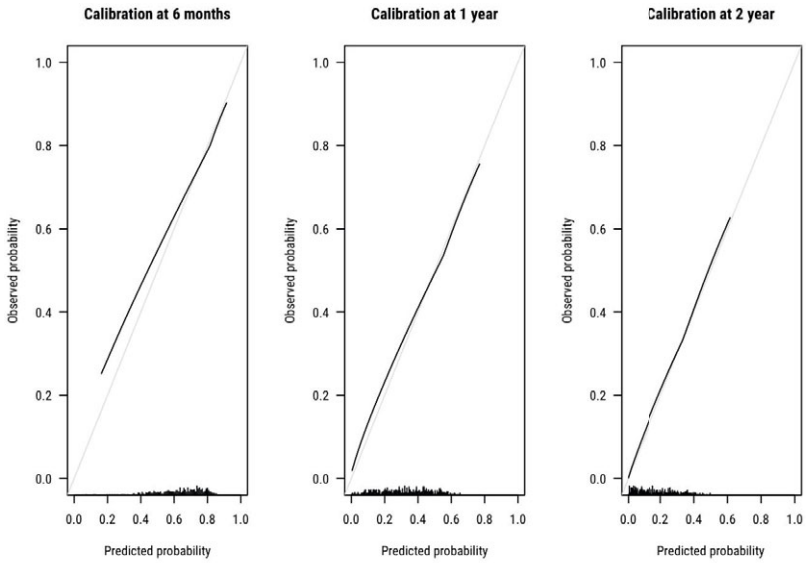

Figure 1. Calibration plots for the predicted systemic recurrence probability at six months (A), one year (B), and two years (C).

Prediction Web Application

The prediction model for recurrence was implemented in a web application to estimate a patient's recurrence probability after CRS-HIPEC at different time points. The application is available at https://colorectalpm.shinyapps.io/recurrence_colorectal_pm/.

DISCUSSION

In the current study, we developed a prediction tool for recurrence after cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with colorectal peritoneal metastases (PM) that could aid in clinical decision-making. PCI and right primary tumor location were the most important significantly associated factors with an increased risk of recurrence. The prediction model that was developed showed fair discriminatory capacity and was well-calibrated, providing accurate risk predictions.

High recurrence rates after CRS-HIPEC in patients with PM underscore the need for the optimization of perioperative treatment strategies. Additional perioperative systemic therapy might reduce the risk of recurrence in these patients. In most countries, perioperative systemic chemotherapy, either in neo- or adjuvant setting, is standard of care in patients undergoing CRS-HIPEC for colorectal PM.[25] The evidence supporting the benefit of this additional therapy is however scarce and randomized controlled trials (RCTs) are missing.[26] In the Netherlands, due to this lack of evidence, systemic therapy is not used as a standard perioperative regimen.[27] This provided the opportunity to initiate the ongoing CAIRO-6 trial, in which patients with isolated colorectal PM are randomized to receive either CRS-HIPEC alone, or CRS-HIPEC with perioperative systemic therapy.[13] The results from this trial will provide valuable information on the benefit of systemic therapy for these patients.

However, not all patients with PM likely benefit from systemic therapy or the addition of this treatment to CRS-HIPEC. Due to associated toxicity and patient burden, it would be preferred to select patients who most likely gain survival benefit. Patients at low risk of recurrence may not derive significant benefit from systemic therapy and could potentially be spared additional treatment. A previous study by Rieser et al. (2021) developed a prediction model to specifically predict early recurrence after CRS-HIPEC (i.e., within eight months).[11] The authors did not specify location of recurrence, but identified BMI, liver lesions, progression on chemotherapy, posi-

tive nodal stage, and PCI as predictors of early recurrence. This model showed fair discriminatory power and has not yet been externally validated but might have added value in patient selection for additional therapy or CRS-HIPEC in general.
An important limitation of this study and most previous studies evaluating risk factors for recurrence after CRS-HIPEC is that a substantial proportion of patients received perioperative systemic therapy, which could have affected the risk of recurrence. For the utility in perioperative patient selection, one would preferably identify risk factors in a cohort of patients who did not receive perioperative systemic therapy. Since perioperative systemic therapy is not standard of care in the Netherlands, the current study presents a relatively large cohort of patients who did not receive additional perioperative therapy. In this cohort, only PCI and primary tumor location were strong predictors for recurrence in this cohort. In contrast to the study of Rieser et al., prognostic factors like nodal stage and synchronous liver metastases were not significantly associated with recurrence.[11] This might be due to selection bias, as these patients might have been treated with perioperative chemotherapy more often.

Although our prediction model shows fair discriminatory capacity, better discrimination would be preferred for individual patient selection and its utility in clinical decision-making. Another strategy to identify patients who most likely benefit from systemic therapy would be to predict the development of extra-peritoneal recurrence (either with or without peritoneal recurrence). Patients with 'systemic disease' are more likely to benefit from systemic treatment compared to patients with local disease only. An additional model predicting extra-peritoneal recurrence identified PCI as the only significant predictor and provided a similar performance compared with the model for any recurrence. Previous studies evaluating the site of recurrence after CRS-HIPEC were not able to establish any risk factor for extra-peritoneal recurrence.[6, 12] This is probably explained by a difference in outcome measures. These previous studies used isolated extra-peritoneal recurrence as an outcome measure, whereas the current study included all extra-peritoneal metastases (with or without peritoneal recurrence), since we argued that both groups would benefit from the addition of systemic therapy. Due to similar performance and the limited ability to identify factors that specifically predict extra-peritoneal recurrence, we concluded that the additional value of this second model in clinical practice would be limited.

If the CAIRO-6 trial shows that the addition of systemic therapy results in an overall survival benefit for patients with colorectal PM undergoing CRS-HIPEC, one could argue that this should become standard of care for all patients. The first results

of the CAIRO-6 trial show that the addition of perioperative systemic therapy has acceptable tolerability, so the burden of this addition might be limited.[28] Nonetheless, we argue that the proposed model could potentially help guide clinical decision-making in selected cases, since this is currently the only tool available for the preoperative prediction of recurrence in patients undergoing CRS- HIPEC. To establish its potential utility, the model should be externally validated, preferably on the CAIRO-6 data. Additionally, new predictors that might optimize patient selection are widely being investigated. A potential biomarker of interest, specifically for the prediction of extra-peritoneal recurrence, is ctDNA. A study by Beagan et al. showed that ctDNA could serve as a preoperative marker of recurrence in a small cohort of patients with colorectal PM.[29] In four out of five patients who experienced extra-peritoneal recurrence, ctDNA was detected preoperatively.

Strengths and limitations

A major strength of this study is that it includes a large cohort of patients with PM undergoing CRS-HIPEC who did not receive perioperative systemic therapy. Hence, the models presented in this study are currently the only available tools to select patients who potentially benefit from perioperative systemic therapy. Nonetheless, their utility in individual patient selection is limited as they do not show optimal discrimination. The sample size calculation to use nine predictors in the models resulted in a sample size of ~500 patients. Although relatively large, our cohort was limited to 408 patients which could have limited the power to find significant results. The retrospective nature of this study could have resulted in selection bias. Although perioperative systemic therapy is currently not standard of care for patients with PM undergoing CRS-HIPEC in the Netherlands, patients with potential risk factors for early recurrence could have received adjuvant systemic therapy more often. Likewise, although not standard of care, induction systemic therapy could have been considered in patients with extensive or borderline resectable disease. These patients were excluded from the current study, as the use of perioperative systemic therapy could have affected the risk of recurrence. Another important limitation due to the retrospective nature was missing data. Missing data was common for some potential predictors such as preoperative CEA and mutational status. To address missing data, multiple imputation was used. This is accompanied by a small risk of bias, but this was deemed to be higher with complete case analysis.

CONCLUSIONS

Based on the developed prediction model the ability to select patients who might benefit from perioperative systemic therapy around CRS-HIPEC based on their risk of recurrence is limited. Since this model is currently the only available tool for pre-operative prediction of recurrence, it could aid in clinical decision-making. The utility of this model must be further evaluated, and future studies should focus on the identification of new risk factors for recurrence to improve patient selection for perioperative systemic therapy.

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DATA SUPPLEMENT

Data supplement 1: Handling of missing data

Multivariate multiple imputation by chained equations (i.e., predictive mean matching) was used in model predictors and outcome to reduce bias due to missing data.1 Multivariate imputation by chained equations was performed using predictive mean matching. This method assumes that data are missing at random (MAR), meaning that any systematic differences between the observed and missing values can be explained by differences in the observed data. An inclusive imputation strategy was performed to satisfy the MAR assumption: baseline, treatment-related and outcome variables were included as predictors for the imputed missing values.2, 3 Twenty imputed datasets were created with 10 iterations per set to comply with the recommendation of one imputation per percent of incomplete observations.4 Convergence of the chained equation procedure was visually evaluated from trace plots of the mean and standard deviation of the imputed data against iteration number. Imputed data was assessed for consistency and plausibility amongst the different datasets. Statistical analyses were performed in each imputed dataset and subsequently Rubin's rule was used to pool results of analyses.

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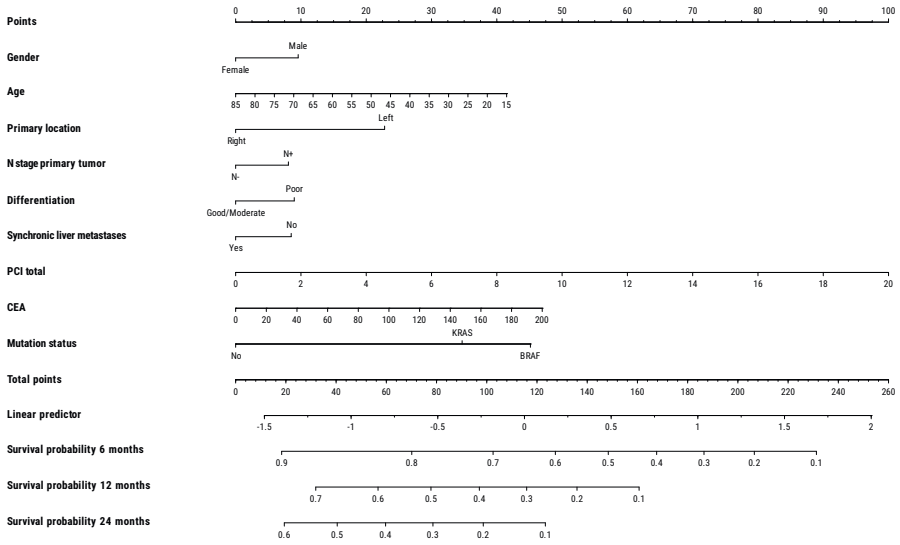
Data supplement 2: supplementary tables and figures

Supplementary table 1. Predictors for extra-peritoneal recurrence after CRS-HIPEC

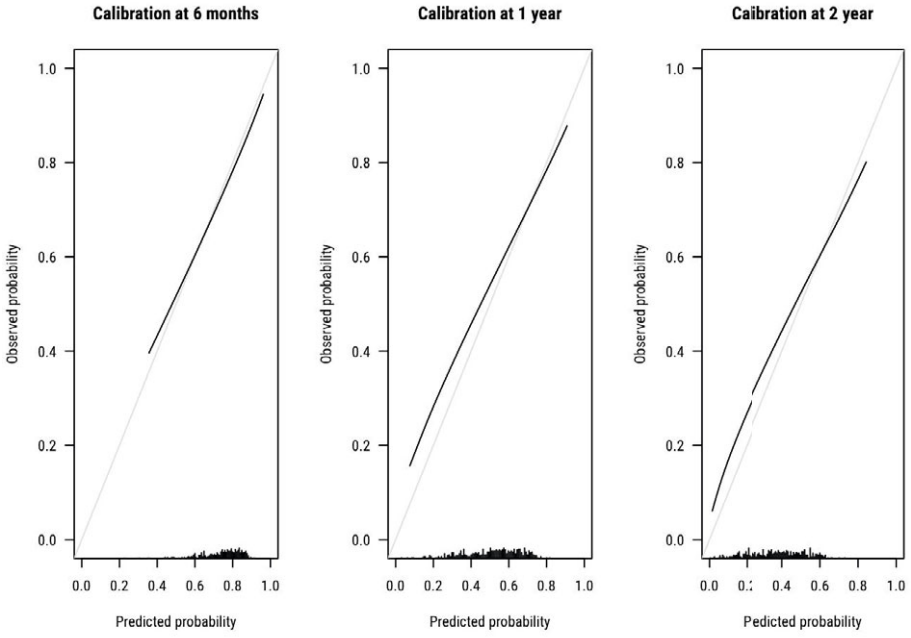
	Univariable HR (95% CI)	**Regression coefficient** (β) [a]	**Multivariable HR** (95% CI) [a]
Gender			
Male	Ref	Ref	Ref
Female	1.158 (0.867 – 1.548)	0.308	1.361 (0.893 – 2.073)
Age (years)	0.995 (0.982 – 1.009)	-0.008	0.992 (0.976 – 1.009)
Primary tumor location			
Right sided	Ref	Ref	Ref
Left sided	0.901 (0.673 – 1.205)	-0.224	0.800 (0.567 – 1.128)
N stage primary tumor [b]			
N-	Ref	Ref	Ref
N+	1.367 (0.963 – 1.941)	0.242	1.274 (0.868 – 1.870)
Synchronous liver metastases [c]			
Yes	1.322 (0.818 – 2.138)	0.145	1.129 (0.6260 – 2.039)
Differentiation primary tumor			
Good/moderate	Ref	Ref	Ref
Poor	1.008 (0.659 – 1.543)	-0.004	0.996 (0.606 – 1.640)
PCI at HIPEC	1.078 (1.043 – 1.114)	0.056	1.057 (1.018 – 1.098)
CEA	1.003 (0.998 – 1.008)	0.002	1.002 (0.997 – 1.007)
Mutational status			
No	Ref	Ref	Ref
BRAF	2.168 (0.670 – 7.014)	0.808	2.244 (0.651 – 7.736)
KRAS	1.734 (0.585 – 5.139)	0.583	1.791 (0.628 – 5.120)

CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; KRAS, kirsten rat sarcoma viral; MMC mitomycin-C; PCI, peritoneal cancer index; Ref, reference.

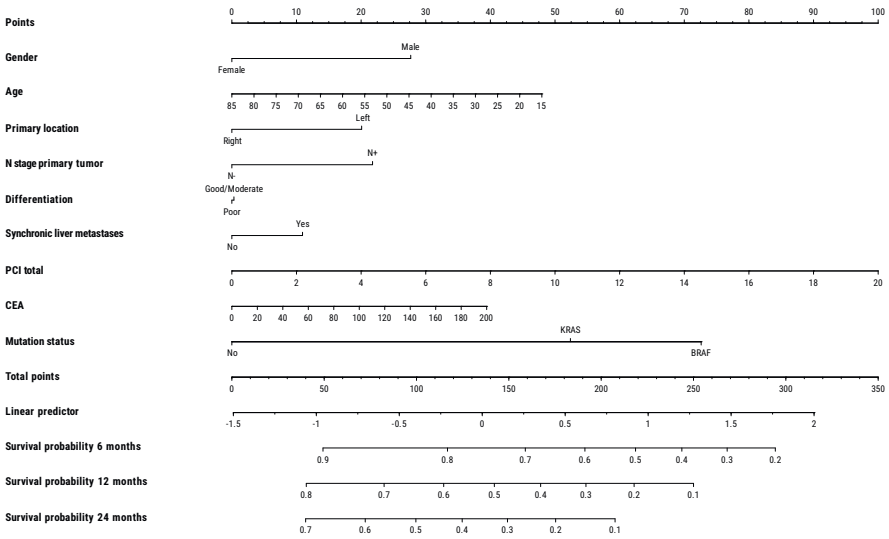
[a] After internal validation, adjustment with shrinkage factor 0.84. [b] Nodal stage was determined by clinical staging for patients with synchronous PM and pathological staging for patients with metachronous PM. [c] Synchronous to colorectal peritoneal metastases



Supplementary figure 1. Risk-prediction nomogram for systemic recurrence after CRS-HIPEC for patients with colorectal PM.



Supplementary figure 2. Calibration plots for the predicted extra-peritoneal recurrence probability at six months (A), one year (B), and two years (C).



Supplementary figure 3. Risk-prediction nomogram for recurrence after CRS-HIPEC for patients with colorectal PM.

Part III

Summary
General discussion and future perspectives

Chapter 11

English summary
Nederlandse samenvatting

The current thesis focuses on two types of peritoneal surface malignancies: peritoneal mesothelioma (PeM) and peritoneal metastases (PM) originating from colorectal carcinoma (CRC). In **part I** of this thesis we aim to improve outcomes of patients with PeM. We explore new therapeutic options to improve outcomes for both patients who undergo cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) and patients who are ineligible for surgery. **Part II** of this thesis focuses on the optimization of patient selection for CRS-HIPEC in patients with colorectal PM.

PART I – PERITONEAL MESOTHELIOMA

Chapter two describes the results of the MESOPEC trial in which we assessed the feasibility of adjuvant treatment with dendritic cell-based immunotherapy (DCBI) after CRS-HIPEC. Feasibility was predefined as administration of at least three adjuvant DC vaccinations in 75% of the patients. In total, 18 patients with epithelioid PeM were included, of whom 16 underwent CRS-HIPEC. We concluded that treatment with adjuvant DCBI after CRS-HIPEC is feasible and safe since all 16 patients received at least three adjuvant DC vaccinations, without the occurrence of severe toxicity. Median progression-free survival (PFS) was 12 months (IQR 5–23) for all patients. Promising survival outcomes and immune modulatory effects of DCBI were observed in a subset of the patients. Comprehensive immunomonitoring showed increased proliferation of circulating natural killer cells and CD4+ T-helper (Th) cells. After treatment, co-stimulatory molecules, including ICOS, HLA-DR, and CD28 were upregulated, predominantly on memory and proliferating Th-cells and minimally on CD8+ cytotoxic T-lymphocytes (CTLs). An increase in CD8+ terminally differentiated effector memory (Temra) cells positively correlated with PFS, whereas co-expression of ICOS and Ki67 on CTLs trended toward a positive correlation. These data provide rationale for future combination treatment strategies.

Chapter three presents the study protocol of the ENSURE trial, together with the results of the first patient who was included in this trial. The primary aim of the ENSURE trail is to assess the feasibility of DCBI administration before and after extended pleurectomy/ decortication (eP/D) in patients with resectable epithelioid pleural mesothelioma. The first patient was treated according to protocol and received seven DC vaccinations (two neo-adjuvant and five adjuvant to eP/D), with an ongoing recurrence-free survival (RFS) of 12 months. No severe postoperative complications or severe DCBI-related adverse events (AEs) were observed. The intratumoral immunological effect of DCBI was assessed by analysis of the tumor

material prior to and after DCBI, together with a biopsy from a delayed type hypersensitivity (DTH) skin test after DCBI injection. CD8+ tumor-infiltrating lymphocytes (TIL) and tertiary lymphoid structures (TLS) appeared to be more abundant post DCBI. T cell receptor (TCR) sequencing showed four overlapping TCR clones between the skin biopsy and the tumor post DCBI that were not present prior to DCBI. This suggests the presence of DCBI-specific/induced T cell infiltration into the tumor. These preliminary clinical results are promising, but full inclusion must be awaited before firm statements can be made.

For patients with PeM who are ineligible for surgery, current anti-tumor treatment options are limited to an often ineffective systemic treatment. In **chapter four**, we present the study protocol of the INTERACT MESO trial. In this trial, patients with PeM who are not eligible for CRS-HIPEC are treated with intraperitoneal (IP) chemotherapy. We hypothesized that local treatment would be a promising approach for these patients since PeM rarely disseminates outside the abdominal cavity. A higher, more effective dose of chemotherapy can directly be delivered at the site of the disease. Limited systemic uptake will likely result in less toxicity compared to systemic chemotherapy. Paclitaxel is a chemotherapeutic agent that is considered favorable for IP use. Before the effectivity of this treatment can be investigated, the maximum tolerable dose of IP paclitaxel monotherapy needs to be determined. For this purpose, the INTERACT MESO trial was designed. Secondary aims are to assess safety and toxicity, feasibility, and the pharmacokinetic profile of this treatment.

Another promising treatment strategy for patients who are ineligible for surgery is targeted therapy. In **chapter five**, we performed a systematic literature search to explore the genomic landscape of PeM aiming to identify potential therapeutic targets. In total, 13 articles were included, comprising 824 patients with PeM. A total of 142 genes were altered in ≥1% of patients, of which 7 genes were altered in ≥10%. *BAP1* was the most commonly altered gene (50%). Other commonly altered genes were *NF2* (25%), *CDKN2A* (23%), *CDKN2B* (17%), *PBRM1* (15%), *TP53* (14%), and *SETD2* (13%). In total, 17% of patients with PeM were carriers of a germline mutation, mainly in *BAP1* (7%). This proportion is higher compared to the pleural variant of mesothelioma and germline mutations might be a larger contributor to the incidence of PeM than previously thought. Based on these data, currently available targeted therapy options are limited. Several targeted agents, such as poly (ADP-ribose) polymerase (PARP), enhancer of zeste homolog 2 (EZH2), and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, were identified that might provide new therapy options in the future.

In **chapter six,** we evaluated the value of next generation sequencing (NGS) of PeM samples in current clinical practice. Foundation Medicine F1CDx NGS was performed on 20 tumor samples of patients treated in the Erasmus MC Cancer Institute. This platform assesses 360 genes that are known to be somatically mutated in solid tumors and provides a genomic signature. NGS was successful in 19 out of 20 cases. Tumor mutational burden (TMB) was low in 10 cases and 11 cases were microsatellite stable. In the other cases, TMB and microsatellite status could not be determined. *BAP1* mutations were detected in 32% of the patients, *CDKN2A/B* and *NF2* mutations in 16%, and *ATM* in 11%. For patients with mutations in *NF2* or *ATM*, potential targeted therapies are available for other tumor types (i.e., protein kinase inhibitors for three *NF2* mutated tumors, and poly ADP-ribose polymerase (PARP) inhibitors for two ATM mutated tumors). These therapies are currently not available for patients with PeM, but ongoing developments might result in new treatment options in the future.

PART II – COLORECTAL PERITONEAL METASTASES

To achieve favorable outcomes of CRS-HIPEC, careful patient selection is essential. In **chapter seven**, we aimed to assess the impact of low skeletal muscle mass (SMM) on outcomes after CRS-HIPEC in patients with colorectal PM or pseudomyxoma peritonei (PMP). SMM was measured on computed tomography (CT) by means of the L3 muscle index. Of 284 included patients, 149 had low SMM. Occurrence of severe postoperative complications did not differ between groups (28.9% for patients with low vs. 34.1% for patients with normal SMM). Low SMM was not associated with postoperative complications (p = 0.344). For patients with colorectal PM, no significant differences were observed in median disease-free (DFS) or overall survival (OS) between patients with low (7 months, IQR 4–14, 33 months, IQR 14–NR, respectively) and patients with normal SMM (8 months, IQR 5–20, 35 months, IQR 18–NR, respectively). Survival outcomes also did not significantly differ between groups for patients with PMP (3-yearDFS 47.3% for patients with low SMM vs. 54.5% for patients with normal SMM, p = 0.676; 3-year OS 70.8% vs. 90.9% respectively, p = 0.172). In conclusion, SMM was not identified as a predictor of severe complications or survival outcomes after CRS-HIPEC in patients with colorectal PM or PMP.

In **chapter eight**, we evaluated the impact of the onset of PM (synchronous vs. metachronous) on survival outcomes after CRS-HIPEC. A retrospective cohort study included 390 patients undergoing CRS-HIPEC for colorectal PM in two aca-

demic centers in the Netherlands between 2010 and 2020. Patients were classified as synchronous (s-PM, i.e., diagnosis at time of presentation, staging, or primary surgery) or metachronous onset (m-PM, i.e., diagnosis during follow-up) of colorectal PM. A total of 179 patients (45.9%) had s-PM. These patients presented more often with a higher TN-stage and poor differentiation/signet ring cell histology. Treatment with perioperative chemotherapy was more common in patients with s-PM. Patients with m-PM experienced more serious postoperative complications (i.e., Clavien-Dindo ≥III). There was no significant difference in DFS between s-PM (median 9 months, IQR 5–15) and m-PM patients (median 8 months, IQR 5–17). OS was significantly shorter for patients with s-PM (median 28 months, IQR 11–48) versus patients with m-PM (median 33 months, IQR 18–66, p = 0.049). In a multivariable analysis, time of onset of PM was not independently associated with OS. We concluded that synchronous onset of colorectal PM is associated with poor tumor characteristics and more advanced disease but is not an independent predictor of survival outcomes after CRS-HIPEC.

Chapter nine provides an overview of treatment and survival outcomes for patients deemed ineligible for CRS-HIPEC. A total of 476 patients with colorectal PM were referred for CRS-HIPEC to the Erasmus MC Cancer Institute from 2014 to 2020. Out of these patients, 227 (48%) were deemed ineligible for this treatment. Data on follow-up therapy was available for 198 patients, of which 73% received systemic treatment. These patients had a median OS of 17 months [IQR 9–25]. For patients receiving best supportive care (BSC) median OS was 4 months [IQR 2–9]. The main reason for ineligibility was extensive PM (42%), with a median OS of 11 months [IQR 5–18]. Patients deemed ineligible due to (extensive) liver (9%) or lung metastases (8%) showed longer OS (median 22 months, IQR 8–27, and 24 months, IQR 12–29, respectively) than patients with extensive PM (median 11 months, IQR 5–18) or distant lymph node metastases (median 14 months, IQR 4–25).

In **chapter ten**, we aimed to develop a prediction model for recurrence after CRS-HIPEC, to identify patients with colorectal PM that might benefit from the addition of systemic therapy. We hypothesized that patients with a high risk of recurrence would gain benefit from this addition, whereas patients with a low risk could be spared this additional treatment. We performed a retrospective cohort study, including 408 patients with colorectal PM that underwent CRS-HIPEC in four Dutch HIPEC centers and did not receive perioperative systemic therapy. The predictors that were entered in the model were sex, age, primary tumor location, differentiation and nodal stage, the presence of synchronous

liver metastases, PCI at CRS-HIPEC, preoperative CEA, and mutational status. The internally validated prediction model for recurrence showed fair discrimination, based on a C-index of 0.64 (95% CI 0.62, 0.66). We concluded that it remains challenging to select patients that would benefit from perioperative systemic therapy in addition to CRS-HIPEC. Since this model is currently the only available tool for pre-operative prediction of recurrence in this population, it could aid in clinical decision making. Future studies should evaluate the utility of the proposed model and should focus on the identification of new predictors.

NEDERLANDSE SAMENVATTING

Dit proefschrift focust op twee soorten maligniteiten van het peritoneum: peritoneaal mesothelioom (PeM) en peritoneale metastasen (PM) afkomstig van het colorectaal carcinoom (CRC). In **deel I** van dit proefschrift richten we ons op het verbeteren van de resultaten voor patiënten met PeM. Hierbij exploreren we nieuwe behandelopties om de overlevingsuitkomsten voor zowel patiënten die cytoreductieve chirurgie (CRS) gecombineerd met hypertherme intraperitoneale chemotherapie (HIPEC) ondergaan te verbeteren, als voor patiënten die niet in aanmerking komen voor chirurgie. **Deel II** van dit proefschrift richt zich op de optimalisatie van patiëntselectie voor CRS-HIPEC bij patiënten met colorectale PM.

DEEL I – PERITONEAAL MESOTHELIOOM

In **hoofdstuk twee** van dit proefschrift worden de resultaten van de MESOPEC studie beschreven. In de MESOPEC studie hebben we de haalbaarheid van adjuvante behandeling met dendritische cel immunotherapie (DCBI) na CRS-HIPEC onderzocht. Haalbaarheid werd gedefinieerd als toediening van ten minste drie adjuvante DC-vaccinaties bij 75% van de patiënten. In totaal werden 18 patiënten met epitheloïd PeM geïncludeerd, waarvan 16 CRS-HIPEC ondergingen. We concludeerden dat behandeling met adjuvante DCBI na CRS-HIPEC haalbaar en veilig is, aangezien alle 16 patiënten ten minste drie adjuvante DC-vaccinaties ontvingen, zonder ernstige toxiciteit. De mediane progressievrije overleving (PFS) was 12 maanden (IQR 5-23) voor alle patiënten. In een deel van de patiënten werden veelbelovende overlevingsresultaten en immuunmodulerende effecten van DCBI geobserveerd. Uitgebreide immunomonitoring toonde een toename in proliferatie van circulerende natural killer cellen en CD4+ T-helper (Th)-cellen aan. Na behandeling was er was een toename te zien in de expressie van co-stimulerende moleculen, waaronder ICOS, HLA-DR en CD28, op geheugen- en prolifererende Th-cellen. Dit was in mindere mate het geval voor CD8+ cytotoxische T-lymfocyten (CTL's). Er was wel sprake van een positieve correlatie tussen de toename van CD8+ terminaal gedifferentieerde effectormemory (Temra) cellen en de PFS. Ten aanzien van de co-expressie van ICOS en Ki67 op CTL's en PFS, was er slechts sprake van een positieve trend. Deze resultaten bieden een basis voor nieuwe combinatietherapieën.

In **hoofdstuk drie** wordt het studieprotocol van de ENSURE-studie gepresenteerd, met daarbij de resultaten van de eerste geïncludeerde patiënt. Het primaire doel

van deze studie is om de haalbaarheid van DCBI-toediening vóór en na uitgebreide pleurectomie/decorticatie (eP/D) te beoordelen in patiënten met resectabel epitheloïd pleuraal mesothelioom. De eerste patiënt werd volgens protocol behandeld en ontving in totaal zeven DC-vaccinaties (twee neoadjuvant en vijf adjuvant aan eP/D) met een lopende PFS van 12 maanden. Er werden geen ernstige postoperatieve complicaties of ernstige DCBI-gerelateerde bijwerkingen waargenomen. Middels analyse van het tumorweefsel vóór en na DCBI, samen met een huidtestbiopt na DCBI-injectie, werd het intratumorale effect van DCBI onderzocht. Na DCBI leek er in de tumor sprake van een toename van CD8+ tumor-infiltrerende lymfocyten (TIL) en tertiaire lymfoïde structuren (TLS). T-celreceptor (TCR) sequencing toonde een overlap aan tussen vier TCR klonen in het huidbiopt en de tumor na DCBI, die niet aanwezig waren vóór DCBI. Dit suggereert aanwezigheid van DCBI-specifieke/geïnduceerde T-celinfiltratie in de tumor. Deze voorlopige klinische resultaten zijn veelbelovend, maar volledige inclusie moet worden afgewacht om harde uitspraken te kunnen doen over deze behandelstrategie.

Voor patiënten met PeM die niet in aanmerking komen voor chirurgie zijn de huidige behandelingsopties beperkt tot een vaak ineffectieve systemische behandeling. In **hoofdstuk vier** presenteren we het studieprotocol van de INTERACT MESO studie. In deze studie worden patiënten met PeM die niet in aanmerking komen voor CRS-HIPEC behandeld met intraperitoneale (IP) chemotherapie. We veronderstellen dat lokale behandeling een veelbelovende aanpak kan zijn voor deze patiënten, omdat PeM zelden buiten de buikholte verspreidt. Een hogere, effectievere dosis chemotherapie kan direct op de plaats van de ziekte worden toegediend. Beperkte systemische opname zal waarschijnlijk resulteren in minder toxiciteit in vergelijking met systemische chemotherapie. Paclitaxel is een chemotherapeutisch middel dat als gunstig wordt beschouwd voor IP-gebruik. Voordat de effectiviteit van deze behandeling kan worden onderzocht, moet de maximaal verdraagbare dosis van IP-paclitaxel monotherapie worden bepaald. Voor dit doel is de INTERACT MESO-studie ontworpen. Secundaire eindpunten zijn het beoordelen van de veiligheid, toxiciteit, haalbaarheid en farmacokinetisch profiel van deze behandeling.

Een andere veelbelovende behandelstrategie voor patiënten die niet in aanmerking komen voor een operatieve behandeling is gerichte therapie. In **hoofdstuk vijf** voerden we een systematische literatuurstudie uit om het mutatielandschap van PeM in kaart te brengen en hiermee potentiële therapeutische targets te identificeren. Er werden 13 artikelen geïncludeerd, met in totaal 824 patiënten met PeM. In totaal waren 142 genen afwijkend in ≥1% van de patiënten, waarvan zeven genen afwijkend waren in ≥10%. *BAP1* was het meest voorkomende aangedane gen (50%).

Andere veelvoorkomende mutaties bevonden zich in *NF2* (25%), *CDKN2A* (23%), *CDKN2B* (17%), *PBRM1* (15%), *TP53* (14%) en *SETD2* (13%). In totaal was 17% van de patiënten met PeM drager van een kiembaanmutatie, voornamelijk in *BAP1* (7%). Dit percentage is hoger in vergelijking met de pleurale variant van mesothelioom. Kiembaanmutaties vormen waarschijnlijk een grotere bijdrage aan de incidentie van PeM dan eerder gedacht. Op basis van deze resultaten zijn de momenteel beschikbare gerichte therapieopties beperkt. Diverse gerichte middelen, zoals poly (ADP-ribose) polymerase (PARP)-remmers, enhancer of zeste homoloog 2 (EZH2)-remmers en cycline-afhankelijke kinase 4/6 (CDK4/6)-remmers, zouden in de toekomst nieuwe behandelopties kunnen bieden.

In **hoofdstuk zes** evalueerden we de waarde van next generation sequencing (NGS) van PeM tumorsample in de huidige klinische praktijk. Op tumormateriaal van 20 patiënten met PeM behandeld in het Erasmus MC Kanker Instituut werd Foundation Medicine F1CDx NGS verricht. Dit platform beoordeelt 360 genen die regelmatig somatisch gemuteerd zijn in solide tumoren en geeft een genetische handtekening. NGS was succesvol in 19 tumorsamples. Tumor mutational burden (TMB) was laag in 10 samples en in 11 samples was de tumor microsatelliet stabiel. In de andere gevallen konden de TMB en microsatellietstatus niet worden bepaald. Van de 19 geanalyseerde tumorsamples, werden BAP1-mutaties gedetecteerd in 32% van de samples, CDKN2A/B- en NF2-mutaties in 16%, en ATM in 11%. Voor patiënten met mutaties in NF2 of ATM zijn potentiële gerichte therapieën beschikbaar voor andere tumortypes (bijv. proteïnekinaseremmers voor drie NF2-gemuteerde tumoren en poly ADP-ribose-polymerase (PARP)-remmers voor twee ATM-gemuteerde tumoren). Deze therapieën zijn momenteel niet beschikbaar voor patiënten met PeM, maar lopende ontwikkelingen kunnen in de toekomst nieuwe behandelopties opleveren.

DEEL II – COLORECTALE PERITONEALE METASTASEN

Om gunstige uitkomsten na CRS-HIPEC te bereiken, is zorgvuldige selectie van patiënten essentieel. In **hoofdstuk zeven** beoordeelden we het effect van een lage skeletspiermassa (SMM) op de resultaten na CRS-HIPEC bij patiënten met colorectale PM of pseudomyxoma peritonei (PMP). SMM werd gemeten op computertomografie (CT) scans aan de hand van de L3-muscle index. Van de 284 geïncludeerde patiënten hadden 149 een lage SMM. Er was geen verschil in het aantal ernstige postoperatieve complicaties tussen de groepen (29% voor patiënten met lage SMM versus 34% voor patiënten met normale SMM). Een lage

SMM was niet geassocieerd met postoperatieve complicaties (p = 0.344). Voor patiënten met colorectale PM werden geen significante verschillen waargenomen in ziektevrije overleving (DFS) of algehele overleving (OS) tussen patiënten met lage (respectievelijk 7 maanden, IQR 4-14 en 33 maanden, IQR 14-NR) en patiënten met normale SMM (respectievelijk 8 maanden, IQR 5-20, en 35 maanden, IQR 18-NR). Voor patiënten met PMP verschilden de overlevingsresultaten niet significant tussen beide groepen (3-jaars DFS voor patiënten met lage SMM was 47% versus 55% voor patiënten met normale SMM, p = 0.676; de 3-jaars OS waren respectievelijk 71% en 91%, p = 0.172). We concludeerden dat een lage SMM geen voorspeller is van ernstige complicaties of overlevingsresultaten na CRS-HIPEC in patiënten met colorectale PM of PMP.

In **hoofdstuk acht** evalueerden we het effect van het moment van het ontstaan van PM (synchroon versus metachroon) op overlevingsresultaten na CRS-HIPEC. Er werd een retrospectieve cohortstudie uitgevoerd, waarin 390 patiënten met colorectale PM werden geïncludeerd die CRS-HIPEC ondergingen tussen 2010 en 2020 in twee academische centra in Nederland. Patiënten werden geclassificeerd als synchroon (s-PM: diagnose op het moment van presentatie, stadiëring of primaire chirurgie) of metachroon ontstaan (m-PM: diagnose tijdens de follow-up) van de colorectale PM. In totaal werden 179 patiënten (46%) geclassificeerd als s-PM. Deze patiënten hadden vaker een hoger TN-stadium en slechte differentiatie/zegelringcel histologie van de primaire tumor. Behandeling met perioperatieve chemotherapie kwam vaker voor bij patiënten met s-PM. Bij patiënten met m-PM was vaker sprake van een ernstigere postoperatieve complicatie (Clavien-Dindo ≥III). Er was geen significant verschil in de mediane DFS tussen s-PM (9 maanden, IQR 5-15) en m-PM-patiënten (8 maanden, IQR 5-17). Mediane OS was significant korter voor patiënten met s-PM (28 maanden, IQR 11-48) in vergelijking met patiënten met m-PM (33 maanden, IQR 18-66, p = 0.049). In een multivariate analyse was het moment van ontstaan van PM niet onafhankelijk geassocieerd met OS. We concludeerden dat het synchroon ontstaan van colorectale PM geassocieerd is met slechte tumorkenmerken en meer gevorderde ziekte, maar geen onafhankelijke voorspeller is van overlevingsresultaten na CRS-HIPEC.

Hoofdstuk negen geeft een overzicht van de behandeling en overlevingsresultaten voor patiënten die niet in aanmerking komen voor CRS-HIPEC. In totaal werden van 2014 tot 2020 476 patiënten met colorectale PM verwezen naar het Erasmus MC Cancer Institute voor CRS-HIPEC. Van deze patiënten werden er 227 (48%) afgewezen. Gegevens over vervolgbehandeling waren beschikbaar voor 198 patiënten, waarvan 73% systemische therapie kreeg. Deze patiënten hadden een

mediane OS van 17 maanden [IQR 9-25]. Voor patiënten die beste ondersteunende zorg (BSC) ontvingen, was de mediane OS 4 maanden [IQR 2-9]. De belangrijkste reden voor afwijzing was uitgebreide lokale ziekte (42%), met een mediane OS van 11 maanden [IQR 5-18]. Patiënten die ongeschikt werden geacht vanwege (uitgebreide) lever (9%) of longmetastasen (8%) hadden een langere mediane OS (respectievelijk 22 maanden, IQR 8-27, en 24 maanden, IQR 12-29) dan patiënten met uitgebreide lokale ziekte (11 maanden, IQR 5-18) of verre lymfekliermetastasen (14 maanden, IQR 4-25).

In **hoofdstuk tien** ontwikkelden we een predictiemodel voor het ontstaan van een recidief, om patiënten met colorectale PM die CRS-HIPEC ondergaan te identificeren die baat zouden kunnen hebben bij toevoeging van systemische therapie. We veronderstelden dat patiënten met een hoog risico op een systemische recidief baat zouden hebben bij deze toevoeging, terwijl dit mogelijk niet het geval is voor patiënten met een laag risico. Voor de ontwikkeling van het model werden data gebruikt van een retrospectief cohort, bestaande uit 408 patiënten met colorectale PM die CRS-HIPEC ondergingen in vier Nederlandse HIPEC-centra zonder perioperatieve systemische therapie. De voorspellers die in het model werden gebruikt waren geslacht, leeftijd, locatie van de primaire tumor, differentiatie- en lymfeklier status, aanwezigheid van synchrone levermetastasen, PCI bij CRS-HIPEC, preoperatieve CEA en mutatiestatus. Het intern gevalideerde voorspellingsmodel voor een recidief toonde een redelijke discriminatie, gebaseerd op een C-index van 0.64 (95% CI 0.62, 0.66). De waarde van dit model lijkt beperkt in het selecteren van patiënten die baat hebben bij perioperatieve systemische therapie naast CRS-HIPEC. Omdat het model momenteel de enige beschikbare tool is voor het preoperatief voorspellen van een recidief in deze populatie kan het bijdragen aan de klinische besluitvorming. Vervolgstudies moeten zich richten op de evaluatie van de bruikbaarheid van deze modellen en de identificatie van nieuwe predictie factoren.

Chapter 12

General discussion and future perspectives

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The current thesis focused primarily on two types of peritoneal surface malignancies (PSM): peritoneal mesothelioma (PeM), a type of primary PSM, and peritoneal metastases (PM) originating from colorectal carcinoma (CRC), a common cause of secondary PSM. Despite significant differences, they share an aggressive nature, resulting in unfavorable prognoses. Cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has the potential to improve survival, but recurrence rates are high and not all patients are eligible for this extensive treatment. For those ineligible, the current treatment options are limited.

In part I of this thesis we aimed to improve outcomes of patients with PeM. We focused on improving outcomes after CRS-HIPEC and the exploration of new therapeutic options. Part II of this thesis focused on the optimization of patient selection for CRS-HIPEC in patients with colorectal PM.

PART I: PERITONEAL MESOTHELIOMA

The survival rate of patients with PeM in the Netherlands has only slightly improved over the past decades.[1] The introduction of CRS-HIPEC enhanced prognosis for selected patients, but recurrence rates remain high.[2] Due to strict patient selection, the majority of patients with PeM is not eligible for CRS-HIPEC. A large proportion of these patients receive no anti-tumor treatment due to the lack of effective treatment options. Clearly, there is a need to advance both the surgical and the palliative treatment for patients with PeM. The first important step towards this goal was the centralization of mesothelioma care in two officially acknowledged expert centers in The Netherlands: the Dutch Cancer Institute (NKI) and the Erasmus MC Cancer Institute (EMC). This resulted in an increase in annual referrals and the number of patients receiving treatment at these centers.[2]

Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy – Perioperative therapy

To improve the surgical outcomes of patients with PeM, we aimed to explore the feasibility of combining CRS-HIPEC with adjuvant immunotherapy (**chapter 2**).[3] Dendritic cell-based immunotherapy (DCBI) in the form of 'MesoPher' aims to induce an immune response through tumor antigen presentation by DC's.[4] We hypothesized that adjuvant DCBI after CRS-HIPEC could lower the risk of disease recurrence by initiating a long-term anti-tumor immune response. In **chapter 2**,

we demonstrated that DCBI adjuvant to CRS-HIPEC is a feasible treatment strategy. Toxicity related to DCBI was limited, which has also been reported for patients with pleural mesothelioma and pancreatic cancer.[5, 6] In line with preclinical data, the efficacy of DCBI seemed to be related to the tumor load.[7] The presence of proliferating effector T cells correlated to progression-free survival, which was longer in patients in whom complete cytoreduction was performed. This points towards a role of an immunosuppressive tumor microenvironment (TME) in patients with residual disease.

Studies examining the TME of mesothelioma have indeed reported the presence of various regulatory and inhibitory cells, potentially hampering an effective anti-tumor response.[8, 9] Investigation of the TME following DCBI could provide more insight into its effectiveness and possible resistance mechanisms. In **chapter 2,** we solely evaluated the systemic immune response, as DCBI was given as an adjuvant treatment after CRS-HIPEC. To gather more information regarding the efficacy, administering DCBI as a neo-adjuvant therapy could be considered, alongside analyzing tumor biopsies in case of recurrence or progression. For patients with pleural mesothelioma, we are currently performing a phase I trial to determine the feasibility of the combination of neo-adjuvant and adjuvant DCBI with Extended-Pleurectomy/Decortication (NCT05304208). Besides the opportunity to investigate the TME up on DCBI, we hypothesized that this approach might also facilitate complete resection as DCBI has demonstrated the potential to reduce tumor load.[4] In **chapter 3,** we present a case report of the first patient who successfully completed this treatment. An increase in the number of tumor-infiltrating cytotoxic T cells and tertiary lymphoid structures was detected, suggesting the initiation of a DCBI-induced anti-tumor immune response. While these preliminary results are promising, more data must be awaited before firm statements can be made.

Based on the findings from **chapter 2,** it is likely that additional combination strategies will be necessary to enhance the efficacy of DCBI, particularly in cases with residual disease. A promising approach to overcome immune suppression is the combination of DCBI with a checkpoint inhibitor (CPI). Gulijk et al. reported a synergistic effect of concurrent treatment with DCBI and CPI in mice and showed that sequential treatment in patients with pleural mesothelioma was safe.[10] Future clinical studies should also explore the safety and efficacy of concurrent treatment with DCBI and CPI.

Another approach is activation of CD40, which is a key regulator in T cell antitumor immunity. CD40 agonists can induce antitumor T cell responses and can improve

T cell infiltration by altering the TME.[11, 12] The efficacy of CD40 agonists is reliant to the presence of DC primed T cells, providing a rationale for combination with DCBI.[13] Currently, a phase I study is investigating concurrent treatment with DCBI and a CD40 agonist in patients with metastatic pancreatic cancer.[14] If this therapy is found to be feasible and safe, this should be also explored as a perioperative treatment for patients with PeM undergoing CRS-HIPEC, but might also be beneficial for patients who are ineligible for surgery.

Local treatment strategies

The majority of patients with PeM are diagnosed in an advanced stage in which CRS-HIPEC is not feasible.[2] The standard treatment for these patients is systemic chemotherapy, which is often ineffective and is associated with substantial morbidity.[15, 16] This probably explains why the majority of patients receive no anti-tumor treatment at all.[1] One of the strategies that we explored to provide these patients with an effective treatment option is local treatment in the form of intraperitoneal (IP) chemotherapy. PSMs in general are perceived as relatively resistant to systemic therapy because of a peritoneum-blood barrier.[17, 18] Administering chemotherapy directly on-site could therefore be more effective. A recent phase I dose-finding trial explored the use of IP chemotherapy concurrent to a standard systemic regimen in patients with colorectal PM.[19] This study reported similar toxicity compared with systemic treatment and showed promising responses in some patients.[20] We aim to explore this approach in the INTERACT MESO trial, of which the study protocol is presented in **chapter 4**. Since PeM less commonly disseminates systemically compared to CRC, we hypothesized that IP mono-chemotherapy might be a more suitable approach. Omitting systemic chemotherapy will likely reduce toxicity and improve quality of life, which is of utmost importance in a palliative setting. Paclitaxel was chosen as the chemotherapeutic agent, which is considered favorable for IP use because of its large molecular weight and lipophilic properties.[21, 22] Sugarbaker reported favorable outcomes in a small number of patients who underwent CRS-HIPEC followed by IP paclitaxel, including patients with an incomplete cytoreduction.[23, 24] A possible additional advantage of this approach is the drainage of ascites, a common and major quality of life limiting symptom in patients with PeM.[25] This can be performed via the IP access port and avoids repeated drainage interventions. The INTERACT MESO trial has currently enrolled its first patients.

Another noteworthy local treatment strategy is pressurized intraperitoneal aerosol chemotherapy (PIPAC).[20] PIPAC is administered during laparoscopy and intends to enhance drug distribution and penetration into peritoneal tumor deposits. A small

French cohort study conducted PIPAC alternating with systemic chemotherapy in 26 patients with unresectable PeM, with a median of three procedures per patient.[26] Radiological responses were limited, but in 14 patients CRS was performed after PIPAC treatment. This resulted in complete cytoreduction in 13 patients, with a median PFS of almost 34 months. While these outcomes are promising, it should be noted that PIPAC requires serial laparoscopy and is accompanied by the risk of complications, high patient burden, and related costs. An ongoing phase II multicenter RCT is evaluating the efficacy of PIPAC, comparing PIPAC with systemic chemotherapy to systemic chemotherapy alone as first-line treatment for patients with PeM (NCT03875144).[27] This trial will provide essential data on the effectiveness of PIPAC, which is needed to evaluate the possible benefit for patients with PeM.

Systemic treatment strategies

For patients with PeM who develop systemic metastases, or patients who do not respond to local therapy, systemic treatment is indicated. Immunotherapy with CPI has been an important breakthrough for several advanced malignancies. The benefit of CPI for patients with mesothelioma appears less evident.[28] The recent Checkmate 743 trial by Baas et al. showed modest responses to combination CPI therapy with nivolumab and ipilimumab in patients with pleural mesothelioma.[29] For a subset of patients this therapy seems promising, as some long-term responders were observed. The efficacy was more pronounced in patients with a non-epithelioid subtype of mesothelioma, who generally respond poorly to chemotherapy.[30] For patients with the most common, epithelioid, subtype of mesothelioma, the median overall survival (mOS) improved with only two months. Despite this marginal survival benefit, combination CPI therapy is currently the first-line treatment for patients with pleural mesothelioma in the Netherlands.

For the treatment of PeM, CPI therapy is not (yet) included in the current guidelines. Especially for patients with a sarcomatoid subtype of PeM, who are by definition ineligible for surgery, this could be a promising new treatment. For patients with an epithelioid subtype of PeM, it is questionable whether CPI is the appropriate treatment. CPI therapy is accompanied by substantial morbidity.[28] To avoid ineffective treatment with the risk of toxicity, careful patient selection is required. Reliable biomarkers to guide this selection are currently lacking. PD programmed death ligand 1 (PD-L1), tumor mutational burden (TMB), and microsatellite instability (MSI) have been studied, but their value seems limited.[31] In line with previous studies, we found that all PeM tumors in which MSI and TMB could be determined were microsatellite stable (MSS) and showed low TMB (**chapter 6**). [32-36] This might

partially explain the limited efficacy of CPI in mesothelioma. Future studies should explore novel predictive biomarkers, as a subset of patients could gain substantial benefit from CPI therapy.

Targeted therapies

Another type of cancer treatment that requires careful patient selection is targeted therapy. Targeted therapies have significantly changed the prognosis of patients with various malignancies and specific molecular profiles. For rare cancers like PeM, access to these drugs has been very limited due to the absence of data on the mutational landscape and challenges in the conduct of clinical trials.[37] To provide a comprehensive overview of the mutational landscape of PeM and the potential targets for therapy, we performed a scoping review of high-throughput sequencing studies (**chapter 5**). In **chapter 6**, we evaluated the benefit of mutational profiling in current practice by performing genomic characterization for a small cohort of patients with PeM. Based on the findings of these chapters, there are currently no targeted drugs accessible for patients with PeM. We did identify a range of drugs that may provide therapeutic options in the future.

A promising therapy is poly (ADP-ribose) polymerase (PARP) inhibition. PARP inhibitors are approved by the European Medicines Agency (EMA) for the treatment of several advanced malignancies and they target the DNA damage response (DDR) pathway.[38] This pathway plays an important role in the etiology of PeM, involving genes such as *BAP1*, *PBRM1*, and *SETD2* (altered in ~50%, 15%, and 13% of patients respectively).[39] PARP inhibitors could also be effective in *ATM* aberrant tumors. Aberrations in *ATM* were present in two (11%) of the patients in our own cohort but were uncommon in our review. For patients with (pleural) mesothelioma, two trials have investigated the efficacy of PARP inhibitors, reporting conflicting results.[40, 41] One of these trials did not include patients based on their mutational profile, which could have affected the outcomes and underlines the importance of patient selection.[41] An ongoing phase II trial, including patients with PeM and alterations in the DDR, will provide more information on the potential benefit of this therapy (NCT04515836). Another therapy of interest for BAP1-altered malignancies is enhancer of zeste homolog 2 (EZH2) inhibition. Loss of *BAP1* results in upregulation of EZH2 and a recent phase II trial with tazemetostat, an EZH2 inhibitor, showed promising results in a subset of patients with *BAP1*-altered PeM.[42, 43] Tazemetostat got an orphan designation by the EMA for treatment of mesothelioma, but is not yet available for patients in the Netherlands and additional biomarkers to select patients who benefit from this therapy need to be identified.[44]

Two other noteworthy drugs are inhibitors of mammalian target of rapamycin (mTOR) and cyclin-dependent kinase 4/6 (CDK4/6). mTOR inhibitors are approved by the EMA for the treatment of several *NF2-altered* tumors.[44] Although *NF2* alterations are common in mesothelioma (~25% in PeM), clinical trials that are studying the efficacy of mTOR inhibitors are scarce.[45] For patients with *CDKN2A/B* altered tumors (~23%), *CDK4/6* inhibitors could be beneficial.[46-48] This therapy is currently being investigated in one of the arms of the British MiST trial (NCT03654833). This multi-arm trial aims to accelerate the availability of targeted drugs for patients with mesothelioma by stratification based on the molecular profile. A similar, but pan-cancer trial, is the Dutch DRUP trial (NCT02925234). This trial currently only very limited allows inclusion of patients with mesothelioma, but could aid in facilitating access to new treatment options for patients with PeM in the future[49]. Lastly, it should be noted that while targeted therapies offer promising advantages in terms of specificity and efficacy, they are associated with high costs and do not devoid toxicity. The cost-benefit of mutational analysis should therefore be regularly reevaluated.

Besides the therapeutic consequences, molecular profiling adds to the understanding of the etiology underlying PeM. In **chapter 5**, we reported that 17% of the patients were carriers of a germline mutation. This is higher compared to pleural mesothelioma (approximately 7%), supporting the hypothesis of a distinct etiology.[50, 51] The majority of germline mutations in PeM were located in *BAP1* (7%), causing a tumor predisposition syndrome that is associated with an early onset of several malignancies, such as (uveal) melanoma, renal cancer, and PeM.[52] Another significant proportion of PeM cases is caused by rarely occurring germline mutations.

The detection of these germline mutations also poses several challenges, particularly regarding possible screening. According to the current Dutch guideline, referral to a clinical geneticist should be considered for patients with mesothelioma who are below the age of 60, have a history of one or more BAP1-related malignancies, or have family members with BAP1-related malignancies or hereditary breast cancer/melanoma.[53] Distinct guidelines on screening for (peritoneal) mesothelioma in patients with a germline mutation are currently lacking. Further investigation about the role of germline mutations in PeM etiology, especially for the more uncommon types, and the potential role of screening is warranted.

PART II: COLORECTAL PERITONEAL METASTASES

Patients with colorectal PM generally have a worse prognosis compared to patients with systemic colorectal metastases.[54] However, colorectal PM reflect a distinct, 'locoregional', type of metastasis, offering the potential for a treatment with curative intent (CRS-HIPEC). CRS-HIPEC is currently the standard treatment option for selected patients with colorectal PM, with promising survival outcomes in the most recent RCT (mOS 41 months).[55]

Cytoreductive surgery with intraperitoneal chemotherapy – Patient selection

To obtain these outcomes, patient selection is key. Which factors should be used in this patient selection remains an important subject of discussion. According to Dutch guidelines, factors such as fitness for major surgery, the extent of local and systemic disease, and tumor histology should be involved.[56] Yet, a significant proportion of patients do not appear to gain substantial benefit from CRS-HIPEC, reflected by high recurrence rates.[55, 57] Additional prognostic factors have been proposed by literature, but their value remains unclear, warranting further research.[58, 59]

The extent of CRS-HIPEC results in significant morbidity, with severe postoperative complications in about one third of the patients.[55, 60, 61] While this risk is more acceptable in patients with long-term survival benefit, the occurrence of these complications is also associated with impaired survival outcomes.[62, 63] Identification of 'high risk' patients could aid in patient selection for CRS-HIPEC but could also help identify patients who might benefit from interventions like prehabilitation. This can in turn lower the risk of complications, thereby improving short- and long-term outcomes after CRS-HIPEC. A factor that has been associated with complications after general colorectal surgery is sarcopenia, which is mainly defined by the loss of skeletal muscle mass (SMM).[64-66] We hypothesized that low SMM could also be a valid predictor for patients who undergo CRS-HIPEC. In a retrospective cohort study, we showed that low SMM was not associated with long- or short-term outcomes (**chapter 7**). This is probably explained by an already very selected patient population based on fitness for major surgery. Although there are no strict cut-off values to determine this fitness for major surgery, an estimation is made based on factors like age, comorbidities, and performance status to exclude 'frail' patients.[56] Low SMM can be an expression of frailty, yet it is probably not an independent factor to predict surgical outcomes.

Another potential prognostic factor is the timing of the onset of PM.[67-69] We hypothesized that the synchronous onset (i.e. concurrent presentation with the primary tumor) of colorectal PM is a negative prognostic factor. In a retrospective cohort study, we indeed found that synchronous onset was associated with impaired OS compared to metachronous onset, yet it was not an independent prognostic factor (**chapter 8**). Synchronous onset of PM seems to be reflecting a more aggressive tumor biology, but factors like lymph node positivity and poor primary tumor differentiation are more valuable predictors of outcomes after CRS-HIPEC. The time of onset of PM should therefore not be used as a factor in patient selection but could be taken in consideration in the absence of more accurate factors.

As CRS-HIPEC is a local treatment, the presence of systemic metastases is considered a contraindication. In **chapter 9**, we provided an overview of patients with colorectal PM who were deemed ineligible for CRS-HIPEC. Not entirely surprising, patients who were deemed ineligible for CRS-HIPEC due to extensive PM showed impaired survival outcomes compared to patients who were deemed ineligible due to systemic metastases (i.e., liver or lung). This is in line with previous studies and supports the hypothesis that prognosis is mainly determined by the extent of PM and not by the presence of systemic metastases.[54, 70, 71] Selected patients with limited systemic metastases could gain benefit from CRS-HIPEC, possibly combined with systemic chemotherapy.[56, 72]

The role of systemic chemotherapy around CRS-HIPEC poses another topic for discussion. Systemic chemotherapy is considered relatively ineffective for PSM, limiting its value in the prevention of local recurrence.[73] The addition of systemic chemotherapy could be beneficial to eradicate occult metastases, since systemic recurrence after CRS-HIPEC is not uncommon.[74] To avoid unnecessary treatment, identification of patients with a high risk of these occult metastases is needed. Previous studies were unable to identify factors that predict systemic recurrence after CRS-HIPEC, but these studies included patients who received perioperative systemic chemotherapy.[75, 76] In **chapter 10**, we aimed to develop a prediction model for recurrence in a patient population that did not receive perioperative systemic chemotherapy. We could not accurately predict (systemic) recurrence based on factors such as PCI, nodal stage, synchronous liver metastases, tumor differentiation, and mutational status. Although the models could predict the proportion of patients are likely to develop (systemic) recurrence, it could not identify high-risk patients. The latter would be needed to guide patient selection. Based on these results, the addition of systemic chemotherapy might be indicated for all patients with colorectal PM undergoing CRS-HIPEC. The CAIRO-6 trial is investigating this

approach in a randomized setting and will provide more information about the role of perioperative systemic therapy soon (NCT02758951).[77]

While the role of perioperative systemic chemotherapy remains unclear, the efficacy of HIPEC is also being questioned since the results of the PRODIGE 7 trial were published.[55] This trial reported no survival benefit from the addition of HIPEC to CRS, while it did result in a higher postoperative complication rate. As applies to perioperative systemic chemotherapy, the addition of HIPEC might not be beneficial for all patients. This once again underscores that patient selection is key, but there are some additional remarks that deserve some attention. The PRODIGE 7 trial consisted of a highly selected patient population that was heavily pretreated with oxaliplatin-based systemic therapy. Although the mOS was impressive, mPFS was less impressive (i.e., 11 – 13 months), indicating probable selection of patients who benefit from systemic therapy. This systemic chemotherapy regimen could also have induced platinum resistance, affecting the efficacy of the oxaliplatin-based HIPEC.[78, 79] Lastly, oxaliplatin is associated with a higher rate of severe postoperative complications compared to mitomycine-C (MMC).[78] A phase IV randomized clinical trial is currently investigating the benefit of MMC-based HIPEC, which might be a more suitable regimen (NCT05250648).[80] This trial also combines CRS +/- HIPEC with systemic chemotherapy, making it challenging to determine the potential efficacy of HIPEC. For patients who respond well to systemic chemotherapy, the benefit of HIPEC could be limited. However, there might be a subset of patients who do gain benefit from HIPEC. Therefore, future studies should not only focus on refining the criteria for patient selection for CRS but should also focus on selection for perioperative systemic chemotherapy and/or HIPEC.

CONCLUSIONS AND FUTURE PERSPECTIVES

Within this thesis, we aimed to optimize current surgical treatment strategies and identify possible new treatment strategies for patients with PeM and colorectal PM, with the ultimate goal to improve survival outcomes and quality of life. For patients with PeM who are eligible for surgery, we aimed to improve outcomes by demonstrating the feasibility of DCBI as an adjuvant treatment after CRS-HIPEC. The modest response, especially in patients with residual disease, requires the exploration of additional combination strategies. Further optimization of treatment strategies around CRS-HIPEC could enhance its curative potential and might even broaden eligibility for this therapy in the future.

For patients with PeM who are ineligible for surgery, systemic and local treatment strategies should be further explored. The ongoing INTERACT MESO trial will provide valuable insights in the utility of IP paclitaxel monotherapy, holding significant potential for these patients. If proven effective, this treatment approach could potentially even serve as a neo-adjuvant option, enabling CRS-HIPEC for selected patients who initially do not qualify for treatment with curative intent.

For patients with PeM and specific molecular profiles, targeted therapies could offer a solution. To accelerate availability for rare malignancies like PeM, research should focus on molecular alterations rather than tumor type or location. While targeted therapies hold significant potential for selected patients, it is essential to emphasize accurate patient selection as they are accompanied by high costs and potential toxicity.

Patient selection was also the focus of our research for patients with colorectal PM. Our objective was to enhance postoperative outcomes by optimizing patient selection for both CRS-HIPEC as for the addition of perioperative systemic therapy. Although we did not identify any new predictive factors that provide additional value to current patient selection criteria, we believe that future studies should explore novel predictive factors that can be assessed before surgery. These factors should ultimately be used to guide comprehensive treatment strategies, including CRS with either HIPEC and/or systemic chemotherapy. Refinement of patient selection will improve outcomes of this extensive treatment but will also aid in shared decision-making and the avoidance of unnecessary patient burden.

A final strategy that is outside the scope of this thesis, yet deserves some attention, is early-stage diagnosis. The extent of local disease is a critical factor that limits treatment options and impairs survival for patients with PeM and colorectal PM. The diagnosis of PSM in general remains a major challenge, but new methods, such as MRI and 68Ga-FAPI PET/CT, hold significant promise.[81-83] Before implementation in daily care, further investigation is warranted, especially regarding their cost-effectiveness.

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Appendices

List of publications
Contributing authors
PhD Portfolio
Acknowledgements / Dankwoord
About the author

LIST OF PUBLICATIONS

This thesis

Dietz MV, van Kooten JP, van Vugt JLA, Brandt-Kerkhof ARM, Verhoef C, Madsen EVE. The Impact of Low Skeletal Muscle Mass on Short- and Long-Term Outcomes After Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy. Ann Surg Oncol. 2022;29(9):5830-41.

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Dietz MV, Madsen EVE. ASO Author Reflections: The Timing of Onset of Peritoneal Metastases from Colorectal Carcinoma Is Not an Independent Predictor of Survival Outcomes After Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy. Ann Surg Oncol. 2022;29(11):6577-8.

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PHD PORTFOLIO

PhD Candidate:	Michelle Valérie Dietz
Erasmus MC Departments:	Surgical Oncology and Gastrointestinal Surgery, Pulmonary Medicine
PhD Period:	May 2021 – October 2023
Promotor:	Prof. dr. C. Verhoef, Prof. dr. J.G.J.V. Aerts
Co-promotor:	Dr. E.V.E. Madsen

PhD Training	Year	ECTS
Academic & research skills		
ICH-GCP training	2021	1.0
BROK 'Basiscursus regelgeving Klinisch Onderzoek'	2021	1.5
ESP72 Joint Models or Longitudinal and Survival Data	2021	0.7
The basic course on R	2021	1.8
Basistraining Castor	2021	0.3
Biostatistics I (CK020S)	2022	2.0
Scientific Integrity	2022	0.3
Biomedical writing course	2022	1.5
Advanced Immunology	2022	1.0
Logistic Regression	2023	1.4
Paint 3D		
Oral presentations		
Survival outcomes after Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in Patients with Synchronous versus Metachronous onset of Peritoneal Metastases of Colorectal Carcinoma. 40th ESSO, Lissabon, Portugal.	2021	1.0
TEIPP-targeting immunotherapy in patients with relapsed advanced non-small cell lung cancer. 3th ACE TI-IT, Rotterdam, The Netherlands.	2022	1.0
Behandeling en Overleving van patiënten die niet in aanmerking komen voor Cytoreductieve Chirurgie (CRS) met Hypertherme Intraperitoneale Chemotherapie (HIPEC). NVvH Chirurgendagen, The Hague, The Netherlands.	2022	1.0
TEIPP-targeting immunotherapy in patients with relapsed advanced non-small cell lung cancer. Wetenschapsdag longgeneeskunde, Rotterdam, The Netherlands.	2022	1.0
Adjuvante dendritische-cel-immuuntherapie (DCBI) na cytoreductieve chirurgie (CRS) en hypertherme intraperitoneale chemotherapie (HIPEC) voor patiënten met maligne peritoneaal mesothelioom: een open-label klinische fase II-studie. NVvH Chirurgendagen, Veldhoven, The Netherlands.	2023	1.0
Adjuvant dendritic cell-based immunotherapy after surgery and hyperthermic intraperitoneal chemotherapy for malignant peritoneal mesothelioma. 16th IMIG conference, Lille, France.	2023	1.0

	Year	ECTS
Poster presentations		
The Impact of Low Skeletal Muscle Mass on short and long term Outcomes after Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy 40th ESSO, Lisbon, Portugal.	2021	0.5
Molecular aberrations and potential actionable mutations in Malignant Peritoneal Mesothelioma: A scoping review of high throughput sequencing studies 41st ESSO, Bordeaux, France.	2022	0.5
Treatment and Survival Outcomes of Patients with Colorectal Peritoneal Metastases deemed Ineligible for Cytoreductive Surgery (CRS) with Hyperthermic Intraperitoneal Chemotherapy (HIPEC): Results of a Retrospective Study 41st ESSO, Bordeaux, France.	2022	0.5
Adjuvant dendritic cell-based immunotherapy after surgery and hyperthermic intraperitoneal chemotherapy for malignant peritoneal mesothelioma. Erasmus MC Cancer Retreat, Rotterdam, The Netherlands.	2023	0.5
Evaluation of body composition at L3 from computed tomography (CT) images using DAFS software, a 3D fully automated multi-slice multi-organ extraction platform. 16th International Cachexia Conference, Stockholm, Sweden.	2023	0.5
Teaching		
Supervising internship & master thesis Erasmus MC L. Janssen	2022	2.0
Supervising master students	2022	1.0
Surgery lecture medical interns	2023	0.3
Conferences		
40th ESSO Congress, Lissabon, Portugal.	2021	0.9
3rd ACE TI-IT, Rotterdam, The Netherlands.	2022	0.6
NVvH Chirurgendagen, The Hague, the Netherlands.	2022	0.6
Dutch Fluorescence Guided Surgery Symposium, Bussum, The Netherlands	2022	0.3
Wetenschapsdag longgeneeskunde, Rotterdam, the Netherlands.	2022	0.3
41st ESSO Congress, Bordeaux, France.	2022	0.9
1st Erasmus MC Cancer Institute Research Retreat, Rotterdam, the Netherlands.	2023	0.6
NVvH Chirurgendagen, Veldhoven, the Netherlands.	2023	0.6
16th iMig conference, Lille, France.	2023	0.9
Awards	**Year**	
iMig Young Investigator Award	2023	-
Grants	**Year**	**€**
Travel grant Stichting Sacha Swarttouw-Hijmans	2023	1000
Travel grant Erasmus Trustfonds	2023	2000
Travel grant Stichting Prof. Michaël-van Vloten Fonds	2023	2500
Other	**Year**	**ECTS**
Immunology consortium Erasmus MC	2023	1.0

ABOUT THE AUTHOR

Michelle Valérie Dietz, born on November 9th, 1994, in Leiderdorp, the Netherlands, is the eldest daughter of Egon Dietz and Greetje Frankena and the sister of Céline Dietz. Her educational journey began at the Davinci college in Leiden, where she completed her secondary education. After graduating in 2012, she started a bachelor's degree in biomedical sciences at the University of Amsterdam. In 2014, Michelle transitioned to medical training at the Erasmus University Medical Center in Rotterdam, marking the beginning of her immersion in the world of medicine. In 2017, she successfully obtained both bachelor's degrees, paving the way for her to commence a master's degree in medicine. It was during her surgical internship at the Maasstad Hospital in Rotterdam that Michelle developed a profound interest in surgery, a passion that deepened further during her master's thesis at the Department of Surgical Oncology in the Erasmus MC. After she obtained her medical degree in 2021, the opportunity arose to pursue a PhD in the field of peritoneal malignancies. From May 2021 to October 2023, Michelle worked full-time on her PhD-trajectory, as described in this thesis, under the guidance of dr. E.V.E. Madsen, Prof. dr. C. Verhoef, and Prof. dr. J.G.J.V. Aerts. Since October 2023, Michelle has been doing a five-month research internship at the St George hospital in Sydney, Australia, under the esteemed supervision of Prof. dr. David Morris. Following the defense of her thesis, she will commence work as a surgical resident not-in-training in the IJsselland hospital.